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Short communication

Cryptic introduction of the red alga *Polysiphonia morrowii* Harvey (Rhodomelaceae, Rhodophyta) in the North Atlantic Ocean highlighted by a DNA barcoding approach

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ABSTRACT

Macroalgae are prone to be introduced outside of their autochthonous area; however, the difficulty to unequivocally identify them based on morpho-anatomical features, mainly due to the lack of diagnostic characters, often hampered the rapid detection of exogenous species. In the present study, we document that the *Polysiphonia* species that dominates, during spring, the high intertidal level in Brittany is *Polysiphonia morrowii*. We demonstrated the presence of this alien species in the North Eastern Atlantic in light of molecular sequences. This species originally from the Northwest Pacific Ocean has already been reported as introduced in Chile and New Zealand on a molecular ground and has been suspected in North Sea and Mediterranean Sea based on morpho-anatomical ground. Among the 105 individuals of *P. morrowii* collected along the coast of Brittany, three haplotypes were found suggesting several introduction events. In our opinion, the progression of this exogenous species, which has been so far undetected due to its morphological similarities with native species, should be further monitored.

1. Introduction

Interoceanic shipping and human activities (aquaculture, fishing) form an interconnected network between seas and oceans, favoring species dispersal and increasing the risk of introduction into coastal marine ecosystems (Carlton and Geller, 1993). In the marine realm, more than 300 introduced species have been recently listed, being most of them introduced accidentally via shipping and aquaculture activities (Molnar et al., 2008). Moreover, it has been suggested that the number of alien species may be underestimated due to the lack of conspicuous characters to distinguish between sibling species (Knowlton, 1993).

The genus *Polysiphonia* is cosmopolitan and phylogenetically very diverse (ca. 200 taxa currently recognized; Guiry and Guiry, 2011, www.algaebase.org). Many *Polysiphonia* species are prone to phenotypic plasticity (Kim et al., 2000); therefore most of the taxonomic characters used to identify species within the genus are not diagnostic and frequently render accurate species identification problematic, or nearly impossible (Kim and Yang, 2006). Molecular tools, including the barcode, have become, during the last two decades, the most frequently used alternative to morpho-anatomical studies for accurate identification as well as species delineation (e.g. Saunders and Le Gall, 2010). For instance, McIvor et al. (2001) demonstrated that the introduced species *Neosiphonia harveyi* (Harvey) M.S. Kim & I.K. Lee (as *Polysiphonia harveyi*) actually involved two cryptic species introduced into the northern Atlantic Ocean from Japan based on molecular investigations of the *rbc*L gene. This study also showed that *N. harveyi* is present in New Zealand, thereby revealing an invasion that had gone undetected due to its morphological similarity with the native species *P. strictissima* J.D. Hooker & Harvey. Likewise, *Polysiphonia morrowii* has recently been reported as an alien species in the Southern Pacific Ocean (Chile and New Zealand) based on *rbc*L sequences (Kim et al., 2004; Mamoozadeh and Freshwater, 2011). According to the literature, this species is native to the Northwest Pacific Ocean and has been recorded in South Korea (Kim et al., 1994), Japan (Kudo and Masuda, 1992), China (Segi, 1951) and Far East Russia (Perestenko, 1980). Moreover, its presence outside its native range has been suspected on morpho anatomical ground during the last decade in New Zealand (Nelson and Maggs, 1996), the Mediterranean Sea (Curiel et al., 2002; Erduğan et al., 2009; Verlaque, 2001) and putatively in the North Sea as *P. senticulosa* Harvey (Maggs and Stegenga, 1999). During a survey of the genus *Polysiphonia* in Brittany, we have uncovered, in the upper intertidal zone, conspicuous and

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extensive patches of *Polysiphonia* which morpho-anatomical characters are congruent with the description of *P. morrowii* and *P. stricta*. In the present study, we sampled 110 specimens and used a DNA-barcoding approach for their identification at the species level.

2. Materials and methods

2.1. Samples

One hundred and ten specimens of *Polysiphonia* were sampled in four different localities (7–74 specimens per site) along the coast of Brittany, France from Saint Malo to Quiberon (about 450 km apart). Three of these sites were located in the intertidal rocky shore (Saint Malo, Roscoff and Quiberon), while the remaining one occurred on a floating pontoon in the marina of Perros-Guirec. In the laboratory, fresh specimens were observed under a dissecting microscope (Olympus® CKX41) before and after being hand sectioned to study their morphological characteristics: number of pericentral cells, presence of cortication, tetrasporangia as well as male or female reproductive structures, types of connection of the rhizoids and shape of vegetative tips. A fragment of tissue from each individual was preserved in silica gel for molecular analysis. Moreover, at least one specimen from each site was pressed and mounted on a herbarium sheet.

