Research Article

Chrysymenia wrightii (Rhodymeniales, Rhodophyta) - a new non-native species for the European Atlantic Coast

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Received: 3 July 2008 / Accepted: 15 September 2008 / Published online: 18 December 2008

Abstract

Chrysymenia wrightii, originally described from Japan, was found for the first time from the European Atlantic coast. It was collected in several subtidal rocky bottom habitats (9-14 meters depth) of the Ría de Arousa (Galicia, NW Spain). In this work, a description of Galician gametophytic and tetrasporophytic plants is provided. They are similar to the Mediterranean and Japanese plants. DNA sequence data of materials from Galicia and Korea were investigated using nuclear SSU and ITS1-5.8S-ITS2 rDNA and plastid rbcL sequences. No genetic variation was observed in the SSU, and only one substitution was detected in ITS and rbcL data between Galician and Korean samples, respectively. Our molecular data indicate that the Galician populations of C. wrightii are probably due to a recent introduction event from the northwest Pacific. Despite the fact that C. wrightii was formerly recorded as a new non-native species from a Mediterranean hotspot (Thau Lagoon, 1987-1989), it was never reported for the European Atlantic coast. After 30 years of its first report in the Mediterranean Sea, the simultaneous occurrence of C. wrightii in four subtidal localities of NW Spain suggests that this species may have colonized the region unnoticed for several years which may be related to its subtidal habitat and short life cycle. Similarly to the Mediterranean Sea, the introduction of C. wrightii on the European Atlantic coasts could be enhanced by the intensive aquaculture widely spread along the Galician coast. Curiously, C. wrightii is apparently restricted to both areas, the Ría de Arousa and the Thau Lagoon. In addition, many non-native species markedly represented in Galicia since the 1980’s were firstly reported in the Ría de Arousa. Consequently, the Ría de Arousa should be considered an outstanding European Atlantic hotspot of introduced marine species similar to the Solent region (South of England) or the Thau Lagoon.

Key words: Chrysymenia wrightii, Spain, non-native species, Atlantic, seaweed, aquaculture

Introduction

The introduction of non-native species is mainly caused by vessels and aquaculture (Stefaris et al. 2005). In the Galician region (NW Spain), fishing and aquaculture industry have a great development. They have favoured the interchange of biological material, which is responsible for changes in the natural communities. During the last years, several non-native species are being locally abundant in the Galician rías. Sargassum muticum (Yendo) Fensholt, Undaria pinnatifida (Harvey) Suringar, Lomentaria hakodatensis Yendo, Grateloupia turuturu Yamada, G. subsectinata Holmes, Gracilaria vermiculophylla (Ohmi) Papenfuss, Heterosiphonia japonica Yendo, Dasya sessilis Yamada, Neosiphipha harveyi (J. Bailey) M.S. Kim, H.-G. Choi, Guiry & G.W. Saunders and Ulva pertusa Kjellman have been reported along the Galician coasts, and most of them are widely spread along the coast and the Galician rías (Pérez-Cirera et al. 1989; Santiago Caamaño 1990; López Rodríguez et al. 1991; ICES 1992; Cremades Ugarte 1995; Bábara and Cremades 1996; 2004, Veiga et al. 1998; Pérez-Ruzafa et al. 2002; Rueness 2005; Bábara and Cremades 2004, 2005; De Clerck et al. 2005; Barreiro et al. 2006; Cremades Ugarte et al. 2006; Freire et al. 2006; Peña and Bábara 2006; Baamonde López et al. 2007 and López Figueroa et al. 2007).

Although Chrysymenia wrightii (Harvey) Yamada was reported as non-native seaweed for the European coast (Thau Lagoon, Mediterranean
Figure 1. *Chrysymenia wrightii* in Galicia (NW Spain), localization of the non-native plants along the Ría de Arousa (Galicia, Spain).

