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Research Article

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

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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 *rbc*L sequences. No genetic variation was observed in the SSU, and only one substitution was detected in ITS and *rbc*L 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 (Streftaris 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. subpectinata* Holmes, *Gracilaria vermiculophylla* (Ohmi) Papenfuss, *Heterosiphonia japonica* Yendo, *Dasya sessilis* Yamada, *Neosiphonia 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; Bárbara and Cremades 1996; 2004, Veiga et al. 1998; Pérez-Ruzafa et al. 2002; Rueness 2005; Bárbara et al. 2002, 2003, 2005; De Clerck et al. 2005; Barreiro et al. 2006; Cremades Ugarte et al. 2006; Freire et al. 2006; Peña and Bárbara 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

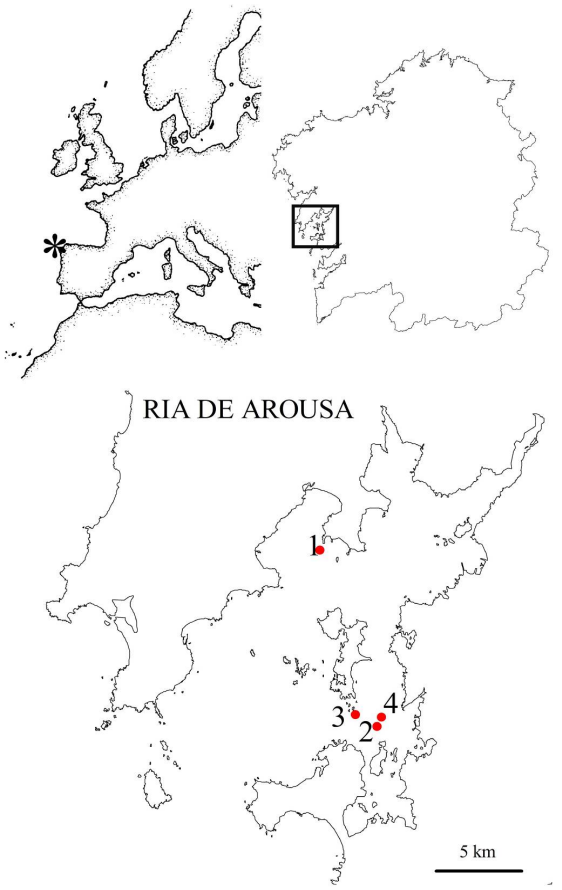

Figure 1. *Chrysymenia wrightii* in Galicia (NW Spain), localization of the non-native plants along the Ría de Arousa (Galicia, Spain).

Sea) (Ben Maïz et al. 1987), it has not been detected on the European Atlantic coast. In the NW of Iberian Peninsula, no *Chrysymenia* species are known. Solely, *C. ventricosa* was reported in the Cantabric Sea (Gorostiaga et al. 2004; Llera González and Álvarez Raboso 2007) whereas the rest of the Iberian records for this warm-temperate species are known from the South of Spain (Conde et al. 1996) and the Mediterranean Sea (Ballesteros 1989). During current surveys in subtidal maërl beds in the Galician rías, several specimens of the northwest Pacific species *C. wrightii* were collected from rocky bottom areas. The aim of this work is to add *C. wrightii* as a new non-native species to the European Atlantic seaweeds catalogue and describe its morphology, habitat, current distribution and extent of its populations, as well as the genetic variation of the Atlantic and Pacific populations.

Materials and methods

Plants were first collected (October 2005) by SCUBA diving in a subtidal rocky bottom habitat covered with mud (9-12 meters depth) of the Ría de Arousa during the explorations of the Galician maërl beds. From a new subtidal exploration along this ría (September and October 2007) we found *C. wrightii* in the original site examined in 2005 and in three new localities, Cambados, Islote Galiñeiro and Tragove (Figure 1, Annex 1) which are located 11 km from the first locality. Samples were preserved in 4% formalin seawater at 4°C in darkness. In order to study reproductive structures, sterile plants collected in Cabo Cruz (September 2007) were cultured in sterile seawater (31-32 PSU) at 16ºC and 4:20 light:dark photoperiod. Plants were dissected by hand with a stainless steel razor blade. Herbarium specimens were deposited at the herbarium of the University of Santiago de Compostela (SANT-Algae).

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 *rbc*L 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 *rbc*L. 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 Biosytems [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).