2.2. Molecular analyses

DNA was extracted from 5 to 10 mg of dry algal tissue using the Nucleospin® Multi-96 plant kit (Macherey-Nagel) according to the manufacturer's protocol and the *rbc*L gene was amplified on an Eppendorf thermocycler following Guillemin et al. (2008). Briefly, reaction mixture contained 0.5× PCR buffer (Abgene), 125 μM each dNTP, 1 pmol each primer, 2.5 mM $MgCl_2$, 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 of 72 °C for 7 min. Finally, PCR products were purified and sequenced by LGC genomics (Berlin, Germany). The obtained sequences were corrected and aligned using Codoncode Aligner v. 3.7 (www.codoncode.com).

Species identification was attempted by searching the National Center for Biotechnology Information (NCBI) reference database using the basic local alignment search tool (BLAST). To complete the BLAST, a tree-based approach (Ross et al., 2008) was used with sequences downloaded from GenBank (http://www.ncbi.nlm.nih.gov) including 19 specimens belonging to the genera *Polysiphonia*, *Neosiphonia* and *Vertebrata*; one outgroup species (*Laurencia natalensis*) and a selection of sequences of *P. morrowii* generated in the present study in order to include all the haplotypes obtained for each of the four localities sampled in Brittany (Table 1). All sequences were truncated to 1225 sites at the 5′ and/or 3′ end because the sequences downloaded from GenBank were incomplete. Models of nucleotide evolution were determined using Modeltest (Posada and Crandall, 1998). Tree-based methods were performed using Phylogeny.fr (www.phylogeny.fr/version2_cgi/index.cgi, Dereeper et al., 2008). Maximum-likelihood (ML) and Bayesian inference (BI) analyses used a GTR + I + G model of sequence evolution and the following parameters derived from Modeltest: Base composition = (A: 0.3097, G: 0.2197, C: 0.1411, T: 0.3295), rate matrix = (2.7880, 6.2016, 5.7235, 0.9990, 27.6721), gamma shape =2.69, proportion of invariable sites = 0.552.

3. Results

Specimens collected in the higher intertidal on rocky to sandy substrates formed extensive, dense and conspicuous patches in spring while plants disappeared almost completely in summer. Individuals formed tufts and were highly branched. Plants ranged from 15 to 30 cm in length. The morphological observations revealed a structural organization of four pericentral cells without cortication, sharply pointed vegetative tips, straight series of tetrasporangia and rhizoids not separated from the central axis by any cross walls. Only tetrasporophyte and vegetative individuals were observed.

A total of 110 *rbc*L sequences were generated (Table 1) and the alignment covered 1225 bp with 430 variable sites (35.4%). Two distinct groups were revealed: one with five identical sequences (4.5%) obtained from samples of Saint Malo and perfectly matched (100% similarity) a GenBank sequence of *Polysiphonia stricta* and the second group was composed of 105 sequences that closely matched (99–100% similarity) *P. morrowii* sequences in the database. Three different haplotypes were found in Brittany. The nucleotide sequence identity between pairs of haplotypes ranged from 99.76% for H2/H3 to 99.92% for H1/H3. The haplotype H1 was found in all selected location in Brittany, whereas H2 and H3 were only observed in Roscoff and Quiberon (Fig. 1). Phylogenetic relationships were inferred using *rbc*L gene by ML and BI analyses. Both reconstruction methods showed similar topology (Fig. 1). The sequences generated in the present study were resolved in the lineage containing the *P. morrowii* sequences from GenBank. This lineage was supported by relatively strong support values (79% for the ML tree and 99% for the BI tree). *P. stricta* joined *P. pacifica* and were resolved as a sister group of *P. morrowii*. These three species and *P. atlantica* were resolved as a sister group to the remaining *Polysiphonia* species included in the present analysis.

4. Discussion

Our results based on molecular sequences demonstrated unequivocally the presence of the alien species *P. morrowii* in the North East Atlantic. These non-native species display morphological similarities with the native European species *P. stricta*. Maggs and Stegenga (1999) pointed out that *P. morrowii* could be distinguished from *P. stricta* by its sharply pointed vegetative tips. Nevertheless, this feature was not included among consistent characters for *Polysiphonia* species identification (Stuercke and Freshwater, 2008). Furthermore, we observed a few specimens without pointed vegetative tips which turned to be *P. morrowii* in light of our molecular results. The morphological similarity among both species, together with the fact that they share the same habitats, makes species identification feasible only for well-trained phycologists. In contrast, a molecular approach, such as the one employed in the present study, offers a rapid and accurate alternative (e.g. Destombe et al., 2010).

The observation of *P. morrowii* individuals in the marina of Perros-Guirec and close to the largest aquaculture areas in Brittany (i.e. Roscoff, Saint-Malo, Quiberon) suggests that *P. morrowii* was introduced via vessels and oyster transport (Boudouresque et al., 2011) which is in agreement with the fact that most alien seaweed in Europe have been introduced over the last four decades via aquaculture activities—primarily those involving the Pacific oysters, *Crassostrea gigas* (Farnham, 1980; Mineur et al., 2009). Although few sequences are available from the putative area of origin (South Korea with *rbc*L haplotypes H1, H3 and H4), the presence of different haplotypes in Brittany (H1, H2 and H3) suggests multiple introduction events. Intraspecific *rbc*L divergence within