Diversity of tropical macroalgae in the New Zealand marine aquarium trade

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Caulerpa racemosa var. *macrophysa* on an imported *Zoanthus* sp., colony from one of my coral holding tanks

Abstract

Exotic species often slip through international borders undetected. Many perish but for some species introduction to a foreign land or sea brings release from natural enemies and a chance to establish a population. Increased global trade has increased the frequency of species introductions through stowaways and lapses in biosecurity. Once an invader is established there is the opportunity for explosive population growth at the detriment of native species and humans. The marine aquarium trade is a significant vector of species introductions, including algal introductions. The most publicized introduction from aquaria was the release of the green alga *Caulerpa taxifolia* from the Oceanographic Museum in Monaco. *C. taxifolia* introduction had large negative impacts on the invaded ecosystem.

Molecular barcoding of tropical macroalgae entering the New Zealand aquarium trade was implemented using various molecular markers (*cox1*, *cox3*, *rbcL*, *tufA*, LSU). Both NCBI Blast searches and maximum-likelihood phylogenies were used to identify the isolates. A total of 62 species of tropical macroalgae were identified from coral rocks imported into New Zealand, plus samples from reef hobbyists. Some species found are known as invasive elsewhere, for example, *Caulerpa cylindracea*, *C. racemosa*, *C. sertularioides*, *Ulva ohnoi* and *Chaetomorpha vieillardii*. All three major groups of algae were well represented in my findings with 26 species of red algae, 24 species of green algae and 12 species of brown algae. Temperature tolerance limits are the largest determinant in survival in algae, while salinity and pH are less significant. Temperature tolerance of tropical algae to the minimum Sea Surface Temperature in Auckland (14°C) and Wellington (11°C) was tested. My results show that one species *Chaetomorpha vieillardii* can survive at Auckland minimum winter sea surface temperatures. Our findings have important implications for New Zealand biosecurity, as not only are a large

diversity of exotic macroalgae entering the New Zealand marine aquarium trade
unregulated, but there is also the potential for them to survive in New Zealand waters.

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1. Introduction

1.1 *Species introductions and ecological hypothesis on the effects of invasions*

Species introductions can have a detrimental effect on the environment (Gozlan *et. al.* 2009). Humans facilitate many species introductions through their movement (anthropogenic introductions) (Heike *et. al.* 2014). Species introductions can be considered a more significant environmental stressor than eutrophication and toxic chemical release (Chapman 1995). Introducing non-native freshwater fish to new habitats has a variety of detrimental impacts on the environment, including increased predation. For example, predation from the introduced fish *Salmo trutta* (Linnaeus 1758) and *Oncorhynchus mykiss* (Walbaum 1792) reduce recruitment of juvenile *Galaxias vulgaris* (Stokell 1949) in New Zealand rivers (Woodford and McIntosh 2010), leading to habitat degradation and increased competition for resources with native biota. The largest predictor variable of native fish decline in Iberian streams was invasive fish introduction (Hermoso *et. al.* 2011). Hybridization of introduced species with closely related native species may occur post-introduction. For example, Nile Tilapia hybridized with native *Oreochromis* (Günther 1889) in Zambia (Deines *et. al.* 2014). Another threat from species introduction is disease transmission. For example, the intracellular fish parasite *Sphaerothecum destruens* was introduced to Europe from China carried by *Pseudorasbora parva* (Temminck and Schlegel 1846), the introduction was followed by mortality in a large range of native host fish (Andreou *et. al.* 2012). These introductions may even prevent proper ecosystem function (Gozlan *et. al.* 2009). Terrestrial systems also suffer from species introductions. For example, the Argentine ant, *Linepithema humile* (Mayr 1868), is a well-documented introduced species to many countries and has displaced native ants and altered invertebrate community structure (Wild 2004).

An introduced species is one transported to a non-native habitat by natural pathways, humans or human activity (anthropogenic introductions) (Alpert 2006). The absence of population regulatory forces can lead to exponential population growth of introduced species to the detriment of native flora and fauna, making an introduced species an invasive species (Briggs 2012). There are differing views on the definition of invasive species. One view is that an invasive species is “any introduced non-native that causes harm to the economy, environment or human health post-introduction” (U.S. Executive Order 13112 1999). Another view is that the invader does not need to cause harm but population size and spread beyond the location of introduction is a criterion for being invasive (Moutou and Pastoret 2010). An introduced species that remains at low population density with little to no detriment to the ecosystem remains simply an introduced species (Moutou and Pastoret 2010).

There have been many hypotheses on factors that facilitate invasion of an introduced species. Prominent are: a) enemy release; b) propagule pressure; c) competitive exclusion; and d) disturbance. The enemy release hypothesis states that introduction of a foreign species to a habitat may release it from the predation and competition pressures present in its native range (Liu and Stiling 2006). For example, enemy release has been proposed for the spread of *Acer platanoides* (L.) “Norway maple” introduced across North America. Norway maple had three times lower rates of herbivory in its introduced range versus its native range in Europe. Norway maple also had lower fungal damage in its introduced range (Adams *et. al.* 2009). Another example of enemy release is the lower occurrence of herbivore damage in North American introduced populations than European populations of Klamath weed, *Hypericum perforatum* (L.) (Vila *et. al.* 2005).

The propagule pressure hypothesis for facilitation of invasion success explains why some species do not successfully establish post-introduction even though the habitat is suitable (Eschtruth and Battles 2011). Propagule pressure is frequently used to explain the variation in invasion success in plant communities (Richardson and Pyšek 2006). The more seeds (larger propagule amounts) introduced to the non-native habitat, the more likely the species can establish. There is often interaction between propagule pressure and disturbance in predicting introduction success. For example, experimental increases in propagule numbers and disturbance of habitat in the brown alga *Sargassum muticum* (Yendo) Fensholt increased recruitment success (Britton-Simmons and Abbott 2008) in Washington, USA.

Competitive exclusion may occur when introduced species displace native species (Bøhn *et. al.* 2008). The introduced mud snail *Ilyanassa obsoleta* (Say 1822) feeds on the eggs of the native mud snail *Cerithidea californica* (Haldeman 1840) and competitively excludes it from tidal creeks in San Francisco (Race 1982). Competitive exclusion does not always occur with introductions. For example, in the St Francis River crayfish, *Orconectes quadruncus* (Creaser 1933), habitat use did not alter when sympatric with the invasive woodland crayfish *Orconectes hylas* (Faxton 1890) (Westhoff and Rabeni 2013). The impact of an invasive species' introduction on an ecosystem is dependent on present species interactions. For example, if an invader displaces an important habitat species but doesn't fulfill the same ecosystem services then the overall ecological impacts may be severe. On the other hand, if an invasive species fills the ecological role of the displaced species then the impacts are less severe (Rodriguez 2006). For example, the introduced Woodland crayfish displaces the St Francis river crayfish but has the same role as the St Francis river crayfish being an omnivorous

scavenger of fallen plant materials and insects and is prey for fish and birds (Westhoff and Rabeni 2013).

Disturbance events or environmental change can and will increase the risk of species invasion (Stachowicz *et. al.* 2002). If a habitat is available and if it is not promptly filled by a native species then there is the chance that an introduced species will colonize that habitat. In the Chilean Andes, disturbance was the largest determinant of plant invasion success regardless of elevation or temperature (Lenbrechts *et. al.* 2016). 40-56% of the variation in the study's model for plant invasion success was explained by disturbance, while propagule pressure only explained 10% of the variation.

Increasing global sea surface temperature increases risk of invasion of non-indigenous marine species from warmer climates (Stachowicz *et. al.* 2002). Global warming causes species range shifts latitudinally and may facilitate invasion, for example, as tropical species range further pole wards (Stohlgren *et. al.* 2005; Occhipinti-Ambrogi 2007). The largest threat to biotic communities may not be from increasing annual mean temperature but the maximum and minimum temperatures giving invaders a head start over native species (Stachowicz *et. al.* 2002). Global warming may also facilitate a dominance shift of non-native species leading to global biotic homogenisation (Stachowicz *et. al.* 2002).

1.2 Marine introduction

Increased international shipping brings higher risk of transporting marine species between continents. Ballast water frequently supports live organisms from various taxa including planktonic stages (Hyun *et. al.* 2017). Whole coastal planktonic assemblages can be transported across oceanic barriers to similar habitats (Geller and Carlton 1993). Homogenization of coastal communities may occur through the hybridization of closely

related native and introduced fauna (Carlton 1979). Bays and estuaries used as ports are especially vulnerable ecosystems (Geller and Carlton 1993).

Hull fouling is estimated to have introduced 74% of the nonindigenous marine invertebrates to the Hawaiian Islands (Eldredge and Carlton, 2002) and 42% of the 26 unintentionally introduced marine species to Japan (Otani 2006). Unintentional introductions mediated by aquaculture occur through fouling aquaculture equipment, cages, lines and floats provide surfaces for recruitment and can be used as stepping-stones for invasive species (Campbell *et. al.* 1017; Kim 2007).

Aquaculture mediated species introductions often occur from intentional release of aquaculture species for future harvest. For example, Chinook salmon to New Zealand (McDowall 1994; Quinn *et. al.* 1996), Tilapia cichlids *Oreochromis* spp. introduced to Asia (Gozlan *et. al.* 2009) and shellfish culture (Ruiz *et. al.* 1999). These non-native species introduced for commercial purposes, often have dire consequences for native biodiversity (De Leaniz *et. al.* 2010). Salmonoid introduction to the southern hemisphere was an attempt by governments and fisheries to capitalize on what they felt was an underutilized niche as the majority of native freshwater fish were small and not of interest by sports fishermen (De Leaniz *et. al.* 2010). Salmonoids are currently one of the largest threats to native Galaxids in New Zealand (De Leaniz *et. al.* 2010). Fisheries mediated introduction of coarse fish to New Zealand for recreational purposes; for example, Rudd (*Scardinius erythrophthalmus* (Linnaeus 1758)), Perch (*Perca fluviatilis* (Linnaeus 1758)) and Tench (*Tinca tinca* (Linnaeus 1758)) has played a part in the decrease in lake water clarity and quality (Rowe 2007). Perch are also suspected in the increased frequency of cyanobacterial blooms in the Karori Reservoir in Wellington due to top down control on zooplankton, the primary prey of juvenile perch (Smith and Lester

2007). Introduced fish are also predators on native fish and have also threatened breeding grounds (Rowe 2007).

1.3 Aquarium trade mediated introductions

The aquarium trade is a significant vector of species introductions (Gertzen *et. al.* 2008; Semmens *et. al.* 2004). However, the trade has received little attention and risks have not been well studied (Calado and Chapman 2006). One third of aquatic invasive species that have had large detrimental impact to native biodiversity are either ornamental or aquarium species (Padilla and Williams 2004a; 2004b). For example, Koi carp (*Cyprinus carpio*) (Linnaeus 1758); goldfish (*Carassius auratus*) (Linnaeus 1758); and plants such as *Elodea canadensis* (Michx); *Egeria densa* (Planch); *Lagarisiphon major* (Ridl) and *Ceratophyllum demersum* (L.) (oxygen weeds) have been introduced to New Zealand waterways as aquarium or pond discards (Chapman 1996).

1.4 Biosecurity

The Ministry of Primary Industries (MPI) takes biosecurity threats seriously as there are large implications for New Zealand biodiversity and economic production if a species capable of disrupting the ecosystem, or interfering with economically important species becomes established (MAF Biosecurity New Zealand 2009a; 2009b). There are also large economic costs in loss of production and for pest eradication or control post-invasion (Goldson *et. al.* 2015). Online trading of flora and fauna has long been a risk to New Zealand's native biota as introductions are inevitable (Derraik and Phillips 2010).

Biosecurity risk assessment for the importation of live fish, invertebrates and aquarium plants to New Zealand is primarily based around introduction of pathogenic microbes. For example, the bacteria *Aeromonas salmonicida*, *Flavobacterium*

psychrophilium or viruses: Cyprinid herpesvirus-3, *Macrobrachium rosenbergii* *nodavirus* (MrNV) are considered risk species (MAF Biosecurity New Zealand 2009a; 2009b). Dried, preserved and live plants and algae can be imported for research purposes (MAF Biosecurity New Zealand 2010). Live specimens imported for research use are to be maintained in containment facilities.

In New Zealand, livestock importers are allowed to import corals, invertebrates, fish and aquarium plants from all countries under strict protocols set out by MPI to reduce risk of introducing foreign diseases (MAF Biosecurity New Zealand 2009a; 2009b). On importation Convention of International Trade in Endangered Species (CITES) permits need to be provided for listed species of corals and other invertebrates along with environmental protection authority (EPA) permits (MAF Biosecurity New Zealand 2009a; 2009b). Tests for viruses must be done before livestock is released for sale and all mortalities reported to MPI. If 20% mortality occurs during quarantine then laboratory testing for pathogens needs to be carried out (MAF Biosecurity New Zealand 2009a; 2009b). Corals, bivalves and the rocks that they are attached to harbor algal spores and juveniles; many too small or slow growing to be visualized during the 4-6 week quarantine period. Many algae survive and grow once sold to hobbyists or pet shops (personal observations).

1.5 *Algal introduction*

Macroalgae are frequently introduced world-wide both intentionally for commercial purposes and unintentionally. Vectors of unintentional introduction include ballast water release (Carlton and Galler 1993), hull fouling (Mineur *et. al.* 2007), plus aquaculture equipment fouling, for example, equipment fouled by the brown alga

Undaria pinnatifida (Harvey) Suringar (James and Shears 2016), and the green alga *Codium fragile* var. *tomentisoides* (Van Goor) P.C.Silva (Dromgoole 1975). *U. pinnatifida* was also transported between mussel farms in New Zealand on equipment (Forrest and Blakemore 2006). Intentional introductions mostly occur from algal mariculture. Algal mariculture is an important industry in developing nations for food production and export (Halling *et. al.* 2013; Pickering *et. al.* 2007). Potential candidates for mariculture are often introduced to new locations when algae of high suitability for aquaculture are lacking (Halling *et. al.* 2013; Pickering *et. al.* 2007). A review in 2007 found that 277 species of algae are introduced and some algal families contain many more successful invaders than others. For example, the green algal families Caulerpaceae and Codiaceae, the brown algae of the Fucaceae and Alariaceae, and the red algal families Ceramiaceae and Rhodomelaceae are particularly prevalent (Williams and Smith 2007).

The red alga *Acanthophora spicifera* (M.Vahl) Børgesen introduced to Pearl Harbor, Hawaii in 1952 invaded habitat previously dominated by *Laurencia nidifica* J.Agardh and outcompetes many species, including *Hypnea cervicornis* (J.Agardh) E.Y.Dawson. *Hypnea musciformis* (Wulfen) J.V.Lamouroux was introduced to Hawaii from Florida in 1974 for production of carrageenan. It has since partially displaced the native *Hypnea cervicornis* (Russel 1992). The introduced *Gracilaria salicornia* (C.Agardh) E.Y.Dawson had a range expansion of 280 meters per year in Hawaii (Cox 1999). *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva and *Kappaphycus striatum* (F. Schmitz) P.C.Silva are also introduced species in Hawaii that have spread on average 250 meters per year since their introduction to Kane'ohe Bay, O'ahu (Cox 1999; Conkin and Smith 2005).

Undaria pinnatifida (Laminariales) is native to Japan, Korea and China and was found in Wellington Harbor, New Zealand in the 1980's (Hay and Luckens 1987). *U. pinnatifida* has been introduced to Baja California (Aguilar-Rosas 2013), Argentina (Meretta *et. al.* 2012), France for aquaculture (Castric-Fey *et. al.* 1993; James *et. al.* 2016), Spain (Peteiro *et. al.* 2016), and Australia (Campbell and Burrage 1998; Sanderson 1990).

Green algae species from the genus *Caulerpa* have been introduced to various locales outside their native range. *C. taxifolia* (M.Vahl) C.Agardh and *C. racemosa* (Forsskål) J.Agardh have been introduced to the Mediterranean Sea (Piazzi and Ceccherelli 2002; Piazzi *et. al.* 2003). *C. brachypus f. parvifolia* (Harvey) A.B.Cribb is a recent introduction to Florida (Lapointe and Bedford 2010).

Some algal introductions can be directly attributed to the aquarium trade. The most publicized introduction is *Caulerpa taxifolia* released from the Oceanographic Museum, Monaco (Baulch 1999; Meinesz and Hesse 1991). It is common practice to maintain macroalgae in sumps or refugia as a method of trapping nutrients from aquaria. As the alga grows it is fragmented and sections are discarded to remove the nutrients from the aquarium. Species kept include *Caulerpa racemosa* (Forsskål) J.Agardh, *Caulerpa serrulata* (Forsskål) J.Agardh and *C. taxifolia* (Zaleski and Murray 2006). The filamentous green alga *Chaetomorpha* sp. previously thought to be a safe alternative to *Caulerpa* for growing in aquaria may be of high introduction risk due to its wide range of temperature tolerance, fast growth and ease of fragmentation (Odom 2014). *Chaetomorpha vieillardii* (Kützinger) M.J.Wynne has invasive potential as shown in Guam (University of Guam https://cnas-re.uog.edu/wp-content/uploads/2018/09/Invasive_speciesGuam.pdf).

1.6 Algal introduction impacts

Algal introductions, if successful, may have negative consequences for the native biotic community. Impacts of an invasive algal introduction include decreased abundance and species richness in native seaweeds (Levin *et. al.* 2002). Green algae from the order Caulerpales are well known for their invasive nature in the Mediterranean and Australia (Wiedenmann *et. al.* 2001; Piazzzi and Ceccherelli 2002; Piazzzi *et. al.* 2003). There are numerous impacts of *Caulerpa* sp. on native communities from shading (Meinesz *et. al.* 2001). Mediterranean introduced genotypes of *Caulerpa taxifolia* and *Caulerpa cylindracea* Sonder are strong competitors of native seaweeds (Piazzzi *et. al.* 2003; Piazzzi *et. al.* 2016) and sea grasses (Ceccherelli and Cinelli 1997; Ceccherelli *et. al.* 2002). *C. taxifolia* can have effects on seagrasses, for example, *Posidonia oceanica* (L.) Delile has decreased leaf size and increased terpenoid concentrations when in competition with *C. taxifolia*. *Caulerpa taxifolia*, on the other hand, increases its frond size when competing with the seagrass *P. oceanica* (Pergent-Martini *et. al.* 2008). *Caulerpa taxifolia* shows a change in morphology from thick to thin stolons and rachis, and an increase in fragmentation in invasive populations when introduced to non-native locations (Glasby 2013; Wright 2005). Fragmentation assists asexual reproduction in *Caulerpa* species and subsequent range expansion (Wright 2005). The green alga *C. taxifolia* also influences the substrate chemistry impacting biodiversity. *C. taxifolia* fragments had C:N ratio between 14-7 (Taylor *et. al.* 2010). This variation in C:N, compared to the C:N ratios for the following native detritus producers *Avicennia marina* (Forsskål) Vierh C:N ratio: 26.3 ± 0.1 ; *Posidonia australis* Hook.F: 23.0 ± 0.1 ; *Zostera capricorni* Asch: 22.8 ± 0.3 (Taylor *et. al.* 2010), and the toxins associated with *C. taxifolia* detritus, had negative impacts on substrate micro-invertebrates (Ceccherelli and Cinelli 1997; Taylor *et. al.*

2010). The decline in seagrass meadows the world over increases the threat that invasive algal introduction have on remaining seagrasses (Orth *et. al.* 2006).

