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On the occurrence of *Uronema marinum* Womersley (Chaetophorales, Chlorophyta) in the north-western lagoons of the Adriatic Sea, Mediterranean Sea (Italy)

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Abstract

We study the occurrence of the alien macroalga *Uronema marinum* in the lagoon of Venice, the lagoons and ponds of the Po Delta and in Pialassa della Baiona in Emilia-Romagna. *Uronema marinum* was identified for the first time in the summer of 2012, although it has been present since 2008, at least. This species, originally described from South Australia and Western Australia and probably imported with the Manila clam *Tapes philippinarum,* is prevalently associated with thalli of another introduced species, *Agardhiella subulata,* and the invasive *Gracilaria vermiculophylla*, which also have a Pacific origin and have recently colonized the same lagoon areas. *Uronema marinum* is currently widespread in the whole lagoon surfaces, but is particularly abundant in stagnant waters, rich in nutrients, where Gracilariaceae and Solieriaceae prevail on Ulvaceae.

Keywords: Alien macroalgae, *Uronema marinum*, eutrophic environments, Italian lagoons, Adriatic Sea, Mediterranean Sea.

Introduction

The latest lists of alien species in the Mediterranean Sea (Zenetos *et al.,* 2010, 2012) place this region amongst the most affected by biological invasions. In December 2010, the inventory of marine non indigenous species (NIS) included 955 taxa (except phytoplankton). That number increased to 986 in December 2012, despite the exclusion of 62 taxa from the previous list. One hundred and twenty-eight taxa were macrophytes ranking this group in fourth place after Mollusca (215), Crustacea (159) and Polychaeta (132). However, in the Adriatic Sea, the 52 alien macrophytes are the largest NIS group, about as many as the introduced Mollusca and Polichaeta together (27 taxa each). Colonization affects the North Adriatic coastal lagoons mainly, Venice Lagoon in particular where new species are continuously reported (Sfriso *et al*., 2010, 2012; Occhipinti Ambrogi *et al*., 2011; Wolf *et al*., 2011). Most of them are introduced via aquaculture or shellfish trade. Alien macroalgae are in fact used to keep imported fish and/or molluscs fresh and at the end of the working day they are discharged into the lagoon canals. Records in Venice Lagoon show that most of the NIS species, such as *Sargassum muticum* (Yendo) Fensholt, *Undaria pinnatifida* (Harvey) Suringar and *Desmarestia viridis* (O.F.Müller) Lamouroux, are from canals adjacent to wholesale fish markets. Other species, including a number of small epiphytes are associated with the introduction and intensive farming of the Manila clam *Tapes philippinarum* Adams & Reeve.

The aim of this paper is to study a species of Pacific origin for the first time in Europe, and specifically in the north-western Adriatic lagoons. Its occurrence has been correlated with certain water column and surface sediment parameters and the presence of other common NIS that have also successfully colonized these environments. The new alien is rather similar in morphology to *Okellya curvata* (Printz) Leliaert & Rueness which occurs on the European Atlantic coast (Leliaert *et al.*, 2009).

Materials and Methods

a) Macrophyte sampling

The new species was recorded in the course of surveys carried out in the Po Delta lagoons and ponds (17 sites sampled in May-July and October 2008, 2009, 2010), in Pialassa della Baiona and Sacca di Goro in the Emilia-Romagna Region (6 sites sampled in June and October 2009) and in Venice Lagoon (29 sites sampled in June and October 2010, Fig. 1). These surveys were carried out to evaluate the ecological status of the transitional environments by studying the submerged flora (Sfriso *et al.,* 2007, 2009), according to the Water Framework Directive (2000/60/EC).
Samples were preserved in 4% formaldehyde up to laboratory identification using a stereo zoom microscope E-1654ZT45 (Euromex microscopes, Holland) and a light microscope-353Ph (Optika microscopes, Italy).

