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Recognition and distribution of *Polysiphonia morrowii* (Rhodomelaceae, Rhodophyta) in New Zealand

Abstract: *Polysiphonia morrowii*, a species native to the North Pacific, is believed to have been introduced to Chile, Turkey, Italy and France. This species was first recorded from New Zealand in 2011, based on collections made in 2004 from two sites on South Island, but no commentary was provided in the distribution or characteristics of the material collected. In 2009, as part of a study on macroalgae associated with soft sediment habitats in New Zealand, several samples of *P. morrowii* were collected from Otago Harbour, a location already known to host 17 non-indigenous macroalgal species. However, this species has gone unnoticed in New Zealand. We present the current understanding of the distribution of this species in New Zealand, and the distinguishing features of the specimens collected, to assist with biosecurity and monitoring programmes. Including this report of *P. morrowii*, there are now seven species of *Polysiphonia* and *Neosiphonia* that are regarded as non-indigenous in New Zealand.

Keywords: New Zealand; non-indigenous species; *Polysiphonia morrowii*; Rhodophyta.

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Introduction

The genus *Polysiphonia* Grev. is one of the largest in the Rhodophyta, with approximately 200 species currently recognised and ca. 1000 species and infraspecific taxa named (Guiry and Guiry 2012). Species of *Polysiphonia sensu lato* have a filamentous habit composed of segmented, polysiphonous branches and have a wide range of morphological variation that has led to much debate about the classification of species within the genus (Mamoozadeh and Freshwater 2011). The genus has had a long and confused nomenclatural history, as summarised by Kim et al. (2000). The type species *P. urceolata* (Lightf. *ex* Dillwyn) Grev. is conspecific with *P. stricta* (Dillwyn) Grev. (Kim et al. 2000). Kim and Lee (1999) segregated the genus *Neosiphonia* M.-S. Kim *et* I.K. Lee from *Polysiphonia* based on molecular data and a combination of morphological characters; *Neosiphonia* currently includes about 30 species worldwide (Guiry and Guiry 2012).

In an integrated molecular-morphological study, Stuercke and Freshwater (2008) examined characters used for the identification of *Polysiphonia* species and found that of 22 characters tested, five were consistent and useful (number of pericentral cells, rhizoid-pericentral cell connection, relationship of lateral branches to trichoblasts, development of spermatangial axes and arrangement of tetrasporangia). It is clear that for accurate identification of species in this genus, morphological observations need to be supported by molecular tools; the plastid-encoded ribulose-1,5-biphosphate carboxylase/oxygenase large subunit gene (*rbc*L) has proven the most useful in species discrimination (Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2011).

Accurate species identifications are essential when identifying suspected introduced taxa and any biosecurity risks they may pose. The genus *Polysiphonia* includes species reported to be invasive in various parts of the world, e.g., *P. brodiei* (Dillwyn) Spreng. is considered to be native to Europe and has been introduced to the Pacific and Atlantic coasts of North America, Japan and Australasia (Global Invasive Species Database 2012). This species has been assessed in Australia as one of the ten most damaging invasive/non-indigenous marine species (Hayes et al. 2005) based on a combination of human health, economic and environmental criteria.

In New Zealand, no detailed study of the genus *Polysiphonia* has been carried out since the revision of Adams (1991). She recognised 15 species, which were divided into two groups (*Oligosiphonia* with four pericentral cells, and *Polysiphonia* with more than four pericentral cells), provided an illustrated key for identification and reported all of the names recorded for New Zealand and

their synonyms. Nineteen species of *Polysiphonia* and two species of *Neosiphonia* are currently reported to occur in the New Zealand region, and of these, five are regarded as endemic and seven as non-indigenous (Nelson 2012). Occasionally, samples of *Polysiphonia* species collected in New Zealand have been included in molecular studies from other regions. McIvor et al. (2001) first detected the occurrence of *N. harveyi* (J.W. Bailey) M.-S. Kim, H.-G. Choi, Guiry *et* G.W. Saunders in New Zealand, a species that had passed unnoticed because *P. strictissima* Hook.f. *et* Harv. is a morphologically indistinguishable native sibling species. Within a taxonomic and molecular study on *Neosiphonia* and *Polysiphonia* from Florida and Mexico, Mamoozadeh and Freshwater (2011) included samples of *P. constricta* Womersley, *P. pernacola* N.M. Adams, *P. isogona* Harv. and *P. strictissima* from New Zealand. In addition they recorded the presence of *P. morrowii* Harv. Although this was the first report of this species for New Zealand, Mamoozadeh and Freshwater (2011) did not comment on this new record. During a study of macroalgal diversity associated with soft sediment habitats, we collected several samples of *P. morrowii* and reported its occurrence in Otago Harbour in a report prepared for the Ministry of Fisheries of New Zealand (Neill et al. 2012).