Impacts of *Caulerpa taxifolia* introduction on seagrass are not always detrimental. For example, the Australian seagrass *Posidonia australis* J.D.Hooker was unaffected by *C. taxifolia* introduction, showing that the impacts of *C. taxifolia* invasion on seagrasses may be dependent on seagrass species or other variables (Glasby 2013). The shading of *Caulerpa cylindracea* by the canopy of *Posidonia oceanica* prevents successful invasion of established *P. oceanica* beds (Ceccherelli and Cinelli 1999; Bernardeau-Esteller 2015).

Herbivores often show preference to native alga over introduced species. *Fucus vesiculosus* (L.) is preferred to *Sargassum muticum* (Yendo) Fensholt by the snail *Littorina littorea* (Linnaeus 1758) and the urchin *Psammechinus miliaris* (P.L.S. Müller 1771) in the North Sea (Schwartz *et. al.* 2016a; 2016b), while in some introductions herbivores prefer the introduced to native species (Vermeji *et. al.* 2009). Native herbivores may or may not be affected by anti-herbivory toxins in the non-native species. The Mediterranean urchin *Paracentrotus lividus* showed significant loss of spines and longer righting times when fed *C. taxifolia* exclusively compared to control algae (Boudouresque *et. al.* 1996). This may be due to the cocktail of toxins used to deter herbivory by many *Caulerpa* species (Erickson *et. al.* 2006). In southeastern Australia a variety of grazers, both fish and invertebrates, had preference to *Sargassum* sp. and *Ulva* sp. over the *Caulerpa* species: *C. scalpelliformis* (R.Brown ex Turner) C.Agardh, *C. filiformis* (Suhr) Hering and *C. taxifolia* (Davis *et. al.* 2005).

The red alga *Kappaphycus* sp. overgrows live corals killing them. It frequently reaches biomasses over 10kg/m² comprising up to 50% percentage cover on reefs (Conkin and Smith 2005). *Kappaphycus* sp. outcompetes native algae, can regenerate from small fragments and is of little interest to native herbivores.

In South Australia, the introduced brown alga *Undaria pinnatifida* often establishes when canopies of native *Phyllospora* sp. are disturbed (Valentine and Johnson 2004). In France, *Undaria pinnatifida* show preference to artificial substrates free of other benthic organisms rather than rocky substrate, this may be due to it being less competitive than native kelps (Floc'h and Jean-Yves 1996). Most studies show *U. pinnatifida* as having little to no negative impact on native biodiversity (Floc'h and Jean-Yves 1996; Valentine and Johnson 2004). *U. pinnatifida* occurrence however reduced native species richness in Nuevo Gulf in Patagonia Argentina (Casas *et. al.* 2004). This is evidence that although *U. pinnatifida* was introduced to many locations including France, Australia and New Zealand and has established it is often simply an introduced species rather than becoming invasive.

1.7 Algal taxonomy

Algal taxonomy is an important discipline within phycology. Taxonomic knowledge is required for research in ecology, physiology, bio-assessment and algal genetics (Manoylov 2014). Taxonomic identification is regarded as the most common hypothesis testing practice in science (Manoylov 2014). The study of morphological characteristics of species is important for determining phylogenic relationships between taxa. Marine macroalgae are diverse organisms and are in three major groups; green algae (Chlorophyta), Red algae (Rhodophyta), and Brown algae (Ochrophyta, class Phaeophyceae). Red and green algae have primary plastids and the brown algae have secondary plastids derived from a red alga (Larkum and Vest 2003).

Many algae species are polymorphic (Verbruggen *et. al.* 2014) making it difficult to morphologically identify to species level with any accuracy. For example, the brown

algal genus *Sargassum* has many polymorphic species (Mattio and Payri 2011). The *Caulerpa racemosa*-*Caulerpa peltata* species complex (Sauvage *et. al.* 2013) has morphologies ranging from umbrella-shaped branch tips to rounded tips plus intermediate morphologies making morphological identification difficult (Verbruggen *et. al.* 2014).

Algal cryptic and pseudo-cryptic diversity is another constant issue for morphological taxonomy. Cryptic species are those with very little to no morphological differentiation between species but are genetically distinct. For example, the species of the *Rhipsalis* group within the genus *Halimeda* have more than one species that overlap morphologically (Verbruggen *et. al.* 2005). The red algal species complex *Bostrychia radicans* Montagne/*Bostrychia moritziana* Sonder ex Kützing J.Agardh contains seven distinct lineages (Zuccarello and West 2003) that are morphologically indistinguishable. Molecular methods are required to identify cryptic species (Sun *et. al.* 2012).

1.8 Molecular identification and barcoding

Molecular barcoding is a powerful tool for delimiting species in both terrestrial and marine environments (Herbert *et. al.* 2003). Molecular barcoding is the use of individual or multiple gene sequences (often protein-coding) to identify a specimen to genus or species level. The technique assists morphological species identification, increasing certainty in taxonomic conclusions. Molecular species delimitation has been utilized in the study of brown algae (Ni-Ni-Win *et. al.* 2011; Vieira *et. al.* 2014; Buchanan and Zuccarello 2018), red algae (Broom *et. al.* 2002; Cianciola *et. al.* 2010; Muangmai *et. al.* 2014) and green algae (Verbruggen *et. al.* 2007; Hyang and Myung 2015; Lee and Kim 2015).

Both polymorphic and cryptic species make morphological identification difficult. Polymorphic species have plastic morphology so one species can show many distinct

morphologies increasing the chance of misidentification, such as many *Caulerpa* species (Gacia *et. al.* 1996; Verbruggen *et. al.* 2014). For example, *Lobophora* a genus of brown algae previously thought to consist of only a few species (J.V.Lamouroux) Womersley ex E.C.Oliveira, has many genetically distinct entities uncovered by molecular techniques (Vieira *et. al.* 2014). *Padina* another genus of brown algae also had many cryptic species identified using molecular barcoding (Ni-Ni Win *et. al.* 2011). An example of barcoding-assisted green algal taxonomy is the refuting the hypothesis that *C. mexicana* (Sonder ex Kützing) and *C. taxifolia* were morphological variants of the same species (Olsen 1998).

1.9 Physiological tolerance

Physiological tolerance limits the distribution of all species. The ability of a species to withstand changes in the environmental conditions makes it more or less adaptable to habitat change during introduction (Zerebecki and Sorte 2011). Invasive species often have broader physiological tolerance limits than native species giving them an advantage in establishment (Higgins and Richardson 2014). Abiotic factors limiting distribution of algae include temperature, light intensity (Gacia *et. al.* 1996), salinity (Carpelan 1964; Pereira *et. al.* 2017; Bollen *et. al.* 2016), pH (Bollen *et. al.* 2016), water flow and nutrient concentrations. Salinity is a determinant of habitat suitability for aquatic species. For example, in habitats with widely fluctuating salinity, only euryhaline species are present whilst stenohaline species are found in habitats with more consistent salinity as they have tighter tolerance limits (Carpelan 1964). The red alga *Acanthophora spicifera* had tolerance of 25-40 practical salinity units (psu), but no new growth occurred at 15psu and high biomass loss occurred at 50 psu. (Pereira *et. al.* 2017). Light intensity impacts photosynthesis in algae. Too high an intensity induces photoinhibition (Hanelt *et. al.* 1997). Macroalgae tend to tolerate lower light intensity than seagrasses (Burfiend and

Udy 2009) and corals (Fabricus 2005), and this increases their competitive ability in turbid coastal ecosystems (Burfiend *et. al.* 2013).

Temperature is the largest determinant for survival in macroalgae (Graiff *et. al.* 2015), and summer maximum temperature and winter minimum temperature are more important in determining thermal tolerance limits than the mean temperature (Graiff *et. al.* 2015). In New Zealand, *Undaria pinnatifida* sporophytes have temperature tolerances higher than the native kelp *Lessonia variegata* J.Agardh as *U. pinnatifida* is unstressed at 25°C whilst *L. variegata* is severely stressed between 20-25°C (Bollen *et. al.* 2016). This may increase the competitive ability of *U. pinnatifida* with ocean warming (Bollen *et. al.* 2016). The green alga *Valonia utricularis* (Roth) C.Agardh has vastly different temperature tolerance dependent on ecotype; the Mediterranean and Atlantic ecotypes can acclimate and maintain photosynthetic efficiency at 15-20°C whilst the Indian Ocean ecotype undergoes photoinhibition when grown at 15-20°C (Eggert *et. al.* 2003a; 2003b). The lower limit for survival is 5°C for the Mediterranean ecotype of *V. utricularis* and 18°C for the western Pacific ecotype (Eggert *et. al.* 2003). Aquarium release of *Caulerpa taxifolia* has occurred in Japan but establishment has failed, this may be due to winter temperatures being lower than its tolerance limit (Komatsu *et. al.* 2003). There is little evidence that *C. taxifolia* populations have different temperature tolerance (Glasby and Gibson 2007). Both Australian native and invasive *C. taxifolia* have similar growth rates at 15-18°C, but native Australian *C. taxifolia* had higher growth rates than the invasive at 22-25°C (Glasby and Gibson 2007). *C. taxifolia* tolerance to subtropical temperatures makes both the Australian native and the Mediterranean invasive populations a potential biosecurity threat to northern New Zealand in the future. *Caulerpa taxifolia* is listed as an “unwanted organism” under the New Zealand Biosecurity Act 1993, due to its history of invasiveness overseas.

2.0 Materials and Methods

2.1 *Sample collection*

Two pet shops in Wellington New Zealand that sell ornamental tropical marine aquarium fish and invertebrates were visited regularly when new coral orders arrived, either monthly or bimonthly. Many corals were also purchased from five online retailers and importers. Algal recruits were collected from the aragonite rock, bivalve shells and concrete to which the corals were attached. A knife was used to break the substrate at the algal holdfast if one was present. Corals that were purchased were monitored and over time algal specimens that grew from the rocks were sampled. Marine aquarium hobbyists across New Zealand were contacted and some donated algal specimens for identification. Coral species and likely origins were recorded along with when the sample was taken (Table S1). A small sample of alga was retained on silica gel for DNA extraction and as a voucher specimen. In some cases, a live specimen was grown in culture for morphological identification or physiological tolerance testing. There were a few cases where no voucher was prepared as only enough tissue for DNA extraction was present.

2.2 *Culture conditions*

To assess the risk of establishment that escaped aquarium algae poses to New Zealand coastal regions, survival of algal samples was tested at New Zealand minimum sea surface temperatures. NZ minimum sea surface temperatures were determined from National Oceanographic and Atmospheric Administration (NOAA) data for Auckland and Wellington (seatemperature.org). Four culture chambers were set to the NZ minimum sea surface temperatures as follows: minimum temperature in Wellington

winter ($11^{\circ}\text{C}\pm 1^{\circ}\text{C}$), minimum temp in Auckland winter ($14^{\circ}\text{C}\pm 1^{\circ}\text{C}$), and for the other 2 incubators, $20^{\circ}\text{C}\pm 1^{\circ}\text{C}$ a mid range temperature and a control temperature matching my reef aquaria $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$. HOBO loggers were used to track the temperatures (over 3 days) to insure temperature did not fluctuate more than 1°C . Fluorescent tubes provided photosynthetically available radiation (PAR) of $10\text{-}20\ \mu\text{mol}^{-1}\ \text{m}^{-2}\ \text{s}^{-1}$. The light cycle was 16:8 light: dark. As light intensity is not equal throughout the chamber, culture dishes with lids were randomly placed throughout the incubator. Sterile dishes with lids were filled with 200 ml sterile (steam sterilized for 10 minutes at 100°C) seawater with quarter-strength modified Provasoli's enrichment medium (West 2005), salinity of 35 psu. 2 drops of 1 mg/ml germanium dioxide was added per dish to inhibit diatom growth (Lewin 1966).

Algal species identified as frequent arrivals or those traded by hobbyists for nutrient export were selected from aquaculture tanks at $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$ (K. Woodhouse aquaria) for use in the temperature tolerance experiment. The algae samples were cut into 12 approximately equal lengths. Specimens were kept at 25°C for 24 hours post-cutting in dishes to allow for recovery. Samples were then randomly allocated to separate sterile dishes, 3 dishes per algal specimen per temperature were placed randomly in the chamber. No acclimatization procedure was undertaken.

Survival was measured by bleaching of the alga. The alga turning white (loss of pigments) or disintegration was considered as no survival. Specimens were observed weekly for chloroplast constriction or signs of reproduction. Any changes in morphology were recorded. Any remaining pigmentation was considered as 'survival'. Survival/no survival was determined after 2 weeks. Some specimens were kept for up to 4-weeks to confirm survival/death, due to partial bleaching after 2 weeks.

2.3 *Voucher preparation*

Algal specimens collected within the New Zealand marine aquarium trade were inspected under a dissecting microscope in a dish of distilled water to remove detritus and epiphytes using tweezers. Specimens with excessive overlapping branches had branches removed as required to expose the morphology of the alga and then the specimen was pressed on herbarium paper. Specimens were also preserved in 100% ethanol if too small to press, and mounted on microscope slides in 60% Karo (corn syrup)/1% formalin. Aniline blue staining was used to stain the cytoplasm of ethanol preserved specimens due to pigment loss. The edges of the coverslip were sealed with nail polish after 2 weeks of drying to prevent over drying. Herbariums and slides were numbered to identify the specimen. Vouchers were deposited at Te Papa (Table S1). Micrographs were taken using an Olympus BX63 microscope with a DP80 camera (Olympus). Herbarium photographs were taken using an iPhone 7. Figures were annotated in Canvas 5 (Canvas V5.0.1).

2.4 *Molecular identification*

Some algae were identified morphologically, however morphological variability and lack of characters in many algal groups makes DNA barcoding a superior method of identification (Cianciola *et. al.* 2010). When used alongside morphological identification, molecular barcoding increases the certainty in species identification (Manoylov 2014).

2.5 DNA extraction

Each algal specimen was cleaned under a dissecting microscope to remove potential contaminants and ensure that it was a single specimen. A portion of the most recent growth was removed and extracted using either: a modified CTAB protocol (Zuccarello and Lokhorst 2005), or a Chelex extraction protocol for small tissues (Goff and Moon 1993). DNA extracts were stored at -20°C.

Extracted DNA was amplified depending on preliminary identification of the alga and the available databases. Genes used were as follows: for siphonous green algae (*Caulerpa*, *Bryopsis*, *Derbesia* and *Rhipidosiphon*) partial plastid elongation factor A (*tufA*) gene was used, primers *Caul tufA*-forward and *Caul tufA*-reverse were used (Famá *et. al.* 2002), and in some cases partial ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene using primers *rbcL* 712F and *rbcL* 904R (Lagourgue *et. al.* 2018). For species within the Cladophorales, such as *Chaetomorpha*, *Cladophora* and *Valonia*, partial large subunit ribosomal RNA (LSU) using primers C'1 forward and D2 reverse were used (Hassouna *et. al.* 1984). For red algae, partial cytochrome oxidase subunit 1 (*cox1*) gene was used, using primers GazF1 and GazR1 (Saunders 2005), or partial (*rbcL*) gene using primers 765F and 1150R (Freshwater *et. al.* 1994). For brown algae, partial cytochrome oxidase subunit 3 (*cox3*) gene was used with primers *cox3* 44F and *cox3* 739R (Vieira *et. al.* 2014). Primers and amplicon lengths are shown in (Table S2).

2.6 Polymerase chain reaction

The PCR reaction volume was 30 µL and consisted of ~0.1–0.4 µg genomic DNA, 1.25 nmol of each dNTP, 7.5 pmol of each primer, 1× ammonium reaction buffer,

2.5 mM MgCl₂, 4-5 μL of 0.25% bovine serum albumin and 1 unit of *Taq* polymerase (Bioline, Meridian Bioscience Inc. USA)

The standard amplification conditions were as follows: denaturing at 94°C for 5 min, 36 cycles of denaturing at 94°C for 1 min, annealing at 45°C for 30 sec and extension at 72°C for 1 min and a final extension at 72°C for 5 min. Primers and primer specific alterations to the amplification procedure are shown in (Table S2).

Agarose gel electrophoresis was done on the PCR products to identify successful amplification of the target region. Electrophoresis was in 1% agarose with 0.5 TBE, at 100 volts, using 1 μL loading buffer (glucose and bromophenol blue) to 3 μL PCR reaction. Gels were electrophoresed for 30 minutes with a 50bp ladder (Bioline) to determine the size of the amplified DNA. Gels were then stained in ethidium bromide for 10-15 minutes, rinsed in water, and photographed under UV light.

Successful PCR products were prepared for sequencing using Exo Sap-IT (USB Corporation) per PCR product. This involved incubated the PCR product with 0.5U Exo-SapIT at 37°C for 20 min, then heating to 80°C for 10 min. Cleaned products were sequenced commercially (Macrogen Inc. Seoul, Korea). Sequences were edited using the Geneious software package (Geneious Prime.2019.2.1) (<https://www.geneious.com>) to remove primers and low quality sequence.

Sequences were queried against the National Center for Biotechnology Information sequence database (NCBI) Genbank using the Basic Local Alignment Search Tool (BLAST) (Johnson *et. al.* 2008), to confirm that the sequences were of the gene of interest. The Blast top hit was recorded along with the E value and the sequence identity percentage. BLAST searching compares a query sequence with those in the NCBI database to find regions of similarity. BLAST then calculates the statistical significance of the high sequence identity matches, reporting significance as “E” values.

Alignments were built for each gene and genus of algae sampled using the top 5 Blast hits for each of our sample sequences, Genbank sequences for the named species in the genus of the top Blast hit for each sample sequence were included. In some cases, examples of closely related genera were included in the alignment. Alignments were made using MAFFT with scoring matrix 200PAM/K=2, gap open penalty 1.53, offset value 0.123, implemented in Geneious. Alignments of protein coding genes were translated and cut to start at the first codon position and end at third codon position to allow for codon position partitioning.

Maximum Likelihood analyses were implemented using IQ-tree (Trifinopoulos *et. al.* 2016). IQ-tree was used to select the molecular evolution models (Modelfinder) (Kalyaanamoorthy *et. al.* 2017) and construct (ML) trees with 1,000 bootstrap replicates. Genes were partitioned by codon where appropriate. Models selected for each data set were selected using the BIC criterion and are presented in Table S3. Trees were annotated in Figtree v1.4.4 (Rambaut 2010) and Canvas draw (Canvas Draw V5.0.1). Sequences were deposited in Genbank, and accession numbers presented in Table S1.

