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An undescribed species of putative Japanese *Pyropia* first appeared on the central coast of British Columbia, Canada, in 2015☆

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A R T I C L E I N F O

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In 2015, on the central coast of British Columbia, Canada, I documented the occurrence of a putative Japanese species of *Pyropia* that may have arrived with tsunami debris. This find occurred at a site that has been subjected to an annual intensive seaweed survey beginning in the summer of 2011 so there is little doubt that the species was new to the area in 2015. In a molecular phylogeny, this species is basal in a clade of Japanese and other Far East species of *Pyropia* although this particular species has yet to be reported from Japan. In addition to the central coast of British Columbia, the species was previously recorded from the South Island, New Zealand, and from Monterey Bay, California, based on molecular sequences so it is clearly a species that is able to move beyond its native range. The foliose phase of the species was not observed in 2016, but overall *Pyropia* diversity and abundance were down in 2016. However, the cryptic conchocelis phase was identified by sequencing pinkish barnacle shells collected in 2016 near the area where the species was observed in 2015 so it is possible that the species is becoming established on the central coast of British Columbia. Until it becomes established there, however, it is premature to describe it with a type locality in this area; indeed it would be preferable to find it in Japan and describe it from its native habitat. This find highlights the difficulty in documenting possible invasive species from tsunami debris when the Japanese flora and fauna have not been sufficiently characterized to allow for comprehensive comparisons.

1. Introduction

When the 2011 earthquake and tsunami struck Japan, Kou Sasaki of Ofunato lost his fishing boat. The boat drifted across the Pacific and washed ashore near the village of Klemtu on the central coast of British Columbia, Canada. The battered boat was cleaned of the mussels and barnacles growing on it, and people in Klemtu helped refurbish it. Tim McGrady of the Spirit Bear Lodge in Klemtu claimed the boat for use in the lodge's bear-watching operations. Preliminary efforts to find the owner were unsuccessful. When lodge guest Yoshi Karasawa translated the name of the boat — *Twin Pines* — she too was fascinated. She used her contacts in Japan to find the owner. Karasawa then brought Kou Sasaki to Canada, and McGrady brought him to Klemtu for a reunion with his former boat –adapted from a CBC report, 24 Aug 2015.

Sasaki's boat was one of > 150 small boats that washed ashore on North American beaches as part of the 2011 Great Tohoku Earthquake tsunami marine debris (Murray et al. 2015). These and other large floating structures often harbored fouling organisms. The story of Sasaki's boat struck a chord with me after I discovered in 2015 a putative Japanese species of *Pyropia* on a pristine beach on northwest Calvert Island about 100 km south of Klemtu, well within the area of documented Japanese tsunami marine debris (Maximenko et al. 2015). The species resembled other Japanese species of *Pyropia* based on the distinctive disposition of reproductive cells, and I wondered whether the species could have arrived with Japanese tsunami marine debris as an inadvertent passenger in barnacle shells attached to the boat. [The microscopic, perennial sporophyte phase of *Pyropia*, the conchocelis, normally grows in calcareous substrates, including barnacle shells (Martínez, 1990).] Below, I report on efforts to identify this species and associate it with the tsunami debris.

2. Materials and methods

Thalli of *Pyropia* were collected on rock in sand at the low intertidal

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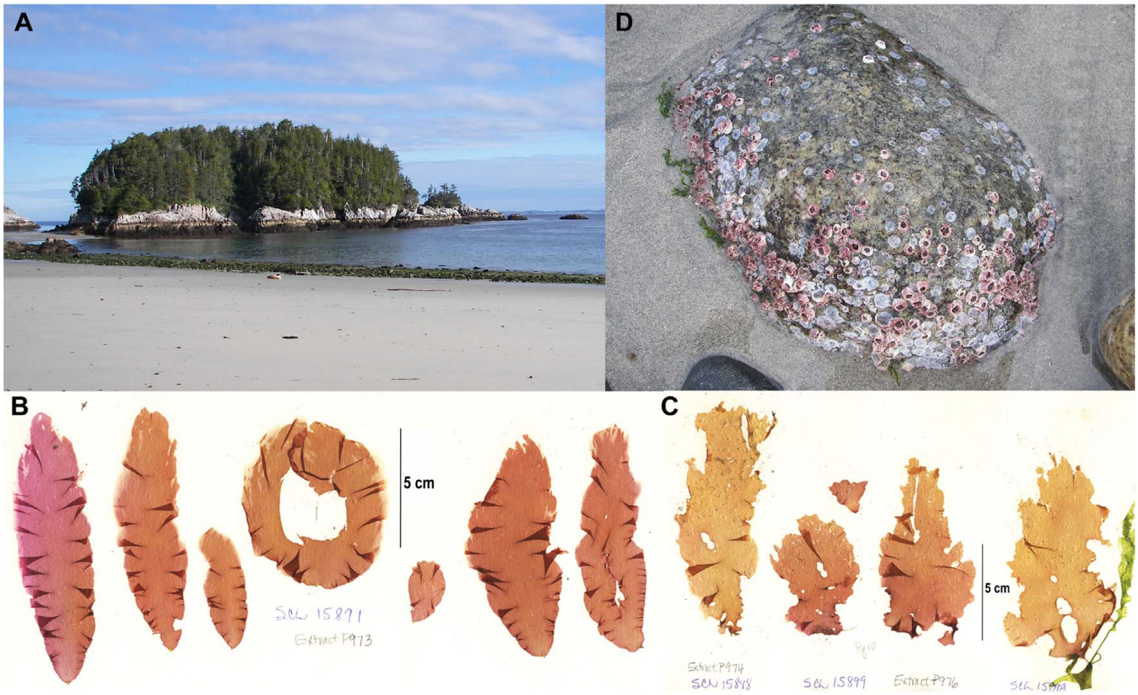

