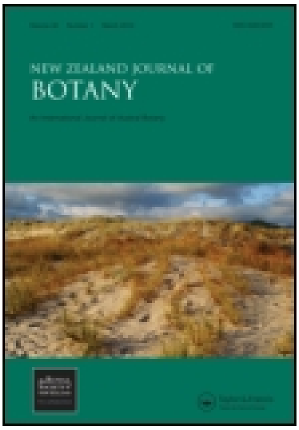

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Schizymenia dubyi (Rhodophyta, Schizymeniaceae), a new introduced species in Argentina

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Schizymenia dubyi (Chauvin ex Duby) J. Agardh is a red alga of the order Nemastomatales that is native to Europe, Asia and Oceania. The first specimen of this seaweed from the Argentinian coast was collected in the Chapadmalal intertidal wave-cut platform (38°12′S, 57°40′W) in September 2008. Taxonomic identification of the species was made on the basis of vegetative and reproductive characters and confirmed by DNA sequence analysis of the *rbc*L gene. The *S. dubyi* samples collected in the field were represented by foliose gametophytes as well as crustose tetrasporophytes. The gametophytes were distributed in patches of c. 50% coverage of the analysed quadrants ($n = 20$). Gametophyte abundance was 3.1 ± 2.5 (mean ± SD) plants per quadrant (0.04 m^2). All these were fertile females. Molecular results placed the specimens from Argentina within the *S. dubyi* clade, closely related to *S. dubyi* from Japan and France.

Keywords: intertidal; invasive species; molecular analysis; Nemastomatales; Northern Patagonia

Introduction

Exotic species that spread beyond their point of introduction and become regionally abundant are termed invasive species (Richardson et al. 2000; Kolar & Lodge 2001). Seaweed invasions may result from intentional aquaculture or accidental introductions, and have been reported worldwide. Well-known examples of invasive seaweeds are *Undaria pinnatifida* (Harvey) Suringar and *Sargassum muticum* (Yendo) Fensholt, among the brown algae, and *Codium fragile* subspecies *tomentosoides* (van Goor) P.C. Silva and *Caulerpa taxifolia* (M. Vahl) C. Agardh, among the green algae. Invasive red algae include *Grateloupia turuturu* Yamada and *Porphyra katadae* A. Miura, among others.

Regional reviews and checklists help to document the arrival of new invasive species in specific locations (Maggs & Stegenga 1999; Boudouresque & Verlaque 2002; Orensanz et al. 2002; Ribera Siguan 2003; Castilla et al. 2005). The Global Invasive Species Database (http://www.issg.org/database) of the International Union for the Conservation of Nature (IUCN) provides extensive data (native/introduced ranges, references) on introduced seaweeds.

Schizymenia dubyi is distributed widely in the Indo-Pacific as well as in the north eastern Atlantic, with the Mediterranean coasts as its southernmost location (Gabriel et al. 2011). To date, it has not been described as an invasive species. The goal of this study is to report the presence of *S. dubyi* on the Mar del Plata

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coast, Argentina, a new location outside its known geographical range. In addition, estimates of the relative abundance of the seaweed are provided.

Materials and methods

The seaweed was identified from 22 samples, collected during spring 2008 in a Chapadmalal rocky intertidal zone (38°12′S, 57°40′W; Fig. 1) in Mar del Plata, Province of Buenos Aires, Argentina. The site is formed by a compact sedimentary rock, Loess Pampeano Soil, sometimes cemented by crystalline calcium carbonate. Its colour is variable and its consistency is soft. The sampling points were located in the low intertidal region, which were exposed during extreme neap tides and covered during high spring tides (Scelzo et al. 1996).

Healthy whole specimens of *S. dubyi* in foliose gametophytic and tetrasporophytic (Haematocelis-phase) phases were collected by hand in the intertidal zone during a very low neap tide. Samples were fixed in 5% formalin in seawater and processed using standard procedures for taxonomic analysis (Ramírez 1982). Samples were deposited at Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN) and Museo Nacional de Historia Natural (MNHN) of Chile, under the catalogue numbers BA 47179–47182 and SGO 158293–158298, respectively.