Figure 2. *Chrysymenia wrightii* in Galicia (NW Spain), habit: **A -** tetrasporophytic plant (Cabo Cruz, October 2005); **B -** gametophytic plant with cystocarps (Islote Galiñeiro, 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, *rbc*L 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, *rbc*L 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, *rbc*L and ITS1-5.8S-ITS2 data were performed usuing 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

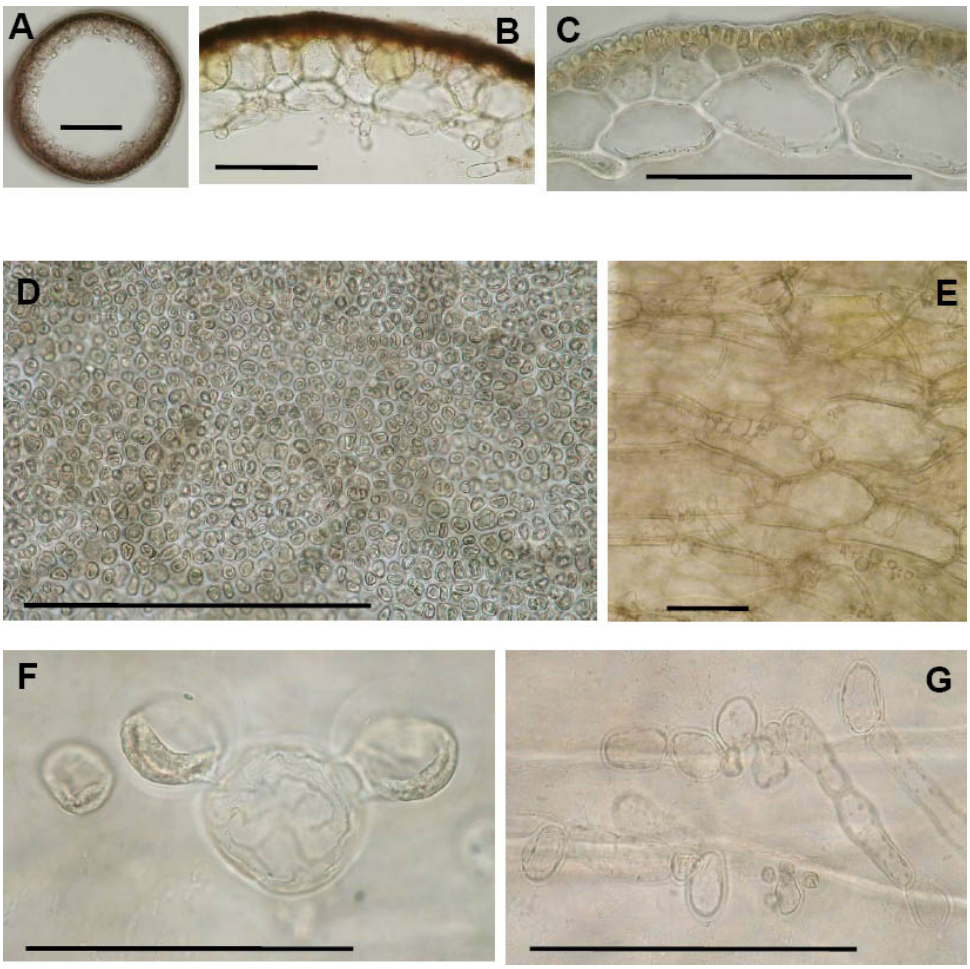

Figure 3. *Chrysymenia wrightii* in Galicia, vegetative structure: **A -** transverse section of an apical branch showing the hollow thallus; **B-C -** cortical, subcortical and hyphaelike filaments in transverse section; **D -** cortical cells in surface view, outer part; **E -** hyphaelike filaments and gland cells in surface view, inner part; **E -** gland cells; **F -** hyphaelike filaments. Scale bar = 200 µm (Photographs by Ignacio Bárbara).

cells (5-8×6-12µm) and the subcortical layer has 3-5 large cells and inner irregular rizoidal cells (Figure 3). The medullary layer of 3-5 cells is loosely and irregularly arranged. Medullary cells are hyaline, elongated (130-400×60-90µm). Gland cells (up to 70 µm diameter) and hyphaelike filaments (2-5 cells) are developed from innermost medullary cells, solitary or in aggregation (Figure 3) as it is commented by Yamada (1932).