Fig. 1. A. North Beach, Calvert Island, where the undescribed *Pyropia* was found on rock growing in sand at low tide level.
B. Herbarium specimens of *Pyropia* sp. 15 June 2015 collection.
C. Herbarium specimens of *Pyropia* sp. 19 June 2015 collection.
D. Pink barnacle shells, North Beach, Calvert Island, from which the conchocelis of *Pyropia* sp. was extracted, amplified and sequenced.

margin of the west end of North Beach, Calvert Island (Fig. 1A, 51° 39.802′ N 128° 08.593′ W), on 15 and 19 June 2015.

Samples were pressed on herbarium paper (Fig. 1B, C) for depositing in the UBC herbarium, and subsamples were desiccated in silica gel for later DNA extraction, amplification, and sequencing. The same location was revisited on 4 June 2016. No *Pyropia* resembling the 2015 collection were observed. Pink barnacle shells were collected on rock in sand at the low intertidal margin of the east end of North Beach, Calvert Island (Fig. 1D, 51° 39.824′ N 128° 08.209′ W) on 04 June 2016; these were desiccated in silica gel for later DNA extraction, amplification, and sequencing.

DNA extraction followed the protocol of Lindstrom and Fredericq (2003) except that 16–31 mg desiccated tissue was extracted after pulverization using liquid nitrogen and a mini-pestle, and 10 μL 0.1 M dithiothreitol was added to the extraction solution. The same extraction solution and protocol were used for the pink barnacle shells after 316 mg of desiccated shell was pulverized using a hammer. PCR amplification and sequencing of the *rbc*L gene were carried out as described in Lindstrom (2008) except that KitoF1 was used as the forward primer rather than F57 (Lindstrom et al. 2015). Sequences were compared to those in GenBank using the Blastn program. A maximum likelihood phylogenetic tree was constructed as in Lindstrom et al. (2015).

3. Results

Sequences of both the foliose and conchocelis phases were nearly identical to GenBank sequence HQ687540 (except for the second bp in the sequence, which may be incorrect, and two C-T transitions in the middle of the sequence); it differed from GenBank sequence JN029002 by four C-T transitions. These sequences were included in a phylogeny of other closely related species from Sutherland et al. (2011) and GenBank blastn searches ($\geq$ 97% identical, conducted 10 Jan 2017); the results are shown in Fig. 2. The Calvert I. specimens are clearly related to the New Zealand and Monterey specimens and probably conspecific, and this species belongs to a clade of species that are endemic to the northwest Pacific, particularly Japan and Korea (*P.* '*spatulata*' from Massachusetts, also in this clade, is likely another introduced northwest Pacific species, C.D. Neefus, pers. comm., 2009).

4. Discussion

The occurrence of a putative Japanese species of *Pyropia* on the central coast of British Columbia in June 2015 highlights the possibility that the species arrived with marine debris, namely in barnacle shells attached to tsunami debris, such as the fishing boat that came ashore near Klemtu. Finding the population at the northwest end of Calvert Island was entirely serendipitous. It required the unlikely conjunction of the right person (an expert in the genus *Pyropia*) being in the right place (low intertidal rock in sand at this particular beach) at the right time (late spring—many species of *Pyropia*, like this one, are ephemeral, occurring on the shore for a very short period of time, which is seasonally very specific). Although it is generally thought that introduced species take a long time to become sufficiently established to be detectable in a local flora, it is also possible that such species can be observed shortly after they arrive if the right set of circumstances exists. Such appears to be the case for this species.

This is not the first instance of a Japanese species of foliose Bangiales being introduced to distant shores. Broom et al. (2002) reported the identity of *Porphyra carolinensis* from the NW Atlantic and *P. lilliputiana* from Australia and New Zealand with Japanese *P. suborbiculata* and surmised that the species had been transported to distant shores as a fouling organism on hulls of seagoing vessels. Neefus et al. (2008) reported the occurrence in the NW Atlantic of two more Japanese species of *Porphrya* that likely arrived via shipping, and Milstein et al. (2015) recognized three species of Asian foliose Bangiales in Brazil. The concurrence of all three studies that fouling of ocean-going vessels was the likely vector in these introductions supports the present hypothesis that the Calvert Island *Pyropia* may also have arrived by the transoceanic crossing of a seagoing vessel, in this instance a vessel that was part of the 2011 Japanese tsunami marine debris.