2.7 *Species determination*

To delimit species using pairwise differences, we used a working hypothesis of 2% difference equals a potentially different species, and <2% is the same species. This hypothesis was used for *cox1* in red algae, *cox3* in brown algae, *rbcL* in red algae, *tufA* = in green algae except for *Caulerpa* spp., and the LSU gene in the Cladophorales except for *Cladophora*. The LSU gene in the Cladophorales has little divergence between some lineages, e.g., there was <1% pairwise difference between species in the *Cladophora albida/serica* clade (Boedeker *et. al.* 2016). We used a hypothesis that >1% pairwise difference indicates a different species and <1% indicates the same species in

Cladophora. For Udotaceae *rbcL* we used with >4% pairwise difference to indicate different species as this is an average difference between species within *Rhipidosiphon* (Coppejans *et. al.* 2011). *TufA* in green algae is conserved, and within the genus *Caulerpa* there is little variation. For example, the pairwise distance values between some species is very low: between *C. taxifolia* and *C. distichophylla* it is 0.24%, *C. brachipus* and *C. subserrata* it is 0.2% distance, however *C. mexicana* have 0.4% within species pairwise difference (Famá *et. al.* 2002). As pairwise distance between *Caulerpa* spp. is low we used a hypothesis of >0.2% pairwise difference indicates a different species and <0.2% pairwise difference the same species.

3.0 Results

3.1 Identification red algae

Hypnea spp.

Hypnea spp. specimens found on coral rocks were branched red-dark thalli with frequent indeterminate branching and branch tips having a single apical cell (not shown). Specimens sectioned had a single layer of small rounded cortical cells and large unpigmented hexagonal shape medullary cells; a central axial cell was also present. Sample A55 had cystocarps present (Fig. 1A, B). Some samples (A69) showed less frequent indeterminate branching of sample (Fig. 1C). Other samples were only small fragments (A95)(Fig. 1c). Micrographs of samples A101, A94, A97 were included in (Fig. S3).

Figure 1. Examples of *Hypnea wyneei*. Herbarium and photomicrographs; A = A55, scale bar = 1mm, B = A55 with cystocarp, scale bar = 400µm, C = A69, scale bar = 2 cm, D = A95, scale bar = 1cm,

Specimens A55, A69, A95 and A97 are within a clade containing *Hypnea wynnei* Nauer, Cassano & M.C.Oliveira (KX426549, KX426547, KX426551), *H. stellulifera* (J.Agardh) Yamagishi & Masuda (HQ422721) and *Hypneocolax stellaris* subsp. *oreintalis* Børgesen (HQ422720) with high bootstrap support (100%)(Fig. 2). Aligned sequences show 0-0.2% differences in *cox1* between the samples and their top Blast hit, *H. wynnei* (KX426549) from Brazil. I designated samples A55, A69, A95 and A97 as *H. wynnei*.

Figure 2. Maximum-likelihood tree of *Hypnea* spp. using *cox1* sequences. Samples A55, A56, A69, A94, A95, A97 and A101 shown. *Cystoclonium purpureum* (HM916901) was used as the outgroup. Only bootstrap values >50% displayed. Scale bar = 0.3 substitutions per site.

Sample A56 is sister to *Hypnea valentiae* (Turner) Montagne (HQ141882) but with low bootstrap support (50%), they are within a clade containing *Hypnea spinella* (C.Agardh) Kützinger with high bootstrap support (91%). A56 has 6.45% pairwise difference to its sister *Hypnea valentiae* (HQ141882) but has 2.8% pairwise difference in *cox1* to *H. spinella* (KU905111) its closest pairwise sequence and top Blast hit from Bahia, Brazil. I designate sample A56 as *Hypnea* sp1. Sample A94 groups with *Hypnea brasiliensis* P.B.Jesus, Nauer and J.M.C.Nunes with high bootstrap support (98%) and this clade also contains a sequence identified as *Hypnea cervicornis* J.Agardh (KM214502). Sample A94 sample has 1.13% pairwise difference in *cox1* to its closest Genbank sequence and top Blast hit *H. brasiliensis* (KU905079), also from Brazil. I designated sample A94 as *H. brasiliensis*. Sample A101 is sister to a sequence designated as Gigartinales sp. (HQ423064) with high support bootstrap, and 100% percent identity to that sequence. I designated sample A101 as *Hypnea* sp2, the same species as Gigartinales sp. (HQ423064).

Chondrophycus spp.

The three *Chondrophycus* sp. samples found on maricultured coral rocks were branching, flattened with a holdfast attaching then to the substrate. Apical pits were present (Fig. 3B, C). Specimen A1 (Fig. 3A) was preserved in ethanol resulting in pigment loss and shriveling, live specimen had dark red pigmentation.

Figure 3. Examples of samples of *Chondrophycus* sp. Photomicrographs. A = A1, preserved specimen, scale bar = 1mm, B = A107, scale bar = 1mm, C = A8 branch tip, scale bar = 400µm

Sample A1 is sister to *Chondrophycus* sp. (KX258817) from the Loyalty Islands, New Caledonia based on *cox1* sequences with moderate bootstrap support (79%)(Fig. 4). The sample has 3.75% difference in *cox1* to *Chondrophycus* sp. (KX258817) its closest pairwise sequence and top Blast hit. I designated sample A1 as *Chondrophycus* sp1. Sample A107 is most closely related to *Chondrophycus* sp. (HQ423056) from Hawaii with moderate bootstrap support (85%). The sample has 7.72% pairwise difference in *cox1* to *Chondrophycus* sp. (HQ423056) its top Blast hit. I designated sample A107 as *Chondrophycus* sp2.

Figure 4. Maximum-likelihood tree of *Chondrophycus* spp. using *cox1* sequences. Samples A1, A8 and A107 shown. *Chondria scintillans* G.Feldmann (KF492717) was used as the outgroup. Only bootstrap values >50% displayed. Scale bar = 0.3 substitutions per site.

Sample A8 is sister to a clade containing A107 and *Chondrophycus* sp. (HQ423056) with low bootstrap support (66%) and 9.3% pairwise difference between A8 and *Chondrophycus* sp. (HQ423056). Sample A8 has 8.33% pairwise difference in *cox1* to the top Blast hit *Chondrophycus* sp. (KX258815), with which it does not group in the phylogenetic analysis. I designated Sample A8 as *Chondrophycus* sp3.

Laurencia spp.

Samples A96 and A102 were found on rock associated with wild collected *Zoanthus* colonies, they had distinctive apical pits and thin intertwining branches, having a matted appearance, branches had multiple attachment points to the substrate. Specimen A96 (Fig. 5A) was preserved in ethanol and has lost the deep red pigmentation present in live specimens. Sample A102 (Fig. 5B) had a purplish pigmentation when fresh.

Figure 5. Examples of samples of *Laurencia* sp. Herbarium and photomicrograph; A = *Laurencia* sp. A96, scale bar = 400 μ m, B = *Laurencia* sp. A102, scale bar = 1cm.

Sample A96 is within a clade based on *cox1* sequences containing *Laurencia nidifica* Hauck with low bootstrap support (<50%) (Fig. 6). A96 has 5.6% pairwise difference to its sisters *Laurencia nidifica* (HQ422750) and 6.04% difference to *L. nidifica* (GU223888). Aligned sequences of *cox1* show 5.52% difference between A96 and *Laurencia saitoi* Perestenko (KM254876) its closest pairwise sequence and top Blast hit from Monterey, California, USA. I designated sample A96 as *Laurencia* sp1.

Figure 6. Maximum likelihood tree of *Laurencia* spp. using *cox1* sequences. Samples A96 and A102 shown. *Chondria scintillans* (KF492717) was used as the outgroup. Only bootstrap support values >50% displayed. Scale bar = 0.3 substitutions per site.

Sample A102 is sister to *Laurencia catarinensis* Cordeiro-Marino and Fujii (KF492718) but is unsupported (<50%), and 6.26% pairwise difference between the sequences. A102 is 5.96% different in *cox1* to its closest pairwise sequence, with which it does not group in the ML tree, and top Blast hit *Laurencia* sp. (GU223893) from Oahu, Hawaii, USA. I designated sample A102 as *Laurencia* sp2.

Pterocladia spp.

Samples of *Pterocladia* either arose from a single holdfast, A68 (Fig. 7A), or were stoloniferous, A93 (Fig. 7B). Samples had a flattened (Fig. 7C) single lobed or branching thallus with rounded tips and dark reddish-brown pigmentation.

Figure 7. Examples of *Pterocladia* spp. photomicrographs; A = A68, scale bar = 2cm, B = A93, scale bar = 400µm, C = A98, scale bar = 1mm.

Samples A68 and A93 were grouped with high bootstrap support (92%) and had identical in *cox1* sequences (Fig. 8). The samples are within a clade containing *Pterocladia caerulescens* (Kützinger) Stantelices & Hommersand (KX557255) their top Blast hit with high support bootstrap (97%). Samples A68 and A93 have 0.37% pairwise difference in *cox1* to their closest Genbank sequence, *P. caerulescens* (KX557255) from Port Dauphin, Madagascar. I designated both A68 and A93 as *Pterocladia caerulescens*.

Figure 8. Maximum likelihood tree of samples of *Pterocliadiella* spp. using *cox1* sequences. Samples A52, A68, A93 and A98 shown. *Gelidium corneum* (Hudson) J.V.Lamouroux (KJ960705) was used as the outgroup. Only bootstrap values >50% displayed. Scale bar = 0.2 substitutions per site.

Sample A52 is within a clade containing *Pterocliadiella phangiae* J.Sohrabipour, P.-E Lim and C.A.Maggs with high bootstrap support (90%). A52 has 9.39% pairwise difference in *cox1* to the closest Genbank sequence and top Blast hit *Pterocliadiella phangiae* (KC209092) from Port Dickson, Malaysia. I designated sample A52 as *Pterocliadiella* sp1. Specimen A98 is sister to *P. beachiae* Freshwater and *P. caerulea* and has 8.29% pairwise difference in *cox1* to the closest Genbank sequence and top Blast hit *P. beachiae* (KT208059) from Espirito Santo, Brazil. I designated sample A98 as *Pterocliadiella* sp2.

Peyssonnelia spp.

The *Peyssonnelia* samples found in this study were encrusting red thalli conforming tightly to the rock substrate (Figs. 9B, C) or growing outwards from the substrate, A104 (Fig. 9D). They have a row of apical cells on the margin of the thallus. Sample A53 (Fig. 9A) was fixed in ethanol resulting in loss of red pigment.

Figure 9. Examples of samples of *Peyssonnelia* spp., herbarium and photomicrographs; A = A53, stained with aniline blue, scale bar = 200 μ m, B = herbarium of A70, scale bar = 1cm, C = A86, scale bar = 1cm, D = A104, scale bar = 400 μ m.

Sample A86 is within a clade, based on *cox1* sequence analysis, containing *Peyssonnelia atropurpurea* P.Crouan and H.Crouan (JX969703), *Peyssonnelia* sp. (KM254486, KM254380, KM254509, KM254687 and KM254773) with moderate bootstrap support (70%) (Fig. 10). Sample A86 is 8.31% different in *cox1* to *Peyssonnelia* sp. (KM254773), its closest pairwise sequence and top Blast hit. I designated sample A86 as *Peyssonnelia* sp1.

Figure 10. Maximum-likelihood tree of *Peyssonnelia* spp. using *cox1* sequences. Samples A53, A70, A86 and A104 shown. *Ramicrusta appressa* (JX969695) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.6 substitutions per site.

Samples A53 and A70 are within a clade containing many sequences with moderate support (77%). Sample A53 has 7.63% pairwise difference in *cox1* to closest Genbank sequence and top Blast hit *Peyssonnelia rubra* (HQ422737). I designate sample A53 as *Peyssonnelia* sp2. Sample A70 has 7.28% difference in *cox1* to *Peyssonnelia* sp. (JX969736); its closest pairwise sequence, and top Blast hit. I designated sample A70 as *Peyssonnelia* sp3. Sample A104 is within a clade, without support, containing *Peyssonnelia* sp. (JX969686, JX969725 and JX969699). A104 has 8.6% pairwise difference in *cox1* to the top Blast hit *Peyssonnelia* sp. (JX969686) however it has 8.3% pairwise difference to sequences *Peyssonnelia* sp. (JX969747, JX969725 and JX969699). I designated sample A104 as *Peyssonnelia* sp4.

Ramicrusta sp.

Samples A84 and A92 were not large enough to preserve as vouchers, as the entire samples were required for DNA extraction. The samples were crustose reddish brown thalii on aragonite substrate.

Samples A84 and A92 (Fig. 11) group with high bootstrap support (100%) and have identical *cox1* sequences. The samples are sister to *Ramicrusta appressa* K.R.Dixon (JX969695, JX969707) with moderate support bootstrap (76%). The closest Genbank sequence and Blast hit for samples A84 and A92 was *Ramicrusta apressa* (JX969695) from Teloa, Vanuatu with 6.2% difference in *cox1* for A84 and 6.61% difference in *cox1* for A92. This difference in identity is due to samples A84 and A92 having different sequence lengths after sequence editing. I designated samples A84 and A92 as *Ramicrusta* sp.

Figure 11. Maximum-likelihood tree of *Ramicrusta* spp. using *cox1* sequences. Samples A84 and A92 shown. *Peyssonnelia rubra* (HQ422737) was used as the outgroup. Only bootstrap support values >50% displayed. Scale bar = 0.2 substitutions per site.

Meredithia sp.

Samples A3 (Fig. 12) had a deep reddish-purple thallus with rounded margins and a ruffled appearance. The voucher specimen was 6mm in diameter and is a small cutting from a 4 cm diameter specimen found on glass at a retailer in Lower Hutt, Wellington.

Figure 12. Sample of *Meredithia* sp. A3. Photomicrograph. Scale bar = 1mm.

Sample A3 groups with *Meredithia* sp. (KC157609) with moderate bootstrap support (83%) (Fig. 13). The sample is grouped with three named species: *M. kraftii*, *M. norfolkensis* and *M. nutleorum* with moderate bootstrap support (73%). Aligned sequences shows 0.45% difference in *cox1* between sample A3 and *Meredithia* sp. (KC157609), its closest pairwise difference and top Blast hit. I designated sample A3 as *Meredithia* sp., the same species as *Meredithia* sp. (KC157609) from Mindoro Oriental, Philippines.

Figure 13. Maximum-likelihood tree of *Meredithia* spp. using *cox1* sequences. Specimen A3 shown. *Kallymenia reniformis* (Turner) J. Agardh (KC157599) was used as the outgroup. Only bootstrap support values >50% displayed. Scale bar = 0.2 substitutions per site.

Leptofauchea sp.

Sample A67 (Fig. 14) was donated by a marine aquarium hobbyist in Auckland (Suppl. Table 1), the specimen had light red pigmentation, a flattened branching thallus, branching was slightly constricted near their bases, tips were rounded, and paler than older tissue. A single holdfast was present.

Figure 14. Sample A67, *Leptofauchea* sp. Herbarium. Scale bar = 2cm.

Sample A67 was within a clade containing *Leptofauchea cocosana* Filloramo and G.W.Saunders (KR140330), *Leptofauchea leptophylla* (Segawa)(KF547030) and samples designated as Rhodymeniales sp. with moderate bootstrap support (75%)(Fig. 15). A67 has 8.65% pairwise difference in *cox1* to the top Blast hit *Leptofauchea leptophylla* (KF547030) from South Korea. I designated sample A67 as *Leptofauchea* sp.

Figure 15. Maximum-likelihood tree of *Leptofauchea* spp. using *cox1* sequences. Sample A67 shown. *Hypnea musciformis* (Wulfen) J.V.Lamouroux (KT428772) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.3 substitutions per site.

Halopeltis sp.

Sample A4 (Fig. 16) was dark red flattened thallus, had a constricted base on some branches, the branches overlap forming a matted clump. Branch tips are mostly rounded. Overall, the live specimen was a matted flat thallus with irregular overlapping branches.

Figure 16. Sample A4, *Halopeltis* sp., herbarium. Scale bar = 2cm.

Sample A4 is within a clade containing *Halopeltis willisii* Freshwater and Saunders, *Halopeltis* sp. (EF101939) and *Halopeltis adnata* G.W.Saunders and C.W.Schneider, with moderate bootstrap support (87%)(Fig. 17). Aligned sequences show 9.24% pairwise difference between sample A4 and *Halopeltis willisii* (HM915686) its closest Genbank sequence and top Blast hit. I designated sample A4 as *Halopeltis* sp.

Figure 17. Maximum-likelihood tree of *Halopeltis* sp. using *cox1* sequences. Sample A4 shown. *Ceratodictyon repens* (Kützing) R.E.Norris (KY656528) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.3 substitutions per site.

Lomentariaceae

Sample A99 (Fig. 18A) had a flattened irregularly branched thallus and a prostrate growth form. Pigmentation was dark reddish brown. Samples A105, A106 (Fig. 18B, C) had subcylindrical irregularly branched thalli that formed a tangled mat-like growth on

the substrate. The branch tips were rounded and dark-red. Specimens had a single holdfast.

Figure 18. Examples of *Gelidiopsis* sp. and *Ceratodictyon repens* herbarium and photomicrographs; A = A99, *Ceratodictyon repens* scale bar =2cm, B = A105, *Gelidiopsis* sp. Scale bar = 1mm, C = A106, *Gelidiopsis* sp. Scale bar = 1mm.

Sample A99 had the highest Blast hit to *Ceratodictyon repens* (Kützing) R.E.Norris (KY656528), and is within a clade containing *Ceratodictyon repens* with high bootstrap support (97%) (Fig. 19). Sample A99 is 0.65% different to the closest Genbank sequence, *Ceratodictyon repens* (KY656528) from Chiriqui, Panama. I designated sample A99 as *Ceratodictyon repens*.

Figure 19. Maximum-likelihood tree of *Ceratodictyon* spp. and *Gelidiopsis* spp. using *cox1* sequences. Samples A99, A105 and A106 shown. *Halopeltis australis* (J.Agardh) G.W.Saunders (HM033024) was used as the outgroup and was removed for clarity. Only bootstrap support values >50 displayed. Scale bar = 0.3 substitutions per site.

Specimens A105 and A106 were identical and grouped with high bootstrap support (97%). Specimens A105 and A106 are within a clade containing *Gelidiopsis* sp. (HQ422790), and *Ceratodictyon scoparium* (Montagne and Millardet) R.E.Norris (HQ422608, KY656519). Both samples A105 and A106 have 0.16% pairwise difference in *cox1* to *Gelidiopsis* sp. (HQ422790), their top Blast hit. As *Gelidiopsis* sp., and *Ceratodictyon* sp. are synonymized (Schneider and Whyne 2007). I designated both samples A105 and A106 as *Ceratodictyon* sp. the same species as *Gelidiopsis* sp. (HQ422790).