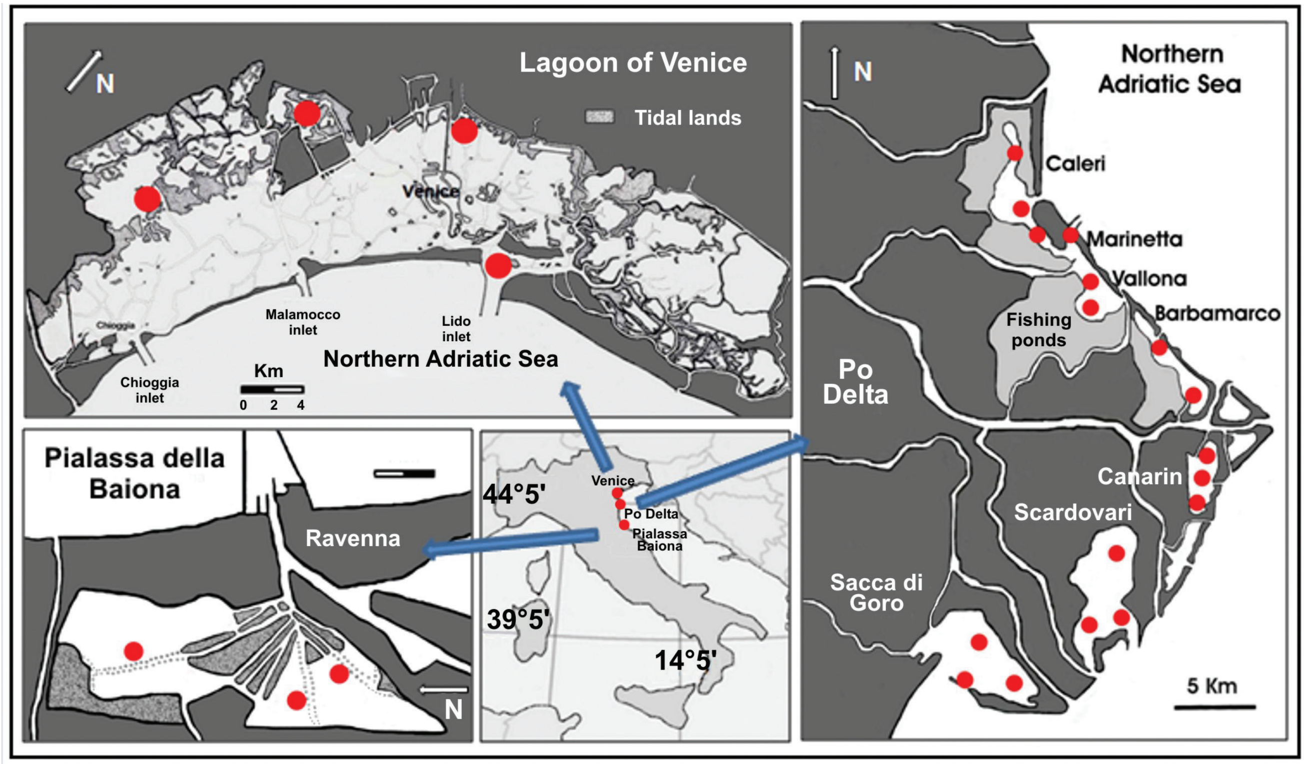

Fig. 1: Maps of the lagoon areas in the Northern Adriatic Sea where *Uronema marinum* was recorded: Venice Lagoon, 2010; Po Delta lagoons, including Sacca di Goro, 2008-2009; Pialassa della Baiona, 2009.

b) Physico-chemical parameters and nutrient concentrations

At each sampling station, temperature and dissolved oxygen (% DO) were measured using a portable instrument (Oxi 330i, Wissenschaftlich-technische Werkstätten GmbH, Weilheim, Germany), whereas water transparency was measured by means of a Secchi disk (25 cm in diameter). Because of the shallow waters (0.5-2.5 m) and the high tidal amplitude (0.5-1 m), water transparency measurements were reported as a percentage of water column visibility; a value of 100% means that the bottom was visible; a value of 50% means that the disk disappeared at half way from the bottom. Salinity was determined in the laboratory by chlorine titration according to Oxner (1962).

Five or six surface samples of the water column (0-100 cm depth), collected by a cylindrical home-made bottle (i.d. 4 cm, height 130 cm), were mixed together and subsamples of 250-500 ml were filtered through GF/F Whatman glass fibre filters (porosity: 0.7 µm) and kept frozen until analysis. DIN (dissolved inorganic nitrogen: sum of ammonium, nitrites and nitrates) concentrations, reactive phosphorus (RP) and silicates (Si) were determined by spectrophotometric analyses according to Strickland & Parsons (1972). The filters were frozen until Chlorophyll-*a* (Chl-*a*) and phaeophytin-*a* (Phaeo-*a*) determination according to Lorenzen's (1967) procedure.

Samples of surface sediment were collected using a Plexiglas corer (i.d. 10 cm). The 5 cm top layers of three cores were carefully homogenized and subsamples of ca. 100 ml were retained frozen and freeze-dried for the determination of fines (fraction <63 µm). Other subsamples were kept fresh for sediment density determination according to the procedures reported in Sfriso *et al.* (2005).

c) Statistical Analysis

The correlations between the presence of *U. marinum*, a number of environmental parameters of the water column and surface sediments, and the presence of those macrophytes that were common to all the lagoons and ponds considered in this study were obtained by the Pearson coefficients at $p < 0.05$ and $p < 0.001$, using Statistica release 10 (Statsoft, Inc. Tulsa, USA).