In order to study the genetic variation between Galician and Korean individuals of *C. wrightii*, several silica-dried specimens from both areas (Annex 2) were compared at the Polar Biodiversity Laboratory, Korea Polar Research Institute (KOPRI). Genomic DNA from samples was extracted using DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). After DNA extraction, the rest of silica-dried samples were deposited at the KOPRI herbarium. The nuclear SSU and ITS rDNA, and plastid *rbcL* were amplified from total genomic DNA using polymerase chain reaction (PCR) and the primer combinations of Saunders and Kraft (1994, 1996) for SSU, Tai et al. (2001) for ITS1-5.8S-ITS2, and Freshwater and Rueness (1994) for *rbcL*. Agarose gel-purification or direct purification with High Pure™ PCR Product Purification Kit (Roche Diagnostics, Indianapolis, IN, USA) or LaboPass PCR Purification Kit (COSMO Genetech, Seoul, Korea) was used to clean PCR products. DNA purified using this method was sequenced using the BigDye™ terminator cycle sequencing ready reaction kit (PE Applied Biosystems [ABI], Foster City, CA, USA). Sequence data were collected using an ABI PRISM 3730 DNA Analyzer, and were edited using the SeqEd DNA sequence editor (ABI) software package. The edited sequences were aligned relative to one another using the SeqPup multiple alignment program (Gilbert 1995) and MacClade 4 program (Maddison and Maddison 2003).
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Figure 2. Chrysymenia wrightii in Galicia (NW Spain), habit: A - tetrasporophytic plant (Cabo Cruz, October 2005); B - gametophytic plant with cystocarps (Isla Galileire, October 2007); C - herbarium material from Cambados, 7-X-2007 (SANT-Algae 19525); D - apical branches, constricted at the base and tapering at the tips. Scale bar A-C = 5 cm, D = 5 mm (Photographs by Ignacio Bárbara).

The final alignment for SSU, rbcL and ITS1-5.8S-ITS2 consisted of three, three and seven taxa (Annex 2), respectively. The sequences of the 1771, 1526 and 703 aligned nucleotide positions of SSU, rbcL and ITS1-5.8S-ITS2 data were edited to remove the 5' and 3' PCR primer regions (Saunders and Kraft 1994; Freshwater and Rueness 1994; Tai et al. 2001), as well as ambiguously aligned regions, to yield 1723, 1448 and 658 base pairs for phylogenetic inference, respectively. Pairwise distance for SSU, rbcL and ITS1-5.8S-ITS2 data were performed using PAUP* 4.0b10 for Macintosh (Swofford 2002).

Results and discussion

Galician plants of Chrysymenia wrightii are similar to the ones described for the Mediterranean coast (Ben Maïz et al. 1987) and the Japanese coast (Yamada 1932; Lee 1978). They are 15-40 cm high, red-brownish in colour, terete and hollow (Figure 2). The thallus is gelatinous and tender, attached by a discoid holdfast and cylindrical axes, 3-4 mm at the base to 1-2 mm at the apex. Plants grow monopodially, 2-4 times branched (alternate, opposite or irregular) with branches constricted at the base and tapering at the tips. The cortical layer consists of 2-3 small
C. Chrysymenia wrightii in Galicia, vegetative structure: A - transverse section of an apical branch showing the hollow thallus; B-C - cortical, subcortical and hyphalike filaments in transverse section; D - cortical cells in surface view, outer part; E - hyphalike filaments and gland cells in surface view, inner part; F - gland cells; G - hyphalike filaments. Scale bar = 200 µm (Photographs by Ignacio Bárbara).

Gametophytic plants are covered by numerous cystocarps along the main axe and 1-2 order branches. Mature cystocarps are subspherical (460-950 (1200) µm in diameter) with a carpospore (80-120 µm in diameter) and carpospores (17-20×20-30 µm in diameter) (Figure 4). Cystocarps match with the descriptions of Lee (1978) and Yamada (1932). Whereas, Ben Maïz et al. (1987) described cystocarps (500-800 µm in diam.) without ostiole for the Mediterranean plants. In Galicia, this feature is observed only in immature cystocarps. Male plants were not observed. Tetrasporangia divided cruciately (28-42×25-30 µm) scattered in the cortical layer and originate from inner cortical cells (Figure 4). Galician plants show a morphological variability in size and diameter of axes. Plants are profuse in branching, second order branches being abundant. In general, gametophytic plants are more irregular in form and branching compared to the tetrasporophytic plants, because adventitious short branches (similar in form to
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Figure 4. Chrysymenia wrightii in Galicia, reproductive structure: A - tetrasporangial plant in transverse section; B - tetrasporangia under cortical cells, surface view; C - cystocarp subspherical with carpostome; D - mature sphaerical carpospores; E - adventitious short branches from cystocarps (arrows). Scale bar A, C-D = 200 µm, B = 50 µm, E = 2 cm (Photographs by Ignacio Bárbara).

