The first record of *Grateloupia subpectinata* from the New Zealand region and comparison with *G. prolifera*, a species endemic to the Chatham Islands

**Abstract:** A finely pinnately branched species of *Grateloupia* not seen previously in New Zealand was collected in Tauranga, New Zealand, growing on a tug boat. Molecular sequencing data (*rbcl*) revealed it to be *Grateloupia subpectinata*, a species first described from Japan, and also reported as native to Korea and China, and introduced to Britain, France, and Australia. The only species of *Grateloupia* with morphology similar to *G. subpectinata* in the New Zealand region is *G. prolifera* from the Chatham Islands. A sequence of this species was obtained for comparative purposes and found to be distinct from *G. subpectinata.*

**Keywords:** *Grateloupia prolifera; G. subpectinata; Halymeniales; introduction; New Zealand.*

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**Introduction**

*Grateloupia* (Halymeniales, Rhodophyta) is a very large and diverse genus encompassing species with morphologies that range from foliose blades to finely pinnately branched fronds. Identification of species of *Grateloupia* is often difficult because morphology can be highly variable within species and, in addition, a number of species share similar morphologies. There has been a great deal of taxonomic confusion, with some long-standing problems in relation to the application of names. The generitype of *Grateloupia* is *G. filicina* (J.V. Lamour) C. Agardh, originally described from the Adriatic Sea. Although a number of species with similar finely pinnately branched thalli have been described from tropical to cold temperate regions, in many instances the name *G. filicina* has been applied subsequently and other names reduced to synonymy.

The species *Grateloupia subpectinata* Holmes (1912) was described from material collected in Japan, but was reduced to synonymy with *G. prolongata* J. Agardh by Yendo (1914), and then treated as a synonym of *G. filicina* by Okamura (1936). Studies in the northwest Pacific, using both molecular and morphological data to examine *Grateloupia* that have been assigned to *G. filicina*, have resulted in the resurrection of *G. catenata* Yendo (Wang et al. 2000) and the recognition of a new species, *G. asiatica* Kawaguchi et al. (2001). Faye et al. (2004) compared material of *Grateloupia* similar to *G. asiatica* but distinguished by reproductive and vegetative features as well as *rbcl* sequences, and concluded by reinstating *G. subpectinata.* After examining *Grateloupia* and *Dermocorynus* in the north-eastern Atlantic, Wilkes et al. (2005) concluded that the Australian *G. filicina* var. *luxurians* A. Gepp et E.S. Gepp, which was apparently introduced to Britain before 1947, warranted recognition as a distinct species, *G. luxurians* (A. Gepp et E.S. Gepp) R.J. Wilkes, L.M. McIvor et Guiry. De Clerck et al. (2005) examined material referred to *G. filicina* from throughout the world and, with an *rbcl* molecular phylogeny, revealed “a plethora of ‘cryptic’ species.” They concluded that true *G. filicina* was restricted to the Mediterranean basin, and that the “various geographically disjunct populations hitherto attributed to *G. filicina* do not constitute a single monophyletic lineage.” They also recognized *G. luxurians* as a distinct species; however, their combination was published a few months after Wilkes et al. (2005). Verlaque et al. (2005) concluded that *G. luxurians* and *G. subpectinata* were synonymous on the basis of *rbcl* sequence data as well as morphological comparisons between Australian, Mediterranean, and Japanese material.

In New Zealand, the genus *Grateloupia* remains poorly known. Although seven species are currently recognized...
(Nelson 2012), the need for taxonomic investigations of New Zealand members of the genus has been identified for some time (Adams 1994). In recent years, the non-indigenous species *G. turuturu* Yamada has been reported, initially from Wellington Harbor (D'Archino et al. 2007). It has been found subsequently to have spread more widely, largely in harbors and ports and adjacent areas. Although, on the North, South, and Stewart islands of New Zealand, there is no indigenous species of *Grateloupia* with finely pinnately branched fronds, the species *G. prolifera* J. Agardh, considered to be endemic to the Chatham Islands, has a morphology very similar to that of the so-called *G. filicina* group (Adams 1994: 197). Chapman and Parkinson (1974) placed *G. prolifera* into synonymy with *G. prolongata* J. Agardh (type locality, Pacific Mexico), but Adams (1994) considered that retention of the local name was preferable until detailed comparisons were carried out.