Results and Discussion

Thallus morphology and reproductive structures

Filaments of *Uronema marinum*, 100-300 (500 µm) long, were recorded on larger macroalgae such as *Gracilaria vermiculophylla* (Ohmi) Papenfuss, *Agardhiella subulata* (C. Agardh) Kraft & Wynne, *Solieria filiformis* (Kützing) P.W. Gabrielson and species of Cladophorales at the sites shown in Figure 1. Filaments were uniseriate, unbranched and composed of 3-20 (30) close-set subcylindrical cells, more elongated (1.5-4 times) and wider (8-15 µm) in the distal portion (Fig. 2). They were attached to the host by a basal discoid (conical in side view) holdfast of 4-6 µm in diameter formed by an elongation of the basal cell

(Figs. 2F, G). Cell walls were thin and not lamellate. Each cell contained a single parietal, lobed chloroplast with 1, rarely 2, pyrenoids (Figs. 2C-E). Filaments composed of less than 7-10 cells were sterile. In longer thalli, globose-pyriform zooids, 4-6 µm in diameter, similar to those reported by Womersley (1984) and Kraft (2007), were produced after transformation of distal intercalary and apical cells into zooidangia (Fig. 3). The zooids were released in the water column through subapical exit pores (Fig. 2H) leaving empty cells, originally thought to be cells undergoing senescence and breakdown.

Ecology

The first records for the Northern Adriatic date back to 2008, when specimens were observed in the lagoons of the Po Delta and in the confined areas of Venice Lagoon. Thalli were attached to other alien algae, such as *Gracilaria vermiculophylla, Agardhiella subulata* and *Soleria filiformis*, which most likely were introduced by aquaculture (Zenetos *et al.,* 2010; Sfriso *et al.,* 2012). Later, *U. marinum* was also recorded in Venice Lagoon and in Pialassa della Baiona in Emilia-Romagna as an epiphyte on many other species, mostly Cladophorales. It was found in abundance in stagnant and eutrophic areas rich in orthophosphates, conditions that favoured the growth of dense populations, covering larger macroalgae completely. Scattered filaments were also recorded near the lagoon inlets of Venice Lagoon, but they were casual and mostly attached to floating thalli of Gracilariaceae and Solieriaceae of the innermost areas.

Pearson coefficient analysis (48 sites sampled both in late spring and autumn) of the most common environmental parameters and the macrophytes present in all these transitional areas, showed *U. marinum* to have a very high correlation ($p < 0.001$) with *Gracilaria vermiculophylla* and significant correlations ($p < 0.05$) with *Agardhiella subulata*, *Gracilariopsis longissima* (S.G. Gmelin) M. Steentoft, L.M. Irvine & W.F. Farnham and the concentration of reactive phosphorus (Table 1).