Polysiphonia morrowii was originally described from Hakodate, northern Japan (Harvey 1857, Masuda et al. 1995: 199) and is considered to be native to the North Pacific [China (Tseng 1984), Commander Islands (Selivanova and Zhigadlova 1997), Japan (Yoshida et al. 1990), Korea (Kim et al. 1994, 2004, Choi et al. 2001) and Russia (Perestenko 1996)] (Guiry and Guiry 2012). Based on morphological characters, it has been reported as an introduced species in the Mediterranean [France (Verlaque 2001), Italy (Curiel et al. 2002) and Turkey (Erdugan et al. 2009)]. Geoffroy et al. (2012) reported the cryptic introduction of *P. morrowii* in the North Atlantic based on barcoding data.

The aim of this paper is to emphasise the recent recognition of this species in New Zealand, its distribution and the distinguishing features of the specimens collected. This information may assist with ongoing biosecurity and monitoring programs.

Materials and methods

Ten samples of *Polysiphonia morrowi* were collected in Otago Harbour at four sheltered intertidal sites: Harwood (45°48.857′ S, 170°39.987′ E), Torpedo Bay (45°47.858′ S, 170°37.677′ E), Te Ngaru (45°47.222′ S, 170°40.622′ E) and Otakou (45°47.910′ S, 170°42.863′ E) in late summer and early spring (February, October 2009) attached to wood and also growing unattached. Specimens were pressed, and the vouchers were deposited in the Museum of New Zealand Te Papa Tongarewa Herbarium (WELT) (Thiers 2012), subsamples were dried in silica gel for molecular analysis and the remaining samples fixed in 5% formaldehyde-seawater for morphological observations. Photomicrographs were taken on a digital photomicroscope (Zeiss Axiocam on Axiovert 200 inverted microscope, Oberkochen, Germany).

DNA extraction was performed following the CTAB/Proteinase K method of Zuccarello and Lokhorst (2005). The plastid-encoded large subunit of the ribulose bisphosphate carboxylase/oxygenase gene (*rbc*L) was amplified and sequenced using primers F57/JrSR, or F57/R753 (Freshwater and Rueness 1994, Broom et al. 2010). The amplified products were checked for correct length, purity and yield on 1% agarose gels stained with ethidium bromide. PCR products were cleaned using ExoSAP-IT (USB, Cleveland, OH, USA) and sequenced using standard methods. Sequences were compared to sequences held in GenBank using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/).

Selected specimens

Akaroa Harbour: Onuku, 23 October 2004, *Freshwater and Hommersand*, WNC2007-s001, WNC2007-s002 WELTA032414 (tetrasporophyte). Otago Harbour: Harwood, February 2009, *Nelson*, WELTA032587 (tetrasprophyte), WELTA032403 (tetrasporophyte), WELTA032404 (tetrasporophyte); also, October 2009, *Neill and Nelson*, WELTA032409 (sterile), WELTA032410 (sterile); Te Ngaru, October 2009, *Nelson and Dalen*, WELTA032405 (tetrasporophyte), WELTA032586 (tetrasporophyte); Torpedo Boat Mole, October 2009, *Nelson and Dalen*, WELTA032406 (sterile), WELTA032407 (tetrasporophyte); Otakou township, Wellers Rock Te Umukuri, *Neill and Farr,* WELTA032408 (carposporophyte). Curio Bay: 28 October 2004, *Freshwater and Hommersand*, WNC2007-s020 (NZ04-130) (tetrasporophyte), WNC2007-s022 (NZ04-132) (sterile), WNC2007-s048 (NZ04-140) (tetrasporophyte).

