

RESEARCH ARTICLE

Hidden diversity in high-latitude Southern Hemisphere environments: Reinstatement of the genus *Rama* and description of *Vandenhoeikia* gen. nov. (Cladophoraceae, Ulvophyceae, Chlorophyta), two highly variable genera

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Abstract

The continental coasts and remote islands in the high-latitude Southern Hemisphere, including the subantarctic region, are characterized by many endemic species, high abundance of taxa, and intermediate levels of biodiversity. The macroalgal flora of these locations has received relatively little attention. Filamentous green algae are prolific in the intertidal of southern islands, but the taxonomy, distribution, and evolutionary history of these taxa are yet to be fully explored, mostly due to the difficulty of access to some of these locations. In this study, we examined specimens of the order Cladophorales from various locations in the high-latitude Southern Hemisphere including the subantarctic (the Auckland Islands, Bounty Islands, Campbell Island, Macquarie Island, and Kerguelen Islands), as well as mainland New Zealand, the Chatham Islands, Chile, and Tasmania. The analyses of the rDNA sequences of the samples revealed the existence of two new clades in a phylogeny of the Cladophoraceae. One of these clades is described as the novel genus *Vandenhoeikia* gen. nov., which contains three species that are branched or unbranched. The amended genus *Rama* is reinstated to accommodate the other clade, and contains four species, including the Northern Hemisphere "*Cladophora rupestris*." In *Rama* both branched and unbranched morphologies are found. It is remarkable that gross morphology is not a predictor for generic affiliations in these algae. This study illustrates that much can still be learned about diversity in the Cladophorales and highlights the importance of new collections, especially in novel locations.

KEYWORDS

biogeography, Chile, Cladophorales, New Zealand, phylogeny, rDNA sequences, Southern Ocean, taxonomy

INTRODUCTION

Many parts of the planet remain poorly explored, even more so when it comes to seaweed diversity. One such area is the Southern Ocean (Antarctic Ocean)

and surrounding waters (cold-temperate islands of the major ocean basins). This region harbors extraordinary locations, some of them extremely remote and inhabited by numerous unique taxa (Chown et al., 1998; Convey & Stevens, 2007; Hommersand et al., 2009;

Abbreviations: bp, basepair; ITS, internal transcribed spacer; LSU, large subunit; rDNA, ribosomal DNA; SSU, small subunit.

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Pellizzari et al., 2017). Despite the isolation of many of the locations in the high-latitude Southern Hemisphere, many of the higher-latitude locations are connected via the west wind drift/Antarctic Circumpolar Current (Waters, 2008). While there are endemic seaweed species in these locations, they also share cold-temperate species with southern South America, the southern tip of South Africa, New Zealand, and the islands of the South Atlantic (Adams, 1994). Although some progress has been made in recent years (e.g., Boedeker et al., 2010; Broom et al., 2010; Hommersand, 2007; Nelson et al., 2022; Nelson & Broom, 2010; Oliveira et al., 2020; Pellizzari et al., 2020; Sanches et al., 2016), the algal assemblages of these regions remain understudied, and novel collections and taxonomic treatments would be very useful for appreciating their diversity and evolutionary relationships.

The early expeditions led to the description of numerous algal species, many now of uncertain taxonomic status. In particular, the treatments of Harvey and Hooker (1845) and Harvey (1855) provided the basis of the algal taxonomy of subantarctic environments. Additional early contributions were made by Grunow (1867), Rabenhorst (1878), Reinsch (1888, 1890), Skottsberg (1907), Svedelius (1900), Gain (1912), Cotton (1915), Taylor (1939), and Lindauer (1947).

While old species names are difficult to apply to specimens in many groups of algae, this is a particular challenge in the green algal order Cladophorales due to the lack of diagnostic morphological characters, extensive phenotypic plasticity, cryptic diversity, and parallel or convergent evolution (Boedeker et al., 2012, 2016, 2018; Leliaert et al., 2007; van den Hoek, 1963). The combination of these features has resulted in persistent taxonomic confusion in this group of branched or unbranched filamentous algae at all levels, from species and genera to families and orders. Progress in developing a taxonomy that reflects evolutionary history has been made by the application of molecular methods, and the resulting phylogenies have led to the discovery of polyphyletic genera and subsequent drastic changes in the taxonomy of the order (Boedeker et al., 2012, 2016; Leliaert, Rueness, et al., 2009; Leliaert, Verbruggen, et al., 2009). Instead of the three genera traditionally recognized in the Cladophoraceae based on gross morphology (*Cladophora* with branches, unbranched *Chaetomorpha*, and *Rhizoclonium* unbranched with lateral rhizoids), there are currently seven genera placed in the family based on molecular data: *Cladophora*, *Chaetomorpha*, *Rhizoclonium*, *Lurbica*, *Lychaete*, *Pseudorhizoclonium*, and *Willeella* (Boedeker et al., 2016; Wynne, 2016, 2017). At the species level, one of the challenges is how to connect previously described species to evolutionary lineages in order to decide on species epithets and synonyms, for which diagnostic characters are needed. In morphologically simple organisms, such as, for example, in the

genetically diverse genus *Rhizoclonium*, there is a limit to the number of species that can have a unique morphology and that can be successfully diagnosed based on observable characters (Boedeker et al., 2016, 2018; Verbruggen, 2014). For a comprehensive analysis of any regional flora, the most seriously limiting factor is the level of systematic knowledge (Adams, 1994; Hurd et al., 2004; Nelson, 1994; Parsons, 1985a, 1985b). This is particularly true for remote high-latitude sites where familiarity with the flora is limited and sporadic due their locations, and especially true for the taxonomically challenging order Cladophorales. Previously published species lists have included species names without critically reviewing them or without in-depth taxonomic analysis (e.g., Boraso de Zaixso, 2004; Gallardo et al., 1999; Papenfuss, 1964; Pellizzari et al., 2017).

The genus *Rama* had been described to accommodate subantarctic taxa that combined the morphological features of *Cladophora* and *Rhizoclonium*, mostly unbranched filaments that produce lateral rhizoids, but also true branches in the basal parts of the thalli (Chapman, 1952). The type species, *Rama novae-zelandiae*, was described from Otago, southern New Zealand. It was later synonymized with *Rhizoclonium ambiguum* by Chamberlain (1965). *Rama antarctica* was described from Campbell Island and was also reported from the Snares and Auckland Islands, and it was recognized as a separate species based on its larger cell diameter. This species was accepted by some authors (Boras de Zaixso, 2004) but not by others (Adams, 1994). The third species of the genus, *Rama longiarticulata*, is unbranched but has been included in *Rama* rather than in *Rhizoclonium* because of a true branch that was reported by Agardh (1878) but that was not observed by Chapman (1952). This species has not been mentioned in the literature since. The genus, therefore, was overall not accepted or used.