Lomentaria sp.

Sample A2 (Fig. 20A) was dark red with short, curved branches, frequent indeterminate branching, and the base of each branch had a very slight constriction (Fig.

22B). The branch tips were rounded (Fig. 20B, C). The specimens are hollow in cross section with a single layer of cortical cells, and one layer of small oval medullary cells (not shown).

Figure 20. Examples of *Lomentaria* sp. A2, herbarium and photomicrographs; A = herbarium sample, scale bar = 2cm, B = shows branching and slight constrictions, stained with aniline blue, scale bar = 400µm, C = branch tip, stained with aniline blue, scale bar = 100µm.

Sample A2 is sister to *Lomentaria hakodatensis* Yendo (HQ422881) but with low bootstrap support (50%) (Fig. 21). There is a clade containing *Lomentaria divaricata* (now *Hooperia divaricata* Durant) M.J.Whyne (Whyne *et. al.* 2019) (KU941949) and the above two sequences with high support bootstrap (87%). Sample A2 is 14.12% different in *cox1* to *Lomentaria hakodatensis* (HQ422881). A2 is 13.4% different in *cox1* to *Lomentaria divaricata* (KU941949) from Canada, its closest pairwise sequence and top Blast hit. I designated sample A2 as *Lomentaria* sp.

Figure 21. Maximum-likelihood tree of some of the Lomentariaceae using *cox1* sequences. Sample A2 shown. *Sonderopelta* sp. (JX969710) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.3 substitutions per site.

Chamaeobotrys spp.

Samples (Fig. 22A-C) were small, < 1cm, and were composed of rounded fleshy red vesicles with flattened upper surface. Samples were unbranched, except for A64 (Fig. 22C). A small holdfast was present and attached to the aragonite substrate, vesicles contained mucilage.

Sample A23 is sister to *Chamaeobotrys boergesenii* T.Tanaka (KU726729) based on *rbcL* sequence data, but with low bootstrap support (<50%)(Fig. 23). Sample A23 has a pairwise distance of 13.56% in *rbcL* to its sister *Chamaeobotrys boergesenii* (Weber Bosse) (KU726729) from Western Australia. The sample, however, has 10.7% pairwise difference to its top Blast hit *Chrysomenia brownii* (KU726719). I designated sample A23 as *Chamaeobotrys* sp1.

Samples A9 and A64 grouped together with high support bootstrap (93%). Aligned sequences show 0.31% difference between samples A9 and A64, they are within a clade containing their top Blast hit *Chamaeobotrys* sp. (KU726727) with low bootstrap support. Sample A9 has 4.66% pairwise difference in *rbcL* to *Chamaeobotrys* sp. (KU726727) from New South Wales, Australia and A64 has 4.62% pairwise difference to *Chamaeobotrys* sp. (KU726727). I designated samples A9 and A64 as *Chamaeobotrys* sp2.

Amphiroa sp.

Sample A33 (Fig. 24) was a geniculate coralline alga with thin heavily calcified branches with rounded branch tips. Branches were 1.5-4mm in length and 250µm in diameter. The specimen when collected was pink with pale branch tips.

Figure 24. Sample *Amphiroa* sp. A33, herbarium. Scale bar = 0.5cm.

Sample A33 is within a clade, based on *cox1* sequence data, containing uncultured Corallinales (GQ917303, GQ917507) and *Amphiroa valonioides* Yendo (HQ422698) and *Amphiroa bowerbankii* (LC071730) without support bootstrap (< 50%)(Fig. 25). Aligned sequences show that A33 has 9.95% pairwise difference in *cox1* to the top Blast hit and closest Genbank sequence *Amphiroa anceps* A.Mann (LC071723) from Eastern Cape, South Africa and *A. anceps* (LC071724, LC071725). I designated sample A33 as *Amphiroa* sp.

Figure 25. Maximum-likelihood tree of *Amphiroa* spp. using *cox1* sequences. Sample A33 shown. *Sporolithon durum* (NC 023454) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar shows 0.5 substitutions per site.

3.2 Identification of Green algae

Ulva ohnoi

One sample of *Ulva ohnoi* M.Hiraoka and S.Shimada was found free living in a holding tank at a pet shop in Lower Hutt; the sample was a ruffled light-green thallus. Preservation in ethanol resulted in loss of pigment (Fig. 26).

Figure 26. Sample *Ulva ohnoi* A42, photomicrograph. Scale bar = 1 mm.

Specimen A42 is within a clade, based on *tufA* sequence data, containing *Ulva ohnoi*, *Ulva beytensis*. M.Hiraoka and S.Shimada *Ulva reticulata* Forsskål and *Ulva taeniata* Setchell with moderate bootstrap support (81%)(Fig. 27). Aligned sequences showed that A42 is identical in *tufA* to the top Blast hit *Ulva ohnoi* (KF195523) from Australia and *U. ohnoi* (MF614793) from Gabes, Tunisia. I designated specimen A42 as *Ulva ohnoi*.

Figure 27. Maximum-likelihood tree of *Ulva* spp. using *tufA* gene sequences. Specimen A42 shown. *Ulvaria obscura* (Kützinger) Gayral ex Bliding (HQ610408) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.03 substitutions per site.

Chaetomorpha spp.

Three samples identified as *Chaetomorpha* sp. and *Lurbica* sp. were found during this study. Sample A80 (Fig. 28A) is formed of large sized cells, 400µm in diameter and 600µm-1mm in length, filaments are dark green and are tangled in rounded balls. The sample was unattached to the substrate. Specimen A82 (Fig. 30B) has thin unbranched upright filaments up to 6 cm long and is attached to substrate.

Figure 28. Samples of *Chaetomorpha* spp. Herbarium; A = *Chaetomorpha spiralis* A80, scale bar = 2cm, B = *Chaetomorpha* sp. A82 Scale bar = 2cm.

Sample A80 is within a clade, based on LSU sequences, containing *Chaetomorpha* sp. (LT607220) *Chaetomorpha* cf. *inflata* Kützinger (LT607071), *C. vieillardii* (LT607222, LT607223), *Chaetomorpha spiralis* Okamura (LT607221), and sample A112 (Fig. 29) with high support (100%). Both A80 and A112 are within a larger clade with high bootstrap support 100% that also contains *Chaetomorpha basiretrorsa* (LT607219). A80 has 0.4% difference in LSU to *Chaetomorpha spiralis* (LT607221), its closest pairwise sequence and top Blast hit. A80 has 0.5% pairwise difference to *C. vieillardii* (LT607222). I designated sample A80 as *Chaetomorpha vieillardii*. A112 has 100% identity to the top Blast hit *Chaetomorpha spiralis* (LT607221) from Mombasa, Kenya and a sample of *Chaetomorpha vieillardii* (LT607222) from Zanzibar, Tanzania. I designated sample A112 as *Chaetomorpha vieillardii*.

Figure 29. Maximum-likelihood tree of some Cladophorales using LSU gene sequences. Samples A80, A82, A112 shown. *Cladophoropsis membranacea* (AM503489) was used as the outgroup taxa. Only bootstrap support values >50 displayed. Scale bar = 0.04 substitutions per site.

Sample A82 is within a clade containing *Chaetomorpha cf. restricta* (Suhr) Kützinger, *Cladophorales sp.* and *Lurbica zinkwasii* Boedeker with high support bootstrap (97%). The aligned sequences show that A82 has 3.37% pairwise difference in LSU to all samples in the clade including the top Blast hit *Chaetomorpha cf. restricta* (LT607144) I designated sample A82 as *Lurbica sp.*

Cladophora spp.

Various *Cladophora* and samples were found on imported coral rocks sample. A19 (Fig. 30A) is branched with large cells 0.4-1.5mm in width and 1-2mm in length whole specimen had a fan shape. Sample A34 (Fig. 30B) is branched with long narrow cells 0.35-1mm in length. Sample A38 (Fig. 30C) has cells 40-60 μ m in diameter and 95-130 μ m in length. Sample A113 (Fig. 30D) is an unbranched filament with cells 60 μ m in width and 50-75 μ m in length. Micrographs of samples of A39, A115 and A114 were included in (Fig. S5).

Figure 30. Examples of *Cladophora* spp. Herbariums and photomicrographs; A = A19 scale bar = 1mm, B = A34 scale bar = 400 μ m, C = A38 stained with aniline blue, scale bar = 200 μ m, D = A113, scale 200 μ m.

Samples A19 and A39 are in a clade, based on LSU sequences with moderate support bootstrap (77%)(Fig. 31), and showed only 0.13% sequence difference to each other. They grouped with *Lychaete dotyana* (W.J.Gilbert) M.J.Wynne with high support bootstrap (100%). Both A19 and A39 have high similarity to the closest Genbank sequence *Lychaete dotyana* (LT904846) from Reunion Island, Indian Ocean. A19 has 0.47% difference and A39 has 0.35% difference with *L. dotyana* (LT904846) their closest pairwise sequence. I designated samples A19 and A39 as *Lychaete dotyana*.

Figure 31. Maximum-likelihood tree of some Cladophorales using LSU gene sequences. Samples A19, A38, A39, A113, A114, A115 shown. *Valoniopsis pachynema* (G.Martens) Børgeson (AJ544741) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.05 substitutions per site.

Both samples A113 and A115 had identical sequences of LSU and formed a well supported group (99%). The samples grouped with sample A38 but with lower bootstrap support (63%). These samples were in a clade with *Cladophora montagneana* Kützinger (now *Willeella brachyclados* (Montagne) M.J.Wynne) with low bootstrap support (69%).

Samples A38, A113 and A115 all have 2.8% difference in LSU to their closest Genbank sequence and top Blast hit, *Cladophora montagneana* (LT607098) from Western Australia. I designated samples A38, A113 and A115 as *Willeella* sp.

Samples A114 and A34 are sister to many of the species in the data set. A114 and A34 have 1.5% pairwise difference to each other, and are likely the same species. A114 has 6.62% pairwise difference in LSU to its top Blast hit, *Lychaete feredayi* (Harvey) M.J.Wynne (FM205030) from Kaikoura, New Zealand. I designated sample A114 as *Lychaete* sp. Specimen A34 has 6.32% difference in LSU to *Lychaete feredayi* (FM205030) its closest pairwise sequence and top Blast hit. I designated sample A34 as *Lychaete* sp.

Cladophoropsis sp.

Samples A36 and A43 (Fig. 32A-C) are tufts of rigid branching filaments, mostly 140-160 μm in diameter and up to 4 cm in length for A36, and up to 300 μm for sample A43, arising from a stolon. The stolon has cells of varying sizes up to 400 μm in diameter and 450 μm in length. Secondary attachment cells (tenaculæ) are present on branches, and rhizoids are present on stolons. Live specimen (Fig. 32C) had deep green pigmentation and a shiny appearance. Filament length and diameter is highly variable.

Figure 32. Examples of samples of *Cladophoropsis* sp., Photomicrographs; A = A36, B = A43, C = A36 live specimen grown in aquaria. Scale bars = 1mm.

Specimens A36 and A43 are within a large clade, based on LSU sequence data, containing *Cladophoropsis membranacea* Hofman Bang ex C.Agardh) Børgesen (AM503489), *Cladophoropsis philippinensis* W.R.Taylor, *Boodlea composita* (Harvey), *Boodlea montagnei* (Harvey ex J.E.Gray) Egerod, *Boodlea saimensis* Reinbold, *Cladophoropsis* sp., *Phyllodictyon anastomosans* (Harvey) Kraft and M.J.Wynne with moderate bootstrap support (74%) (Fig. 33). Sample A36 has 0.19% pairwise difference to the top Blast hit, *Cladophoropsis membranacea* (AM503489), and the following sequences: *Cladophoropsis* sp. (AM503489), *Boodlea saimensis* (AM503422), *Cladophoropsis philippensis* (AM503490), *Phyllodictyon anastomosans* (AM503520). Sample A43 has 0.14% pairwise difference in LSU to the top Blast hit *Cladophoropsis membranacea* (AM503489) and the following sequences: *Cladophoropsis* sp. (AM503489), *Boodlea saimensis* (AM503422), *Cladophoropsis philippensis* (AM503490), *Phyllodictyon anastomosans* (AM503520). I designated samples A36 and A43 as *Cladophoropsis* sp.

Figure 33. Maximum-likelihood tree of some species within the Siphonocladales using LSU gene sequences. Samples A36 and A43 shown. *Valoniopsis pachynema* (AJ544741) was chosen as the outgroup. Only bootstrap support >50 displayed. Scale bar = 0.008 substitutions per site

Valonia spp.

Six samples were found of rigid siphonous vesicles. Some specimens were irregularly branched, attached to the substrate by single holdfast, with green pigmentation and sometimes with a metallic appearance. Samples A27 and A54 (Fig. 34A) were solitary single vesicles. Sample A27 was large, 3cm in diameter. A73, A74, A116 (Fig. 34B, C) were in small clusters, sometimes branched.

Figure 34. Examples of samples of *Valonia* spp. Herbarium; A = A54 *Valonia ventricosa* scale bar = 1mm, B = A116 *Valonia macrophysa*, scale bar = 0.5cm, C = A73 *Valonia macrophysa*, scale bar = 0.5cm, D = A75 *Valonia fastigiata*, scale bar = 0.5cm.

Specimens A27 and A54 are within a clade, based on LSU sequence data, containing sequences named *Ventricaria ventricosa* (J.Agardh) J.L.Olsen and J.A.West (now *Valonia ventricosa* J.Agardh) with high bootstrap support (99%)(Fig. 35). Both A27 and A54 have identical sequences to the top Blast hit, *V. ventricosa* (AM503545) from Tahiti. I designated both A27 and A54 as *Valonia ventricosa*.

difference to the top Blast hit *V. macrophysa* (AM503536) from Kwa Zulu Natal, South Africa, whilst A73 has 0.4% difference to *V. macrophysa* (AM503536). I designated samples A73, A74 and A116 as *Valonia macrophysa*.

Bryopsis sp.

Thalli of sample A100 (Fig. 36) form dense soft tufts, up to 4 cm high, carpeting coral rock and mariculture plugs. Individual plants are like plumules, irregularly branched with a bare basal section of varying lengths. The upper section has distichous branching.

Figure 36. Sample *Bryopsis* sp. A100. Herbarium. Scale bar = 2cm.

Sample A100 is sister to and within a clade, based on *tufA* sequence data, of several *Bryopsis* samples: *Bryopsis* sp. (NC037363), *Bryopsis* sp. 28 (LK022446), *Bryopsis* sp. 24 (LK022445), *Bryopsis* sp. 21 (LK022440), *Bryopsis* sp. 20 (LK022452) and *Bryopsidaceae* sp. (KU361838), but without support bootstrap (< 50) (Fig. 37). The sample has 12.91% difference in *tufA* to the closest Genbank sequence and Blast hit, *Bryopsis* sp. (MK125425), that is not part of the clade containing sample A100. I

designated sample A100 as *Bryopsis* sp. most closely similar to *Bryopsis* sp. (MK125425) from Mallacoota, Victoria, Australia.

Figure 37. Maximum-likelihood tree of *Bryopsis* spp. using *tufA* gene sequences. Sample A100 shown. *Derbesia marina* (Lyngbye) Solier (HQ610250) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.3 substitutions per site.

Derbesia sp.

Thalli of sample A35 (Fig. 38A, B) are siphonous unbranched filaments 60-100µm in diameter and up to 3cm in length. The live specimen formed a dense turf of bright green filaments growing on coral rubble and across glass.

Figure 38. Sample of *Derbesia* sp. A35. Photomicrographs; A = unstained, B = stained with aniline blue. Scale bars =1mm.

Sample A35 groups, based on *tufA* sequences data, with *Derbesia* sp.

(KU361859) with high support bootstrap (93%) and in a clade (90% bootstrap support) with another *Derbesia* sp. sample (KU361860) (Fig. 39). The sample has 0.42% pairwise difference in *tufA* to *Derbesia* sp. (KU361859) its top Blast hit. I designated sample A35 as the same species as *Derbesia* sp. (KU361859) from Walton Rocks beach, Florida, USA.

Figure 39. Maximum-likelihood tree of *Derbesia* sp. using *tufA* gene sequences. Sample

A35 shown. *Bryopsis plumosa* (Hudson) C.Agardh (JQ755423) was used as the outgroup and was removed for clarity. Only bootstrap support values >50 displayed. Scale bar = 0.3 substitutions per site.

Halimeda sp.

Sample A88 (Fig. 40); Solitary specimen on coral rock, erect, branched with segments in a single plane. Each segment bears a single daughter segment except for the 5th segment that bears 5 daughter segments. Segments are lightly calcified but flexible and rubbery in texture when alive, light green pigmentation when fresh.

Figure 40. Sample *Halimeda* sp. A88. Herbarium. Scale bar = 2cm.

Sample A88 (Fig. 41) is sister, based on *rbcL* sequences, to many of the species in the dataset. Sample A88 has 3.8% pairwise difference in *rbcL* to *Halimeda discoidea* Decaisne (AB700354, AB038488) and *Halimeda magnidisca* J.M.Noble (FJ624530). A88 has 4% pairwise difference in *rbcL* to its top Blast hit *Halimeda* aff. *tuna* (KY570457). I designated sample A88 as *Halimeda* sp., possibly most similar to *H. discoidea*.

Figure 41. Maximum-likelihood tree of *Halimeda* sp. using partial *rbcL* gene sequences. Sample A88 shown. *Udotea cyathiformis* L. (MG784864) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.4 substitutions per site.

Rhipidosiphon spp.

Three specimens of *Rhipidosiphon* spp. were found, A20, A66 and A76, (Fig. 42).

They were green thalli erect of various sizes with rhizoidal holdfast, dichotomously branched siphons in a single plane on top of a short stipe, whole specimen looks like a fan. Sample A20 (Fig. 42A) appears to be lightly calcified. Sample A76 (Fig. 42B) has a stipe diameter of 120µm, cells close to the stipe are 35- 50µm in diameter, cells near edge of fan are 20µm in diameter. The whole specimen is 5mm in height including the stipe and 6 mm in diameter across the widest point of the fan shaped structure. Sample A66 (Fig. 42C) is 4mm in height including the stipe, and retains the deep green pigmentation from the live specimen.

Figure 42. Examples of samples of *Udotea* sp. Photomicrographs; A = A20, B = A76, and C = A66. Scale bars = 1mm.

Sample A20 (Fig. 43) is sister, based on *rbcL* sequences to *Udotea glaucescens* Harvey ex J.Agardh (FJ432650) with moderate support bootstrap (70%). A20 is 1.3% different in *rbcL* to *Udotea glaucescens* (FJ432650) its closest pairwise sequence. Sample A20 is 4.3% different to the top Blast hit *Rhipidosiphon* sp. (AB700348). I designated A20 as *Udotea glaucescens*.