Results

The distribution of *Polysiphonia morrowii* in New Zealand is illustrated in Figure 1A and B. Of the samples collected in late summer and early spring from intertidal sites in

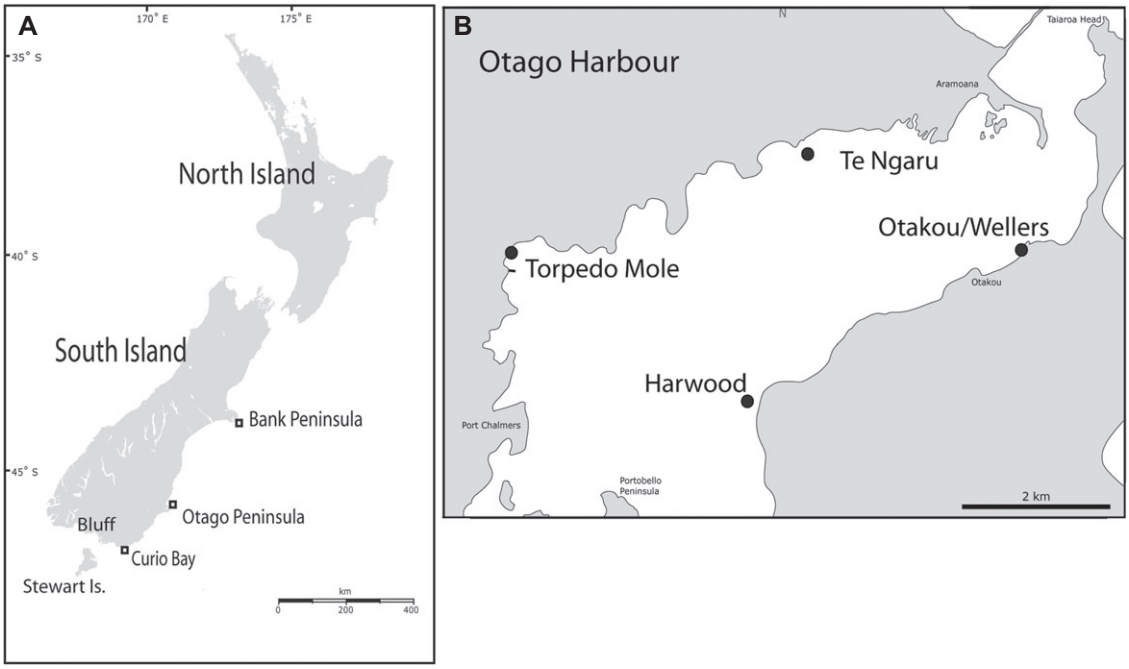

Figure 1 *Polysiphonia morrowii*: distribution of the species. (A) Map of New Zealand showing the locations (squares) where the species was found. (B) Detail of the distribution in Otago Harbour (circles).

Otago Harbour, two were confirmed to be *P. morrowii* using molecular sequence data, and the additional seven samples were confirmed morphologically. The first 650 bp of the *rbc*L sequence is identical in samples WELTA032404 (650 bp) (GenBank KC152487) and WELTA032587 (1388 bp) (GenBank KC152488) from Otago Harbour. The complete sequence, from WELTA032587, had a two base-pair difference with samples of *P. morrowii* from Korea (AY958161), Chile (AY396029) and samples from Curio Bay (HM573579) and Akaroa Harbour (HM573583) (Mamoozedah and Freshwater 2011). This sequence also corresponds to haplotype 1 found by Geoffrey et al. (2012). The maximum-likelihood analysis based on the *rbc*L data, which includes New Zealand samples of *P. morrowii*, can be found in Neill et al. (2012).

Morphological observations

Thalli were blackish, grew in dense tufts (Figure 2), 3–9 cm high and consisted of primary upright and prostrate axes that lacked cortication (Figure 3) and possessed four pericentral cells (Figure 4). Branches were slender, 60–90 µm wide, irregularly alternate and terminated with sharp points (Figures 3 and 6). Prostrate filaments were attached to substrata with adventitious unicellular rhizoids without septation (Figure 5). Cystocarps were urceolate and 150–300 µm wide with broad ostioles (Figure 5). The tetrasporangia were in straight series of up to eight (Figure 7). Occasionally, adventitious recurved branches were observed originating from the prostrate axis (Figure 8).

Collections were made in October (spring) and February (late summer). A total of 11 samples were examined, of which seven were tetrasporophyes found in October and February, one was a carposporophyte found in October and the remaining three were sterile. The specimens from Curio Bay and Akaroa Harbour were also tetrasporic.