In this study, we focused on sampled members of the Cladophoraceae from subantarctic environments to gain, with the aid of molecular data, an understanding of their taxonomy and distribution in these remote areas. We reinstate and emend the genus *Rama* and describe a novel genus, *Vandenhoekia* gen. nov.

MATERIALS AND METHODS

Taxon sampling and morphological identification

Specimens for this study were collected from various subantarctic locations—including Auckland Islands (NZL), Bounty Islands (NZL), Campbell Island (NZL; see Figure S1 in the Supporting Information), Macquarie Island (AUS), Kerguelen Islands (FRA), Falkland Islands—and from mainland New Zealand, the Chatham Islands (NZL), cold-temperate Chile,

and Tasmania (AUS; [Table S1](#) in the Supporting Information). The samples were preserved in silica gel, ethanol, or formaldehyde or as pressed herbarium specimens or a combination of those methods. Specimens were identified morphologically using a light microscope according to various taxonomic treatments (namely, Adams, [1994](#); Chapman, [1952](#), [1956](#); Hoffmann & Santelices, [1997](#); Ricker, [1987](#); Santelices, [1989](#); van den Hoek, [1963](#), [1982](#); van den Hoek & Chihara, [2000](#); Womersley, [1984](#)) and deposited in the herbarium of the Museum of New Zealand Te Papa Tongarewa (WELT). These samples were complemented with previously published molecular data of the Cladophorales to establish their phylogenetic position ([Table S1](#)). Some samples of the new genus were collected as single specimens. Due to the unique nature of these lineages and to further the named diversity in the Cladophorales plus the difficulty of collection in some of these locations, we propose names based on these genetically unique samples. Naming species and genera based on single or few specimens from hard to access locations is useful for cataloging diversity (Cabrera et al., [2022](#); Schneider et al., [2019](#)).

Molecular markers and phylogenetic analyses

Total genomic DNA was isolated, using the Chelex method (Goff & Moon, [1993](#)), from 35 specimens. Molecular phylogenetic analyses were based on nuclear-encoded small subunit (SSU) and partial large subunit (LSU) rDNA gene sequences as well as on the typically more variable internally transcribed spacer ITS1 and ITS2 rDNA regions. The first approximately 590 nucleotides of the LSU rDNA gene were amplified using the universal primers C'1 forward and D2 reverse (Hassouna et al., [1984](#); Leliaert et al., [2003](#)). About 1,700 base pairs (bp) of the SSU rRNA gene were amplified using the primer pairs SR1-SS11H and SSU897-18SC2 (Leliaert et al., [2007](#)). The whole ITS rDNA region was amplified with the primers 9F and 7R (Hayakawa et al., [2012](#)), resulting in approximately 1,000 bp-long fragments. For some samples, the ITS1 and ITS2 rDNA regions were amplified separately. The ITS1 region was amplified using the primer pair ITS5-ITS2 (White et al., [1990](#)), and the ITS2 region was amplified using the primer pair ITS3-ITS4 (White et al., [1990](#)).

Polymerase chain reaction (PCR) amplifications were carried out with an initial denaturation step of 94°C for 5 min followed by 30–34 cycles of 1 min at 94°C, 1 min at 56–57°C for the LSU and SSU primers or at 59–63°C for the primers for the whole ITS rDNA region (9F and 7R) or at 50–54°C for the ITS1 and ITS2 primers, and 1 min at 72°C, and a final extension

step of 5 min at 72°C. The reaction volume was 25 µL and consisted of approximately 0.1–0.4 µg genomic DNA, 1.25 nmol of each dNTP, 6 µmol of each primer, 1 × reaction buffer, 1–2.5 mM MgCl₂, 0.005% BSA, up to 5 µL of 5 M betaine, and 1 U of *Taq* polymerase (Bioline, Meridian Biosciences Inc, USA). The annealing temperature for the ITS primers as well as the amounts of MgCl₂ and betaine varied depending on the amplification success of particular specimens. Amplifications were checked for correct size by electrophoresis in 1% agarose gels. Polymerase chain reaction products were purified with Montage PCR filter units (Millipore) or with ExoSAP-IT® (USB Corporation). Cleaned PCR products were commercially sequenced (Macrogen, Seoul, Korea). The obtained sequences have been deposited in GenBank ([Table S1](#)).

The LSU, SSU, and ITS rDNA sequences were aligned separately with MUSCLE (Edgar, [2004](#)) using the default settings and then edited by eye in Se-AL v2.0a11 (Rambaut, [2007](#)). A concatenated dataset of LSU and SSU rDNA gene sequences of 86 specimens consisting of newly generated sequences and additional sequences downloaded from GenBank was assembled to establish phylogenetic relationships within the family Cladophoraceae (listed in [Table S1](#)). The genus *Lychaete* was used as an outgroup. Subsequently, a second dataset of LSU, ITS, and SSU rDNA sequences of 34 specimens was assembled that included the samples that formed a clade with *Cladophora rupestris* in the previous analysis plus additional samples that had very similar LSU rDNA gene sequences. Both datasets were analyzed using Bayesian inference (BI) and maximum likelihood (ML). Corrected pairwise distances were calculated in MEGA X (Kumar et al., [2018](#)) for the LSU, SSU, and ITS rDNA partitions separately.

The two concatenated datasets were partitioned into LSU and SSU rDNA, and into LSU, SSU, and ITS rDNA, respectively, applying unlinked models of nucleotide evolution to each partition, with GTR+I+G as the initial model for each partition. Bayesian inference was performed with MrBayes v3.2.7 (Ronquist & Huelsenbeck, [2003](#)) and consisted of two parallel runs of one cold and three incrementally heated chains each, and 10 million generations sampled every 1,000 generations. Posterior probabilities were obtained using a Metropolis-coupled Markov chain Monte Carlo approach. The average standard deviation of the split frequencies of the two parallel runs indicated that the tree samples became increasingly similar and that a stationary distribution was reached. The log files of the runs were also checked with Tracer v1.4.1 (Rambaut & Drummond, [2007](#)), and a burn-in sample of 2,500 trees was removed from the 10,000 retained trees before calculating the majority rule consensus trees in MrBayes. Maximum likelihood

analyses were performed using IQTREE2 (Minh et al., 2020); the best-fit model of each gene sequence was determined with ModelFinder (Kalyaanamoorthy et al., 2017). The LSU and SSU rDNA gene data set used DNA substitution models LSU: TIM2e+I+G4 (LSU) and TIM3e+I+G4 (SSU). The LSU, SSU, and ITS rDNA sequence data set used DNA substitution models K2P (LSU + SSU) and GTR+F+I+G4 (ITS). Support for individual internal branches was determined by non-parametric bootstrap (500 replicates; Felsenstein, 1985). The trees were visualized with FigTree v.1.2.2. (Rambaut, 2008).