Figure 43. Maximum-likelihood tree of *Udoteaceae* using partial *rbcL* gene sequences. Samples A20, A76, A66 shown. *Halimeda macroloba* Decaisne (FJ624513) was used as the outgroup. Only bootstrap support values >50 displayed. Scale bar = 0.02 substitutions per site.

Samples A76 and A66 group together with moderate support bootstrap (75%).

The samples have 2.8% difference in *rbcL*. A76 and A66 are within a clade containing sample A20 and *Udotea glaucescens* (FJ432650) without support bootstrap (<50 %). A76 is 4.7% different to the top Blast hit *Udotea* sp. (MH591113) from Zanzibar, Tanzania. I designate sample A76 as *Udotea* sp. Sample A66 has 4% pairwise difference in *rbcL* to the closest Genbank sequence and top Blast hit *Udotea* sp. (MH591113). I designated A66 as *Udotea* sp.

Caulerpa spp.

A maximum-likelihood tree was built containing a total of 77 *tufA* gene sequences of *Caulerpa* spp. Samples A25, A27, A40, A41, A45 A79, A91, A111 were included. *Caulerpella ambigua* Okamura (KM186521) was used as the outgroup. The following trees for *Caulerpa* spp. are subsets from this full tree (Figure S1).

Caulerpa cylindracea

Sample A25 (Fig. 44) is a siphonous green alga with a thick stolon, 2.5mm diameter, thick assimilators, 2 mm diameter and 4-5 cm length, with one row of elongate ramuli with rounded tips on opposite sides of the assimilator. Some assimilators have slightly enlargement above their attachment to the stolon, many rhizoids are present along the stolon, up to 2 cm length.

Figure 44. Sample A25 *Caulerpa cylindracea*. Herbarium voucher. Scale bar = 2cm.

Specimen A25 is within a clade, based on the full maximum-likelihood tree (Fig. S1) of *Caulerpa* spp. *tufA* sequence data, containing *Caulerpa okamurae* Weber Bosse (KX809677), *Caulerpa cylindracea* and *Caulerpa racemosa* with high support bootstrap (99%). A25 is identical to all the sequences in the clade including the top Blast hit *Caulerpa cylindracea* (FM956048). I designated specimen A25 as *Caulerpa cylindracea*.

Figure 45. Clade removed from the full *tufA* maximum-likelihood tree of *Caulerpa* spp. Specimen A25 shown. Only bootstrap support values >50 displayed. Scale bar = 0.1 substitutions per site. X shows where the clade was removed from the full tree.

Caulerpa lentillifera

Sample A26 (Fig. 46) is a siphonous green alga with thin assimilators, 1-1.5mm diameter, 2-6 cm long, with rounded ramuli, 1-1.5 mm diameter. Assimilators have ramuli along entire length except for 5-7 mm from attachment to the infrequently branched stolon, many short rhizoids are present along the length of the stolon.

Figure 46. Sample A26 *Caulerpa lentillifera*. Herbarium voucher. Scale bar = 2cm.

Sample A26 is within a clade, based on the full maximum-likelihood tree (Fig. S1) of *Caulerpa* spp. *tufA* containing *Caulerpa lentillifera* J.Agardh and *Caulerpa microphysa* (Weber Bosse) W.R.Taylor with high support bootstrap (100%)(Fig. 47). Sample A26 is identical to all Genbank sequences in the clade including its top Blast hit *Caulerpa lentillifera* (NC039377), and excluding *Caulerpa microphysa* (DQ652518). I designated A26 as *Caulerpa lentillifera*.

Figure 47. Clade from the full *tufA* maximum-likelihood tree of *Caulerpa* spp. using *tufA* sequence data. Specimen A26 shown. Only bootstrap support values >50 displayed. Scale bar = 0.1 substitutions per site. X shows where the clade was removed from the full tree.

Caulerpa sp.

Sample A41 (Fig. 48A) is a siphonous green alga with thin assimilators <0.5mm diameter, <2 cm long and few flattened mushroom-shaped ramuli, with thin stolons, <0.5 mm in diameter, and has many short rhizoids, <7mm length.

Figure 48. Samples of *Caulerpa* spp. Herbariums; A = *Caulerpa selago* A41 scale bar = 2cm. B = *Caulerpa chemnitzia* A91 scale bar = 2cm.

Sample A91 (Fig. 48B) is a siphonous green alga with thin assimilators, 0.5mm diameter, <2 cm long and some flattened mushroom-shaped ramuli, with thin stolons, <0.5 mm in diameter, and has many short rhizoids, <7mm length.

Sample A41 is sister, based on the full maximum-likelihood tree (Fig. S1) of *Caulerpa* spp. *tufA* to *Caulerpa selago* (Turner) C.Agardh (FM956061) from Indonesia with moderate support bootstrap (78%) (Fig. 49). A41 is identical in *tufA* to its sister *Caulerpa selago* (FM956061) and its top Blast hit *Caulerpa racemosa* (DQ652466). The difference in phylogenetic placement may be due to different sequence lengths. I designated sample A41 as *Caulerpa selago*.

Figure 49. Clade from the full *tufA* maximum-likelihood tree of *Caulerpa* spp. using *tufA* sequence data. Specimens A41 and A91 shown. Only bootstrap support values >50 displayed. Scale bar = 0.025 substitutions per site. X shows where the clade was removed from the full tree.

The clade containing sample A91, also contains sequences named *Caulerpa peltata* J.V.Lamouroux (KC153510, AJ512415), 7 sequences of *C. chemnitzia* (Esper) J.V.Lamouroux, *C. racemosa* (AJ417950, DQ652466), *C. selago* (FM956061) and sample A41, excluding *C. racemosa* (AJ417950) with high support bootstrap (86%). Sample A91 has 0.1% pairwise difference to the top Blast hit *C. chemnitzia* (KJ957093) and *C. chemnitzia* KF256103, KU761457, KU761456) and *C. peltata* (KC153510). *C.*

peltata has been synonymised with *C. chemnitzia* (Belton *et. al.* 2014). I designated sample A91 as *Caulerpa chemnitzia*.

Caulerpa sp.

Sample A40 (Fig. 50) is a siphonous green alga with flattened or slightly rounded mushroom shape ramuli, thin assimilators, 0.70mm diameter, with few ramuli, <7, the stolon is 0.7 mm diameter. Rhizoids, <1 cm length, are frequent along the stolon, rhizoids are larger close to the assimilators.

Figure 50. Sample *Caulerpa racemosa* var. *macrophysa* A40. Herbarium voucher. Scale bar = 2cm.

Sample A40 is within a clade, based on the full maximum-likelihood tree (Fig. S1) of *Caulerpa* spp. *tufA* sequence, containing *Caulerpa racemosa* f. *macrophysa* (Sonder ex Kützing) Svedelius with full bootstrap support (Fig. 51). A40 is identical in *tufA* to the top Blast hit *Caulerpa racemosa* f. *macrophysa* (KJ957088) and sequences *Caulerpa racemosa* f. *macrophysa* (KJ957087, KJ957085, KJ957086, KJ957084). The entity *Caulerpa racemosa* f. *macrophysa* has been synonymised with *Caulerpa racemosa*

var. *macrophysa* (Sonder ex Kützing) W.R.Taylor. I designated specimen A40 as *Caulerpa racemosa* var. *macrophysa*.

Figure 51. Clade from the full *tufA* maximum-likelihood tree of *Caulerpa* spp. using *tufA* sequence data. Sample A40 shown. Only bootstrap support values >50 displayed. Scale bar = 0.1 substitutions per site. X shows where the clade was removed from the full tree.

Caulerpa serrulata

Sample A45 (Fig. 52) is a siphonous green alga with a thick stolon, 2mm in diameter, short assimilators with flat dichotomous branching blades with serrated margins. The short rhizoids are spaced irregularly along the stolon.

Figure 52. Sample *Caulerpa serrulata* A45 herbarium. Scale bar = 2cm.

Sample A45 is within a clade, based on the full maximum-likelihood tree (Fig. S1) of *Caulerpa* spp. *tufA* sequences, containing mostly sequences from samples identified as *Caulerpa serrulata* and but also a samples identified as *Caulerpa cupressoides* (Vahl) C.Agardh (DQ652336) with moderate support bootstrap (81%)(Fig. 53). Sample A45 has 0.12% pairwise difference from the top Blast hit *Caulerpa serrulata* (KJ957119, KF314147, DQ652346). I designated sample A45 as *Caulerpa serrulata*.

Figure 53. Clade from the full *tufA* maximum-likelihood tree of *Caulerpa* spp. using *tufA* sequence data. Sample A45 shown. Only bootstrap support values >50 displayed. Scale bar = 0.025 substitutions per site. X shows where the clade was removed from the full tree.

Caulerpa spp.

Sample A79 (Fig. 54A) is a siphonous green alga with flattened mushroom shaped ramuli, assimilators are thin, <0.5mm diameter and <2 cm long, with few ramuli, the stolon is thin, <0.5 mm diameter, and has many short rhizoids, <7mm length.

Figure 54. Samples A79 and A111, *Caulerpa* sp., herbariums A = A79, B = A111. Scale bar = 2cm

Sample A111 (Fig. 54B) is a siphonous green alga with flattened mushroom shaped ramuli, assimilators are thin, <0.5mm diameter and <2 cm long, with few ramuli. The stolon is thin, <0.5 mm diameter, and has many short rhizoids, <7mm length.

Sample A79 is within a basal section, based on the full maximum-likelihood tree (Fig. S1) of *Caulerpa* spp. *tufA* sequence data, containing uncultured Ulvophyceae (GU592622, GU592621, GU592606), sample A111, *Caulerpa nummularia* Harvey ex J.Agardh (JN851138, KF256098) and *Caulerpa racemosa* (DQ652426) with no bootstrap support (< 50%)(Fig. S1). Sample A79 is identical in *tufA* to the top Blast hit *Caulerpa racemosa* (DQ652426), and sequences named uncultured Ulvophyceae (GU592606) and *Caulerpa nummularia* (JN851138). I designated sample A79 as *Caulerpa racemosa*. Sample A111 has 0.13% pairwise difference to the top Blast hit *Caulerpa nummularia* (JN851138) from Australia. I designated sample A111 as *Caulerpa nummularia*.

Caulerpa sertularioides

Sample A78 is a siphonous green alga with feather like assimilators that are between 1.5 and 2.5 cm in length and 1 mm in diameter. The assimilators are dark green and are frequent along the stolon. The stolon is moderately thick 1mm in diameter. Cylindrical pinnules, ending in sharp points are present on opposite sides of the assimilator, giving the alga a feathery appearance. Rhizoids are present on short off branches from the stolon. This sample was not sequenced so we have no molecular identification. I designated specimen, A78, as *Caulerpa sertularioides* (S.G.Gemlin) M.Howe.

Figure 55. Herbarium of sample A78 *Caulerpa* sp. Scale bar = 2 cm.

3.3 Identification brown algae

Dictyopteris sp. and *Dictyota* sp.

Sample A109 (Fig. 56A) is a dichotomously branched brown alga with a visible midrib. Branch tips are pale and there are two clusters of apical cells and a midrib, typical of *Dictyopteris* species. Sample A110 (Fig. 56B) is dichotomously branched with no midrib visible. Sample A5 (Fig. 56C) was not sequenced but has morphology typical of *Dictyopteris* sp. (dichotomous branching thallus, a visible midrib and 1 cell layer thick). Micrographs of samples A59 unsequenced and A62 *Dictyota* sp1 were included in (Fig. S4).

Figure 56. Samples of *Dictyota* sp. and *Dictyopteris* spp. Photomicrographs; A = *Dictyopteris* sp. A109 scale bar = 1mm. B = *Dictyota* sp. A110 scale bar = 1mm, C = *Dictyopteris* sp. A5 unsequenced *Dictyopteris* sp., scale bar = 400 μ m.

Sample A62 is sister to its top Blast hit *Dictyota friabilis* Setchell (GU290244) with moderate bootstrap support (88%)(Fig. 57). The sample is most similar to *Dictyota friabilis* (GU290244) from Oahu Hawaii with 6.24% pairwise difference. I designated sample A62 as *Dictyota* sp1, most closely related to *Dictyota friabilis* (GU290244). Sample A110 does not cluster with any other sequence. A110 is 10.03% different from *Dictyota friabilis* (GU290244) its closest pairwise sequence and top Blast hit. I designated sample A110 as *Dictyota* sp2.

Figure 57. Maximum-likelihood tree of *Dictyota* spp. using *cox3* gene sequences. Samples A62, A109 and A110 shown. *Zonaria flabellata* (JQ364040) was used as the outgroup. Only bootstrap support >50 displayed. Scale bar = 0.4 substitutions per site.

Sample A109 is sister, based on *cox3* sequences, to *Dictyopteris polypodioides* (A.P.Candolle) J.V.Lamouroux (EU681445) with high bootstrap support (94%). The sample is 19.26% pairwise different in *cox3* to *Dictyopteris polypodioides* (EU681445) its top Blast hit. I designated sample A109 as *Dictyopteris* sp.

Turbinaria ornata

No voucher specimen of A6 is available as the whole sample was required for DNA extraction.

Sample A6 (Fig. 58) groups, based on *cox3* sequences, with *Turbinaria ornata* (Turner) J.Agardh (KN501562) with high bootstrap support (97%). These are in a larger clade with another *Turbinaria ornata* sample (KY935437) with full bootstrap (100%). Sample A6 has 0.73% pairwise difference in *cox3* to the closest Genbank sequence and top Blast hit *Turbinaria ornata* (KM501562). I designated sample A6 as *Turbinaria ornata*.

Figure 58. Maximum-likelihood tree of *Turbinaria ornata* using *cox3* sequences. Sample A6 shown. *Sargassum hemiphyllum* (Turner) C.Agardh (JF931744) was used as the outgroup, but was removed for clarity. Only bootstrap support values >50 displayed. Scale bar = 0.04 substitutions per site.

Lobophora asiatica.

All *Lobophora* spp. samples found in this study had similar external morphology with a flattened thallus attached to the substrate with rhizoids, light to dark brown pigmentation with a darker ring of apical cells on the growing margin of the thallus. Most samples were small so were preserved on microscope slides. Both samples A7 and A32 were round flattened thalli with light brown pigmentation. A noticeable pale ring and darker apical cell margin was present. Rhizoidial holdfasts anchored the specimens to aragonite substrate. A7 was collected from a *Zoanthus* sp. colony (Fig. 59A) whilst A32 was collected from a separate *Zoanthus* sp. colony (Fig. 59B).

Figure 59. Samples of *Lobophora asiatica* Z.Sun, Ji.Tanaka & H. Kawai photomicrographs; A = A7, scale bar = 400μm, B = A32, scale bar = 1mm.

A maximum-likelihood tree was built using a total of 116 *Lobophora* spp. *cox3* sequences of samples A7, A32, A46, A57, A58, A60, A61 and A108. *Zonaria flabellata* (JQ364040) was used as the outgroup. (Fig. S2). The following trees are subcades removed from the main *Lobophora* spp. tree

Samples A7 and A32 (Figs. 59, 60) group based on *cox3* sequences with high bootstrap support (92%), aligned sequences show that the samples are identical in *cox3*. There is a clade, containing *Lobophora asiatica* Z.Sun (KU353339, KU353338, AB665365), with moderate bootstrap support (77%). A7 and A32 both are 0.31% different to *Lobophora asiatica* (KU353339), their closest pairwise sequence and top Blast hit. I designated samples A7 and A32 as *Lobophora asiatica*.

Figure 60. Subclade from the full maximum-likelihood tree of *Lobophora* spp. using *cox3* sequences. Specimens A7 and A32 shown. Only bootstrap support values >50 displayed. Scale bar = 0.1 substitutions per site. X shows where the subclade was removed from the full tree.

Lobophora sp.

Sample A46 was a small thin flattened thallus, with light brown pigmentation and a dark apical margin (Fig. 61). The voucher specimen was 7 mm in diameter and was collected from a *Caulastrea tumida* (Matthai 1928) skeleton imported from Australia.

Figure 61. Sample of *Lobophora* sp. 24 A46. Photomicrograph. Scale bar = 400µm.

Sample A46 (Figs. 61, 62) is sister to *Lobophora* sp. 24 (KU353341) with high support bootstrap (95%) and is within a clade containing *Lobophora* sp. 24 (KU353342) and *Lobophora* sp. with high bootstrap support (100%). Sample A46 is 0.62% different to the top Blast hit *Lobophora* sp. 24 (KU353342) from Weizhou Island, Beigang, China, and a sequence named *Lobophora* sp. (AB665382), that are within the clade but not sister to A46. Sample A46 has 0.77% pairwise difference to its sister *Lobophora* sp. 24 (KU353341) from Trang area, Ko Vaen, Thailand. I designated sample A46 as *Lobophora* sp. 24

Figure 62. Subclade from the full maximum-likelihood tree of *Lobophora* spp. using *cox3* sequences. Specimen A46 shown. Only bootstrap support values >50 displayed. Scale bar = 0.05 substitutions per site. X shows where the subclade was removed from the full tree.

Lobophora sp.

Sample A58 was a flattened thallus with brown pigmentation (Fig. 63). The voucher specimen was a fragment from 1.5 cm diameter specimen collected from a *Galaxea* sp. colony from Australia.

Figure 63. Sample *Lobophora abscondita* A58 photomicrograph. Scale bar = 1mm

Sample A58 (Figs. 63, 64) is within a clade containing *Lobophora abscondita* C.W.Vieira, Payri and De Clerck with moderate bootstrap support (83%), the sample is 0.62% different from *Lobophora abscondita* (KU353163, KM487773) its closest pairwise sequences. The top Blast hit is to *Lobophora abscondita* (KU353163). I designated sample A58 as *Lobophora abscondita*.

Figure 64. Subclade from the full maximum-likelihood tree of *Lobophora* spp. using *cox3* sequences. Specimen A58 shown. Only bootstrap support values >50 displayed. Scale bar = 0.05 substitutions per site. X shows where the subclade was removed from the full tree.

Lobophora sp1.

Sample A60 (Fig. 65) was a small plating thallus with dark brown pigmentation and a margin of apical cells. The sample was 8 mm in diameter when collected but fragmented when removed from its substrate (a *Favia speciosa* (Dana 1846) colony imported from Australia).

Figure 65. Sample A60 *Lobophora* sp. photomicrograph. Scale bar = 1mm

Specimen A60 (Figs. 65, 66) is sister to *Lobophora* sp. 14 (KU353279) with low bootstrap support (55%), and is within a clade containing *Lobophora petila* C.W.Vieira, Payri and De Clerck (KU353322, KU353320, KU353319), *Lobophora* sp. 30 (KU353283, KU353282) and *Lobophora* sp. 29 (KU353290) with high support bootstrap (100%). Sample A60 has 6.79% pairwise difference to its sister *Lobophora* sp. 14 (KU353279), however A60 is closer in pairwise difference to its top Blast hit *Lobophora petila* (KU353322) from Papua New Guinea with 5.73% difference. I designated sample A60 as *Lobophora* sp1, most similar to *Lobophora petila*.