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 carpostome (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 Mediterra-nean 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

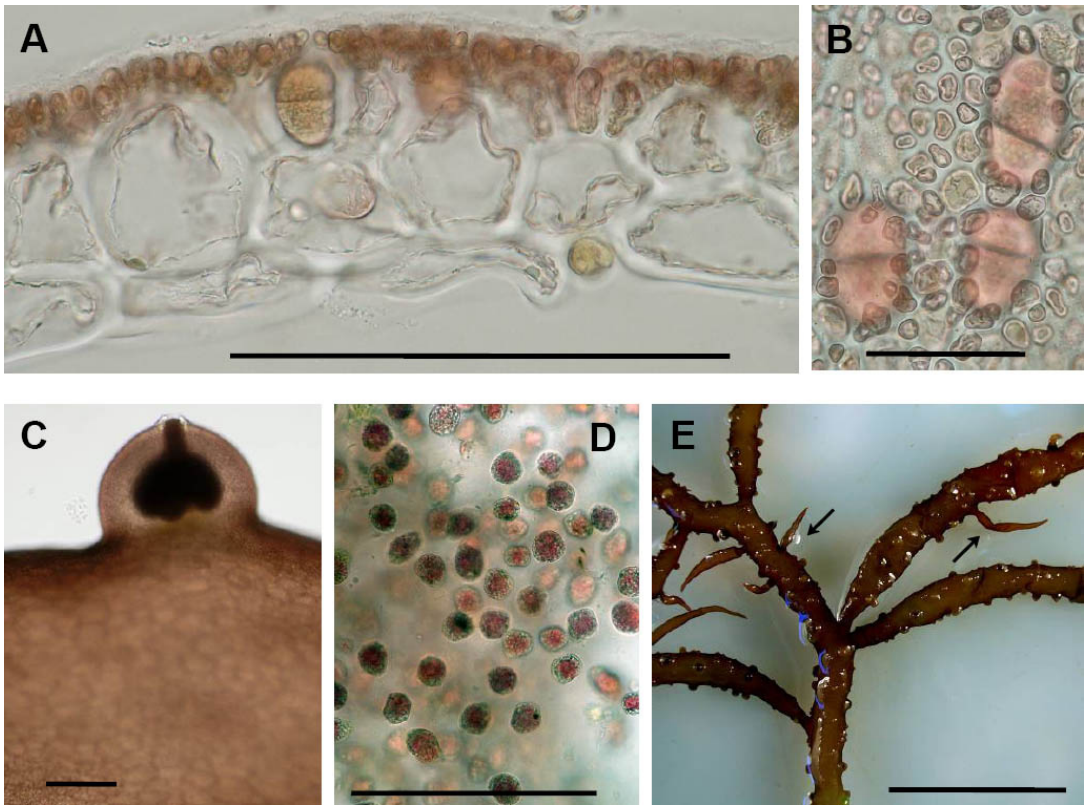

Figure 4. *Chrysymenia wrightii* in Galicia, reproductive structure: **A -** tetrasporangial plant in transverse section; **B -** tetrasporangia under cortical cells, surface view; **C -** cystocarp subspherical with carpostome; **D -** mature sphaerical carpospores; **E -** adventitious short branches 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 *rbc*L 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 *rbc*L (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