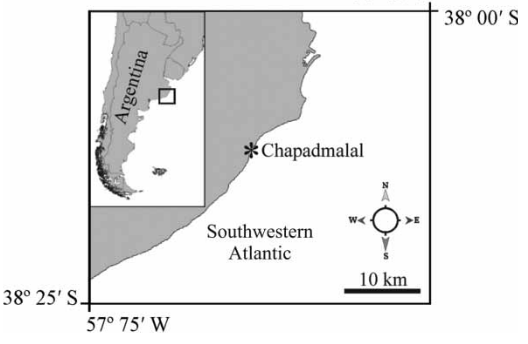

Figure 1 Study area showing the location where *Schizymenia dubyi* was collected.

The taxonomic identity of *S. dubyi* was revised based on the vegetative and reproductive characters. Species were identified using a key for Bangiophyceae and Florideophyceae red algae species (Womersley & Kraft 1994), as well as by consulting marine algae herbarium international collections from the MNHN and MACN, which possess *S. dubyi* species with identical morphology from Australia. No examples of the genus *Schizymenia* were found in the MACN herbarium collection for Argentina.

For phylogenetic analyses, the gametophytes (foliose phase) used in molecular studies were desiccated in silica gel and partial *rbc*L genes were sequenced. Total DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Tokyo, Japan). *Rbc*L gene sequencing procedures were followed as described in Suzuki et al. (2010). *Rbc*L sequences from seven taxa were aligned using ClustalX (Thompson et al. 1997). The length of the alignment used for phylogenetic analyses was 1083 sites. Because *Platoma* was noted as a sister group to *Schizymenia* in previous *rbc*L trees (Gavio et al. 2005; Gabriel et al. 2010), two sequences of the genus *Platoma* were used as outgroup for the analyses.

Bayesian analysis was performed using MrBayes 3.1.2 software (Ronquist & Huelsenbeck 2003). The substitution model of *rbc*L gene sequences was a codon position-specific rate model: first codon position, $\mathrm{GTR}+\mathrm{I}+\mathrm{G}$; second codon position, F81 model; third codon position, $\mathrm{HKY}+\mathrm{I}+\mathrm{G}$ calculated by the hierarchical likelihood ratio tests (hLRT) using MrModeltest 2.2 software (Nylander 2004). Four Markov chain Monte Carlo (MCMC) iterations were performed for 1 000 000 generations, keeping one tree every 100 generations. The first 25% of the generations were discarded as burn-in, and the remaining trees were used to calculate a 50% majority-rule tree and to determine the posterior probabilities (PP) of the individual branches. The average standard deviation of split frequencies of the two MCMC iteration runs was < 0.01 for each analysis, indicating convergence. Maximum likelihood (ML) analysis was performed using RAxML v. 7.2.6 (Stamatakis 2006) implementing a GTRGAMMAI model. Bootstrap percentages (BP) in the ML analyses were calculated based on 1000 replications of heuristic searches. Maximum parsimony (MP) and neighbour joining (NJ) analyses were performed using PAUP* 4.0b10 (Swofford 2002). MP and NJ analyses were subjected to bootstrap resampling (5000 replicates with 100 random additions, 5000 replicates) to estimate robustness (Felsenstein 1985).

Twenty pictures of the sample site were randomly taken during September 2008 to estimate gametophyte abundance (presence or absence and, when present, number of individuals in quadrants of 0.04 m^2). The images were analysed with Image-j software.

Results

The algal community studied is characterised by a predominance of the native red algae Ceramiaceae (*Ceramium* sp.), Corallinaceae (*Jania* sp., *Bossiella* sp.) and Rhodomelaceae (*Polysiphonia* sp.), and some green macroalgae (*Ulva* spp., *Bryopsis* sp.). The *S. dubyi* foliose plants collected from this community represented gametophytes with cystocarps (Fig. 2). Tetrasporophytes were also found close to these. All the gametophyte thalli were fertile females. They are maroon to orange in colour, gelatinous and slippery, up to 40 cm long, 20 cm wide and 1.5 mm thick. The habit of this plant was variable in morphology, with most of the fronds ovalate–lanceolate in shape and with wavy, serrated edges. The thallus was attached to the substrate by a compact and conspicuous holdfast.

Figure 2 *Schizymenia dubyi* individuals on the intertidal rocky shore in Chapadmalal region (Mar del Plata, Argentina).

