Transitional Waters Bulletin
TWB, Transit. Waters Bull. 5 (2011), n. 1, 1-7
ISSN 1825-229X, DOI 10.1285/i1825229Xv5n1p1
http://siba-ese.unisalento.it

RESEARCH ARTICLE

A cryptic alien seaweed spreading in Mediterranean coastal lagoons.

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Abstract

1 - Introductions of exotic macroalgae have increased recently on European shores. Each introduction involves at least one vector of transfer. For macroalgae, the potential vectors are aquaculture (intentional or accidental introduction), fouling on hulls, ballast water, aquarium trading, fishing nets.

2 - Coastal lagoons, including Venice and Thau Lagoon, developed into major hotspots of marine macrophyte introductions in the Mediterranean Sea. Moreover, in shallow lagoons and estuaries, eutrophication processes have resulted in the development of macroalgal biomass.

3 - The most characteristic species of these macroalgal communities include members of the ulvophycean genus *Ulva* L. In foliose Ulvales, simple morphology and anatomy, rampant convergence, remarkable degrees of phenotypic plasticity in response to environmental factors tend to confound attempts at identification and make cryptic introduction difficult to detect.

4 - During a survey of macroalgal biodiversity in Venice Lagoon, among the pool of exotic species found, there was an *Ulva* differing from the Atlantic and Mediterranean species in both vegetative and reproductive features. Detailed observations consented us to identify the taxon as *Ulva pertusa* Kjellman, previously reported in the Mediterranean only for Thau Lagoon.

Keywords: Chlorophyta, cryptic species, introduced species, macroalgae, *Ulva*, Venice Lagoon.

Introduction

Introduced species are considered to be one of the greatest threat to native marine biodiversity and resource values of the world's oceans (Schaffelke *et al.*, 2006).

According to a work of the CIESM, 125 species of alien macrophytes are still present in the Mediterranean Sea (Zenetos *et al.*, 2010), making it the sea that receives the greater number of introduced macrophytes on a global level (Schaffelke *et al.*, 2006).

Marine macroalgae are currently transported either associated with other organisms intentionally introduced (translocations for aquaculture, aquarium or live seafood trade) or accidental introduced (mainly as hull-fouling) (Hewitt *et al.*, 2007).

Major vectors include shipping (fouling on hulls, ballast water), trans-oceanic canals and aquaculture activities (Verlaque *et al.*, 2007a).

However, the success of introduced species depends on the combination of several factors, such as the intensity of the phenomenon, abiotic and biotic characteristics of the receiving environment, eco-physiological characteristics of the introduced species. Coastal habitats such as harbors, estuaries and lagoons, appear more sensitive as the intensity and frequency of carriers is very high. The Mediterranean coastal lagoons with the highest percentage of introduced macrophytes appear to be Thau Lagoon (86.5% of total Mediterranean introductions) and the Venice Lagoon (34.6%) (Verlaque and Boudouresque, 2005).

Moreover, simple morphology and anatomy of macroalgae, their rampant convergence, remarkable degrees of phenotypic plasticity in response to environmental factors tend to confound attempts at identification and make cryptic introduction difficult to detect. Members of Ulvaceae are a major component within macroalgal communities in transitional environments. Particularly, the cosmopolitan genus *Ulva* Linnaeus (Chlorophyta, Ulvales) is represented by species distributed in all oceans and estuaries of the world (Guiry and Guiry, 2011).

Species belonging to the genus *Ulva* have foliose or cylindrical thalli. In transverse section, blades are either monostromatic, when formed by a single layer of cells, or distromatic, when formed by a two-cell layer. Blades can be irregularly lobed, cuneate, linear, lanceolate, oblanceolate, or deeply divided into linear lacinae. Some species show regular perforations such as *U. reticulata* Forsskål, or marginal teeth such as *U. rigida* C.Agardh and *U. taeniata* (Setchell) Setchell and N.L.Gardner (Guiry and Guiry, 2011). Thalli are typically annual, but the holdfast is frequently perennial and gives rise to new blades during each growing season (Fritsch, 1948).

Twenty-one *Ulva* species have been recorded in the Mediterranean Sea (Gallardo *et al.*, 1993, Furnari *et al.*, 2010): *Ulva bifrons* Ardré, *Ulva clathrata* (Roth) C.Agardh, *Ulva compressa* Linnaeus, *Ulva curvata* (Kützing) De Toni, *Ulva fasciata* Delile, *Ulva flexuosa* Wulfen, *Ulva intestinalis* Linnaeus, *Ulva kylinii* (Bliding) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope et J.R.Waaland, *Ulva laetevirens* Areschoug, *Ulva linearis* P.J.L Dangeard, *Ulva linza* Linnaeus, *Ulva neapolitana* Bliding, *Ulva pertusa* Kjellman, *Ulva prolifera* O.F.Müller, *Ulva pseudolinza* (R.P.T.Koeman et Hoek) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope et J.R.Waaland, *Ulva radiata* (J.Agardh) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope et J.R.Waaland, *Ulva ralfsii* (Harvey) Le Jolis, *Ulva rigida*, *Ulva rotundata* Bliding, *Ulva simplex* (K.L.Vinogradova) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope et J.R.Waaland, *Ulva torta* (Mertens) Trevisan.