In 2011 and 2012, a species of *Grateloupia* was collected from a tug boat used to tow a barge from Australia. This article reports on the identity of this material and compares it with *G. prolifera* from the Chatham Islands.

### Materials and methods

A macroalgal sample (WELT A032656) was collected on 7 November 2011, from the tug boat *Katea* (from the waterline, approximately midship, portside) while the vessel was berthed in Tauranga Harbor (37.6712°S, 176.1762°E). The *Katea* had travelled from Australia to bring a barge to New Zealand to assist with the consequences of a major shipwreck on Astrolabe Reef, off shore from Tauranga. No algae were detected on the barge. These vessels were inspected as part of surveillance carried out on behalf of the Ministry for Primary Industries (MPI) by divers from National Institute of Water and Atmospheric Research (NIWA). All algae detected on the hull of the tug were treated with heated seawater while at Astrolabe Reef in an attempt to eradicate the *Grateloupia* that had been identified as a species that was new to New Zealand. Unfortunately, this treatment method proved to be ineffective, and significant regrowth of the alga was observed during a follow-up inspection of the tug on 24 August 2012, by NIWA divers on behalf of MPI. The second sample, consisting of several tufts of algae (WELT A032659), was collected from the tug from the waterline on the portside. This boat was again “heat treated” while at Astrolabe Reef in an attempt to eradicate the alga.

Specimens were pressed, and the vouchers were deposited in the Museum of New Zealand Te Papa Tongarewa Herbarium (WELT A032656 [MITS 70063], WELT A032659, [MITS 70235]) (Thiers 2013). Subsamples were preserved in 4% formalin/seawater or dried in silica gel for molecular analysis. Sections were made by hand and were stained with 1% aniline blue acidified with 1% HCl and mounted in 50% Karo syrup (CPC International Inc., Englewood Cliffs, NJ, USA). Photomicrographs were taken on a BX53 (Olympus, Tokyo, Japan) with SC100 (Olympus, Münster, Germany) digital camera.

In total, 13 specimens of *Grateloupia subpectinata* and 1 specimen of *G. prolifera* were collected from Korea, Japan, and New Zealand. Information for specimens is given in Table 1. Voucher specimens are housed in WELT

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Collection data</th>
<th>Voucher</th>
<th>GenBank accession</th>
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<tbody>
<tr>
<td><em>Grateloupia subpectinata</em></td>
<td>Holmes</td>
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<td>WELT A032659</td>
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<td></td>
<td></td>
<td>Yangyang, Korea; 27 Oct 2012</td>
<td>SKKU9</td>
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<td></td>
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<td>Yangyang, Korea; 27 Oct 2012</td>
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<td></td>
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<td>Kangneung, Korea; 19 Feb 2011</td>
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<td>Kangneung, Korea; 27 Oct 2012</td>
<td>SKKU17</td>
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<tr>
<td></td>
<td></td>
<td>Donghae, Korea; 14 Mar 2010</td>
<td>SH13</td>
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<td>G278</td>
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<tr>
<td></td>
<td></td>
<td>Yura, Japan; 29 Mar 2007</td>
<td>G722</td>
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<tr>
<td><em>G. prolifera</em> J. Agardh</td>
<td>Owenga, Chatham Islands, New Zealand;</td>
<td>WELT A024215</td>
<td>KF156728</td>
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<tr>
<td></td>
<td>31 Jan 2006</td>
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and the herbarium of Chungnam National University, Daejeon, Korea (Thiers 2013). DNA extraction, PCR amplification, and sequencing procedures followed Boo et al. (2009). For amplification and sequencing reaction of the rbcL, rbcLF145–rbcLR898 and rbcLF765–rbcLR1442 were used (Kim et al. 2010). Electropherogram outputs from each sample were edited using Chromas version 1.45. Sequences were collated and aligned using Se-Al version 2.0a11 (Rambaut 2002). Maximum likelihood (ML) analyses were conducted using RAxML (Stamatakis 2006) with the GTR+Γ evolutionary model. We analyzed 63 sequences of Halymeniaceae in the construction of the ML tree, including 32 of G. subpectinata (13 new, 19 published), 1 of G. prolifera from the Chatham Islands, 25 of Grateloupia, and 2 of Yonagunia as putative relatives, with three outgroup species [Glaphyrosiphon intestinalis (Harv.) Leister et W.A. Nelson, Halymenia dilatata Zanardini, and Polyopes lancifolius (Harv.) Kawaguchi et Wang]. We performed 200 independent tree inferences with the “number of run” option with default optimized subtree pruning and regrafting rearrangement and 25 distinct rate categories to identify the best tree. To generate bootstrap values for the best phylogeny, we used 1000 replications under the same model settings.