Schizymenia dubyi is generally characterised by a multiaxial thallus formed by a cortex of six to seven cell layers. This thallus is differentiated into an external cortex and an internal subcortex. The cortex is formed by two to three layers of round to subsquare cells, 20 µm in diameter and abundant glandular cells distributed along its length (Fig. 3). These cells are of variable morphology. Most of the cells are round to oval with an elongate stem, 120–200 µm in length and 40 µm in diameter. The subcortex is composed of three to four layers of rounded cells, 40 µm in diameter, which form bundles supported by filaments originating from a lax stringy bone.

The medulla is formed by thin filaments that cross the thallus periclinaly and anticlinaly (Fig. 3). The gametophytes have differential

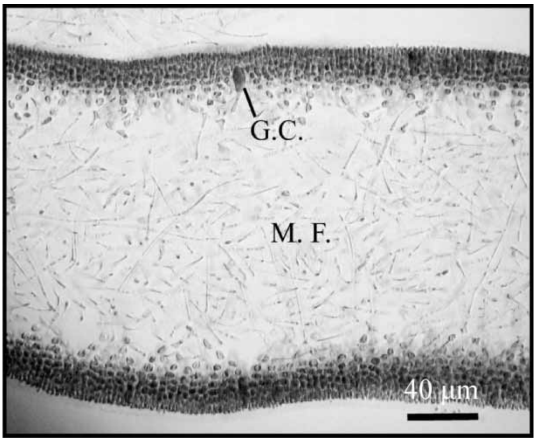

Figure 3 *Schizymenia dubyi* cross-sections of filamentous medulla showing glandular cells on the cortex (G.C.), periclinals and anticlinal medular filaments (M.F.).

reproductive cells in the external cortex. Such cells develop from a structure in the form of an ampulla in separate branches (condition not procarpic) (Fig. 4). After fertilisation, cystocarps develop a specialised opening or carpostome through the cortex, with a delicate pericarp surrounding the mass of carpospores (Fig. 5).

Tetrasporophyte plants develop a crust, a dark red thallus of c. 185 μm in height. The crust presents a single-layer hypothallus of cells, 8 m in diameter and 20 μm in height, a perithallus of six to eight cell layers with sub-square-shaped cells, 10 μm in length and 6 μm in diameter. The epithallus is comprised of several layers of well-pigmented spherical cells, 2.4 μm in diameter (Fig. 6).

Molecular analysis of the *rbc*L gene was conducted on seven taxa in the Schizymeniaceae (Fig. 7). *Schizymenia dubyi* from Argentina, France, Japan and the Pacific coast of the USA formed a clade with full statistical support (1.00 PP and 100% BP). The sequence differences among the *S. dubyi* clade were 0.5% (5 bp).

In the sampled upper intertidal, gametophytes of *S. dubyi* were distributed in patches of c. 50% presence in the analysed quadrants. Abundance was estimated to be 3.1 ± 2.5 (mean ± SD) plants per quadrant (0.04 m^2).

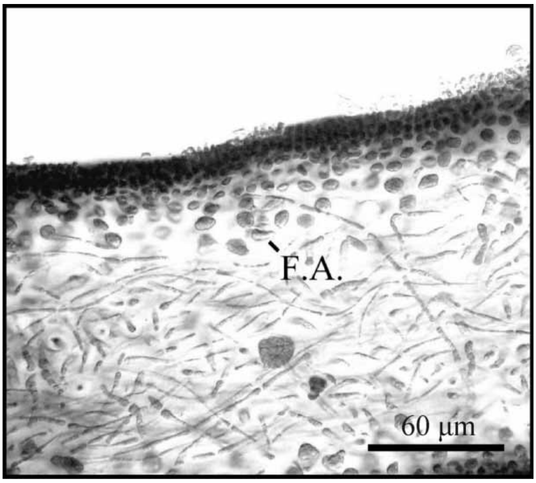

Figure 4 *Schizymenia dubyi* filamentous ampulla (F.A.) where the reproductive cells develop.

