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First record of the invasive red alga *Polysiphonia morrowii* Harvey (Rhodomelaceae, Rhodophyta) on the Patagonian shores of the Southwestern Atlantic

Abstract: Seaweed invasions are recognized as one of the major threats to biological diversity and coastal resources. Nevertheless, the number of introduced macroalgae has been underestimated due to the increasing number of cryptic invasions. *Polysiphonia morrowii* is native to the North Pacific and several cryptic introductions of this species have been reported over the past decade. In this study, we recorded the presence of the invasive *P. morrowii* in the Southwestern Atlantic (Patagonia Argentina) using a morphological and molecular approach for the identification at species level. *Polysiphonia morrowii* specimens from Argentina showed no morphological differences from members of invasive populations in France. We found two different haplotypes, suggesting that *P. morrowii* was probably introduced into this area by multiple introduction events. The increasing number of shipping and aquaculture activities worldwide and the ability of this species to be a successful invader suggest that *P. morrowii* is likely to be a cosmopolitan invasive species and that further management planning is required for its early detection and control.

Keywords: cryptic invasions; *Polysiphonia morrowii*; Southwestern Atlantic.

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Introduction

Macroalgae have an important ecological role for the organization of natural communities, providing food for many benthic faunal species and also offering protection (Kelaheer et al. 2007). The introduction of marine macroalgae is a matter of concern because they may modify both ecosystem structure and function by monopolizing space, developing into ecosystem engineers, and changing food webs (Thresher 2000, Irigoyen et al. 2011). Macroalgae comprise between 10% and 40% of the total alien species in marine habitats (Schaffelke et al. 2006); however, the actual number of invasive macroalgae in marine environments has been seriously underestimated because of the prevalence of morphologically similar species (pseudo-sibling species, Knowlton 1993) and those that cannot be distinguished morphologically but differ genetically (sibling species, Bickford et al. 2007). The recognition of an increasing number of sibling and pseudo-sibling introduced species reflects both the lack of earlier detailed studies of morphological characters as well as recent molecular studies (McIvor et al. 2001).

Filamentous algae are one of the main groups of invasive algae (Williams and Smith 2007). Examples of cryptic introductions within this group are those of the genus *Polysiphonia* Grev. (McIvor et al. 2001, Streftaris et al. 2005). This genus, which comprises over 200 species, is phylogenetically diverse and is widely distributed throughout the world (Guiry and Guiry 2013). *Polysiphonia morrowii* Harvey is native to the Northwest Pacific Ocean (e.g., Segi 1951, Kim et al. 1994) and, over the past decade, it has been introduced into the Mediterranean Sea, New Zealand, the Southwest Pacific coast of Chile and probably the North Sea (as *Polysiphonia senticulosa* Harvey; Kim et al. 1994, Maggs and Stegenga 1999, Verlaque 2001, Curiel et al. 2002, Erdügan et al. 2009, D'Archino et al. 2012). This species has recently been reported in Northern France, where it had not been detected previously because of its morphological similarity to the native species *Polysiphonia stricta* (Dillwyn) Greville (Geoffroy et al. 2012).

In Argentina, the genus *Polysiphonia* is represented by five morphological species distributed along the coast from 37°S to 54°S (Liuzzi et al. 2011). These species grow mainly during the cold season (from May to August) and inhabit rocky intertidal shores and the shallow subtidal (Boraso and Zaixso 2008). Three of these species inhabit rocky shores from North Patagonia: *Polysiphonia hassleri* W.R. Taylor, *Polysiphonia brodiei* (Dillwin) Sprengel, and *Polysiphonia abscissa* J.D. Hooker et Harvey. The first two species are characterized mainly by having more than four pericentral cells. However, *P. abscissa* has four pericentral cells identical to *P. morrowii* and, because both species are morphologically similar (Bustamante and Ramírez 2009), an unnoticed cryptic invasion could have happened in Argentina. This work reports the presence of *P. morrowii* on the Patagonian shores of the Southwestern Atlantic for the first time. This identification was made using both morphological and DNA sequence analyses comparing populations from Argentina and France where this species was also introduced.