**Results**

**Morphology**

The thalli collected from the tug boat were erect, 5–7 cm high (Figures 1 and 2), gelatinous to fleshy, and composed of several axes departing from a discoid holdfast (Figure 2), terete below becoming flattened/compressed and tapering at the apices. Axes were simple to subdichotomously branched (Figure 1), with marginal proliferations (Figure 2) that have acute tips and constricted, terete

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**Figures 1–5** Grateloupia subpectinata collected in Tauranga, New Zealand. (1) Herbarium voucher (WELT A032659). Scale bar, 2 cm. (2) Liquid-preserved sample (WELT A032656). Scale bar, 0.5 cm. (3) Close-up image to show constriction at base of axes (WELT A032656). Scale bar, 0.1 cm. (4) Transverse section (WELT A032659). Scale bar, 100 μm. (5) Detail of the cortex (WELT A032659). Scale bar, 25 μm.
bases (Figure 3). The multiaxial structure consisted of a compact cortex of anticlinal rows of rounded cells (up to 10) slightly increasing in size and a loosely arranged filamentous medulla (Figure 4). The inner cortical cells were stellate (Figure 5).

An exemplar specimen of Grateloupia prolifera is illustrated showing the characteristic *G. filicina*-like morphology (Figure 6). This species is commonly found on open shores on the Chatham Islands in the low intertidal zone.

**Sequence data and analyses**

In total, 1329 bp of *rbcL* were aligned for the analysis. In the ML tree, *Grateloupia subpectinata* was divided into three distinct clades (Figure 7). Clade 1 included samples from Korea and Japan (west side); clade 2 included samples from the Pacific side of Japan only; and clade 3 included samples from various geographical regions, i.e., Korea, China (where this species is considered to be native), and Australia, France, Spain, and New Zealand. Sequence data lodged in GenBank attributed to *G. luxurians* specimens from Australia were found to be separated into two clades, within the *G. subpectinata* clade (AJ868489-Williamstown, Port Philip Bay, J. West, 9 May 2003; De Clerck et al. 2005) and in a clade distinct from *G. subpectinata* (AY435175-Williamstown, Port Philip Bay, M. Guiry, 13 March 2002; Wilkes et al. 2005).

Thirty sequences (13 new, 17 published) of *G. subpectinata* were compared with 27 GenBank sequences of *G. turuturu*, another species of *Grateloupia* originating in the northwest Pacific and with a widespread introduced range. The analyzed alignment included 505 bp of *rbcL* (not all positions were available across all sequences). The maximum pairwise distance within *G. subpectinata* was 6 bp (1.19%); however, it was 2 bp (0.40%) in *G. turuturu*. Five mutations of *G. subpectinata* were found in third codon positions, and all were synonymous mutations. However, one mutation was in the first codon position and it was a non-synonymous mutation. In *G. turuturu*, one mutation was synonymous in the first codon position, and the other was non-synonymous at the third codon position.

**Discussion**

This report provides the first record in New Zealand of the non-indigenous species *Grateloupia subpectinata*, as well as the first molecular sequence data for *G. prolifera*, confirming that it is a distinct species. The identification of *G. subpectinata* was reliant on the molecular sequence data. Although recent studies have shown that there are some morphological and anatomical characters that are valuable in species delimitation of *G. subpectinata* and morphologically similar but distinct species, not all samples will be fully developed and include the features essential for species confirmation. Samples obtained as part of surveillance exercises in harbors and ports (e.g., from vessel hulls, wharf pilings, sea walls) are often sub-optimal, i.e., immature or sterile thalli, or only with one phase (tetrasporophyte rather than gametophyte) present.