Discussion

Polysiphonia morrowii is on the list of the "100 Worst Invasive Species" in the Mediterranean, together with *Grateloupia turuturu* Yamada and *Undaria pinnatifida* (Harv.) Suringar (Streftaris and Zenetos 2006). This species has been reported to have varying morphology as well as wide environmental tolerances. In the extreme southern region of Chile (Punta Arenas), where it has been reported as introduced, *P. morrowii* thalli were reported to reach ca. 20 cm in height (Kim et al. 2004), and in the Mediterranean (Island of Venice), thalli reached up to 50 cm in height. In its native region, thallus height is reported to range from 16 to 25 cm in Korea (Yoon 1986, Kim et al. 1994) and up to 35 cm in

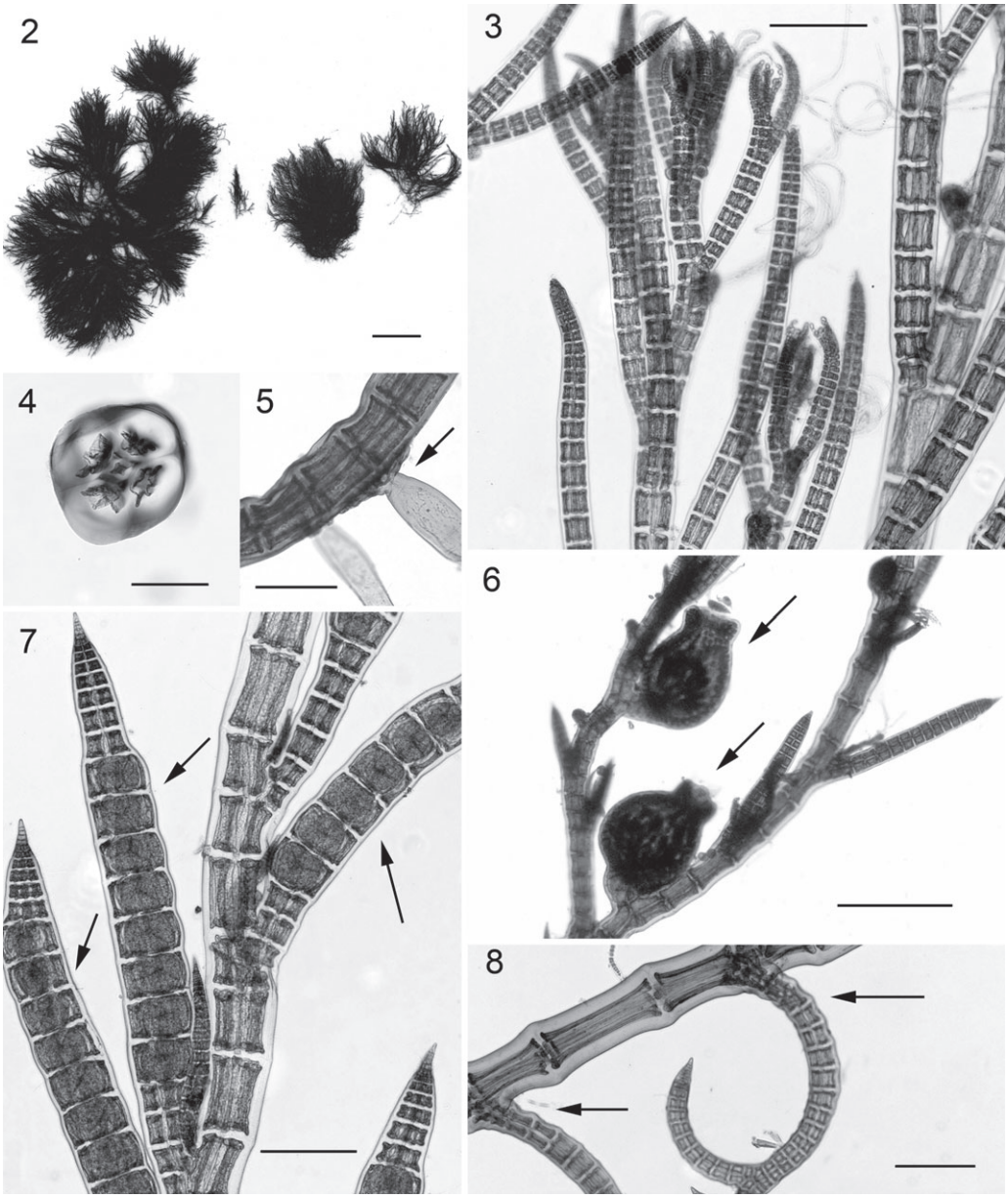

Figure 2 *Polysiphonia morrowii.*
Habit (WELTA032404), collected from Harwood, Otago Harbour (scale bar, 1 cm). (3) Apical part of ecorticate erect branches (scale bar, 200 μm) WELTA032406. (4) Axis cross-section showing four pericentral cells (scale bar, 100 μm) WELTA032587. (5) Unicellular rhizoids without septation (arrow) (scale bar, 100 μm) WELTA032404. (6) Urceolate mature cystocarps (arrows) (scale bar, 500 μm) WELTA032408. (7) Tetrasporangia in straight series (arrows) (scale bar, 100 μm) WELTA032405. (8) Prostrate axis with adventitious recurved branches (arrows) (scale bar,100 μm) WELTA032406.

Japan (Kudo and Masuda 1992). The thallus height of New Zealand samples was much smaller (3–9 cm). New Zealand specimens were all characterised by sharply pointed vegetative tips; however, this character was not stable in specimens from France, where few specimens without pointed vegetative tips were found to be *P. morrowii* based on molecular data (Geoffrey 2012). This character was also not included among consistent characters for *Polysiphonia* species identification by Stuercke and Freshwater (2008).

In the New Zealand populations, and also in Chile (Kim et al. 1994) and in the Lagoon of Venice (Curiel et al. 2002), the tetrasporic stage predominated over the sexual phases. In Turkey in the eastern Mediterranean, *P. morrowii* has been recorded growing in rocky intertidal areas with both tetrasporic and sexual stages (Erdugan et al. 2009). In Korea, specimens in the wild are reported to complete a life cycle within four months, with all reproductive stages concurrently present (Lee and Lee 1991, Kim et al. 2004).

This species survives at temperatures between 6°C and 25.5°C and a salinity range of 23–37 (Curiel et al. 2002). The temperature in Otago Harbour ranges between approximately 6°C and 17°C (Kregting et al. 2008) and salinity is generally 29–33, although this can vary near freshwater inflows (Innes et al. 2010).