Materials and methods

Sampling and morphological analysis

Ten specimens were collected from the low intertidal zone in each of three different localities along the coast of Nuevo Gulf (Patagonia, Argentina): Casino (42°36'S, 64°49'W), Punta Ameghino (42°36'S, 64°52'W), and Las Charas (42°30'S, 64°36'W) (total n=30). Field collections were carried out in August 2009 at Casino and in August 2011 at Las Charas and Punta Ameghino. Additional specimens were sampled from Las Charas in August 2012 (n=25) in order to describe the cystocarpic stage. To compare the morphological characters of *Polysiphonia morrowii* from Argentina with another invaded area, specimens from France (n=5) were collected from rocky and sandy intertidal habitats in Roscoff (48°43'N, 3°59'W) between February 2011 and April 2012. Specimens collected in the field were identified and preserved in 5% formalin seawater solution buffered with borax. Permanent slides were made for microscope observation as described in Tsuda and Abbott (1985) and a fragment of tissue from each individual was preserved in silica gel for molecular analysis. At least one specimen from each site was pressed and mounted on a herbarium sheet and deposited at the Herbarium of the Museo Argentino de Ciencias Naturales Bernardino Rivadavia (BA 47525) and the Instituto de Botánica Darwinion (SI 167782, barcode number 046530). Additional samples from France were deposited at the Roscoff Marine Station, MNHN (P572, P575, P576, P1190, and P1194).

Morphological characters studied for identification of species were those that were uniformly consistent and only vary among species. These are the number of pericentral cells, rhizoid-pericentral cell connection, relationship of lateral branches to trichoblasts, and the arrangement of tetrasporangia (Stuercke and Freshwater 2008). Additional characters were selected to compare morphological variation between *P. morrowii* populations from Argentina and France. These characters were based on the previous descriptions of *P. morrowii* in the literature (e.g., Kudo and Masuda 1981, 1992, Kim et al. 1994, Curiel et al. 2002) and were total length, diameter, length, and length/diameter (L/D) ratio of segments measured on the upper, middle, and lower zone of the upright axis, and the diameter, total length, and L/D ratio of the rhizoids. For each morphological character, five specimens were examined from each population (Argentina and France) and three measurements per specimen were made (except for total length, with one measurement per plant). The null hypothesis of no difference between the specimens of Argentina and France in the morphological characters measured was evaluated with a t-test (Zar 1999). Homogeneity of variance assumptions were evaluated with F tests (Zar 1999). Independent t-tests were performed for each character studied.

Molecular analyses

DNA was extracted from 5 to 10 mg of dry algal tissue using the Nucleospin® Multi-96 plant kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol and the *rbcL* gene was amplified on an Eppendorf thermocycler following Guillemin et al. (2008). Briefly, the reaction mixture contained 0.5× PCR buffer (Abgene, Epsom, UK), 125 μM of each dNTP, 1 pmol of each primer, 2.5 mM MgCl₂, 1 U Taq polymerase (Abgene), and 3 μl of DNA (1:25 dilution); PCR cycling included an initial denaturing step at 94°C for 3 min, followed by 35 cycles at 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s, with a final elongation step at 72°C for 7 min. Finally, PCR products were purified and sequenced using an external facility, Eurofins MWG Operon (Ebersberg, Germany). The sequences obtained were corrected and analyzed following the protocol used in Geoffroy et al. (2012).

Results

Morphological analysis

Specimens examined from Argentina were composed of upright axes and prostrate filaments attached to the

substratum by adventitious rhizoids. Upright axes had four pericentral cells without cortication (Figure 2), the unicellular rhizoids were connected with a pericentral cell (Figure 3), and the ultimate branches arose alternately with sharply pointed apices (Figures 4 and 5). The tetrasporangia were arranged in straight series (Figure 5) and cystocarps were urceolate and 120–180 μm wide (Figure 6). Trichoblasts were absent in the material studied and male specimens were not collected. There were no significant differences between *Polysiphonia morrowii* populations from Argentina and France in any of the morphological characters studied (Table 1).