Apical branches) grow from the pericarp of senescent cystocarps. This growth process is quite peculiar since reproductive structures are usually the last growth stadium, but this species apparently have a high cell activity. Profusion in branching is a good strategy for non-native and invasive species providing lots of vegetative propagules by fragmentation. Although we have not observed new individuals of C. wrightii growing from the cystocarpic branches, they could provide an excellent way of spreading along the Atlantic coast of Europe as it was pointed out for other non-native species with effective dispersal by vegetative propagation (Eno et al. 1997; Bjaerke and Rueness 2004; Husa and Sjötun 2006).

Three SSU rDNA, three rbcL and seven ITS1-5.8S-ITS2 rDNA sequences were completed from seven samples of two different localities in Galicia and three different sites in Korea and were deposited in GenBank (Annex 2). No ambiguities were observed in the sequence data. Comparisons with sequences retrieved from GenBank showed that the SSU rDNA sequences were identical with that of C. wrightii from Japan (Saunders et al. 1999; AF117129). All sequence data for two samples (CH1407 and CH1536) from Galicia were identical to each other. No genetic variation was seen in the SSU, and only one substitution was observed in rbcL (T→C in position 914; data not shown) and ITS data (A→G in position 423; Figure 5) between Galician and all Korean samples, respectively. In addition a deletion (T in position 169) and an insertion (T in position 538) were also observed in the ITS1 and ITS2 region, respectively between Galician and four Korean ones (CH1727, CH1728, CH1729 and CH1774; Figure 5). Only one deletion (T in position 169) was observed between the sample from Samchuk.
(CH1770) and the other four samples from Korea (Figure 5). Our molecular data indicate that the *C. wrightii* populations in Galicia are probably due to a recent introduction event from somewhere of the northwest Pacific near Korean coast.

In the Mediterranean Sea the vector of introduction for *Chrysymenia wrightii* is likely to be mollusc culture (Verlaque 2001; Cormaci et al. 2004). Taking into account that intensive aquaculture is widely spread on the Galician coast, the introduction of *C. wrightii* was likely enhanced by this vector. In the NW Spain, aquaculture has played an important role in the introduction of other non-native species especially since the 1980’s. Examples include *Sargassum muticum*, *Undaria pinnatifida*, *Lomentaria hakodatensis*, *Grateloupia turuturu*, *G. subpectinata*, *Gracilaria vermiculophylla*, *Heterosiphonia japonica*, *Dasya sessilis* and *Ulva pertusa*.

*Chrysymenia wrightii* was added as a new non-native species for the European coast (Thau Lagoon, Mediterranean Sea), based on collections from 1978 to 1985 (Ben Maïz et al. 1987). Subsequently, the species is regularly found in the same area (which is a hotspot of introduced species) (Verlaque 2001, Verlaque et al. 2007) Many other non-native Japanese species such as *Saccharina japonica* (J.E. Areschoug) C.E. Lane, C. Mayes, Druehl and G.W. Saunders, *Ahnfeltiopsis flabelliformis* (Harvey) Masuda and *Sphaerotrichia firma* (E. Gepp) Zinova are equally restricted to the Thau Lagoon with earlier introductions from 1971 to 1988 (Verlaque 2001; Verlaque et al. 2007), although, *S. japonica* has not been detected in the Thau Lagoon since 1989 (Verlaque 2001). In Galicia, *Grateloupia subpectinata* Holmes is an example of a non-native species which has not expanded along the coast. Hitherto, it was only known in 6 localities of the Ría de Arousa (López Rodríguez et al. 1991; Bárbara et al. 2002) and since the 1990’s it has not colonized new localities in other Galician rías. At the present, *C. wrightii* is restricted to both the Mediterranean coast (Thau Lagoon) and the Atlantic coast (4 localities in the Ría de Arousa). Taking into account that the Ría de Arousa provides the new record of *C. wrightii* on the Atlantic coasts, as it happened with other non-native species which are now widely distributed, we conclude that the Ría de Arousa should be considered as an interesting European Atlantic hotspot for the introduction of marine species such as the Solent region in England (Farnham 1980) and the Thau Lagoon in the Mediterranean France (Verlaque 2001).
The present record of Chrysymenia wrightii in the Ría de Arousa occurred 30 years after its detection in the Mediterranean. The subtidal habitat (9-14 meters depth) where the Galician populations were observed could have favoured an unnoticed occurrence for several years. In contrast in the Mediterranean Sea it could have been detected easier because it occurs at shallow localities, from 0.5 to 6 m depth (Ben Maïz et al. 1987). The short life cycle of C. wrightii could further complicate its detection. On the Japanese coasts it is described as an annual species (Lee 1978) which appears for a short period, May to October, whereas in the Mediterranean Sea it was found from January to November (Ben Maïz et al. 1987). However, in Galicia it was only collected in September and October despite many subtidal explorations (2006-2007) carried out along the Ría de Arousa in different months, habitats and water depths. Based on both features that complicate the detection of C. wrightii in Galicia (subtidal habitat and short life cycle), it would be possible that C. wrightii has been introduced earlier in the Ría de Arousa, 1-2 decades ago or more, but it has been unnoticed. Arguably, C. wrightii could also occur unnoticed nowadays along other Atlantic coasts which may only be proven with further subtidal surveys. New studies could also verify if the present record is an isolated case of introduction that will not thrive along the Atlantic Iberian Peninsula or if it has already become an established member of the Galician non-native flora or even if C. wrightii could be considered a future invasive species. Currently C. wrightii occurs in two areas of the Ría de Arousa: (a) locality 1 (1 ha surface) and (b) localities 2-4 (100 ha potential surface). Both are located in the vicinity of aquaculture sites where non-native species like Undaria pinnatifida or Grateloupia turuturu were firstly recorded for the Spanish coast.