RESULTS

Molecular analyses

The concatenated alignment of LSU and SSU rDNA genes was 2,324 bp in length. The second alignment consisting of LSU, SSU, and ITS rDNA sequences was 3,158 bp long.

The tree of the Cladophoraceae had a well-supported backbone and was overall well resolved (Figure 1). Support values for BI and ML analyses were comparable. The phylogeny agreed with previously published trees of the family (e.g., Boedeker et al., 2016) but also showed the existence of two novel clades with high support.

One new clade was sister to *Willeella*, *Lurbica*, and *Pseudorhizoclonium* (82% bootstrap support, 0.99 posterior probability). Three specimens were grouped together in this clade with high support (100% ML, 1.0 BI), each displaying the morphology of separate genera, namely, *Chaetomorpha*, *Rhizoclonium*, and *Willeella/Cladophora* (Figure 1). The specimen with a *Rhizoclonium* morphology from mainland New Zealand (C80) was sister to a specimen with a *Chaetomorpha*-like morphology from Tasmania (L09). The specimen with a *Willeella/Cladophora*-like morphology was sister to the other two and was positioned on a long branch.

The other novel clade grouped with *Chaetomorpha* and *Cladophora/Rhizoclonium*, but the relationships among these three clades were not supported (Figure 1). This clade contained the North Atlantic species *Cladophora rupestris* (clade 1) plus unpublished sequences of specimens with a wide range of morphologies from high-latitude southern locations (south of 42° S; New Zealand, Chile, and subantarctic islands). *Cladophora rupestris* was sister to three other lineages (Figures 1 and 2). The first lineage (clade 2) contained specimens identified morphologically as *Rhizoclonium ambiguum*, *Rhizoclonium* sp., *Chaetomorpha* sp., *Cladophora incompta*, and *Cladophora* sp. (Figure 2). These specimens were

collected from mainland New Zealand, the Chatham Islands, the New Zealand subantarctic islands, and Macquarie Island. Clade 3 was comprised of specimens identified as *Rhizoclonium ambiguum* or *Rhizoclonium* sp., or *Chaetomorpha* sp., *Cladophora* sp., and *Cladophora falklandica*, originating from Campbell Island, Chile, and the Falkland Islands. Clade 4 contained only specimens with *Cladophora*-like morphologies from New Zealand. For specimen P63, only LSU rDNA gene data were available, and this sample did not group with either clade 3 or 4 (Figure 2). Pairwise sequence comparisons in clade 3 revealed identical SSU rDNA gene sequences. No variation within lineages was detected in LSU rDNA gene sequences, while the sequence divergence between clades ranged from 2%–3% between *Cladophora rupestris* and the rest, up to 1.7% between clade 2 and clades 3 and 4, and 0.4% LSU rDNA gene sequence divergence between clades 3 and 4 (Table S2 in the Supporting Information). In ITS rDNA gene region sequences *Cladophora rupestris* was 7%–10% different from the other lineages, and up to 5% sequence divergence was found between lineages 2 and 3 (see Table S3 in the Supporting Information; no ITS rDNA region data for lineage 4).

Our data clearly show that two novel lineages are found within the Cladophoraceae, each containing divergent sequences that suggest multiple species. One lineage had no prior name, and we propose a new genus and three new species to accommodate these samples:

Vandenhoeikia Boedeker gen. nov.

Based on morphology alone the three species in this genus would be placed in different genera of the Cladophorales. Morphologies range from curly unbranched *Rhizoclonium*-like filaments 60–90 µm in diameter, robust unbranched filaments 70–400 µm in diameter with long basal cells and a *Chaetomorpha*-like habit to densely branched, sturdy *Cladophora*-like thalli with long unbranched apical parts and mostly opposite ramuli. Distinct from other genera in the Cladophoraceae in rDNA sequences (GenBank Accession Numbers: LSU rDNA gene OR197082–OR197083, OR197102, OR197093; SSU OR197127–OR197128). Samples of *Vandenhoeikia* vary by 0.02–0.06 in LSU rDNA gene (Table S4 in the Supporting Information).

EPONYM: Named to honor the Dutch phycologist Christiaan van den Hoek for his many significant contributions to our understanding of the Cladophoraceae.

TYPE SPECIES: *Vandenhoeikia subantarctica* Boedeker sp. nov.

HOLOTYPE: WELT A03500, from Bounty Islands, New Zealand.