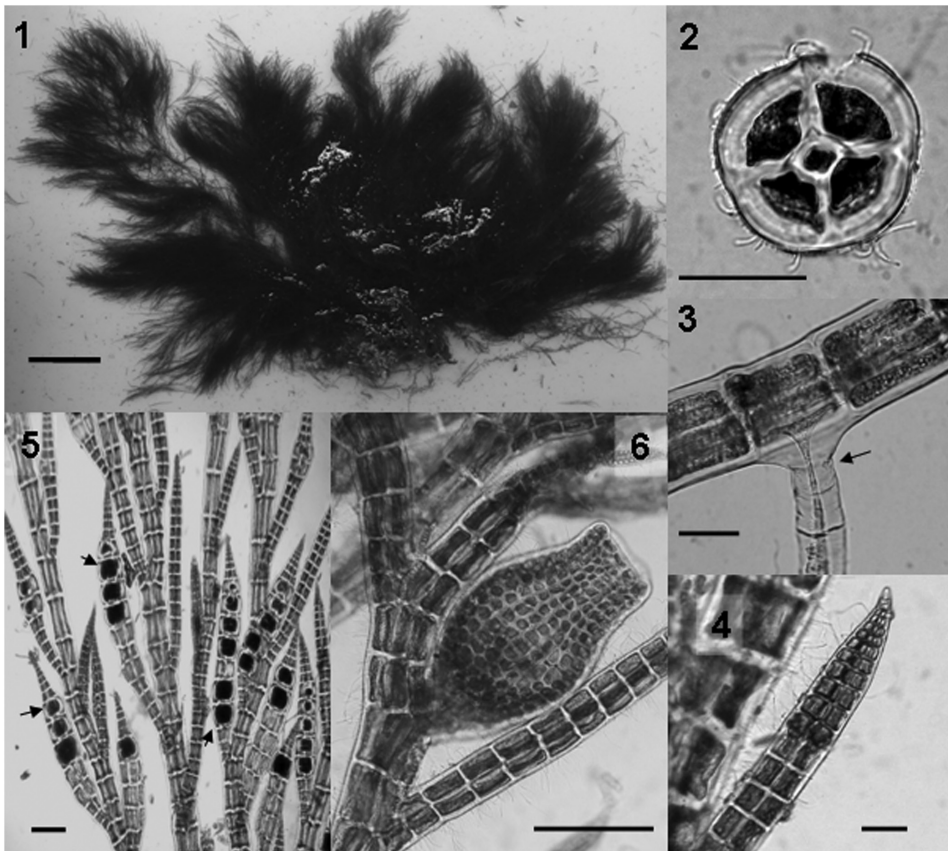
Molecular analysis

A total of 11 *rbcl* sequences were generated (GenBank accession numbers: KF468803, KF468804, KF468805, KF468806, KF468807). All sequences were closely matched (99–100% similarity) with *Polysiphonia morrowii*

sequences in GenBank (JF706219, JF706220, JF097796, AY396033, AY396031, AY396029, AY396027, HM573579). There were two different haplotypes in the sequences from Argentina. The most common was haplotype C1 found at Casino, Punta Ameghino, and Las Charas (Golfo Nuevo) and also present in Korea, New Zealand, Chile, and France (see Geoffroy et al. 2012), but the haplotype C5 was new and was found only at Casino and Las Charas (Golfo Nuevo, Argentina).

Discussion

This work represents the first report of the exotic red alga *Polysiphonia morrowii* on the Patagonian shores of the Southwestern Atlantic. The morphological characters we studied here corresponded to the lectotype of *P. morrowii* designated by Masuda et al. (1995) and were also in agreement with other morphological descriptions made



Figures 1–6 *Polysiphonia morrowii*. (1) Herbarium specimen collected in Las Charas (BA 47525). (2) Transverse section showing four pericentral cells without cortication surrounding the central axial cell. (3) Adventitious rhizoid in open connection with pericentral cell without septation (arrow). (4) Sharply pointed branchlet from the apical zone. (5) Branchlets with tetrasporangia in straight series (arrow). (6) Mature urceolate cystocarp. Scale bars: A, 10 mm; B–D, 50 μm ; E, F, 100 μm .

Table 1 Morphological comparison between specimens of *Polysiphonia morrowii* from Argentina and France.¹

Variables	Argentina Mean±SD	France Mean±SD	T	p-Value
Total length (cm)	15.20±1.30	18.80±3.96	-1.93	0.09
Diameter of segments in upright axis (µm)				
Upper	70.00±13.30	76.00±23.26	-0.50	0.63
Middle	115.33±41.74	123.67±37.75	-0.33	0.75
Lower	85.33±18.03	69.60±11.96	1.62	0.14
Length of segments in upright axis (µm)				
Upper	139.33±30.30	148.00±31.58	-0.44	0.67
Middle	522.67±322.72	627.67±272.68	-0.56	0.59
Lower	104.67±6.91	91.40±16.34	1.67	0.13
Length/diameter ratio of segments (L/D)				
Upper	2.01±0.30	2.10±0.85	-0.23	0.82
Middle	4.39±1.58	4.99±2.02	-0.53	0.61
Lower	1.27±0.28	1.32±0.10	-0.32	0.75
Rhizoid				
Diameter (µm)	38.67±6.06	35.73±11.00	0.52	0.61
Length (µm)	354.00±135.10	283.80±143.50	0.80	0.45
L/D ratio	9.03±1.73	8.33±3.83	0.38	0.72

¹Total length (cm), diameter (µm), length (µm), and length/diameter (L/D) ratio of segments measured on the upper, middle, and lower zones of the upright axis, and diameter (µm), total length (µm), and L/D ratio of rhizoids. T, value from t-test; p-value, probability value; SD, standard deviation.

by Kudo and Masuda (1992) on this species. The morphological identification we made was greatly complemented and confirmed by the molecular sequences we performed and compared with the invasive populations from France, where researchers are currently characterizing the recent invasion and rapid spread of this species (see Geoffroy et al. 2012). The molecular and morphological descriptions of the specimens of *P. morrowii* from Argentina consistently show strikingly similar characteristics with the invasive population from France (Table 1). Furthermore, some of the morphological characters, such as the L/D ratio in the upper and middle part of the thallus, as well as the rhizoid diameter in specimens from invasive populations, showed similar values to the native populations from Korea previously studied by Kim et al. (1994).