This species also highlights one of the difficulties in recognizing

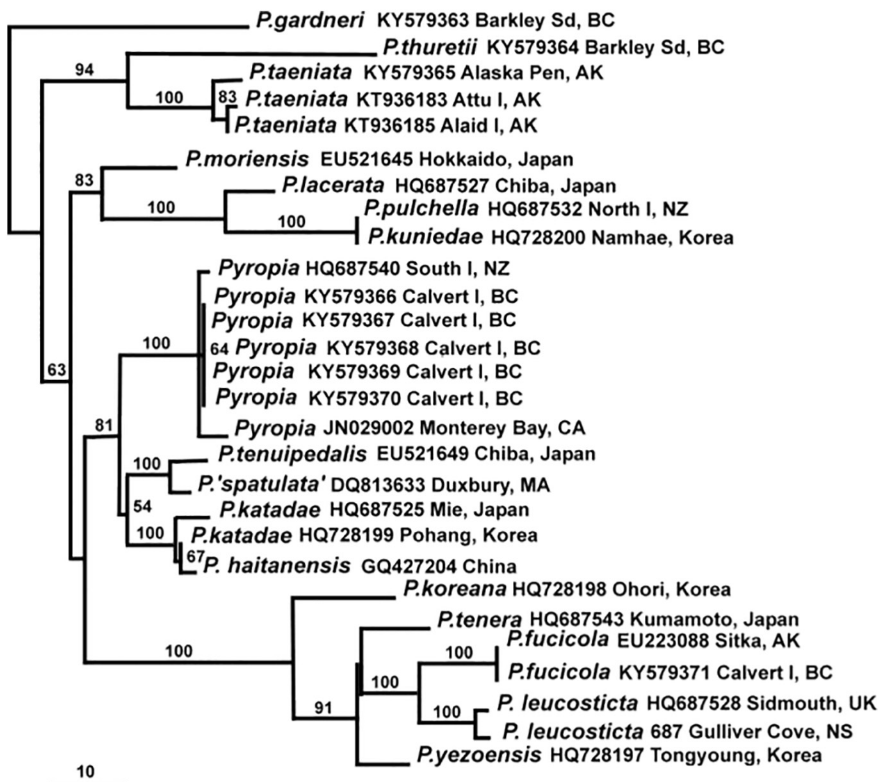

Fig. 2. Maximum likelihood tree of *rbcL* sequences from specimens 97% or more identical to the sequences from Calvert Island. –Ln likelihood = 4157.05970. Numbers above branches are ML bootstrap values.

introduced species. This species has yet to be reported from Japan. It has been found in disparate locations (southern South Island, New Zealand, and Monterey, California), where populations are also thought to have been introduced. All of these specimens occur in a clade of Japanese and other Far East *Pyropias*, which suggests that this species is also native to Japan (closely related *Pyropia* species are known to have strong geographic signatures—Sutherland et al. 2011; Lindstrom et al. 2015). Thus, one of the lessons of the tsunami is the need for a better characterization of the local biota that may provide source material for invasions via marine debris. This lack of knowledge of the Japanese marine algal flora also led West et al. (2016) to demure on the origin of a new genus and species of Stylonematophyceae found on plastic Japanese tsunami debris that washed ashore in Oregon and Washington in 2015 and 2016. A similar level of taxonomic uncertainty was noted by Calder et al. (2014), who examined the hydroids on Japanese tsunami derelict boats and docks that washed ashore in Oregon and Washington in 2012 and 2013. It is important that the present undescribed species of *Pyropia* be documented in Japan, and it is preferable that it be described from its native habitat there. The observation that all three introductions (New Zealand, California, and British Columbia) have distinctive genotypes suggests that these introductions may have originated from different parts of Japan. Study of this species in Japan should allow us to determine whether the Calvert Island material originated from the Tohoku area and would strengthen the argument that it arrived on tsunami debris.

Not all species introductions lead to invasions. In fact, there is a range of effects that non-indigenous species can have on a community, from non-detectable to catastrophic (Inderjit et al., 2006; Schaffelke et al. 2006). In some instances, non-indigenous species can persist at low levels for a period before increasing in abundance and becoming truly invasive (Stockwell et al. 2003). The present find of a non-indigenous *Pyropia* on the central coast of British Columbia appears to fall into the non-detectable effect. Initially we thought we might never see the species again as it was not observed when we visited Calvert Island in early June 2016. However, sequencing of pinkish barnacle shells from the eastern end of North Beach revealed them to harbor the conchocelis of this species based on identical DNA sequences. Thus, it is possible that this species will become an established part of the local seaweed flora over time.

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