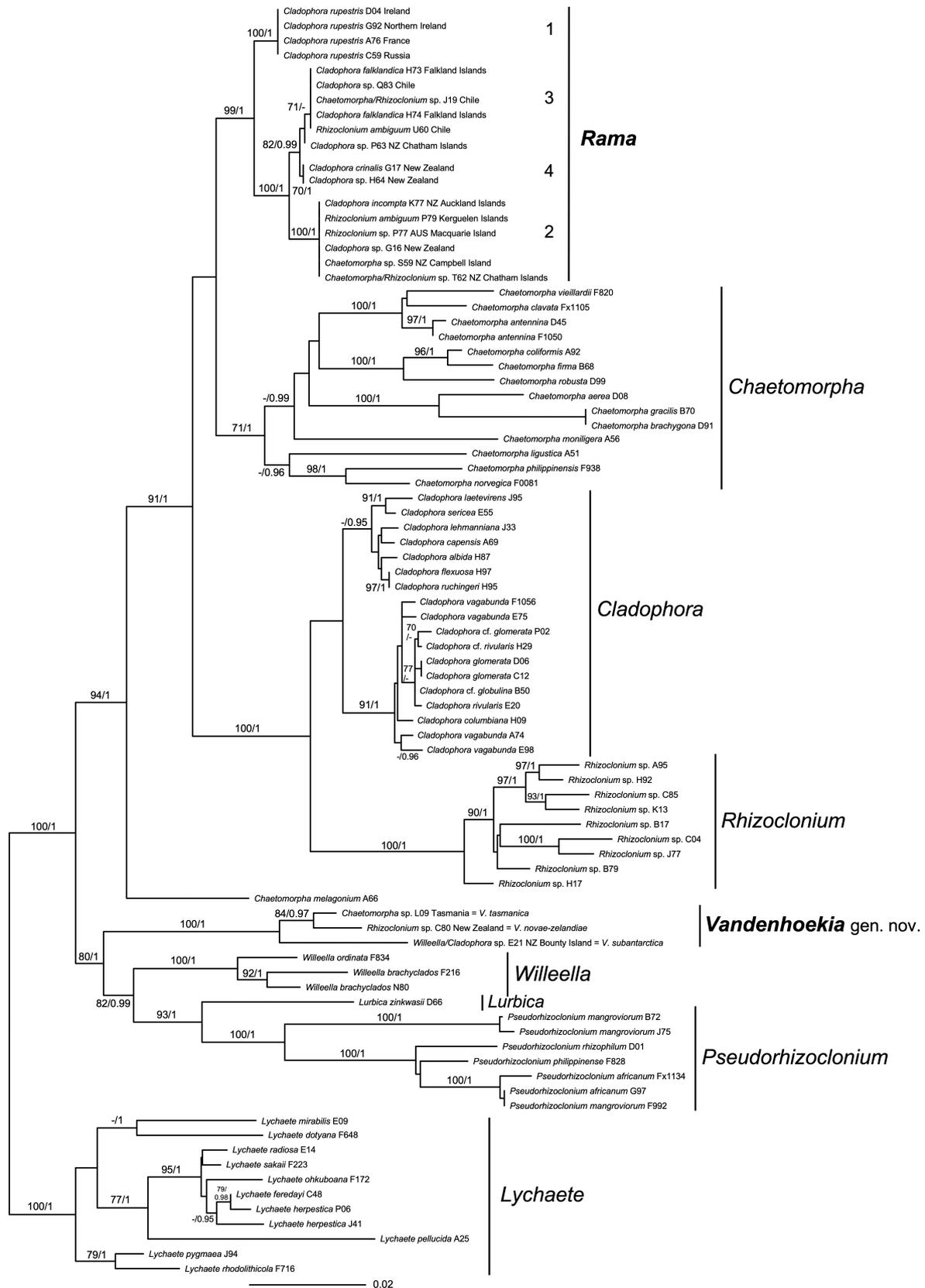


FIGURE 1 Maximum likelihood (ML) phylogram inferred of the Cladophoraceae from concatenated small-subunit (SSU) rDNA gene and partial large-subunit (LSU) rDNA gene sequences. Maximum-likelihood bootstrap values are followed by posterior probabilities from Bayesian inference (BI) on branches. Only bootstrap values above 50% and posterior probabilities above 0.9 are shown. The genus *Lychaete* was used as an outgroup. Sample codes refer to GenBank numbers and samples presented in Boedeker et al. (2016). The scale bar indicates substitutions per site.

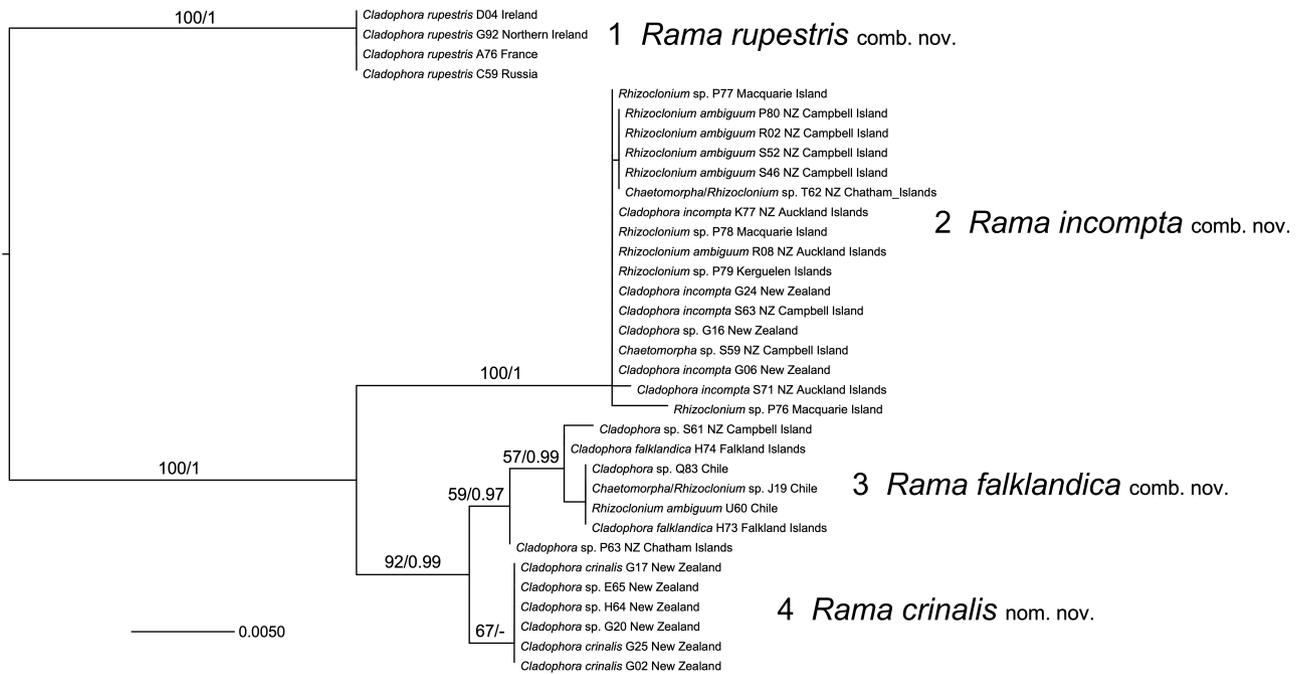


FIGURE 2 Maximum likelihood (ML) phylogram of the genus *Rama* inferred from concatenated rDNA gene sequences of the small-subunit (SSU), the internal transcribed spacer (ITS) region, and the partial large-subunit (LSU). Maximum-likelihood bootstrap values are followed by posterior probabilities from Bayesian inference (BI) on branches. Only bootstrap values above 50% and posterior probabilities above 0.9 are shown. *Cladophora rupestris* was used as outgroup. The scale bar indicates substitutions per site.

Vandenhoeikia subantarctica Boedeker sp. nov.

Figure 3a–c

DESCRIPTION: Light green, robust plants up to 10 cm tall, with several main filaments arising from one holdfast, 250–300 μm in diameter. Rigid, long unbranched apical parts of the main filaments. Second-order branches short, tapering, at acute angles, mostly opposite, sometimes alternate, 120–250 μm in diameter. Cells 2 to 4 times as long as wide. Apical cells conical, 10–120 μm in diameter. Subtidal. Distinct from other species with a *Cladophora*-like morphology in rDNA gene sequences.

ETYMOLOGY: This species is named based on the collection location of the single specimen, the subantarctic Bounty Islands, New Zealand.

HOLOTYPE: WELT A035003. On semi-exposed subtidal rocks in 10 m depth between Tunnel Island and Proclamation Island, Bounty Islands, New Zealand, 08 April 2005, coll. Lou Hunt.