Polysiphonia morrowii has been reported growing on a wide variety of substrata, including rocks, wooden piles, ropes, mussels, crabs and tunicates. In the Lagoon of Venice, it grows in association with other introduced species, such as *Sargassum muticum* (Yendo) Fensholt and *Undaria pinnatifida* (Curiel et al. 2002). Geoffroy et al. (2012) reported *P. morrowii* from localities close to the largest aquaculture areas in Brittany (i.e., Roscoff, Saint-Malo, Quiberon) and suggested that the species was introduced via vessels and the transportation of oysters. Among the 105 individuals of *P. morrowii* collected along the coast of Brittany, three haplotypes were found, suggesting several introduction events, which is in agreement with the fact that most alien seaweeds in Europe have been introduced over the last four decades via aquaculture activities, primarily those involving the Pacific oyster *Crassostrea gigas* Thunberg. Kim et al. (2004) considered *Undaria pinnatifida*, which is native to the northwestern Pacific Ocean and grows in Argentina (Casas and Piriz 1996, Meretta et al. 2012), as a possible vector of introduction, although *Undaria* has not been recorded in Chile. In New Zealand, *Undaria pinnatifida* is well established and has spread widely after its first record in 1987 (Hay and Luckens 1987).

In New Zealand, *P. morrowii* has been collected from soft sediment habitats as well as on rocky reefs and growing on various substrata including rock, wood and shell. Kim et al. (2004) commented that *P. morrowii* "possesses a number of biological features such as the ability to colonise a variety of substrata in intertidal habitats, rapid growth, and a short life history, which might give it a competitive edge over some native algal species".

There has been some debate as to whether *P. morrowii* and *P. senticulosa* Harv. are conspecific or closely related taxa of the northeastern and northwestern Pacific, respectively (Kudo and Masuda 1981, 1992, Kim et al. 1994). *Polysiphonia senticulosa* is native to the northeastern Pacific and has been reported as an introduced species in both Australasia (Nelson and Maggs 1996) and The Netherlands (Stegenga et al. 2007). Curiel et al. (2002) considered *P. senticulosa* to be conspecific with *P. morrowii* as they refer to Womersley (1979) and Nelson and Maggs (1996) reported *P. morrowii* for Australia and New Zealand, respectively. However, these two reports from the southern hemisphere were for *P. senticulosa*, a species that had been initially reported as *P. pungens* Hollenberg (Womersley 1979). Both Womersley (2003) and Nelson and Maggs (1996) followed the treatment of Kudo and Masuda (1988), who synonymised *P. pungens* with *P. senticulosa*. Samples identified as *P. senticulosa* from Wellington were very different in habit from the specimens identified as *P. morrowii*, being reddish and in large fluffy tufts up to 50 cm in height. Molecular data are not available for this entity, and it has not been recently collected. Further investigation is required to compare sequences of *P. morrowii* with sequence data from *P. senticulosa* collected in New Zealand and with material of *P. pungens* and *P. senticulosa* from the North Pacific. At present, these data are not available.