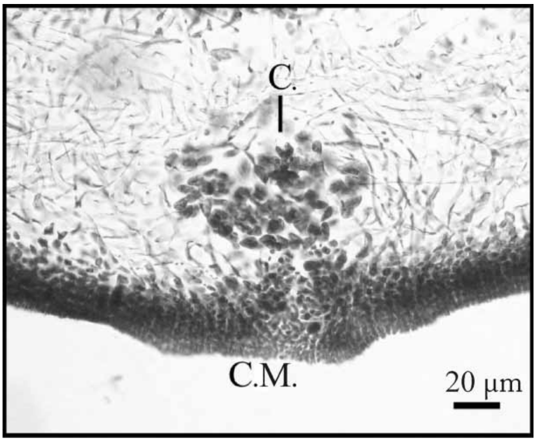

Figure 5 *Schizymenia dubyi* cross-sections of cystocarps showing specialised opening or carpostome (C.) and mass of carpospores (C.M.).

Discussion

This study reports for the first time the presence of the red algae *S. dubyi* on the Argentinian coast. Until now, the distribution of *S. dubyi* was only known to be along the North Atlantic coast from Iceland (Caram & Jónsson 1972), the north of England to Morocco, Madeira and

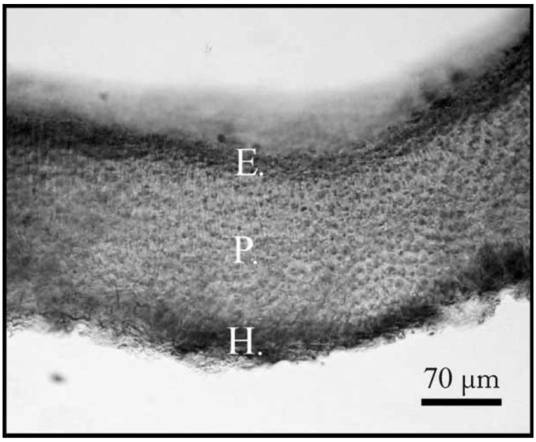

Figure 6 *Schizymenia dubyi* cross-sections of a tetraesporophyte showing hypothallus (H), epithallus (E) and perithallus (P).