SEQUENCE DATA: Holotype: GenBank LSU: OR197093.

Vandenhoeikia tasmanica Boedeker sp. nov.

Figure 3d

DESCRIPTION: Unbranched, dark green filaments 70–400 μm in diameter with intercalary cells 1 or 2 times

as long as wide. The basal cell is very conspicuous and can be several mm long, ending in a branched holdfast. Subtidal. Distinct from other species with a *Chaetomorpha*-like morphology in rDNA gene sequences (GenBank: OR197082, OR197083, OR197127).

ETYMOLOGY: This species is named for its collection locality, Tasmania, Australia.

HOLOTYPE: WELT A034971. Attached on rock at 17 m depth, Kelp Forest, Eaglehawk Neck, Tasman Peninsula, Tasmania, 25 January 2007, coll. C. Boedeker.

SEQUENCE DATA: Holotype: GenBank LSU rDNA gene: OR197082, SSU rDNA gene: OR197127.

NOTE: An additional paratype with identical LSU and SSU rDNA gene sequences was collected from Deep Glen Bay, also on the Tasman Peninsula, at 15 m depth (LSU: OR197083).

SEQUENCE DATA: Holotype: GenBank LSU rDNA gene: OR197082; SSU rDNA gene: OR197127.

Vandenhoeikia novae-zelandiae Boedeker sp. nov.

Figure 3e

DESCRIPTION: Dark green filaments 60–90 μm in diameter, with cells 2 or 3 times as long as wide. Filaments lacking rhizoids and branches, rigid and characteristically curled. Attachment structures unknown. Distinct from other species with a *Rhizoclonium*-like morphology in rDNA gene sequences.



FIGURE 3 Morphology in *Vandenhoekia* gen. nov. (a–c) *Vandenhoekia subantarctica* sp. nov.: (a) herbarium specimen showing the coarse habit and long unbranched apical parts, (b, c) opposite branching at acute angles, unbranched side branches and pointed apical cells; (d) *Vandenhoekia tasmanica* sp. nov., lower parts of unbranched thalli with very long basal cells; (e) *Vandenhoekia novae-zelandiae* sp. nov., unbranched filaments. Scalebars (a) 1 cm; (b–d) 500 μm ; and (e) 200 μm .

ETYMOLOGY: This species is named after its only known collection site, New Zealand.

HOLOTYPE: WELT A034999. Island Bay, Wellington, New Zealand, 23 April 2005, coll. G. C. Zuccarello.

SEQUENCE DATA: Holotype: GenBank: LSU rDNA gene: OR197102, SSU rDNA gene: OR197128.

Based on our phylogenetic results and descriptions of similar algae from the Southern Ocean, we resurrect

the genus *Rama* V.J. Chapman for the clade containing *Cladophora rupestris* and its congeners (three new combinations and one new name).

Rama V.J. Chapman, 1952

Dark green intertidal plants with thick, rather robust filaments. The morphological variation is very wide, ranging from densely branched upright thalli, basally branched prostrate plants with lateral rhizoids and long unbranched apical parts, to unbranched filaments with or without rhizoids. Rhizoids can have haptera and anastomose, leading to entangled mats of thalli. Cell walls thick and lamellate, up to 12 µm across in apical parts and second-order branches, up to 50 µm in main axes. Growth by intercalary cell divisions. When densely branched, axes lined with branches of different length or lined with short, pointed ramuli, branching angles acute, polytomies common. Reproduction insufficiently known in most species, zoospores and gametes produced in terminal parts of thalli, typically in chains of thick-walled sporangia or gametangia that release zoospores or gametes through a lateral pore in the apical third of the cell.

Distinct from other genera in the Cladophoraceae in rDNA gene sequences (GenBank Accession Numbers: LSU OR197084–OR197092, OR197094–OR197101, OR197103–OR197117; SSU: OR197118–OR197126; ITS: OR197195–OR197217).

HOLOTYPE SPECIES: *Rama novae-zelandiae* (J. Agardh) V.J. Chapman, 1952: 56.

Currently accepted name for the type species: *Rama incompta* (Hooker f. & Harvey) Boedeker, M.J. Wynne & Zuccarello comb. nov.

Rama incompta (Hooker f. & Harvey) Boedeker, M.J. Wynne & Zuccarello comb. nov.

BASIONYM: *Conferva incompta* Hooker f. & Harvey, 1845, Lond. J. Bot. 4: 294.

HOMOTYPIC SYNONYM: *Cladophora incompta* (Hooker f. & Harvey) Hooker f. & Harvey 1847: 496.

HETEROTYPIC SYNONYMS: *Rama novae-zelandiae* (J. Agardh) V.J. Chapman, 1952: 56.

Cladophora novae-zelandiae (J. Agardh) De Toni De & Toni, 1889: 332.

Lychaete novae-zelandiae J. Agardh, 1878 (1877): 2.
Rhizoclonium ambiguum (Hooker f. & Harvey) Kützing, 1849: 387.

Conferva ambigua Hooker & Harvey, 1845: 295.

Cladophora incompta f. *tenuis* Reinbold, 1908: 187.

Rama antarctica V.J. Chapman, 1952: 57.

Rhizoclonium pachydermum Kjellman subsp. *maclovianum* G.W.F. Carlson, 1913: 53.

DESCRIPTION: Thalli sparsely branched, prostrate filaments forming entangled turfs. If branched, branching

is restricted to the basal parts of the thallus, and the apical parts are long and unbranched. Thalli sometimes entirely unbranched. Lateral rhizoids can be present, sometimes ending in anastomosing haptera. Diameter of filaments 60–130 µm. Cells short, 1 to 2 times as long as wide, with thick cell walls. Apical cells rounded.

Rama falklandica (Hooker f. & Harvey) Boedeker, M.J. Wynne & Zuccarello comb. nov.

BASIONYM: *Conferva falklandica* Hooker & Harvey, 1845, Lond. J. Bot. 4: 294.

HOMOTYPIC SYNONYMS: *Cladophora falklandica* (Hooker f. & Harvey) Hooker f. & Harvey 1847: 495.

HETEROTYPIC SYNONYMS: *Conferva simpliciuscula* Hooker & Harvey, 1845: 295.

Cladophora simpliciuscula (Hooker f. & Harvey) Hooker f. & Harvey 1847: 496 nom. illeg., non Kützing, 1843: 262.

Cladophora stuartii Harvey, 1859: 340.

Cladophora subsimplex Kützing, 1849: 411.