Figure 66. Subclade from the full maximum-likelihood tree of *Lobophora* spp. using *cox3* gene sequences. Specimen A60 shown. Only bootstrap support values >50 displayed. Scale bar = 0.2 substitutions per site. X shows where the subclade was removed from the full tree.

Lobophora sp.

Sample A108 was a small flattened thallus with a dark brown pigmentation, fading to a pale growth margin with dark apical cells (Fig. 67). Voucher specimen is 6 mm in diameter and was collected from a dead *Pavona* sp. skeleton from Australia.

Figure 67. Sample *Lobophora* sp. 6 A108. Photomicrograph, scale bar = 1mm.

Sample A108 (Figs. 67, 68) is sister to the top Blast hit *Lobophora* sp. 6 (KU353257) with high support bootstrap (96%), and is within a clade containing

Lobophora sp. 6 (KM487892) with moderate bootstrap support (71%). Sample A108 has 0.17 % pairwise difference to the closest Genbank sequence *Lobophora* sp. 6 (KU353257) from Mangareva, Gambier Islands, French Polynesia. I designated specimen A108 as *Lobophora* sp. 6.

Figure 68. Subclade from the full maximum-likelihood tree of *Lobophora* spp. using *cox3* sequences. Specimen A108 shown. Only bootstrap support values >50 displayed. Scale bar = 0.2 substitutions per site. X shows where the subclade was removed from the full tree.

Lobophora spp.

Sample A57 is a thin flattened thallus, with light brown pigmentation and a dark apical margin. The voucher specimen was ethanol preserved and has lost pigmentation.

Sample A61 is a rounded flat thallus with dark brown pigmentation. The voucher specimen had rhizoidal attachment to substrate and was collected from a *Caulastrea furcata* (Dana 1846) skeleton.

Figure 69. Samples of *Lobophora* spp., herbarium and photomicrographs; A = photomicrograph of sample *Lobophora* sp. A57, scale bar = 400 μ m, B = herbarium photo of A61, scale bar = 1cm.

Sample A57 (Figs. 69A, 70) is sister to *Lobophora* sp. 57 (KU353343), its top Blast hit with high bootstrap support (97%), and with 2.65% pairwise difference. I designated sample A57 as *Lobophora* sp2 most similar to *Lobophora* sp. 57 (KU353343) from Mangareva, French Polynesia.

A61 (Figs. 69B, 70) is sister to its top Blast hit *Lobophora* sp. 10 (KU353273) from Juan de Nova Island with high support bootstrap (100%), and has 0.47% sequence difference between the two sequences. I designated sample A61 as *Lobophora* sp. 10.

Figure 70. Subclade from the full maximum-likelihood tree of *Lobophora* spp. using *cox3* sequences. Specimens A57 and A61 shown. Only bootstrap support values >50 displayed. Scale bar = 0.2 substitutions per site. X shows where the subclade was removed from the full tree.

3.4 Physiological tolerance

A total of 10 algal samples (7 red, 1 brown and 3 green) were tested.

Samples A25, A80 and A62 were maintained for 2-4 weeks in the experimental conditions to determine their survival as they appeared to remain viable and were checked for this extended period (Table 1).

All samples survived at the control temperature of 25°C and also at 20°C during the 2-4 week experimental period. All samples at the Auckland minimum SST of 14°C did not survive at 2-weeks or 4 weeks, except sample *Chaetomorpha vieillardii* (A80) that survived for 4-weeks. All samples at the Wellington minimum SST of 11°C did not survive at 2-weeks or 4-weeks.

Table 1. Survival of 10 macroalgae specimens. Incubation temperatures ($\pm 1^\circ\text{C}$). – = no survival in all replicates, + = 100% survival in all replicates.

Isolate code	Species ID	Incubation Temperature			
		11°C	14°C	20°C	25°C
A23	<i>Chamaebotrys</i> sp1.	-	-	+	+
A106	<i>Ceratodictyon</i> sp.	-	-	+	+
A55	<i>Hypnea wynnei</i>	-	-	+	+
A56	<i>Hypnea</i> sp1.	-	-	+	+
A99	<i>Ceratodictyon repens</i>	-	-	+	+
A102	<i>Laurencia</i> sp2.	-	-	+	+
A36	<i>Cladophoropsis</i> sp.	-	-	+	+
A80	<i>Chaetomorpha vieillardii</i>	-	+	+	+
A25	<i>Caulerpa lentillifera</i>	-	-	+	+
A62	<i>Dictyota</i> sp1.	-	-	+	+

After 1 week at 11°C *Chaetomorpha vieillardii* (A80)(Fig. 71) showed chloroplast constriction and did not recover during the experiment. *Chaetomorpha vieillardii* (A80) at 14°C treatment remained normally pigmented with no signs of bleaching or chloroplast constriction.

Figure 71. Micrographs of *Chaetomorpha vieillardii* at weekly intervals during the first 3 weeks of the 4-week physiological tolerance experiment. A, C and E are at 11°C over weeks 1, 2 and 3 respectively. B, D and F are at 14°C over weeks 1,2 and 3 respectively. Scale bars = 400µm.

4.0 Discussion

4.1 Algal introductions

Our data reveals a large diversity of tropical macroalgae arriving in New Zealand through the marine aquarium trade. A total of 62 different species were identified, although some samples could only be identified to genus level. There is a high diversity of tropical macroalgae in the European marine aquarium trade (Vranken *et. al.* 2018), as well as in Brazil (Torrano-Silva *et. al.* 2013). Macroalgal diversity in New Zealand tropical marine aquaria has been understudied (Smith *et. al.* 2010). The presence of nonindigenous algae in aquaria poses the risk of introduction of potentially invasive species. The impacts of such species introductions can be large, including decrease in native species richness (Casas *et. al.* 2004), biofouling of aquaculture equipment (Fitridge *et. al.* 2012; Pochon *et. al.* 2015), and great effort and cost of eradication attempts.

The aquarium trade is a known vector of algal introductions including the introduction of the cold tolerant *Caulerpa taxifolia* to the Mediterranean from an aquarium at the Oceanographic Museum, Monaco (Baulch 1999; Meinesz and Hesse 1991). Other *Caulerpa* spp. introduced outside their native range includes *C. chemnitzia* (Sghaier *et. al.* 2015), *C. cylindracea* (Bernardeau-Esteller 2015) and *C. racemosa* (Nyberg and Wallentinus 2005; Piazzzi *et. al.* 2003). Our results show that at least seven different species of *Caulerpa* spp. are present in New Zealand aquaria. Species found include: *C. chemnitzia*, *C. cylindracea*, *C. lentillifera*, *C. nummularia*, *C. racemosa*, *C. racemosa* var. *macrophysa* and *C. sertularioides*. Some *Caulerpa* species have caused great detriment to native ecosystems due to shading and outcompeting native species (Meinesz *et. al.* 2001; Piazzzi *et. al.* 2003; Piazzzi *et. al.* 2016). *Caulerpa* spp. can regenerate from small fragments and can be difficult to eradicate once established in aquaria (personal observations) and in natural ecosystems (Ceccherelli and Cinelli 1999;

Smith and Walters 2002). A previously study has recorded the presence of a non-invasive strain of *Caulerpa taxifolia* and *Caulerpa racemosa* in the New Zealand Aquarium trade (Smith *et. al.* 2010).

Thau Lagoon in Hérault, France is a hotspot for introduced species (Verlaque 2001). The main vector of species introductions to Thau Lagoon was shellfish spat importation from Japan (Verlaque 2001). A few of the many introduced species are: *Derbesia rhizophora* Yamada, *Grateloupia* cf. *turuturu* Yamada, *Laurencia okamurae* Yamada, *Lomentaria hakodatensis* Yamada, *Ulva pertusa* Kjellman, *Undaria pinnatifida* and *Valonia utricularis* var. *aegagropila* (C.Agardh) Hauck (Verlaque 2001). Many are congeners to species found in the New Zealand aquarium trade. Our research found representatives of the genera *Chondrophycus*, *Derbesia*, *Laurencia*, *Lomentatia*, *Ulva* and *Valonia*, which are known as introduced or invasive species. *Valonia* spp. has been documented in overseas aquarium trades also. *Valonia aegagropila* is present in aquaria in Brazil (Torrano-Silva *et. al.* 2013) and *V. macrophysa* is present in European aquaria (Vranken *et. al.* 2018). *Ulva ohnoi* is an invasive green alga native to the Indo- Pacific that was introduced to the Mediterranean (Flagella *et. al.* 2010). *Ulva ohnoi* is also present in Japan where it is known for its green tide blooms (Hiraoka *et. al.* 2004). One specimen of *Ulva ohnoi* was found at a Wellington aquarium livestock retailer. *Ulva ohnoi* has recently been identified in temperate Australia (Kirkendale *et. al.* 2013), this is evidence that *U. ohnoi* can survive in lower temperatures and would likely survive in northern New Zealand if released from aquaria.

Hypnea Lamouroux is another genus of red alga that is known as an introduced species. I found 4 different species of *Hypnea* in the New Zealand marine aquarium trade. One species, *H. wyneei*, was recorded four times. *Hypnea musciformis* is a common species introduced to non-native locations for mariculture (Russel 1992). *Hypnea cornuta*

(Kützinger) J. Agardh was introduced to Taranto, Italy (Cecere *et. al.* 2004; Petrocelli *et. al.* 2015). *Hypnea flexicaulis* Y. Yamagishi and M. Masuda was introduced to Venice, Italy likely through a shellfish or ship vector (Wolf *et. al.* 2011), and has recently been identified in New Zealand (D'Archino *et. al.* 2015).

Our results also show the presence of at least three tropical Cladophorales in New Zealand marine aquaria. The species found are *Willeella* sp. and *Lychaete dotyana*, and two samples designated only as *Lychaete* sp. Both *Willeella* and *Lychaete* are recent segregates of *Cladophora*. The green algal genus *Cladophora* is known to contain some introduced species, including *Cladophora ruchingeri* (C. Agardh) Kützinger a recent introduced green alga to the Marlborough Sounds in New Zealand (Pochon *et. al.* 2015). *Cladophora ruchingeri* is invasive and results in blooms that may clog mussel aquaculture equipment. Species of the genera *Chaetomorpha* and *Lurbica* Boedeker were also found with two occurrences of *Chaetomorpha vieillardii*, a potential invasive and one occurrence of *Lurbica* sp. The Cladophorales phylogeny shows very little genetic difference between *Chaetomorpha spiralis*, *Chaetomorpha vieillardii* and *Chaetomorpha* cf. *inflata*. Boedeker *et. al.* (2016) found that some sequences of *C. vieillardii* and *C. spiralis* were identical in LSU, and it appears that *C. spiralis* and *C. vieillardii* may be the same or similar species. Sequences labeled as different species in Genbank with little to no pairwise difference makes it difficult to distinguish between species.

The genus *Chaetomorpha* contains bloom-forming strains such as the invasive strain of *Chaetomorpha viellardii* present in Guam (University of Guam https://cnas-re.uog.edu/wp-content/uploads/2018/09/Invasive_speciesGuam.pdf). *C. vieillardii* has been found for sale in the European aquarium trade along with another unidentified species (Vranken *et. al.* 2018). Another species of *Chaetomorpha*, *C. spiralis* has been found in aquaria in Brazil (Torrano-Silva *et. al.* 2013). With knowledge on the potential

for invasiveness in *Chaetomorpha* spp., methods of safe disposal and implications of unsafe disposal have been discussed (Odom *et. al.* 2014). It has been found that *Chaetomorpha* sp. can survive extended periods ~6 days in freshwater, this means that algal tissue or fragments in aquarium water may survive and reach the ocean if disposed of into storm water drains (Odom *et. al.* 2014).

4.2 Temperature tolerance

Our temperature tolerance testing of 10 algal samples to New Zealand minimum winter temperatures in Auckland and Wellington found that only a *Chaetomorpha vieillardii* sample (A80) tolerated 14°C. Cold tolerance in *Chaetomorpha* spp. has previously been shown in *Chaetomorpha crassa* (C.Agardh) Kützinger, *Chaetomorpha moniligera* Kjellman and *Chaetomorpha spiralis* with growth at 15°C and little growth at 10°C, although it is still able to survive (Gao *et. al.* 2018.). Growth at low temperatures makes *Chaetomorpha* spp. a potential risk to New Zealand if released. Other studies have found cold tolerance in tropical algae. The Australian native and invasive *Caulerpa taxifolia* can survive and grow at temperature between 15-18°C (Galasby and Gibson 2007). The Mediterranean ecotype of the tropical alga *Valonia utricularis* has a lower temperature limit of 5°C (Eggert *et. al.* 2003) making it capable of surviving across most of New Zealand. We cannot confirm that *V. utricularis* is present in the New Zealand aquarium trade as we did not find this particular species, however three species of *Valonia* were found. Marine aquaria are often kept at lower temperatures than the temperature of natural reefs, potentially selecting for more cold tolerant strains as has been suggested with the invasive strain of *Caulerpa taxifolia* (Meinesz and Boudouresque 1996).

4.3 Implications of propagule pressure on algal introductions

Propagule pressure is an important determinant of introduction success (Duggan *et. al.* 2006). There are two aspects of propagule pressure; the initial number of individuals introduced and the frequency of subsequent immigration (Drake and Lodge 2004). The more individuals introduced (larger propagule numbers) the higher the probability of establishment. An example of propagule pressure in a natural system is the large populations of the introduced alga *Undaria pinnatifida* in marinas, facilitating its spillover to nearby rocky reef ecosystems (Epstein and Smale 2018). Our results have found multiple occurrences of certain species, such as *Hypnea wynnnei*. Two of the three *Valonia* species were also found occurred multiple times, with two occurrences of *V. ventricosa* and three occurrences of *V. macrophysa*. Species in the Peyssonneliaceae were found multiple times, four samples of *Peyssonnelia* spp. and two samples of *Ramicrusta* sp. This indicates, if this reflects the importation incidence, that propagule pressure from these species, or other species, in New Zealand aquaria could increase the risks of release and establishment. The importation of corals into New Zealand occurs on a regular basis. The main importer in New Zealand currently imports new stock about once a month. A single importation may contain over 100 coral specimens (personal observation). The frequency of coral arrivals and the large number of corals in the importation increases the chance of encountering a variety of algae species and multiple individuals of the same species. We cannot conclude whether the multiple occurrences of certain species of macroalgae that we found in our study are due to multiple introductions to New Zealand or the reproduction of macroalgae in the importers aquaria and their subsequent recruitment to different coral rocks. Our results do indicate that some species are

prevalent in aquaria and so propagule pressure, and chance release, from personal aquaria and retail is still a concern.

4.4 *Algal reproduction in aquaria*

The ability of an alga to survive and reproduce in aquaria could have implications for its potential as an introduced species. Many algae have the capability to regenerate from small fragments or holdfasts making eradication from an aquarium difficult. *Hypnea wynnnei* had cystocarps in the sample we collected, showing the potential for sexual reproduction. Sexual reproduction allows for increased genetic variation in populations. Sexual reproduction increases the spread of the alga between hobbyists' tanks, through spores, when trading corals and fish. The fluctuation in water parameters that occurs in aquaria may provide selection pressures, selecting for weedy strains and developing strains more tolerant to changes in environmental conditions and potentially being more invasive. Sexual recombination produces offspring with more resistance to certain environmental conditions. This has been shown in sexual strains of *Escherichia coli* Migula (Peabody *et. al.* 2017) and yeast (Goddard *et. al.* 2005). Another example of sexual recombination under selection producing a more tolerant strain is a 39-fold increase in imidacloprid resistance occurring in the ladybeetle *Propylaea japonica* (Thunberg) after 20 generations under laboratory experiments (Tang *et. al.* 2015).

4.5 *Polymorphic species*

Polymorphic species have always been an issue for taxonomists. Many algal genera contain polymorphic species; examples include *Sargassum* (Mattio and Payri 2011), *Halimeda* (Verbruggen *et. al.* 2005), and *Caulerpa* (Gacia *et. al.* 1996; Sauvage *et. al.* 2013). *Caulerpa*, for instance, can be highly polymorphic, making species assignment

problematic (Belton *et. al.* 2014; De Senerpont Domis *et. al.* 2003). One of our samples *Caulerpa nummularia* (A111) had flattened mushroom shaped ramuli when grown under low intensity lighting in a refugium, once relocated to higher light the morphology of new ramuli changed to flattened with serrations. Molecular barcoding therefore is an effective way of distinguishing species that have overlap in morphological boundaries (Verbruggen 2014; Verbruggen *et. al.* 2007; 2005). There are cases where molecular identification is difficult or not possible, such as old herbarium specimens where the DNA is degraded, or the specimen is heavily degraded (e.g., with formalin fixation, or rotting) before being dried (Särkinen *et. al.* 2012). One example of this is a sample of *Caulerpa sertularioides* donated as a live specimen from a hobbyist in Auckland. Amplification of the *tufA* gene was not successful due to decay in transport. Morphological identification however identified it as most like *C. sertularioides*.

4.6 Cryptic diversity

Cryptic diversity is another issue that makes morphological identification difficult due to little or no morphological differences between some species (Cianciola *et. al.* 2010). Two brown algal genera well-known for cryptic diversity are *Lobophora* (Schultz *et. al.* 2015; Vieira *et. al.* (2014), and *Padina* (Ni-Ni Win *et. al.* 2011). A recent study identified many genetically distinct entities of *Lobophora* spp. in New Caledonia that can be considered species (Vieira *et. al.* 2014). Our results show that at least seven different species of *Lobophora* have been found in the New Zealand aquarium trade. One species *Lobophora asiatica* was found twice, showing repeat occurrence. There were a few samples that may be new species of *Lobophora*. Sample A60 designated by us as *Lobophora* sp1 and sample A57, designated as *Lobophora* sp2, are different from any other *Lobophora* species sequenced so far. Red algal genera containing cryptic diversity

have also been found by molecular barcoding (e.g., Cianciola *et. al.* 2010; Zuccarello *et. al.* 2005; Zuccarello and West 2002; 2003). This shows the importance of molecular identification in accurately identifying algae.