Historical reports and our molecular data indicate that the C. wrightii populations in Galicia were recently introduced and originate from somewhere in the northwest Pacific and may have used the Mediterranean coast as a stepping stone. To complete the information about this non-native species, extensive samplings of plants and more variable genetic markers could more clearly locate the source populations and further identify the most likely dispersal vector(s).

Acknowledgements

We thank Dr. Marc Verlaque for verifying the identifying of the first Galician plants (October 2005) and his revision of the manuscript. We thank to Carmen Lema for helping in the last field work. We are also grateful to Dr. Hyung-Seop Kim for kindly providing some samples. Contribution to the projects PGIDIT03PXIB10301PR (Xunta de Galicia) and CGL2006-03576/BOS (Ministerio de Educación y Ciencia, FEDER). This work was supported by a grant from the Basic Research Program of the Korea Science and Engineering Foundation (R01-2006-000-10312-0) and a grant from the Basic Research Program of the Korea Polar Research Institute project PE08060.

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### Annexe 1. Galician samples of *Chrysymenia wrightii*.

<table>
<thead>
<tr>
<th>Map reference</th>
<th>Location</th>
<th>Geographic coordinates</th>
<th>Collection date</th>
<th>Depth</th>
<th>Substrate</th>
<th>Observations</th>
<th>Collector</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Ría de Arousa, Cabo Cruz</td>
<td>42°36'32&quot; N 008°53'19&quot; W</td>
<td>3-X-2005</td>
<td>9-12 m</td>
<td>rocky</td>
<td>Tetrasporophytic plants living together to <em>Bonnemaisonia clavata</em> Hamel and <em>Scinaia interrupta</em> (A. DC.) Wynne</td>
<td>I. Bárbara, P. Díaz and V. Peña</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11-IX-2007</td>
<td>12 m</td>
<td>rocky with mud</td>
<td>Gametophytic plants</td>
<td>I. Bárbara, V. Peña and C. Lema</td>
</tr>
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<td>2</td>
<td>Ría de Arousa, Cambados</td>
<td>42°30'39&quot; N 008°50'47&quot; W</td>
<td>7-X-2007</td>
<td>14 m</td>
<td>rocky with mud</td>
<td>Sterile plants, living together to <em>Dasya sessilis</em> Yamada</td>
<td>I. Bárbara, V. Peña and C. Lema</td>
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<td>3</td>
<td>Ría de Arousa, Islote Galiñeiro</td>
<td>42°31'02&quot; N 008°51'44&quot; W</td>
<td>7-X-2007</td>
<td>8 m</td>
<td>rocky with mud</td>
<td>Gametophytic and tetrasporophytic plants</td>
<td>I. Bárbara, V. Peña and C. Lema</td>
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<td>4</td>
<td>Ría de Arousa, Travoge</td>
<td>42°30'58&quot; N 008°50'35&quot; W</td>
<td>7-X-2007</td>
<td>12 m</td>
<td>rocky with mud</td>
<td>Sterile plants.</td>
<td>I. Bárbara, C. Lema and V. Peña</td>
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### Annexe 2. Collection information for the samples used in the molecular study.

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