Species-level identification in the genus *Ulva* is difficult, notably because of intraspecific variability and the paucity of morphological and anatomical characters (Hamel, 1931, Bliding, 1968, Steffensen, 1976, Hoeksema and van den Hoek, 1983, Koeman, 1985, Baamonde Lopez *et al.*, 2007). Moreover, many of such characters vary with age and with environmental conditions associated with geographical locations, habitat and seasonality (Steffensen, 1976, Mshigeni and Kajumulo, 1979, Phillips, 1984, Tanner, 1986, Phillips, 1988, Woolcott and King Korean, 1993).

In the Mediterranean two *Ulva* species are reported as introduced, *Ulva fasciata* Delile and *Ulva pertusa* Kjellman. The former, a cosmopolitan species thriving in warm seas, is present along the south coasts of the Western Mediterranean Sea, the Adriatic Sea and in the eastern Mediterranean basin (Gallardo *et al.*, 1993, Verlaque and Boudouresque, 2005). The latter is an Indo-Pacific species, reported in the Mediterranean only in Thau Lagoon (Verlaque *et al.*, 2002, Verlaque and Boudouresque, 2005).

Materials and Methods

During 2010 seasonal samplings were conducted in Venice Lagoon. It is the largest Mediterranean transitional environment: it has a total surface of about 550 km^2, mean depth is about 1.0-1.2 m, with the exception of the main waterways which are up to 50 m deep. It extends for about 50 km along the northwest coast of the Adriatic Sea, to which it is connected through three wide sea entrances: Lido, Malamocco and Chioggia inlets.

Thalli of *Ulva* sp. were collected in Venice along the Fondamente Nove canal, at Celestia (45°26'19,1"N, 12°20'58,4"E), preserved in 4% formalin in seawater and pressed as herbarium sheets. Voucher specimens are housed in the Phycological section of the *Herbarium Messanensis* (MS, http://sweetgum.nybg.org/ih/).

Samples were sectioned by hand with a razorblade. Transverse and longitudinal sections were cut from the rhizoidal, basal, middle and apical regions and observed under the light microscope Diaplan Leica equipped with a Leica DFC 500 camera (Leica Microsystems, Italy).

Results

Observed specimens were laminar and lobed thalli, 15-20 cm long, bright dark green, with numerous perforations of variable size and irregular shape (figures 1-2).

Blades were distromatic (figure 3) and undulate at the margin. In surface view, cells were rounded, irregularly arranged or regularly ordered only in small groups, each cell with 1-3 pyrenoids (figure 4).

Figure 1. Herbarium specimens of *Ulva pertusa* from Venice lagoon. Scale bars: 2 cm.

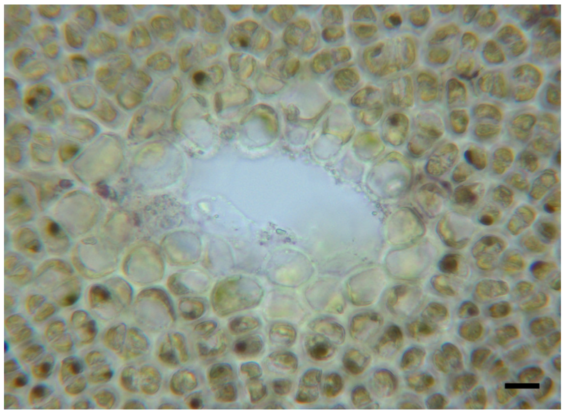

Figure 2. Perforation of the blade. Scale bar: 20 µm.