Although it is too early to make hypotheses about the vector of introduction for *P. morrowii*, we found this red alga growing on the coast of Nuevo Gulf (Argentina), near the marine commercial harbor of Puerto Madryn. Given that several macroalgae have been transported across the world through ballast water and hull fouling for more than 100 years (Hewitt et al. 2007), we consider these vectors as the most likely introduction sources for *P. morrowii* in Argentina. However, we must also mention the existence of other important potential vectors. For example, *P. morrowii* could have been transported and introduced as fouling on other species of commercial importance, such as the Pacific oyster *Crassostrea gigas* (Thunberg;

introduced by 1982), and/or the large brown alga *Undaria pinnatifida* (Harvey) Suringar (Verlaque 2001, Kim et al. 2004, Geoffroy et al. 2012). These two invasive species have been successfully introduced to the Argentine coast accidentally and for commercial purposes over past decades (Orensanz et al. 2002, Meretta et al. 2012). Interestingly, Kim et al. (2004) predicted that *U. pinnatifida*, which is native to the Northwest Pacific Ocean, could be an introduction vector for *P. morrowii* in the Southwest Pacific (Chile). An increasing number of macroalgal introductions in Europe, including *P. morrowii*, have occurred with the massive importation of *C. gigas* from Japan (Verlaque 2001). This oyster has also been introduced to Chile, Peru, Brazil, and Argentina (Orensanz et al. 2002). Its introduction into Argentina resulted from an uncontrolled event in 1982 of a transplanted batch that had probably originated in Coquimbo (Chile, Orensanz et al. 2002). Our results based on molecular sequences showed the presence of two different haplotypes of *P. morrowii* (C1 and C5) in the localities studied in Argentina. We found a common haplotype (C1) in Argentina that is also present in Korea, France, Chile, and New Zealand (Geoffroy et al. 2012) and another haplotype (C5) was found only in Argentina. This evidence strongly suggests the occurrence of multiple introduction events along this vast region covering the Southern Atlantic and Pacific coasts of South America. In fact, considering the increasing number of anthropogenic activities worldwide, such as shipping and aquaculture,

together with the ability of *P. morrowii* to be a successful invader (e.g., to colonize a variety of substrata, rapid growth, vegetative propagation, and short life cycle), we predict that this species is soon likely to be a cosmopolitan invasive species. Therefore, it is critical to encourage ecological research focused on determining the ecological impact *P. morrowii* is having on invaded ecosystems, in order to provide sound baseline information to enable effective early detection plans worldwide.

A recent study performed in Chile proposed that *P. morrowii* should be distinguished from *Polysiphonia abscissa* primarily by differences in their size (Bustamante and Ramírez 2009). In Chile, the total length of *P. abscissa* does not exceed 5 cm, but *P. morrowii* individuals are up to 25 cm. In contrast, in Argentina the reported total length of *P. abscissa* was up to 14 cm (Boraso and Zaixso 2008), which is three times the length observed by Bustamante and Ramírez (2009). However, as noted by Kudo and Masuda (1981), the total length of *P. morrowii* might vary considerably depending on abiotic conditions of the habitat and its age. In our study, the mean total length measured in individuals of the invasive populations of Argentina and France was two to three times higher than the total length observed in native populations from Korea (Kim et al. 1994). In other areas where this species has also been reported as introduced, the total length varies even more, from 3 cm in New Zealand (D'Archino et al. 2012) to 50 cm in the Mediterranean Sea (Curiel et al. 2002). These studies do not support length as being a consistent character for the taxonomic identification of *Polysiphonia* species (see Stuercke and Freshwater 2008). Indeed, the similar morphologies of *P. morrowii* and *P. abscissa* may have even resulted in the invasion

of *P. morrowii* along the Argentinean coast being overlooked: we think this species could have displaced the native *P. abscissa* (Liuzzi et al. 2011), as has happened in other regions with sibling species (Geller 1999, Bortolus 2008). Therefore, further molecular research will be critical to clarify taxonomic problems where there are two or more species of *Polysiphonia* with similar morphological characters overlapping their geographic ranges of distribution, such as the Patagonian coast of Argentina and Chile.

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