DESCRIPTION: Stolon-like basal filaments forming an entangled basal stratum; basal filaments can produce lateral rhizoids; upright filaments unbranched or bearing rows of long unbranched ramuli or many very short thorn-like ramuli or bearing clusters of laterals from several adjacent cells. Intercalary growth. Apical cells tapering, 2 to 6 times as long as wide, (20)–30–50–(60) µm in diameter; cells in main axes two to 6 times as long as wide, 40–100 µm in diameter, thick lamellate walls. Terminal chains of gametangia or sporangia.

Rama rupestris (Linnaeus) Boedeker, M.J. Wynne & Zuccarello comb. nov.

LECTOTYPE: Linnaeus, 1753: 1167. Spencer et al. (2009, p. 245) lectotype of *Conferva rupestris* to be the illustration in Dillenius (1742), Hist. Musc. t. 5, f. 29. 1742, with the voucher in Herb. Dillenius (OXF) “*Conferva marina trichodes ramosior*,” lower lefthand specimen.

BASIONYM: *Conferva rupestris* Linnaeus, 1753: *Species plantarum*, Holmiae (Stockholm), 1167.

HOMOTYPIC SYNONYM: *Cladophora rupestris* (Linnaeus) Kützing, 1843: 270.

For a complete list of homotypic and heterotypic synonyms for this species, see van den Hoek (1963) and AlgaeBase (Guiry & Guiry, 2023).

Rama crinalis Boedeker, M.J. Wynne & Zuccarello nom. nov.

HOLOTYPE: *Cladophora crinalis* Harvey in J.D. Hooker 1855, Fl. Nov. Zel. 2: 263, nom. illeg., coll. W. Colenso, New Zealand; in Herb. Harvey, TCD (Womersley, 1984). Non *Cladophora crinalis* Kützing, 1843: 263.

HETEROTYPIC SYNONYM: *Cladophora crinalis* Harvey var. *eramosa* V.J. Chapman, 1956: 450.

DESCRIPTION: Thalli attached to the substratum by long basal cells, sometimes with rhizoids. Rhizoids can be produced by other cells in the basal parts as well. Irregular organization with intercalary growth, basal parts of filaments sparsely branched, typically producing dichotomies and polytomies. Secondary branches either long and unbranched, or numerous, short and thorn-like. Branches tapering, apical cells pointed. Cells 2 to 4 times as long as wide; diameter in main axes 70 µm, in apical cell 25–30 µm, cell walls thick. Terminal sporangia in chains, consisting of rounded cells 1 to 2 times as long as wide, 100 µm in diameter.

DISCUSSION

The recognition of the new genus *Vandenhoeikia* and the reinstatement of *Rama* in the Cladophoraceae were determined by molecular analyses in this study, adding to our appreciation of the diversity in this family. It is striking that the gross morphology of specimens is no predictor of generic affiliation, as branched and unbranched morphologies have evolved multiple times. In the newly discovered genus *Vandenhoeikia*, the three known taxa have such different morphologies that they would be placed in three separate existing genera without knowledge of their sequence data. Such cases of convergent evolution have been demonstrated repeatedly in the Cladophorales (Boedeker et al., 2012, 2016, 2018; Leliaert, Verbruggen, et al., 2009; Sherwood et al., 2019). In the genus *Rama*, individual taxa also display such extensive phenotypic plasticity that different growth forms could be placed in three different existing genera. Phenotypic plasticity is remarkable in many taxa of the Cladophorales, while others are morphologically well defined and stable (Boedeker et al., 2016; Leliaert & Boedeker, 2007; Leliaert, Verbruggen, et al., 2009). Morphological changes have also been demonstrated in culture studies under varying environmental conditions (Aroca et al., 2020; Leliaert, D'hondt, et al., 2011; van den Hoek, 1963). This showcases that morphological identifications and species lists illustrating local diversity must be viewed skeptically for members of this order (e.g., Féral et al., 2021; Laing, 1927; Papenfuss, 1964).

Conversely, the findings of this study illustrate that this group of macroalgae is still very much under-sampled, especially in remote locations but not just in those locations. Increased sampling of the Cladophorales over the last few years has led to the discovery of several new genera (e.g., Boedeker et al., 2012, 2016, 2018; Sherwood et al., 2019) and even families (Boedeker et al., 2012; Leliaert, Ruess, et al., 2009), and the diversity seen in molecular phylogenies of the Cladophorales must be seen as

vastly under-represented. The algal flora in the high-latitude Southern Hemisphere locations is particularly poorly explored. Many species of *Chaetomorpha* and *Cladophora* described based on collections from the early expeditions to subantarctic regions have never since been investigated again and have unknown taxonomic affinities. Novel collections from southern environments could reveal more unexplored diversity and illuminate the evolutionary history of the Cladophoraceae and other algal groups.

The new genus *Vandenhoeikia* is part of a clade that contains four genera with few species, all of them described or re-instated in recent years based on molecular data (Boedeker et al., 2016; Sherwood et al., 2019). Most taxa in this clade, including the three species of *Vandenhoeikia*, are on long branches in molecular phylogenies. Although this could be the result of long evolutionary time spans without diversification or of many extinctions, most probably this indicates the likelihood of many yet undiscovered species that would break up these long branches and further illustrates a lack of sampling and how more sampling would lead to a better appreciation of its true diversity. Morphologically, the unbranched *V. novae-zelandiae* is similar to the (sub)tropical genus *Pseudorhizoclonium*, while *V. subantarctica* shows some morphological affinity to the closely related genus *Willeella*. While *V. subantarctica* has a very similar morphology to *Cladophora polyacantha* (which recently has been proposed as a synonym of *W. brachyclados*; Wynne, 2016), the type of this species is from French Guyana (Montagne, 1850) and thus most likely represents a different species. Records of *Cladophora polyacantha* reported from New Zealand (Dunedin, Stewart Island; see Chapman, 1954) most likely represent *V. subantarctica* instead. Also, subantarctic records of the South African species *Cladophora virgata* might actually be *V. subantarctica* (Adams, 1994; Montagne, 1845). The third species of *Vandenhoeikia*, *V. tasmanica*, is one of very few known records of *Chaetomorpha*-like algae from the subtidal; the only other species is the poorly known *Chaetomorpha mawsonii*, which is endemic to Antarctica, the Balleny Islands, and the south Shetland Islands (Pellizzari et al., 2017; Wiencke & Clayton, 2002). It would be very interesting to see whether molecular data would place this species within *Vandenhoeikia*.

With *Rama* we are also confronted with a situation where the different species could be placed into separate, distantly related genera based on morphology. Even more remarkably, the same is true for different phenotypes within a single species, as illustrated in Figure 4g–j. Cryptic diversity, parallel evolution, and intraspecific morphological variation have been well documented in the Cladophorales (e.g., Boedeker et al., 2018; Leliaert, Payo, et al., 2011; Leliaert, Verbruggen, et al., 2009). Unbranched filaments have evolved independently at least seven times in the Cladophorales, and *Rhizoclonium*-like morphologies at

least five times (see Boedeker et al., 2012, 2016, 2018; Hanyuda et al., 2002; this study).