The studies of *Grateloupia* species that are pinnately branched have provided very clear evidence that gross external morphology is not sufficient for species separation, with a number of species sharing a *G. filicina*-like morphology (e.g., *G. asiatica*, *G. capensis* De Clerck, *G. catenata*, *G. luxurians*, *G. orientalis* S.-M. Lin et H.-Y. Liang, *G. prolifera*, *G. prolongata*, *G. subpectinata*). The results of this study indicate that further attention needs to be directed to the identity of *G. luxurians*. The type locality of *G. luxurians* is in New South Wales, and the two sequences in GenBank attributed to Australian samples of this taxon differ. Both samples (AJ868489 and AY435175) were collected from the same site in Victoria but on two separate occasions. It is not clear which, if either, of the sequences currently available is correctly applied to this species.

![Figure 6](image_url) Herbarium sheet of *Grateloupia prolifera*, Chatham Islands, New Zealand (WELT A018095). Scale bar, 1.5 cm.
Figure 7  Maximum likelihood analysis of rbcL data from 63 samples from the Halymeniales, with three clades distinguished within Grateloupia subpectinata. The global distributions of members of the three G. subpectinata clades are indicated by the use of three different symbols. [Note that the G. subpectinata sample from Williamstown, Australia (AJ868489) is lodged in GenBank as G. luxurians, but differs from the other Australian sequence of G. luxurians (AY435175).]
The sequence data obtained for *G. prolifera* were found to be most closely related to a sequence from the New Zealand endemic species *G. stipitata* J. Agardh, a species with a solid main stem bearing long, hollow, inflated side blades. While *G. prolifera* is restricted in its distribution to the Chatham Islands, *G. stipitata* has a more widespread distribution in the New Zealand region, found in the southern North I, South, Stewart, and Snares islands. Our results place these two New Zealand endemic species in a well-supported separate clade. In the analysis presented by Gargiulo et al. (2013), *G. stipitata* is also closely related to two species of *Prionitis* from South Africa.

The surveillance exercise that resulted in the discovery of *G. subpectinata* in Tauranga was focused on the examination of vessels transported to New Zealand from Australia. This species has not been found yet by surveillance exercises examining the port region of the harbor, so it may or may not have “jumped ship” onto shorelines. It has been found after approximately 10 months on the vessel hull despite attempts to remove it after the first collections were made.

*Grateloupia subpectinata* is native to the north-western Pacific, i.e., Korea, China, and Japan (Lee et al. 2009, Boo and Ko 2012, Guiry and Guiry 2013), and is closely related to *G. turuturu* (Figure 7), which shares the same native range. These two species have been reported as recent introductions to various regions: *G. turuturu* has been introduced to Australia, Europe, New Zealand, and North and South America, and *G. subpectinata* to Australia and Europe (e.g., Gavio and Fredericq 2002, Verlaque et al. 2005, D’Archino et al. 2007). In the comparative analysis of *G. subpectinata* and *G. turuturu*, sequence data from *G. subpectinata* showed greater diversity (up to 1.19%) than was found in the *G. turuturu* material examined (0.4%). The three distinct clades in *G. subpectinata*, shown in Figure 7, reflect some geographical patterns. In the native distribution range, all samples from the Pacific side of Japan were grouped together as clade 2, and the other samples, i.e., Korea, China, and the west side of Japan, were grouped as clade 1 or 3 with no geographical relationship. All samples from regions where this species has been introduced were grouped as clade 3 with some samples from geographically very distant native regions. There was no equivalent separation of native and non-native clades in the data investigated for *G. turuturu* (data not shown).

About 90 species of *Grateloupia* are currently recognized (Guiry and Guiry 2013), although the circumscription of the genus has been receiving attention recently with the establishment of new genera (e.g., Hommersand et al. 2010) and the resurrection of previously recognized genera (Gargiulo et al. 2013), based on a re-analysis of reproductive development and anatomy, as well as molecular sequence data. Gargiulo et al. (2013) concluded that *G. subpectinata* should be placed in a segregate genus, and that the clade containing *G. stipitata* (as well as two species previously placed in *Prionitis* from South Africa) required further attention.

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