Cryptic diversity needs to be taken into account to correctly determine the diversity of algae entering the New Zealand marine aquarium trade as more species may be entering than recognized by morphological characters. Cryptic species can have different physiological tolerance characteristics as shown by Muangmai (2015) in a study of temperature tolerance of cryptic species of *Bostrychia intricata* (Bory) Montagne. Cryptic species can also have different habitat preferences. For example, on the rocky shores of Moa Point, Wellington, *Bostrychia intricata* cryptic species N2 had strict preference to wave exposed locations whereas cryptic species N4 was found in mostly wave protected locations, cryptic species N5 was found in both exposed and sheltered locations in almost equal proportions (Muangmai 2015). Another example of cryptic species having differing physiological tolerance is the temporal replacement of two cryptic species of *Dictyota* sp. at the Canary Islands (Tronholm *et. al.* 2010). *Dictyota dichotoma* (Hudson) J.V.Lamouroux was previously thought to be present year round but *D. dichotoma* reaches highest abundance at temperatures of 21°C-22°C in summer then dies back to resting stages in winter, being replaced by *Dictyota cymatophila* (A.Tronholm) M.Sanson and J.Afonso-Carrillo. The cryptic species *D. cymatophila* reaches highest abundance during winter temperatures of 17°C-18°C, dying back to resting stages in summer. The variation in temperature tolerance between cryptic species suggests that some cryptic species of algae will be better suited to New Zealand winter minimum SST than others and therefore pose more of a biosecurity risk than others.

4.7 *Utility of BLAST and Maximum-Likelihood Phylogenies in molecular identification*

We used Blast searching along with maximum-likelihood (ML) phylogenies to determine the identity of our samples. The Blast search tool on the NCBI website is useful for assisting molecular taxonomy. Blast compares the sequence in question with those in specific databases and reports those with regions of high similarity to the entire sequence. Blast is biased to finding the most similar sequence over the longest stretch of nucleotides. BLAST will score a longer but lower similarity match higher than a shorter, more similar match (Duffy *et. al.* 2010). This can however lead to misidentification of the closest match in some cases. You can determine if the sequence is of the target gene from Blast search results and in many cases determine the genus and species that the sample was from by the top Blast hits (Dudu *et. al.* 2016; Kool *et. al.* 2012).

Maximum-likelihood (ML) is a robust method of building a phylogeny and provides statistical support for the final tree (Yuan *et. al.* 2013). ML allows for the selection of substitution models to take into account varying rates of evolution across both lineages and sites (Felsenstein 1981; Fukami-Kobayashi and Tateno 1991; Hamberg 2011). The main pitfall of ML is that it is computationally costly (Hamberg 2011)

Molecular identification from Blast searches alone can be done but is not necessarily as accurate as building a ML phylogeny from aligned sequences (Ross *et. al.* 2008). Other studies have utilized both Blast and ML analysis to effectively identify species (Freshwater *et. al.* 2017; Raja *et. al.* 2017). From our results, the top Blast hit and sequences of high identity to the samples were often in the ML clade containing our samples, and in many cases the top Blast hit was sister to our sample. There were cases however where the Blast top hit and the sequence sister to our sample in the phylogeny were different. For example, Sample A8 (*Chondrophycus* sp3) had a larger pairwise

different in *cox1* to its sister *Chondrophycus* sp. (HQ423056) and less of a pairwise difference in *cox1* to its top Blast hit *Chondrophycus* sp. (KX258815).

4.8 Limitations of public database sequences

Some taxonomic groups are understudied therefore have inadequate sequences available in Genbank for effective species level identification of new isolates. This is apparent in the ITS1 gene in fungi (Nilsson *et. al.* 2005; 2006) and the *rbcL* gene in diatoms (An *et. al.* 2018). Some of our samples, that could only be identified to genus level, had large pairwise distances to the closest Genbank sequences. Sample A2, a branching red alga, is 13.4% different in *cox1* to its closest Genbank sequence, *Lomentaria divaricata* from Canada. Sample A4 had 9.24 % pairwise difference in *cox1* to *Halopeltis willisii* from North Carolina USA, another temperate species. Samples A2 and A4 are either of described species that have not been sequenced using *cox1*, or may be undescribed species. An inability to identify a sample to species level has implications for determining if the alga is an introduced species or not, and its physiological tolerance characteristics.

The names of sequences in Genbank must be taken with some caution. For example, within our *Caulerpa serrulata* clade there is a sequence named *Caulerpa cupressoides* (DQ652336) from Indonesia; this may have been a misidentification as it has 0.5% pairwise difference in *tufA*, or 3 base pairs, to our sample (A45) and other samples of *Caulerpa serrulata*. Another limitation of Genbank sequences is that some sequences have not been updated when species or generic names change. An example of sequences that have not been updated to the most recent names were the five sequences named *Caulerpa racemosa* f. *macrophysa* an entity that has been synonymised with *Caulerpa racemosa* var. *macrophysa* (Taylor 1928). Also, sequences were named

Caulerpa peltata an entity that has been synonymized with *Caulerpa chemnitzia* (Belton *et. al.* 2014). Our Cladophorales tree (Fig. 31) also has species present that have been formally renamed but not updated on Genbank by the collector. *Cladophora montagneana* has been renamed *Willeella brachyclados* (Whynne 2016).

Misidentification and incorrect naming of sequences submitted to Genbank is a common occurrence, 65% of over 600 *Ganoderma* spp. fungal sequences surveyed were misidentified or ambiguously labeled (Jargalmaa *et. al.* 2017). The LSU alignment for samples A36 and A43 had sequences with the same pairwise difference to samples named as different species, making it difficult to identify our samples to species level. The LSU gene may also not be highly variable in Siphonocladales preventing us from delimiting species, however, using LSU effectively identified three species of *Valonia* a genus within the Siphonocladales. Other studies have found LSU as an effective marker for identifying species within the Siphonocladales (Leliaert *et. al.* 2009; 2007). Another possibility is that the samples uploaded to Genbank were not all correctly identified before being deposited.

Not all sequences on Genbank have been given species names, many sequences have only been designated with a genus name or even just a family name, for example (*Chondrophycus* sp., *Laurencia* sp.) or order names (Gigartinales sp., Rhodymeniales sp.). This can make it difficult in determining the species that you have sequenced especially if there are few sequences of close identity to the query sequence in Genbank. Species level identification is important to determine whether an isolate is from an introduced species or an already naturalized species.

4.9 Regulation and prevention of algal introductions from aquaria

This study found a large diversity of tropical macroalgae entering the New Zealand Aquarium trade on imported corals. Some of the species identified have proven invasive overseas, such as *Caulerpa racemosa* and *Caulerpa cylindracea* with detrimental effects to native ecosystems when released (Piazzi *et. al.* 2016; 2003). These findings have important implications for New Zealand biosecurity, as there are currently no measures in place to prevent invasive algal species from passing through quarantine undetected as spores or germlings to reach hobbyists aquariums. Strict monitoring of marine aquarium livestock during quarantine and a longer quarantine period would be needed to detect the presence of exotic macroalgal hitchhikers. The outlawing of the sale of certain algal genera has been suggested (Smith *et. al.* 2010), to prevent the spread of invasive algae between aquaria, for example, *Caulerpa* spp. in Florida (Stam *et. al.* 2006). A ban of the sale of nine *Caulerpa* spp. is in place in California USA, with a genus wide ban in San Diego (Diaz *et. al.* 2012). Banning sale and the propagation of potentially invasive algae will need enforcement and education for it to succeed. The ban on *Caulerpa* spp. sales in California USA has so far not ended the sale of the banned *Caulerpa* species due to lack of enforcement (Diaz *et. al.* 2012). On the other hand, many tropical algae cannot tolerate present day winter minimum SST in Auckland and Wellington, New Zealand.

Many aquarists are unaware of the impacts of releasing aquarium specimens or wastewater into natural environments (Martin and Coetzee 2011; Duggan *et. al.* 2006; Gertzen *et. al.* 2008). The finding of an alga with cold tolerance to Auckland minimum winter temperature is alarming as northern New Zealand has a sea surface temperature that rarely declines to or below 14°C. As this strain of *Chaetomorpha vieillardii* can tolerate low temperature, grows into very large unattached balls and can reproduce from

single cell fragments (Odom *et. al.* 2014), it is a biosecurity threat in northern New Zealand. The general public needs to have access to information on the proper disposal of unwanted aquarium livestock and wastewater to reduce the risk of an unintentional release of an alga into New Zealand ecosystems.

5.0 Limitations and future research

5.1 Limitations

Having access to more marine aquarium livestock importers and a larger sample size of coral rocks would have been useful. We did however find a very large diversity of species 62 from our 78 samples. Not knowing the collection location of many of the marine invertebrates that our samples came from was a limitation of our study. Collection location information would have been useful for determining habitat compatibility to New Zealand, as some of the corals kept in New Zealand aquaria are in fact subtropical and I assume that the associated algae will also have low temperature tolerance.

Some of our samples, predominately red algae, could only be identified to genus using the genes chosen for this study. Perhaps a different DNA sequence may have had better resolution or have more sequences available, *cox1* versus *rbcL* for example. In the majority of cases a single gene was effective. Use of a second gene may have increased resolution in identifying some species. Use of two genes was implemented in Boedeker *et. al.* (2017) and Pochon *et. al.* (2015) for more accurate species delimitation. Use of multiple genes relies on the algal group being well studied with good sequence data for each gene, which is not the case for many algal groups.

5.2 Future research

Testing a broader range of physiological tolerance parameters, for example, salinity and pH might be helpful in determining introduction risk of tropical algae to different habitats within New Zealand. Our study used survival/death of tropical macroalgal samples at different temperatures as a measure of introduction risk. Survival/death was adequate for this study but doesn't take into account whether or not the surviving algae are stressed. Some studies used Pulse Amplitude Modulation (PAM) Fluorimetry to determine photosynthetic yield as a measure of stress in algae (Flagella *et. al.* 2008; Graiff *et. al.* 2015). Use of PAM in future physiological tolerance experiments will help in determining stress and potential acclimatization in the algae tested.

A survey, along with dried sample collection for DNA extraction, for algae kept in aquarium refugia or reported by marine aquarium hobbyists would be useful, to better gauge the prevalence of certain algal genera across the entire New Zealand aquarium trade. Sampling from coral rocks that have been freshly imported into New Zealand and are in quarantine would be useful as we will achieve a larger sample size and collect algae that may otherwise detach, be removed or die back while in quarantine. We will also be able to observe if there are signs that the algae are reproducing during quarantine, rather than in the importation step of custody.

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Supplimentary information

Table S1. Algal samples collected from New Zealand marine aquaria. A total of 15 brown algae, 33 red algae and 34 green algae. Top blast hit and percent identity to the blast hit shown. Substrate coral species that the alga was collected from shown.

Sample	Voucher ID Welt	Genbank accession	Name	Gene	Sequence length	Top Blast hit, and Genbank Accession Number	% Identity Blast	Substrate	Collection date
A5	TBA	-	<i>Dictyopteris</i> sp.	-	-			Live rock	18-Jun-2017
A46	TBA	TBA	<i>Lobophora</i> sp.	<i>cox3</i>	701	<i>Lobophora</i> sp. 24, KU353342	99.4	<i>Caulastrea tumida</i>	3-Aug-2017
A6	TBA	TBA	<i>Turbinaria ornata</i>	<i>cox3</i>	683	<i>Turbinaria ornata</i> , KM501562	99.3	dead Faviid	5-Jun-2017
A47	TBA	TBA	<i>Lobophora</i> sp.	<i>cox3</i>				<i>Caulastrea tumida</i>	3-Aug-2017
A7	TBA	TBA	<i>Lobophora asiatica</i>	<i>cox3</i>	696	<i>Lobophora</i> sp., AB665369	99.8	<i>Zoanthus</i> sp.	8-Jun-2017
A32	TBA	TBA	<i>Lobophora asiatica</i>	<i>cox3</i>	677	<i>Lobophora</i> sp., AB665365	99.6	<i>Zoanthus</i> sp. rock	8-Jun-2017
A108	TBA	TBA	<i>Lobophora</i> sp.	<i>cox3</i>	700	<i>Lobophora</i> sp. 6, KU353257	99.8	<i>Pavona</i> sp. skeleton	8-Jun-2017
A57	TBA	TBA	<i>Lobophora</i> sp.	<i>cox3</i>	702	<i>Lobophora</i> sp. 57, KU353343	97.4	Maricultured <i>Seriatopora hystrix</i>	12-Jun-2017
A58	TBA	TBA	<i>Lobophora abscondita</i>	<i>cox3</i>	687	<i>Lobophora abscondita</i> , KU353163	99.4	<i>Galaxea</i> sp.	12-Jun-2017
A59	TBA	TBA	<i>Dictyopteris</i> sp.					Hammer coral	12-Jun-2017
A60	TBA	TBA	<i>Lobophora</i> sp.	<i>cox3</i>	705	<i>Lobophora petila</i> , KU353324	94.3	<i>Favia speciosa</i>	5-Dec-2017
A61	TBA	TBA	<i>Lobophora</i> sp.	<i>cox3</i>	675	<i>Lobophora</i> sp. 10, KU353273	99.5	<i>Caulastrea furcata</i>	5-Dec-2017
A62	TBA	TBA	<i>Dictyota friabilis</i>	<i>cox3</i>	692	<i>Dictyota friabilis</i> , GU290244	93.6	<i>Favites</i> sp.	5-Dec-2017
A109	TBA	TBA	<i>Dictyopteris</i> sp.	<i>cox3</i>	636	<i>Dictyopteris polypodioides</i> , EU681445	80.7	<i>Zoanthus</i> sp.	8-Aug-2018
A110	TBA	TBA	<i>Dictyota</i> sp.	<i>cox3</i>	645	<i>Dictyota friabilis</i> , GU290244	90.3	<i>Zoanthus</i> sp.	8-Aug-2018
A1	TBA	TBA	<i>Chondrophycus</i> sp.	<i>cox1</i>	614	<i>Chondrophycus</i> sp., KX258817	96.3	Maricultured <i>Acropora</i> sp	4-Aug-2017
A2	TBA	TBA	<i>Lomentaria</i> sp.	<i>cox1</i>	684	<i>Lomentaria divaricata</i> , KU941949	86.6	Retailers tank on sand bed	10-Aug-2017

A3	TBA	TBA	<i>Meredithia</i> sp.	<i>cox1</i>	681	<i>Meredithia</i> sp., KC157609	99.5	Retailers tank attached to glass	10-Aug-2017
A107	TBA	TBA	<i>Chondrophycus</i> sp.	<i>cox1</i>	634	<i>Chondrophycus</i> sp., HQ423056	92.3	Maricultured <i>Acropora</i> sp.	14-Aug-2017
A106	TBA	TBA	<i>Ceratodictyon</i> sp.	<i>cox1</i>	680	<i>Gelidiopsis</i> sp., HQ422790	99.8	starburst polyp rock	14-Aug-2017
A4	TBA	TBA	<i>Halopeltis</i> sp.	<i>cox1</i>	684	<i>Halopeltis willisii</i> , HM915686	90.8	<i>Acropora</i> sp.	15-Aug-2017
A8	TBA	TBA	<i>Chondrophycus</i> sp.	<i>cox1</i>	668	<i>Chondrophycus</i> sp., KX258815	91.7	<i>Acropora</i> sp.	15-Aug-2017
A9	TBA	TBA	<i>Chamaeobotrys</i> sp2	<i>rbcL</i>	322	<i>Chamaeobotrys</i> sp., KU726727	91.1	Fragment tile	15-Aug-2017
A105	TBA	TBA	<i>Ceratodictyon</i> sp.	<i>cox1</i>	681	<i>Gelidiopsis</i> sp., HQ422790	99.8	<i>Acropora</i> sp	15-Aug-2017
A23	TBA	TBA	<i>Chamaeobotrys</i> sp1	<i>rbcL</i>	317	<i>Chrysomenia brownii</i> , KU726719	91.3	<i>Zoanthus</i> sp. rock	20-Aug-2017
A104	TBA	TBA	<i>Peyssonnelia</i> sp.	<i>cox1</i>	682	<i>Peyssonnelia</i> sp., JX969686	91.4	Personal reef on rocks	
A33	TBA	TBA	<i>Amphiroa</i> sp.	<i>cox1</i>	682	<i>Amphiroa anceps</i> , LC071723	91.1	<i>Zoanthus</i> sp. rock	20-Aug-2017
A70	TBA	TBA	<i>Peyssonnelia</i> sp.	<i>cox1</i>	589	<i>Peyssonnelia</i> sp., JX969736	92.7	<i>Zoanthus</i> sp. rock	5-Jan-2018
A52	TBA	TBA	<i>Pterocladia</i> sp.	<i>cox1</i>	685	<i>Pterocladia phangiae</i> , KC209092	90.6	<i>Caulastrea</i> sp.	13-Dec-2017
A53	TBA	TBA	<i>Peyssonnelia</i> sp.	<i>cox1</i>	685	<i>Peyssonnelia rubra</i> , HQ422737	92.4	<i>Caulastrea</i> sp.	13-Dec-2017
A98	TBA	TBA	<i>Pterocladia</i> sp.	<i>cox1</i>	591	<i>Pterocladia beachiae</i> , KT208059	91.7	<i>Galaxea</i> sp.	19-Sept-2017
A97	TBA	TBA	<i>Hypnea wynnei</i>	<i>cox1</i>	540	<i>Hypnea</i> sp., KX426549	99.8	Maricultured <i>Seriatopora hystrix</i>	19-Sept-2017
A96	TBA	TBA	<i>Laurencia</i> sp.	<i>cox1</i>	583	<i>Laurencia saitoi</i> , KM254876	94.5	<i>Zoanthus</i> sp. rock	9-Nov-2-17
A95	TBA	TBA	<i>Hypnea wynnei</i>	<i>cox1</i>	598	<i>Hypnea</i> sp., KX426549	99.8	Maricultured <i>Acropora</i> sp.	13-Dec-2-17
A64	TBA	TBA	<i>Chamaeobotrys</i> sp2	<i>rbcL</i>	325	<i>Chamaeobotrys</i> sp., KU726727	91.6	<i>Caulastrea</i> sp.	13-Dec-2-17
A67	TBA	TBA	<i>Leptofaucha</i> sp.	<i>cox1</i>	627	<i>Leptofaucha leptophylla</i> , KF547030	91.4	Hobbyist in Auckland	9-Jan-2018
A68	TBA	TBA	<i>Pterocladia caerulescens</i>	<i>cox1</i>	607	<i>Pterocladia caerulescens</i> , KX557255	99.6	Hobbyist in Auckland	9-Jan-2018
A69	TBA	TBA	<i>Hypnea wynnei</i>	<i>cox1</i>	596	<i>Hypnea</i> sp., KX426549	100	Hobbyist in Auckland	9-Jan-2018
A94	TBA	TBA	<i>Hypnea</i> sp.	<i>cox1</i>	597	<i>Hypnea brasiliensis</i> , KU905079	98.9	<i>Zoanthus</i> sp. rock	1-Mar-2018