Rama rupestris, or *Cladophora rupestris* as this species was previously known, had already been shown in

earlier molecular phylogenies of the Cladophoraceae not to be part of *Cladophora* (Boedeker et al., 2016). The close relationship with the three other *Rama* species is surprising both from a morphological as well as from

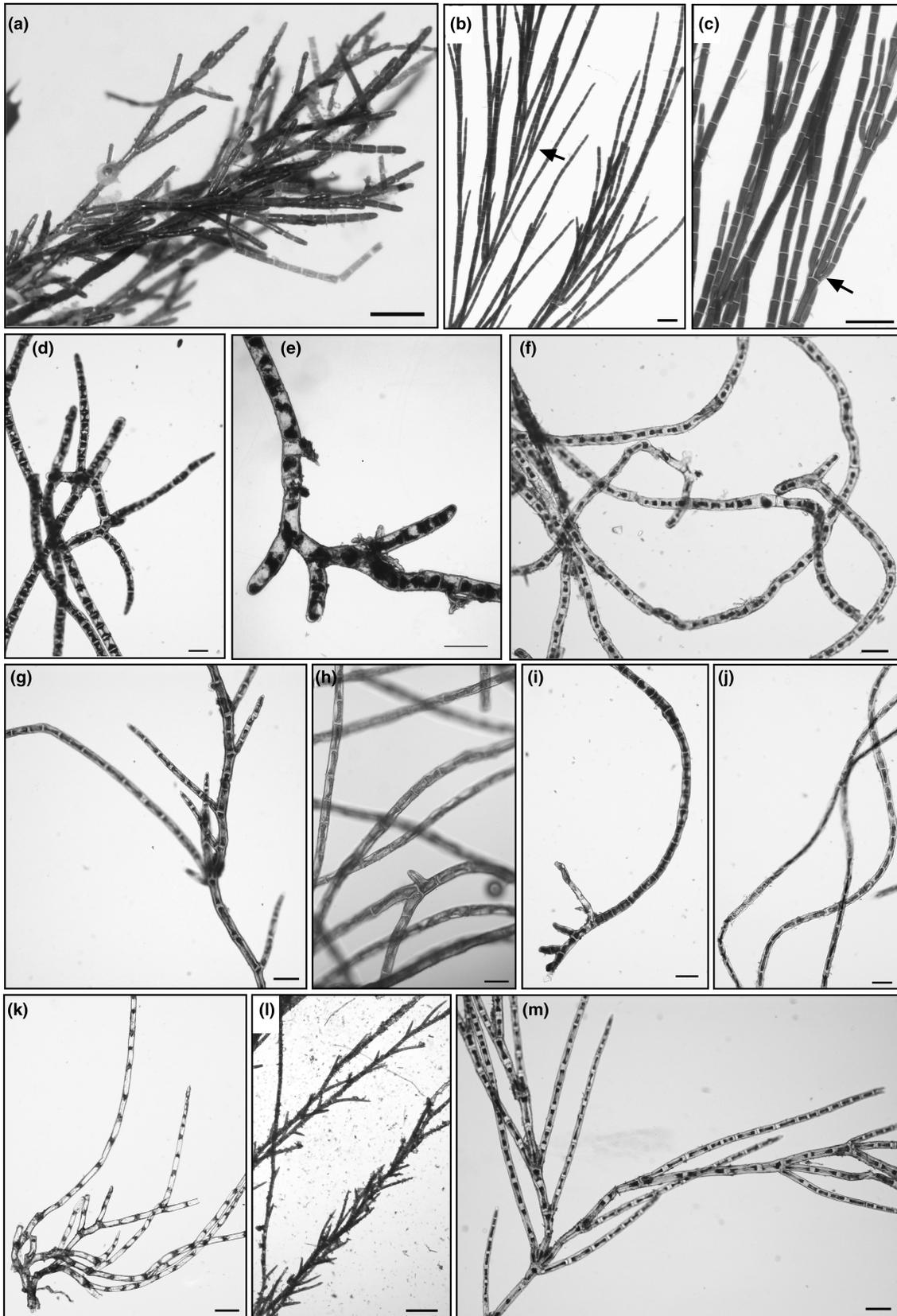


FIGURE 4 Morphology in the genus *Rama*. (a–c) *Rama rupestris* comb. nov.: (a) coarse, densely branched filaments; (b) long unbranched side branches at acute angles (arrow); (c) opposite or polytomous branching (arrow); (d–f) *Rama incompta* comb. nov.: (d) thick filaments with short cells and short basal branches; (e) thick filament with short cells and short side branches and lateral rhizoids; (f) mostly unbranched thin filaments with one rhizoid and one short side branch; (g–j) *Rama falklandica* comb. nov.: (g) branched basal part of thallus with thick cell walls, short tapering side branches and one long unbranched side branch; (h) mostly unbranched filaments with one dichotomy and one developing side branch or rhizoid; (i) mostly unbranched filament with two short side branches and one lateral rhizoid; (j) unbranched filaments; (k) *Rama* sp.: densely branched basal part with long unbranched distal branches; (l, m): *Rama crinalis* nom. nov.: (l) filaments lined with short pointy side branches at acute angles; (M) basal part with polytomies and unbranched side branches. Scale bars (a–c, l) 500 µm; (d–g, i–k, m) 200 µm; and (h) 100 µm.

a biogeographical perspective. *Rama rupestris* has a narrow morphological range and is a good morphospecies, and it is readily identified in the field by the stiff, blueish-green thalli consisting of branches with frequent polychotomies and thick cell walls. This species occurs in cold-temperate waters of the Northern Hemisphere, in the North Atlantic and Mediterranean (van den Hoek, 1963) and in the Northwest Pacific (Sakai, 1964; van den Hoek & Chihara, 2000). Although some records from the Southern Hemisphere seem doubtful (e.g., from subtropical Lord Howe Island; Kraft, 2007), other specimens identified as *Cladophora rupestris* from Tierra del Fuego and Kerguelen Islands (Papenfuss, 1964) and from New Zealand and Gough Island (Chamberlain, 1965; van den Hoek, 1979) might indeed belong to the genus *Rama*. Sequence data from these locations would be very insightful, and if the southern specimens were indeed confirmed as conspecific with the northern hemisphere *R. rupestris*, then this would represent another rare case of bipolar distributions with interesting paleogeographical implications. Other algae with disjunct bipolar distributions that have been confirmed with molecular data are *Desmarestia viridis/willii* and *Acrosiphonia arcta* (van Oppen et al., 1993), and it has been shown that these biogeographical patterns are of relatively recent origin and might relate to the last glacial maximum. *Cladophora pachyderma* (Kjellman) Brand (= *Rhizoclonium pachydermum* Kjellman) is not a good example of a bipolar species, despite being described from the arctic, with subsp. *maclovianum* G.W.F. Carlson described from the Falkland Islands. *Rhizoclonium pachydermum* has been shown to be a freshwater species (Christensen, 1991) and has been placed in *Rhizoclonium* based on molecular data (Zhao et al., 2014). However, *Rhizoclonium pachydermum* subsp. *maclovianum* is marine (Carlson, 1913) and is synonymized here with *Rama incompta*.