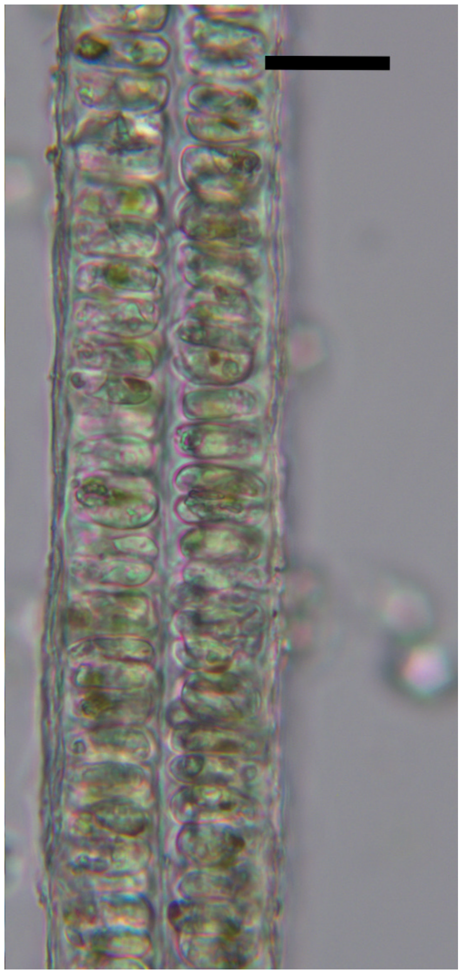

Figure 3. Cross section of the distromatic blade. Scale bar: 40 µm.

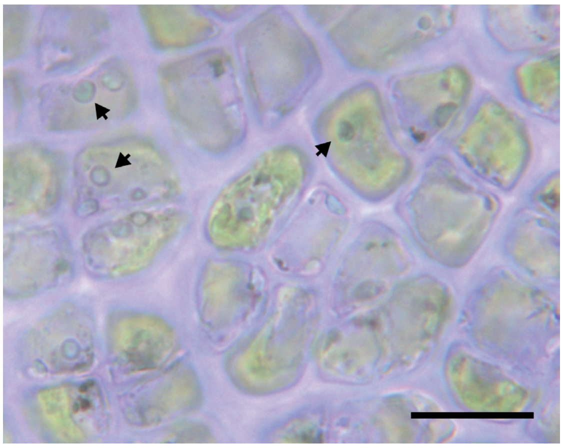

Figure 4. Surface view of the thallus showing cells with 1-3 pyrenoids (arrows). Scale bar: 20 µm.

In longitudinal sections of the basal parts of the blade, cells were markedly larger and oblong, and different rhizoidal filaments grew inside of the distromatic layer (figure 5). Basal regions of thalli were filled with a polysaccharide matrix, and holdfast surface was concentrically wrinkled (figure 6). Basing on the above characters collected samples could be ascribed to *Ulva pertusa* Kjellman.

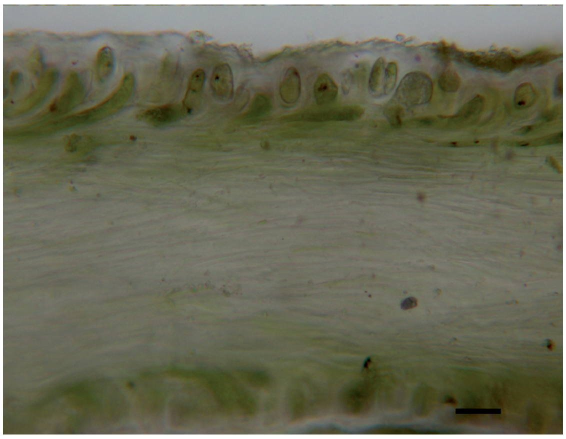

Figure 5. Longitudinal section of the basal part of the thallus filled with a polysaccharide matrix showing rhizoidal cells. Scale bar: 40 µm.

Discussion

Ulva pertusa Kjellman is native of the Indo-Pacific region and is widely distributed along the coasts of China, Japan, Korea and the Philippines (Okamura, 1921, Dong and Tseng, 1984, Lee and Kang, 1986, Silva *et al.*, 1996, Yoshida, 1998). In Europe, it has been recently reported in the Mediterranean France (Verlaque *et al.*, 2002), the Netherlands (Stegenga *et al.*, 2007, Gittenberger *et al.*, 2010) and Atlantic Spain (Baamonde Lòpez *et al.*, 2007). This is a marine species tolerant of a wide range of temperature (da 0° a 30°C) (Tokida, 1954, Wei and Chin, 1983) and salinity (Iwamoto, 1960, Floreto *et al.*, 1994).

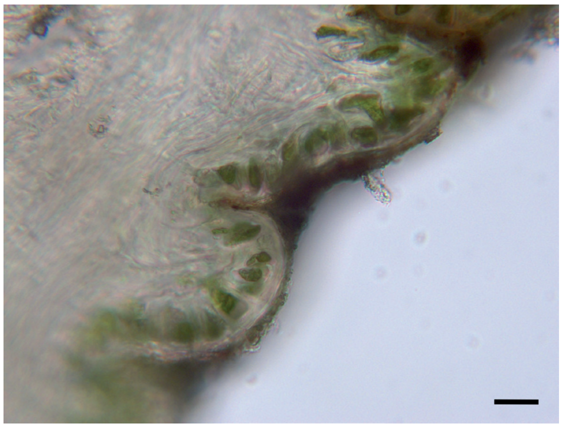

Figure 6. Longitudinal section showing the holdfast surface concentrically wrinkled. Scale bar: 40 µm.