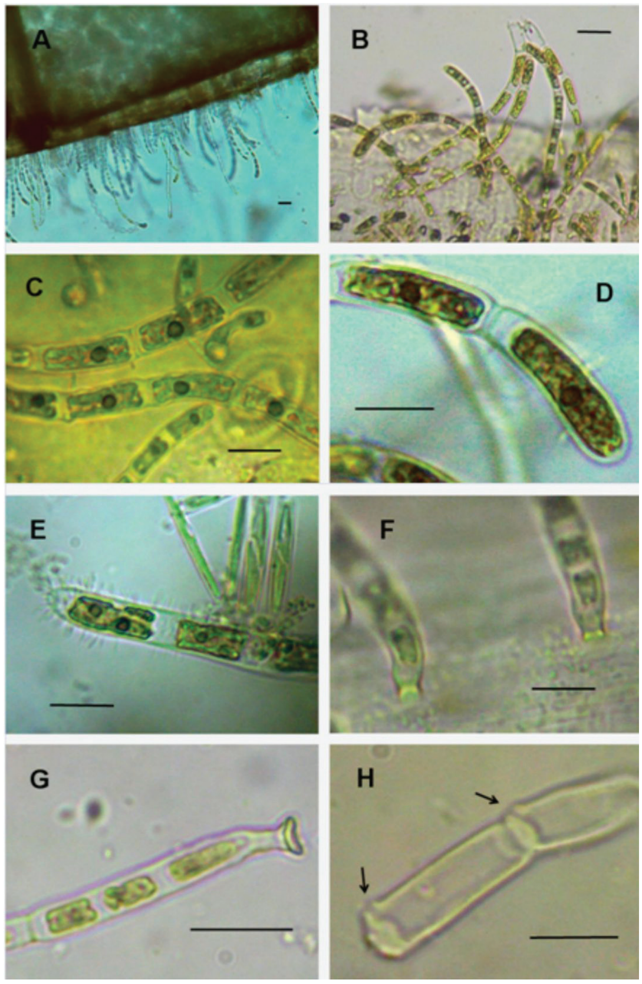

Fig. 2: *Uronema marinum.* A) Epiphytic filaments on *Cladophora* cells; B) Curved filaments with some empty apical cells; C) Cells each containing a parietal and lobed chloroplast with one pyrenoid; D) Apical and subapical cells containing a single pyrenoid; E) Apical cell with a parietal, lobed chloroplast and two pyrenoids; F) Filament attached by a basal holdfast formed by an elongation of the basal cell; G) Highlight on the basal holdfast; H) Empty zooidangia. Arrows show the subapical exit pores. Material in A-E, H stained with iodine solution. A, B: scale bar 30 µm. C-H: scale bar 15 µm.

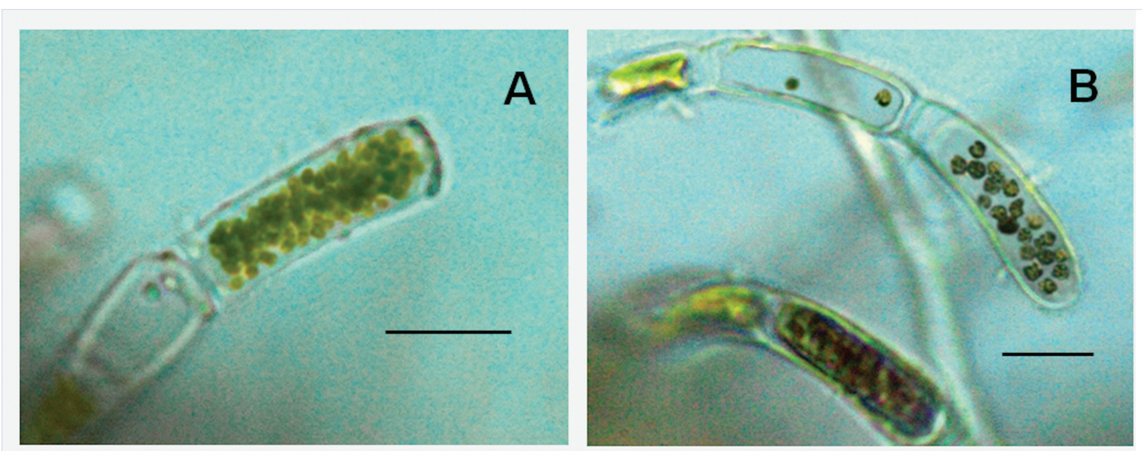

Fig. 3: *Uronema marinum.* A, B) Apical and subapical zooidangia with zooids. Scale bar: 20µm. Material stained with iodine solution.

Table 1. Pearson coefficients between U. marinum, some environmental parameters (A) and the most common macrophytes (B) in 48 sampling sites sampled in spring and autumn (p <0.05 per r ≥|0.28|; p <0.001 per r ≥|0.43|).

A)	**Sal**	**DO**	**Secchi**	**Si**	**RP**	**NH_4^+**	**NO_2^-**	**NO_3^-**	**DIN**	**Chl-*a***	**Phaeo-*a***	**Chl-*a* tot**	**Fines**	**Density**
Uronema marinum	-0.05	0.09	0.11	-0.20	**0.31**	-0.08	0.17	0.20	0.19	0.10	**0.30**	0.15	-0.23	0.09
Gracilaria vermiculophylla	-0.25	-0.12	-0.14	0.09	0.23	0.20	-0.04	0.36	**0.34**	-0.06	0.11	-0.02	-0.17	-0.11
Agardhiella subulata	0.00	0.07	-0.05	0.06	**0.32**	-0.14	0.21	0.32	**0.28**	0.13	0.29	0.17	0.13	0.14
Solieria filiformis	0.17	0.10	-0.03	-0.17	**0.34**	-0.23	0.22	-0.07	-0.06	0.08	0.15	0.10	0.06	0.17
Chaetomorpha aerea	-0.06	-0.08	0.08	-0.20	-0.25	0.09	-0.13	**-0.28**	-0.24	-0.19	-0.15	-0.19	-0.13	-0.13
Chaetomorpha ligustica	0.12	-0.21	0.17	-0.23	-0.06	**0.38**	0.23	-0.13	0.10	-0.22	-0.22	-0.23	-0.23	0.17
Enteromorpha multiramosa	0.00	0.03	0.03	-0.22	-0.15	-0.19	0.03	-0.03	-0.08	0.16	**0.32**	0.20	0.09	0.00
Gracilaria gracilis	-0.01	0.11	0.06	0.02	-0.05	0.04	0.05	-0.23	-0.15	0.06	0.01	0.05	-0.22	0.03
Gracilariopsis longissima	-0.05	0.01	0.03	-0.22	0.04	-0.12	0.00	-0.09	-0.11	0.07	0.19	0.11	0.15	-0.06
Polysiphonia denudata	0.19	0.05	0.08	0.06	0.04	-0.11	0.00	0.25	0.16	-0.18	-0.15	-0.18	0.08	0.11