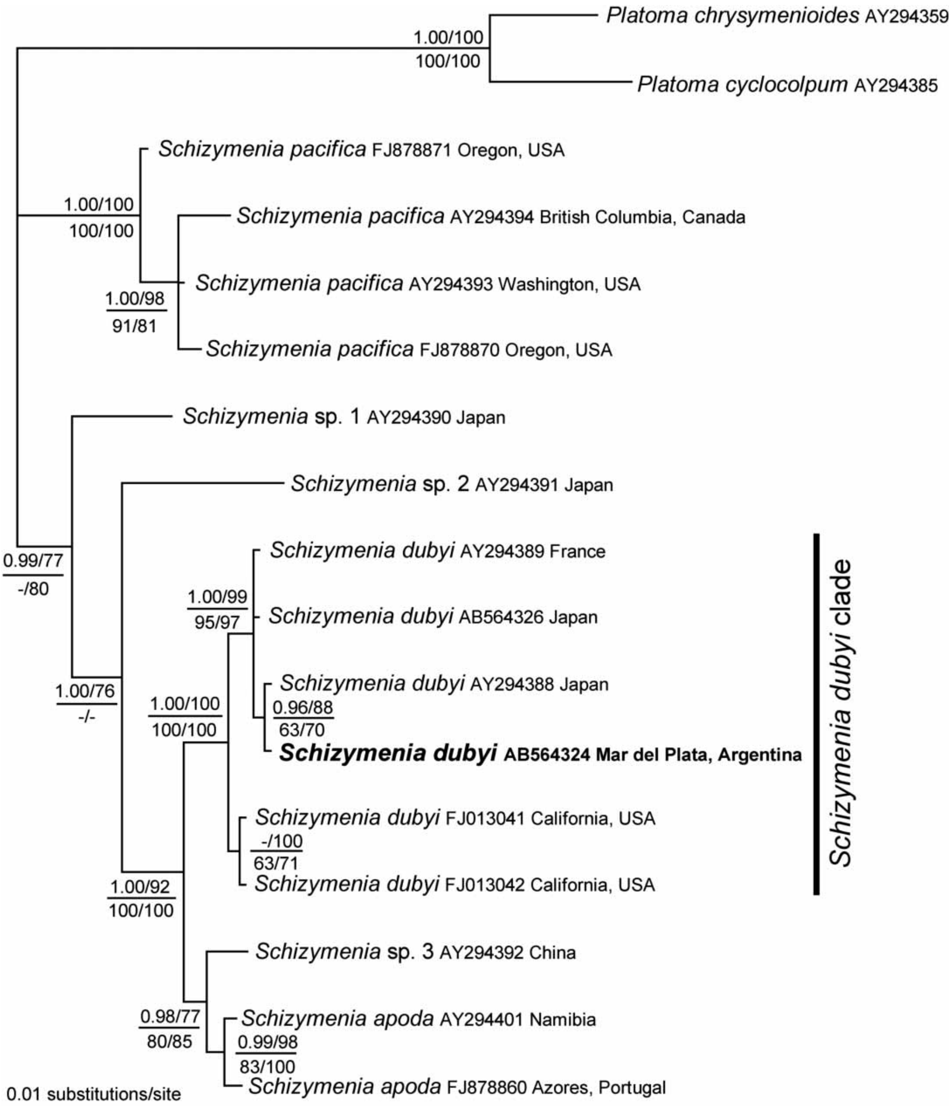

Figure 7 Bayesian tree based on *rbc*L gene sequences. The corresponding posterior probabilities (> 0.94) are shown (top left). Numbers shown at the top right indicate bootstrap values (> 50%) from maximum likelihood. Numbers shown at the bottom indicate bootstrap values (> 50%) from maximum parsimony (left) and neighbour joining (right). In bold, samples of the exotic population detected in Argentina.

the Atlantic Islands (Levring 1974; Neto et al. 2001; John et al. 2004), throughout the Mediterranean (France, Italy & Spain), and in the North Pacific (Japan, Korea, China, North America) up to the Australian coast (Womersley & Kraft 1994). *Rbc*L analyses show that the specimens from Argentina are included in the *S. dubyi* clade and are close to *S. dubyi* from Japan and France. This result indicates the possible origin of the specimens that landed on the Argentinian coast, but the introduction vector of *S. dubyi* is still unknown. However, the most probable colonisation path of *S. dubyi* would be its attachment to the hull of transoceanic vessels or as sporelings transported within ballast water or attached to ballast rocks in the international port of Mar del Plata. Its introduction for aquaculture purposes is improbable because this activity is rare or non-existent in Argentina, and *S. dubyi* is not considered to be an economically important species, unlike *Porphyra*, *Undaria* or *Laminaria* which are extensively cultured in Japan. Accidental introduction associated with cultured shellfish is also improbable because in San Antonio Oeste the closest mussel farms are c. 1000 km to the south.

The accidental or deliberate introduction of marine exotic species may result in severe ecological disturbance to native communities (Bax et al. 2001; Piriz & Casas 2001; Grosholz 2002), and specifically seaweed assemblages, where competition for light and substrate can lead to the local exclusion of native species (DeWreede 1996). For this reason, introduced algae have been mentioned as one of the major problems throughout the world's oceans, altering natural communities and causing significant economic losses (Mathieson et al. 2003). The heteromorphic life cycle of *S. dubyi* enables this species to be a potential successful occupant of different ecological niches, since the crustose phase (tetrasporophyte thallus) can avoid herbivory (Megan & Steneck 2001), and the foliose phase (gametophyte thallus) settles and survives easily, which probably explains its wide distribution.

It has been documented that 31 exotic species have become established on the Argentinian coast (Orensanz et al. 2002). In the case of the algae *Undaria pinnatifida*, an important decrease in the specific richness and diversity of the local algal community has been shown (Casas et al. 2004), while Irigoyen et al. (2011) observed a reduced abundance of some fish species and a higher benthic invertebrate richness and diversity attributable to the provision of new habitat structures. Further local algal introduction cases in Argentina include the red alga *Anotrichium furcellatum* (Boraso de Zaixso & Akselmann 2005) and the brown alga *Sporochnus pedunculatus* (Boraso & Negri 1997; Boraso de Zaixso 1999).

Although there is little information on the abundance and dynamics of *S. dubyi* populations in its native areas, Gorostiaga et al. (2004) reported that this species occurs in isolated clusters and is common when compared with other algae in the north of Spain. In this study, gametophytes of *S. dubyi* showed a patchy distribution with an average of 38.75 plants per m^2 at the lower intertidal zone. We have observed individuals of these species during spring tides in the lower intertidal but we were unable to elucidate their distribution pattern. This report will allow future monitoring of *S. dubyi* in order to understand not only the original establishment of this population, but also its impact on the local indigenous algal community.

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