A93	TBA	TBA	<i>Pterocladia caerulea</i>	cox1	594	<i>Pterocladia caerulea</i> , KX557255	99.6	<i>Euphyllia ancora</i>	1-Apr-2018
A86	TBA	TBA	<i>Peyssonnelia</i> sp.	cox1	620	<i>Peyssonnelia</i> sp., KM254773	91.6	<i>Ricordea florida</i> rock	11-Apr-2018
A84	TBA	TBA	<i>Ramirusta</i> sp.	cox1	593	<i>Ramirusta appressa</i> , JX969695	93.4	<i>Microzoanthus</i> sp.rock	4-Aug-2017
A55	TBA	TBA	<i>Hypnea wynnii</i>	cox1	621	<i>Hypnea</i> sp., KX426549	99.8	Retailers tank on egg crate	
A56	TBA	TBA	<i>Hypnea spinella</i>	cox1	626	<i>Hypnea spinella</i> , KU905111	97.2	Retailers tank on egg crate	
A99	TBA	TBA	<i>Ceratodictyon repens</i>	cox1	627	<i>Ceratodictyon repens</i> , KY656528	99.4	Anemone rock	5-Jun-2018
A92	TBA	TBA	<i>Ramirusta</i> sp.	cox1	639	<i>Ramirusta appressa</i> , JX969695	93.8	Maricultured <i>Hydnophora</i> sp.	5-Jun-2018
A101	TBA	TBA	<i>Hypnea</i> sp.	cox1	568	Gigartinales sp., HQ423064	100	Personal reef on T. noae shell	
A102	TBA	TBA	<i>Laurencia</i> sp.	cox1	587	<i>Laurencia</i> sp., GU223893	94	<i>Zoanthus</i> sp. rock	1-Oct-2018
A111	TBA	TBA	<i>Caulerpa nummularia</i>	tufA	918	<i>Caulerpa nummularia</i> , JN851138	99.9	Personal reef on rocks	
A19	TBA	TBA	<i>Lychaete dotyana</i>	LSU	578	<i>Lychaete dotyana</i> , LT904846	99.5	<i>Zoanthus</i> sp. rock	
A20	TBA	TBA	<i>Udotea</i> sp.	rbcL	117	<i>Rhipidosiphon</i> sp. G.008, AB700348	95.7	<i>Zoanthus</i> sp. rock	
A27	TBA	TBA	<i>Valonia ventricosa</i>	LSU	575	<i>Ventricaria ventricosa</i> , AM503545	100	Personal reef on rocks	
A36	TBA	TBA	<i>Cladophoropsis</i> sp.	LSU	587	<i>Cladophoropsis membranacea</i> , AM503489	99.8	<i>Zoanthus</i> sp. rock	
A45	TBA	TBA	<i>Caulerpa serrulata</i>	tufA	819	<i>Caulerpa serrulata</i> , KJ957119	100	Donated by reef hobbyist Wellington in sump	
A35	TBA	TBA	<i>Derbesia</i> sp.	tufA	886	<i>Derbesia</i> sp. TS1731, KU361859	99.4	Personal reef on rocks	16-Jul-2017
A43	TBA	TBA	<i>Cladophoropsis</i> sp.	LSU	578	<i>Cladophoropsis membranacea</i> , AM503489	99.8	<i>Zoanthus</i> sp. rock	
A39	TBA	TBA	<i>Lychaete dotyana</i>	LSU	580	<i>Lychaete dotyana</i> , LT904846	99.7	<i>Zoanthus</i> sp. rock	
A26	TBA	TBA	<i>Caulerpa lentillifera</i>	tufA	853	<i>Caulerpa lentillifera</i> , NC_039377	100	<i>Zoanthus</i> sp. rock	7-Jul-2017
A25	TBA	TBA	<i>Caulerpa</i>	tufA	930	<i>Caulerpa cylindracea</i> ,	100	<i>Zoanthus</i> sp. rock	7-Jul-2017

			<i>cylindracea</i>			FM956048			
A41	TBA	TBA	<i>Caulerpa racemosa</i>	<i>tufA</i>	894	<i>Caulerpa racemosa</i> , DQ652466	100	<i>Zoanthus</i> sp. rock	7-Jul-2017
A34	TBA	TBA	<i>Lychaete</i> sp.	LSU	585	<i>Cladophora feredayi</i> , FM205030	93.7	<i>Zoanthus</i> sp. rock	12-Jul-2017
A114	TBA	TBA	<i>Lychaete</i> sp.	LSU	585	<i>Cladophora feredayi</i> , FM205030	93.8	Personal reef on fragment racks	16-Jul-2017
A38	TBA	TBA	<i>Willeella</i> sp.	LSU	587	<i>Cladophora montagneana</i> , LT607098	97.2	Personal reef on fragment racks	16-Jul-2017
A42	TBA	TBA	<i>Ulva ohnoi</i>	<i>tufA</i>	554	<i>Ulva ohnoi</i> , MF614793	100	Retailers tank free living	16-Jul-2017
A40	TBA	TBA	<i>Caulerpa racemosa</i> var. <i>macrophyssa</i>	<i>tufA</i>	895	<i>Caulerpa racemosa</i> , KJ957088	100	<i>Zoanthus</i> sp. rock	16-Jul-2017
A54	TBA	TBA	<i>Valonia ventricosa</i>	LSU	584	<i>Ventricaria ventricosa</i> , AM503545	100	Coral fragment	20-Aug-2017
A76	TBA	TBA	<i>Udotea</i> sp.	<i>rbcL</i>	128	<i>Udotea</i> sp., MH591113	95.3	Retailers tank on rocks	20-Aug-2017
A75	TBA	TBA	<i>Valonia fastigiata</i>	LSU	564	<i>Valonia fastigiata</i> , AM503532	98.38	Retailers tank on rocks	26-Aug-2017
A100	TBA	TBA	<i>Bryopsis</i> sp.	<i>tufA</i>	911	<i>Bryopsis</i> sp. 2, LK022439	87.22	Personal reef on glass	10-Oct-2017
A82	TBA	TBA	<i>Lurbica</i> sp.	LSU	436	<i>Chaetomorpha</i> cf. <i>restricta/linoides</i> , LT607144	97.6	Retailers tank on egg crate	18-Dec-2017
A78	TBA		<i>Caulerpa</i> <i>sertularioides</i>					Hobbyist in Auckland	9-Jan-2018
A80	TBA	TBA	<i>Chaetomorpha</i> <i>vieillardii</i>	LSU	622	<i>Chaetomorpha spiralis</i> , LT607221	99.6	Hobbyist in Auckland	9-Jan-2018
A73	TBA	TBA	<i>Valonia</i> <i>macrophyssa</i>	LSU	582	<i>Valonia macrophyssa</i> , AM503536	100	Hobbyist in Auckland	9-Jan-2018
A79	TBA	TBA	<i>Caulerpa racemosa</i>	<i>tufA</i>	768	<i>Caulerpa racemosa</i> isolate FL108, DQ652426	100	Hobbyist in Auckland	9-Jan-2018
A88	TBA	TBA	<i>Halimeda</i> sp.	<i>rbcL</i>	126	<i>Halimeda</i> aff. <i>tuna</i> , KY570457	96	Hobbyist in Auckland	9-Jan-2018
A91	TBA	TBA	<i>Caulerpa</i> <i>chemnitzia</i>	<i>tufA</i>	878	<i>Caulerpa chemnitzia</i> , KJ957093	99.9	<i>Zoanthus</i> sp. rock	11-Apr-2018
A113	TBA	TBA	<i>Willeella</i> sp.	LSU	588	<i>Cladophora montagneana</i> , LT607098	97.2	<i>Zoanthus</i> sp. rock	11-Apr-2018

A112	TBA	TBA	<i>Chaetomorpha vieillardii</i>	LSU	632	<i>Chaetomorpha spiralis</i> , LT607221	100	<i>Blastomussa vivida</i>	22-Apr-2018
A74	TBA	TBA	<i>Valonia macrophysa</i>	LSU	580	<i>Valonia macrophysa</i> , AM503536	99.8	Hobbyist in Hamilton	
A66	TBA	TBA	<i>Udotea</i> sp.	<i>rbcL</i>	126	<i>Udotea</i> sp., MH591113	96	<i>Zoanthus</i> sp. rock	22-Jun-2018
A116	TBA	TBA	<i>Valonia macrophysa</i>	LSU	583	<i>Valonia macrophysa</i> , AM503536	99.8	Mushroom coral rock	30-Jun-2018
A115	TBA	TBA	<i>Willeella</i> sp.	LSU	600	<i>Cladophora montagneana</i> , LT607098	97.2	Aragonite substrate	

Table S2. Table of the genes and primer pairs used for the different groups of macroalgae. Amplicon size and annealing temperatures are shown.

Algae type	preliminary ID	Gene	Primers used	Approx. amplicon size	Annealing temperature	References
Phaeophyceae	<i>Dictyota</i> sp., <i>Lobophora</i> spp.	<i>cox3</i>	<i>cox3</i> -44F CATCGACAAAATGCCAATACCA <i>cox3</i> -739R CAACGNCAYCCWTTTCATTT	717 bp	45°C	Vieira <i>et. al.</i> 2014
Rhodophyta	<i>Amphiroa</i> sp., <i>Gelidiopsis</i> spp., <i>Hypnea</i> spp., <i>Laurencia</i> spp., <i>Peyssonnelia</i> spp. <i>Pterocladia</i> spp.	<i>cox1</i>	Gaz-F1 TCAACAAATCATAAAGATATTGG GAZ-R1 ACTTCTGGATGTCCAAAAAYCA	710 bp	45°C	Saunders 2005
Rhodophyta	<i>Botryocladia</i> spp.	<i>rbcL</i>	<i>rbcL</i> -765F TGAAAGAGCTGAATTYGCTAA, <i>rbcL</i> -1150R GCATTTTGTCCGCAGTGAATACC	408 bp	50°C	Freshwater <i>et. al.</i> 1994
Ulvophyceae	<i>Bryopsis plumosa</i> , <i>Caulerpa</i> spp., <i>Derbesia</i> sp., <i>Ulva</i> sp.	<i>tufA</i>	CAUL-tufAF TGAAACAGAAMAWCGTCATTATGC CAUL-tufAR CCTTCNCGAATMGCRAAWCGC	852 bp	50°C	Fama <i>et. al.</i> 2002
Ulvophyceae	<i>Halimeda</i> sp., <i>Udotea</i> sp.	<i>rbcL</i>	<i>rbcL</i> -712F CATTAYTTAAATGCWACWGC <i>rbcL</i> -904R CAATAACMGCRGTGCATAGC	190 bp	50°C	Lagourgue <i>et. al.</i> (2018)
Cladophorales	<i>Chaetomorpha</i> spp, <i>Cladophora</i> spp, <i>Cladophoropsis</i> spp. <i>Valonia</i> spp.	LSU 28S	C'1 ACCCGCTGAATTTAAGCATAT D2 TCCGTGTTTCAAGACGG	590 bp	55°C	Hassouna <i>et. al.</i> (1984)

Table S3. Table of best-fit models selected by IQtree Modelfinder for codon partitions. 1, 2, 3 are the codon positions. Selection was based on Bayesian Information Criterion (BIC). Log-likelihoods for the maximum likelihood trees shown.

Dataset	Alignment length	Best fit model for partitions	Informative sites per codon	-log likelihood of ML tree
<i>Hypnea</i> spp. alignment - <i>Cox1</i>	546 bp	1. TIM3e+I+G4 2. HKY+F+I+G4 3. TN+F+G4	1. 31 2. 2 3. 157	3979.3985
<i>Chondrophycus</i> spp. alignment <i>Cox1</i>	660 bp	1. TN+F+I+G4 2. F81+F+I+G4 3. TN+F+G4	1. 17 2. 1 3. 116	2937.3471
<i>Laurencia</i> spp. alignment - <i>Cox1</i>	660 bp	1. HKY+F+I+G4 2. HKY+F+I+G4 3. TN+F+G4	1. 15 2. 1 3. 125	2957.4923
<i>Pterocliadiella</i> spp. alignment - <i>Cox1</i>	543 bp	1. TN+F+G4 2. F81+F+I+G4 3. TN+F+G4	1. 23 2. 1 3. 140	2951.4536
<i>Peyssonnelia</i> spp. alignment - <i>Cox1</i>	663 bp	1. TNe+I+G4 2. HKY+F+I+G4 3. TIM2+F+I+G4	1. 30 2. 1 3. 178	5084.6474
<i>Ramicrusta</i> spp. alignment - <i>Cox1</i>	663 bp	1. TNe+I+G4 2. F81+F+I+G4 3. TIM2+F+G4	1. 27 2. 3 3. 123	2619.0675
<i>Meredithia</i> spp. alignment - <i>Cox1</i>	663 bp	1. K2P+I+G4 2. F81+F+I+G4 3. HKY+F+G4	1. 19 2. 2 3. 104	2548.8707
<i>Leptofauchea</i> spp. alignment - <i>Cox1</i>	663 bp	1. TN+F+I+G4 2. HKY+F+I+G4 3. TN+F+G4	1. 28 2. 1 3. 153	3367.7261
<i>Halopeltis</i> spp. alignment - <i>Cox1</i>	663 bp	1. TIM+F+I+G4 2. F81+F+I+G4 3. TIM+F+G4	1. 38 2. 2 3. 170	3834.0478
<i>Gelidiopsis</i> spp. and <i>Ceratodictyon</i> spp. alignment - <i>Cox1</i>	663 bp	1. TIM3e+G4 2. F81+F+I+G4 3. TN+F+G4	1. 5 2. 1 3. 59	1676.4889
<i>Lomentaria</i> spp. alignment - <i>Cox1</i>	663 bp	1. TN+F+I+G4 2. F81+F+I+G4 3. TPM2u+F+G4	1. 29 2. 1 3. 130	2659.4569
<i>Chamaebotrys</i> spp. alignment - <i>rbcL</i>	329 bp	1. K2P+I+G4 2. HKY+F+G4 3. TNe+G4	1. 2 2. 72 3. 13	2140.1985
<i>Amphiroa</i> spp. alignment - <i>Cox1</i>	648 bp	1. TN+F+I+G4 2. F81+F+I+G4 3. TN+F+G4	1. 45 2. 3 3. 173	4824.3257
<i>Ulva ohnoi</i> alignment - <i>tufA</i>	784 bp	1. TPM3u+F+I+G4 2. K3Pu+F+I+G4 3. TPM3+F+I+G4	1. 28 2. 64 3. 37	3637.052
<i>Chaetomorpha</i> spp. alignment - LSU	584 bp	1. TNe+G4	1. 104	2208.2511
<i>Cladophora</i> spp. LSU	623 bp	1. TIM3e+I+G4	1. 147	2814.9532

<i>Cladophoropsis</i> spp. alignment - LSU	567 bp	1. K2P	1. 11	1090.434
<i>Valonia</i> spp. alignment - LSU	583 bp	1. TN+F+G4	1. 32	1282.2955
<i>Bryopsis</i> spp. alignment - <i>tufA</i>	876 bp	1. TIM2+F+I+G4 2. HKY+F+I+G4 3. GTR+F+G4	1. 24 2. 9 3. 155	3780.6972
<i>Derbesia</i> spp. alignment - <i>tufA</i>	861 bp	1. TIM3+F+G4 2. TPM3u+F+I+G4 3. K3Pu+F+G4	1. 34 2. 13 3. 172	3853.0682
<i>Halimeda</i> spp. alignment - <i>rbcL</i>	135 bp	1. TIMe+G4 2. JC+I+G4 3. HKY+F+G4	1. 6 2. 0 3. 20	601.2735
<i>Udotea</i> spp. alignment - <i>rbcL</i>	153 bp	1. JC+G4 2. JC+I+G4 3. HKY+F+G4	1. 5 2. 0 3. 18	547.8818
<i>Caulerpa</i> spp. alignment - <i>tufA</i>	872 bp	1. F81+F+I+G4 2. GTR+F+G4 3. K3P+I+G4	1. 6 2. 104 3. 33	3715.158
<i>Dictyoya</i> spp. and <i>Dictyopteris</i> sp. alignment - <i>Cox3</i>	645 bp	1. GTR+F+I+G4 2. TPM2u+F+I+G4 3. GTR+F+I+G4	1. 55 2. 14 3. 189	7662.413
<i>Turbinaria ornata</i> alignment - <i>Cox3</i>	681 bp	1. HKY+F+G4 2. F81+F+I+G4 3. HKY+F+G4	1. 16 2. 4 3. 84	1895.4187
<i>Lobophora</i> spp. alignment - <i>Cox3</i>	648 bp	1. GTR+F+I+G4 2. HKY+F+I+G4 3. GTR+F+I+G4	1. 61 2. 16 3. 198	12102.0152

Evolutionary models used.

F81= Felsenstein 1981 (Felsenstein 1981)

GTR= General time Reversible (Tavaré 1986)

HKY= Hasegawa-Kishino-Yano 1985 (Hasegawa *et. al.* 1985)

JC= Jukes-Cantor 1969 (Jukes and Cantor 1969)

K2P= Kimura 2-Parameter (Kimura 1980)

K3Pu= Kimura 3-Parameter, assuming unequal base frequencies

TIM2= Transition model

TIM3= Transition model

TIM3e= Transition model, assuming equal base frequencies

TN= Tamura-Nei 1993 (Tamura and Nei 1993)

TNe= Tamura-Nei 1993 assuming equal base frequencies (Tamura and Nei 1993)

TPM2u= Assuming unequal base frequencies

TPM3u= Assuming unequal base frequencies

F= Empirical codon frequencies counted from the data

I= extent of static, unchanging sites in a dataset

G4= gamma distributed rate variation among sites (Yang 1994)

Figure S1. Maximum-likelihood tree of *Caulerpa* spp. using *tufA* gene sequences. Samples A25, A26, A40, A41, A45, A79, A91 and A111 shown in bold. *Caulerpella ambigua* was used as the outgroup and was removed for clarity marked by X. Only bootstrap support values >50 displayed. Scale bar = 0.2 substitutions per site. Arrows show where the tree was cut.

Figure S2. Full maximum-likelihood tree of 116 *Lobophora* samples based on *cox3* sequences. Samples A7, A32, A46, A57, A58, A60, A61 and A108 are shown in bold. *Zonaria flabellata* (JQ364040) was used as the outgroup. Scale bar =0.2 substitutions per site. Arrows show where the sections of the tree connect.

Figure S3. Samples of *Hypnea* sp. photomicrographs; A = A101, scale bar = 2cm, B = A94, scale bar = 400 μ m, C = A97, scale bar = 400 μ m,

Figure S4. Samples of *Dictyopteris* sp. and *Dictyota* sp. photomicrographs; A = A59 unsequenced, scale bar = 1mm, B = A62 *Dictyota* sp1, scale bar = 1mm.

Figure S5. Samples of *Cladophora* sp. photomicrographs; A = A39, scale bar = 1mm, B = A115, scale bar = 400 μ m, C = A114, scale bar = 400 μ m.