Including this report of *P. morrowii* in New Zealand, there are now seven species of *Polysiphonia* and *Neosiphonia* that are non-indigenous to the region. The other previously recorded species are *P. brodiei, P. constricta, N. japonica* (Harv.) M.S. Kim *et* I.K. Lee*, P. senticulosa, N. sertularioides* (Grateloup) K.W. Nam *et* P.J. Kang and *P. subtilissima* Mont. (Nelson 1999, 2012, Hurd et al. 2004).

When a new record is reported it is a challenge to establish whether the species is native or introduced, and this is particularly true when taxa are morphologically similar to native species, or belong to genera that have not been the subject of modern revisions. To confidently discern between native and introduced species, baseline information on distributions and taxonomy are required. At present, knowledge of the New Zealand macroalgal flora is far from complete. This is especially true for the *Polysiphonia/Neosiphonia* complex, which has received little attention in the 20 years since the revision of Adams (1991). During a study on macroalgal diversity associated with soft sediments (Neill et al. 2012), ten *Polysiphonia* species were found to be genetically distinct and only five were identifiable to known species, including the new record for *P. morrowii*. Given that these data were obtained from the soft sediment habitats of only two harbours, we consider that when samples from a full range of habitats throughout the New Zealand region are studied, the diversity in the *Polysiphonia*/*Neosiphonia* complex will be considerably higher than currently reported.

The documentation of introduced macroalgae in New Zealand began with the checklist of Adams (1983), who noted that there were certain species "confined mainly to harbours and sheltered anchorages, particularly those frequented by whalers, sealers, and traders from the late eighteenth century". However, as Hayden et al. (2009) observed, it is possible that some of the species that arrived on the hulls of early vessels are now so widespread and abundant that they cannot readily

be distinguished as non-native. When a new species is detected, such as *P. morrowii*, with a known record of introduction to other regions, it may be a reasonable assumption to categorise it as non-native. Otago Harbour is known to host 17 introduced macroalgal species, including *P. brodiei*, *P. constricta*, *P. subtilissima* and *N. sertularioides* (Neill et al. 2012), and thus an additional non-indigenous species would not be surprising. This would also be the case for the record found in Akaroa Harbour. However, the samples included in the study of Mamoozadeh and Freshwater (2011) were collected on a rocky reef at Curio Bay in south east Otago, a remote and exposed location with no wharves, jetties, shipping or port facilities nor marine farms nearby, making it difficult to identify potential vectors for introduction. The closest harbour, about 100 km south west of Curio Bay, is Bluff, which receives industrial, fishing and recreational vessel traffic. Bluff has not been surveyed for the presence of *P. morrowii*. Chiswell and Rickard (2011) examined the connectivity of major ports in the New Zealand region, modelling the dispersal times based on a 14-year re-analysis of currents, and using an individual-based particle-tracking method, where the "numerical ocean is seeded with numerical passive particles to simulate larval dispersal". The dominant flow along the south-eastern coast of South Island is the Southland current, and this is "the least variable and most predictable of New Zealand's nearshore currents". Chiswell and Rickard's (*loc. cit.*) model showed a fast progression northwards along the coast, with a mean dispersal distance of 177 km in 10 days. However, based on the work of Chiswell and Rickard (2011), any distribution south from Otago is mostly unlikely. Samples collected in our study in Otago Harbor were found on driftwood or unattached, and thus it is possible that driftwood may have served as a vector for the spread of this species.

It is clear that further work is needed on the *Polysiphonia* and *Neosiphonia* group. Non-indigenous species of *Polysiphonia* represent a biosecurity risk as they can grow rapidly and are often detected several years after their establishment. The understanding of the *Polysiphonia/Neosiphonia* complex in New Zealand will be crucial in discerning between native and non-indigenous species.

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