The specimens of *Rama incompta* used in this study originated from New Zealand and the New Zealand subantarctic islands, as well as from Macquarie Island. Based on published records, the range of this species also includes Kerguelen Islands, Cape Horn/Fuegia, the Falkland Islands, and South Georgia (Papenfuss, 1964). The distribution of *Rama falklandica* is similar, spanning the NZ subantarctic islands, Kerguelen Islands, Cape Horn/Fuegia, the Falkland Islands, and Chile. Differences could be the result of misidentifications and under-sampling. Several other algal taxa

have similar distributions, for example, *Durvillea antarctica*, *Macrocystis pyrifera*, or *Adenocystis utricularis* (Griffiths & Waller, 2016). Such patterns are typically the result of long-distance dispersal rather than vicariance, which has been shown to be rare (Wallis & Trewick, 2009; Waters, 2008). The low intraspecific variation found in the ITS rDNA region sequences of the *Rama* lineages (max. 0.8%) also supports the idea of a distribution shaped by more recent dispersal rather than ancient patterns. The west wind drift or Antarctic Circumpolar Current (ACC) is a major factor in shaping distributions of species around the Southern Ocean, especially through floating kelp and rafting (Fraser et al., 2009; Thiel & Gutow, 2004). *Rama* has not been reported from the Prince Edward Islands, Heard Island, the South Sandwich Islands, Saint Paul and Amsterdam Islands, or the Crozet Islands, despite being located in comparable latitudes and influenced by the ACC. The majority of these locations are located within what was the extent of winter sea ice during the last glacial maximum, and populations could therefore have been removed by ice scour and have not (yet) recolonized (Fraser et al., 2009). However, most likely these species are more widespread than currently known in subantarctic environments. Organisms in high latitudes often have wide ranges (Rapoport, 1982; Santelices & Marquet, 1998), a phenomenon known as Rapoport's rule.

The genetic diversity within *Rama* suggests that the four lineages represent four distinct species, as the interspecific divergence ranges from 5% to 10% in the ITS rDNA region sequences. In other studies of the Cladophorales, ITS rDNA region sequences between species had differed by 7% to 29% in *Boodlea* and *Phyllocladon* (Leliaert Verbruggen, et al., 2009; Leliaert et al., 2008) and by 14% between sister species of *Wittrockiella* originating from polyploid speciation (Boedeker et al., 2017). When it comes to the taxonomy and nomenclature of *Rama*, the main problem lies in applying existing names. This is a common problem in the Cladophorales due to their morphological simplicity, phenotypic plasticity, cryptic diversity, convergent evolution, and, most of all, the vast number of described species. In the absence of sequence data of type specimens, it can be arbitrary to apply names to lineages recovered in molecular phylogenies that lack any distinguishing morphological characters (Boedeker et al., 2016, 2018; De

Clerck et al., 2013; Ryberg & Nilsson, 2018; Škaloud et al., 2018). Frequently, names from the Northern Hemisphere have been applied uncritically to specimens from the Southern Hemisphere in the past. Although a lot of progress has been made in the taxonomy of the Cladophorales in the last 20 years, the issue of identifying and naming both field specimens and novel lineages in phylogenies remains a major difficulty. Several species not treated here might belong to *Rama* and would be worthwhile investigating, for example, *Cladophora aucklandica*, *C. colensoi*, or *C. verticillata*. The two (sub)tropical taxa *C. coelothrix* and *C. repens* that have been recorded from Antarctic sites (Papenfuss, 1964; Pellizzari et al., 2017) are clearly cases of misapplied names and might in fact belong to *Rama*. *Rhizoclonium hookeri*, currently a synonym of *Pseudorhizoclonium africanum*, is also likely to be a member of *Rama* rather of the tropical genus *Pseudorhizoclonium* because its type is from the Kerguelen Islands. One of Chapman's original species of *Rama*, *R. longiarticulata*, has not been treated here because it is believed that this species might actually represent the genus *Rhizoclonium* based on the description of thin filament diameter of less than 50 µm, long cells, lateral rhizoids, and lack of branches (Chapman, 1952).

Here we have discovered new diversity in southern latitudes, resulting in the description of the new genus *Vandehoekia* and the reinstatement of the genus *Rama*. This study illustrates the need for increased sampling and sequencing efforts. Novel discoveries in the Cladophoraceae most certainly can still be made, especially in remote locations, and especially in Africa, around the Southern Ocean, and in freshwater environments. We hope that the ongoing efforts to update the taxonomy and nomenclature will encourage further studies investigating the evolutionary relationships of these simple but complex algae.

AUTHOR CONTRIBUTIONS

Christian Boedeker: Conceptualization (lead); data curation (lead); writing – original draft (lead); writing – review and editing (equal). **Michael J. Wynne:** Data curation (equal); writing – review and editing (equal). **Giuseppe C. Zuccarello:** Funding acquisition (lead); project administration (lead); writing – original draft (equal); writing – review and editing (equal).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Habitat of *Rama*, Campbell Island, NZ. (A) rockpool, dominant in high intertidal, (B) abundant in large patches, (C) occurrence in the high intertidal with Bangiales blade, (D) flattened thalli during low tide, resembling the Atlantic *R. rupestris*.

Table S1. *Rama* and *Vandenhoeikia* specimens including sample location, voucher (WELT) numbers and GenBank accession numbers of the LSU rDNA gene, SSU rDNA gene, and ITS rDNA region sequences.

Table S2. Pairwise differences in *Rama* based on LSU rDNA gene sequences (in %), calculated in MEGA X (Kumar et al., 2018).

Table S3. Pairwise differences in *Rama* based on ITS rDNA gene sequences (in %), calculated in MEGA X (Kumar et al., 2018).

Table S4. Pairwise differences in *Vandenhoeikia* based on LSU rDNA gene sequences (in %), calculated in MEGA X (Kumar et al., 2018).

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