B)	***U. marinum***	***G. vermiculophylla***	***A. subulata***	***S. filiformis***	***C. aerea***	***C. ligustica***	***E. multiramosa***	***G. gracilis***	***Gr longissima***	***P. denudata***
Uronema marinum	1.00									
Gracilaria vermiculophylla	**0.50**	1.00								
Agardhiella subulata	**0.40**	**0.43**	1.00							
Solieria filiformis	0.19	0.00	**0.35**	1.00						
Chaetomorpha aerea	-0.02	0.02	-0.23	0.12	1.00					
Chaetomorpha ligustica	0.09	0.12	-0.04	0.18	**0.52**	1.00				
Enteromorpha multiramosa	0.27	0.20	**0.30**	**0.37**	0.25	**0.34**	1.00			
Gracilaria gracilis	-0.20	-0.16	0.04	-0.08	0.21	0.12	-0.15	1.00		
Gracilariopsis longissima	**0.40**	0.15	0.06	**0.39**	0.24	0.18	**0.35**	-0.17	1.00	
Polysiphonia denudata	-0.08	-0.06	0.13	-0.06	0.03	-0.10	-0.08	-0.15	-0.01	1.00

Captions: Sal = Salinity; DO = dissolved oxygen; Si = silicates; RP = reactive phosphorus; DIN = dissolved inorganic nitrogen; Chl-a = Chlorophyll-a; Phaeo-a = Phaeopigments; Tot = total; Fines = sediment fraction <63µm; Density = sediment density.

Both *G. vermiculophylla* and *A. subulata* originate from the Pacific Ocean (Zenetos et *al*., 2010). *Uronema marinum,* probably has the same origin and may have been introduced simultaneously with one (or both) of these species. *Gracilaria vermiculophylla* and *A. subulata* were also significantly correlated with each other (p < 0.001).

Although *Uronema* filaments most likely occurred for some years, until now they passed unnoticed because of their small size and similarity with young stages of other filamentous green algae such as *Ulothrix implexa* (Kützing) Kützing. Nevertheless, the thallus of *U. marinum* clearly differs; it has determinate growth, curved habit, a basal discoid (conical in side view) holdfast, elongated cells with larger diameter towards the apex, and reproduction also differs.

We also examined older herbarium material of *Gracilaria* Greville*, Chaetomorpha* Kützing and *Gracilar-*

iopsis Dawson specimens, collected between the 80's and today (and deposited at our Department), and found that *Uronema* filaments were only present in samples collected after 2008. However, for the lagoons of the Po Delta and Emilia-Romagna we had no older samples because macroalgae were not studied in these basins before. As regards Venice Lagoon, samples were mainly from other areas but no sample included this species.

In the Pacific, *Uronema marinum* is reported throughout the year from shallow subtidal habitats of southern and western Australia, the South Great Barrier Reef, Micronesia and Hawaii (Womersley, 1984; Abbott & Huisman, 2004; Kraft, 2007). On the European Atlantic coast, between France and western Norway, a second species: *Uronema curvatum* Printz (Rueness, 1992; Maggs & O'Kelly, 2007) has been recognized recently as *Okellya curvata* (Printz) Leliaert & Rueness (Leliaert *et al.,* 2009). It has a similar habit, but its vegetative cells lack pyrenoids.

Conclusions

The finding of *Uronema marinum* confirms the role of the Adriatic lagoons and in particular of Venice Lagoon as a hotspot of introduced species. At present, the number of macroalgal NIS reported in Venice Lagoon is 35, ca. 67% of the total number of alien macrophytes (52 taxa, Zenetos *et al*., 2012) recorded in the entire Adriatic Sea.

The new alien, like the invasive *Gracilaria vermiculophylla* and the widespread *Agardhiella subulata* and *Solieria filiformis* to which *U. marinum* is mainly associated, shows a preference for eutrophicated and localized habitats.

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