Species-level identification in the genus *Ulva* is typically difficult, notably in the view of intraspecific variability and paucity of morphological and anatomical characters used for species discrimination (Baamonde Lòpez *et al.*, 2007).

Six foliose species of *Ulva* are present in Venice Lagoon: *U. pertusa*, *U. curvata*, *U. fasciata*, *U. laetevirens*, *U. rigida*, *U. rotundata*. A comparison of morphological diagnostic characters of the species are presented in Table 1.

Macroscopically, the most constant and distinctive characters in *Ulva pertusa* are the concentric wrinkles present at the base of the blades and the peculiar abundant perforations

Table 1 - Comparison of morphological diagnostic characters of foliose *Ulva* species present in Venice lagoon, according to Bliding, 1968, Koeman and Van Den Hoek, 1980, Dion *et al.*, 1998, Malta *et al.*, 1999, Verlaque *et al.*, 2002, Baamonde Lòpez *et al.*, 2007, Kraft *et al.*, 2010, Sfriso, 2010, and present study.

Species	Diagnostic morphological characters				
U. pertusa	Margin without microscopic marginal teeth	Rounded-rectangular cells (surface view)	1-3 pyrenoids	Rhizoidal cells shape cylindrical	Basal region without central cavity; thallus lobed, more or less perforated, holdfast surface concentrically wrinkled
U. curvata	Margin without microscopic marginal teeth	Rectangular or polygonal cells (surface view)	1 pyrenoid	Rhizoidal cells shape cylindrical	Basal region with a flat central cavity, in surface view with longitudinal ribs; thallus mostly curved, membranaceous, medium-light green
U. fasciata	Margin with tooth-like protuberance	Polygonal or quadrangular cells (surface view)	1-4 pyrenoids	Cells of the inferior zones oblong (similar to *U. pertusa*)	Thallus divided to basal region into 3 to many narrow, relatively flat laciniane
U. laetevirens	Margin with tooth-like protuberance, isolated and rarely branched	From rounded to polygonal cells (surface view)	1-3 pyrenoids	Rhizoidal cells shape cylindrical	Basal region without central cavity
U. rigida	Margin with numerous tooth-like protuberance branched	Rectangular or slightly polygonally rounded cells (surface view)	2-4 pyrenoids	Rhizoidal cells shape rounded	Basal region without central cavity, in surface view with longitudinal ribs; thallus lobed to lanceolate, medium-light green
U. rotundata	Margin without microscopic marginal teeth	Polygonal cells (surface view)	1-3 pyrenoids	Rhizoidal cells shape cylindrical	Thallus lobed, without central cavity

(figures 2, 6). The latter are very different from those resulting from grazing activity on other species, which are usually more homogeneous in size and often scattered.

The introduction of alien seaweeds is increasing worldwide due to maritime traffic, associated with algal fouling of hulls and ballast water, and to importation of shellfish for mariculture (Verlaque *et al.*, 2007b, Manghisi *et al.*, 2010). The hypothesized vector of the introduction of *U. pertusa* in Thau Lagoon and in Galicia was the massively imported oysters *Crassostrea gigas* (Tumberg, 1793) from the Pacific (Verlaque, 2001, Verlaque *et al.*, 2002, Baamonde Lòpez *et al.*, 2007). It is likely that the occurrence of this species in Venice lagoon is also due to the importation of oysters from France.

Ulva pertusa is an opportunist pioneer species, competitive with high rates of nutrient assimilation and propagule production (Baamonde Lòpez et al., 2007).

Conclusions

Venice Lagoon is indeed the richest

in macroalgal diversity among Italian transitional environments. Because of the intense human activities it is also the spot of highest introduction of non indigenous species.

The importation of non-indigenous oysters, such as the Japanese Crassostrea gigas, generates a massive and recurring transfer of livestock between aquaculture sites.

Among alien seaweeds, the presence of *U. pertusa* in Venice Lagoon might be due to anthropogenic factors. Moreover, due to its cryptic nature in comparison with other native entities, it has not been possible to date back the exact timing of introduction.

Acknowledgement

The authors would like to thank Laguna Project SNC who kindly helped in collecting samples. This study was supported by a grant PRA2006/2007 from the University of Messina, Italy to G.G.

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