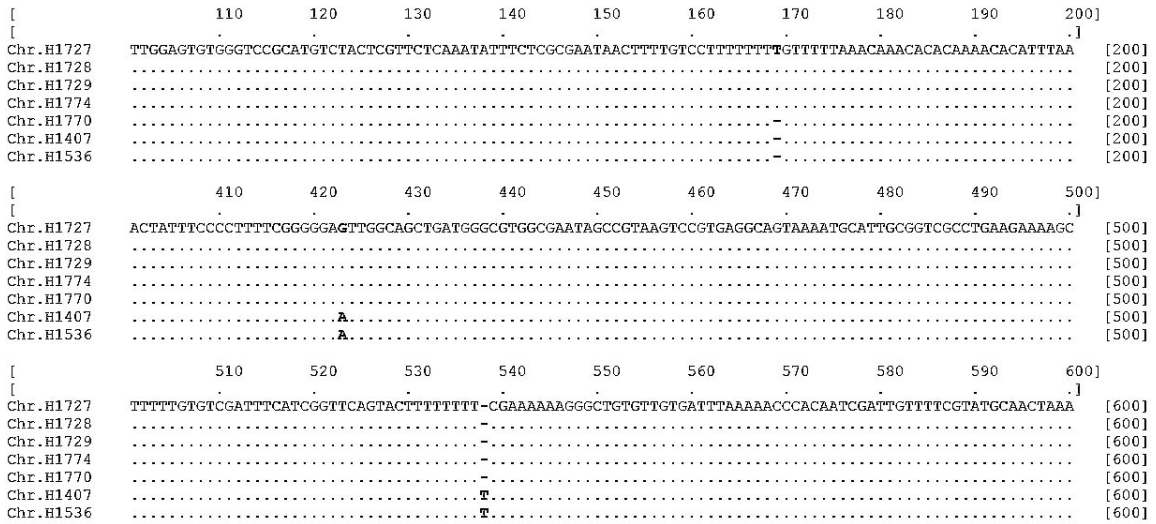

Figure 5. A sub-alignment (positions 101 to 200 and 401 to 600) of ITS1-5.8S-ITS2 rDNA sequences for five Korean (Chr.H1727, Chr.H1728, Chr.H1729, Jangseungpo; Chr.H1774, Yangyang; Chr.H1770, Samchuk) and two Galician samples (Chr.H1407, Boiro; Chr.H1536, Islote Galiñeiro). Bold characters (positions 169, 423 and 538 indicate a deletion, a substitution (G↔A) and an insertion between Korean and Galician samples, respectively). A dash (-) indicates a gap in the multiple alignment; a dot (.) indicates that a taxon has the same character state for that site as in Chr.H1727.

(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 especiallly 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 seaweed 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).

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Annex 1. Galician samples of *Chrysymenia wrightii*.

Map reference	Location	Geographic coordinates		Collection date	Depth	Substrate	Observations	Collector
		Latitude, N	Longitude, W					
1	Ría de Arousa, Cabo Cruz	42 36'32"	008º53'19"	3-X-2005	9-12 m	rocky	Tetrasporophytic plants living together to *Bonnemaisonia clavata* Hamel and *Scinaia interrupta* (A. DC.) Wynne	I. Bárbara, P. Díaz and V. Peña
				11-IX-2007	12 m	rocky with mud	Gametophytic plants	I. Bárbara, V. Peña and C. Lema
2	Ría de Arousa, Cambados	42 30'39"	008º50'47"	7-X-2007	14 m	rocky with mud	Sterile plants, living together to *Dasya sessilis* Yamada	I. Bárbara, V. Peña and C. Lema
3	Ría de Arousa, Islote Galiñeiro	42º31'02"	008º51'44"	7-X-2007	8 m	rocky with mud	Gametophytic and tetrasporophitic plants	I. Bárbara, V. Peña and C. Lema
4	Ría de Arousa, Travoge	42º30'58"	008º50'35"	7-X-2007	12 m	rocky with mud	Sterile plants.	I. Bárbara, C. Lema and V. Peña

Annex 2. Collection information for the samples used in the molecular study.

Samples	Collection Details	GenBank		
		SSU	*rbc*L	ITS
CH1727	-2 m, Jangseungpo, southern coast of Korea, 23-II-2008, J. H. Oak	-	-	EU916720
CH1728	-2 m, Jangseungpo, southern coast of Korea, 23-II-2008, J. H. Oak	EU916714	EU916717	EU916721
CH1729	-2 m, Jangseungpo, southern coast of Korea, 23-II-2008, J. H. Oak	-	-	EU916722
CH1774	Intertidal, Yangyang, eastern coast of Korea, 24-V-2007, S.-M. Kim, C.-J. Kwon and H.-S. Kim	-	-	EU916724
CH1770	Intertidal, Samchuk, eastern coast of Korea, 9-III-2006, S.-M. Kim, C.-J. Kwon and H.-S. Kim	-	-	EU916723
CH1407	SANT-Algae 19058, Boiro, Cabo Cruz, Ría de Arousa, Spain, 3-X-2005, tetrasporophytic plant, I. Bárbara, P. Díaz and V. Peña	EU916712	EU916715	EU916718
CH1536	SANT-Algae 19514, Islote Galiñeiro, Ría de Arousa, Spain, 7-X-2007, tetrasporophytic plant, I. Bárbara, V. Peña and C. Lema	EU916713	EU916716	EU916719