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Research Article

First record of the colonial ascidian *Didemnum vexillum* Kott, 2002 in the Mediterranean: Lagoon of Venice (Italy)

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Abstract

Numerous colonies of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002 have been found in the Lagoon of Venice (Italy) in 2012, overgrowing fouling organisms on maritime structures such as docks, pilings, and pontoons. This is the first record for the Mediterranean Sea. A survey conducted in July 2012 revealed that *D. vexillum* is present in the euhaline and tidally well flushed zones of the lagoon, whereas it was absent at the examined estuarine tracts and at the zones surrounding the saltmarshes. Suitable climatic, physiographic and saline features together with a high volume of international maritime traffic make the Lagoon of Venice a perfect hub for the successful introduction of temperate non-native species.

Key words: *Didemnum vexillum,* Mediterranean, Lagoon of Venice, ascidian, fouling, marinas, invasive species

Introduction

Didemnum vexillum Kott, 2002 (Ascidiacea: Aplousobranchia) is a colonial tunicate able to rapidly colonize hard substrates and overgrow other benthic organisms such as mussels, barnacles, bryozoans and algae. It now has a worldwide distribution and is found mostly in harbors and marinas where it covers a variety of maritime structures such as wharf and mooring pilings, docks, pontoons, ropes and other submerged hard surfaces, but it is also present in outer coastal habitats from the very low intertidal to several tens of meters in depth, down to 81 m (Bullard et al. 2007; Lambert 2009). Colonies are yellowish, ranging in color from cream to orange. The form of the colonies varies with colony age and water movement; in exposed environments they form a thin blanket but in sheltered locations they can form leaf-like ridges or flexible lobes up to a meter in length.

During the last two decades *D. vexillum* has been widely reported from the temperate and cold coasts of North America and Europe as well as from Japan where it is probably native (Bullard et al. 2007; Griffith et al. 2009; Lambert 2009; Stefaniak et al. 2009, 2012; Cohen et al. 2011). In the Southern Hemisphere it has been recorded only from New Zealand. A detailed geo-referenced world-wide distribution of *D. vexillum*, is reported by the NEMESIS database (Fofonoff et al. 2003). Probably the first European record of *D. vexillum* was in North Sea, on the Netherlands coast, in 1991 (Ates 1998; Gittenberger 2007), but it was incorrectly identified as *D. lahillei* (Lambert 2009). The uncertainty in the actual time of invasion and the origin of many alien species, especially for didemnid ascidians, is due to both the lack of wide range surveys and to the difficulty of taxonomic identification at species level.

In this paper, we report the first verified occurrence of *D. vexillum* in the Mediterranean Sea as an established population in the Lagoon of Venice (LoVe), Italy.

Methods

Description of the site

The Lagoon of Venice has a surface area of about 550 km^2, roughly 80% of which is covered by water and about 10% by salt marshes. The mean depth of the water column is about 1.2 m, with only 5% of the lagoon deeper than 5 m (Molinaroli et al. 2009). Following Kjerfve (1994), the LoVe can be defined as a "restricted" coastal lagoon. It is connected to the Adriatic Sea through three inlets (Lido, Malamocco, Chioggia) which allow tidal flushing twice a day. The North Adriatic tides are the largest in the Mediterranean. In the LoVe the mean tidal range is 61 cm (1986-2004), with a maximum estimated at 121 cm. Tides are a main factor in shaping the morphology of the lagoon and in determining such factors as water exchange, dissolved oxygen, salinity, nutrients and sediment distribution. The average input of freshwater from the drainage basin is about 35.5 m^3s^{-1} (recorded in 1999; Zuliani et al. 2005), with the most important tributaries located in the northern basin. A salinity gradient is thus produced, ranging from marine (about 37 psu) to almost freshwater near the bay-head estuaries and freshwater inlets. The mean value is about 30 psu. The majority of the lagoon waters can be classified as mixoeuhaline/mixopolyhaline according to the Venice System (Anonymous, 1959). Salinity is higher during summer and lower during winter due to annual rainfall patterns. Because of the shallowness of the lagoon, water temperature follows strictly the seasonal trends of air temperature, ranging from zero (seldom freezing) to above 30°C.

Samplings

The specimens collected, identified and checked for larvae were hanging on a floating dock in the harbour of the historical Arsenal of Venice (45°26'13" N, 012°21'14" E; WGS84) where the headquarters of the Marine Sciences Institute of the National Research Council of Italy are located. Colonies were first noticed on September 23, 2010, and subsequently sampled on January 26, 2012 and, after larval development, on June 12 and 22, 2012 at different depths from 50 to 100 cm below the water surface. The water temperature was 5°C in January and 20-25°C in June, the salinity ranged from 30 to 35 psu. Samples were preserved both in buffered formaldehyde 4% (formalin 10%) in filtered seawater for morphological analysis and ethyl alcohol 96% analytic grade for future genetic analysis. One of us (GL) confirmed the initial tentative field identification. Parts of the collected colonies are currently deposited at the Institute of Marine Sciences CNR-ISMAR, Venice, Italy. Beside samplings, the development of the colonies was periodically checked.

After the initial finding, extensive surveys were conducted inside the LoVe during July 2012 to assess the occurrence of *D. vexillum*. Estuarine transects from the bayhead of the Dese River to the sea and other minor freshwater inputs were surveyed. Hard substrates in the LoVe suitable to colonization by the species are mainly artificial structures. Floating pontoons of the public boat transport system (ACTV), marinas and navigation piles and ancillary structures (steel chains, ropes etc.) were visually inspected and sampled manually or scraped off with a dip net from the boat.

Results

Taxonomy

Phylum: Chordata, Subphylum: Tunicata, Class: Ascidiacea, Order: Aplousobranchia,
Family: Didemnidae, Genus: *Didemnum*
Species: *Didemnum vexillum* Kott, 2002

Identification of Didemnum vexillum

The collected specimens were unequivocally identified as *Didemnum vexillum* Kott, 2002 based on numerous morphological characters including mature brooded larvae (see Kott 2002; Lambert 2009 for a detailed species description, photos and illustrations). The surface of the tunic exactly matches with the characteristic reticulate pattern described in Lambert (2009). The fresh colonies were pale orange of varying intensity (Figure 1A, B). Preservation in 96% ethyl alcohol extracted most of the colour. The formalin preserved colonies retained a yellowish tan colour, as did *D. vexillum*. The tunic spicules, limited for the most part to the surface layer of tunic but nowhere very dense, were very variable in size and shape, as is characteristic for *D. vexillum*, with a similar size range except for one anomalous large one. In the samples collected in June, there were many mature brooded larvae in the inner tunic matrix below the zooids, with the distinctive 6 lateral ampullae per side, and

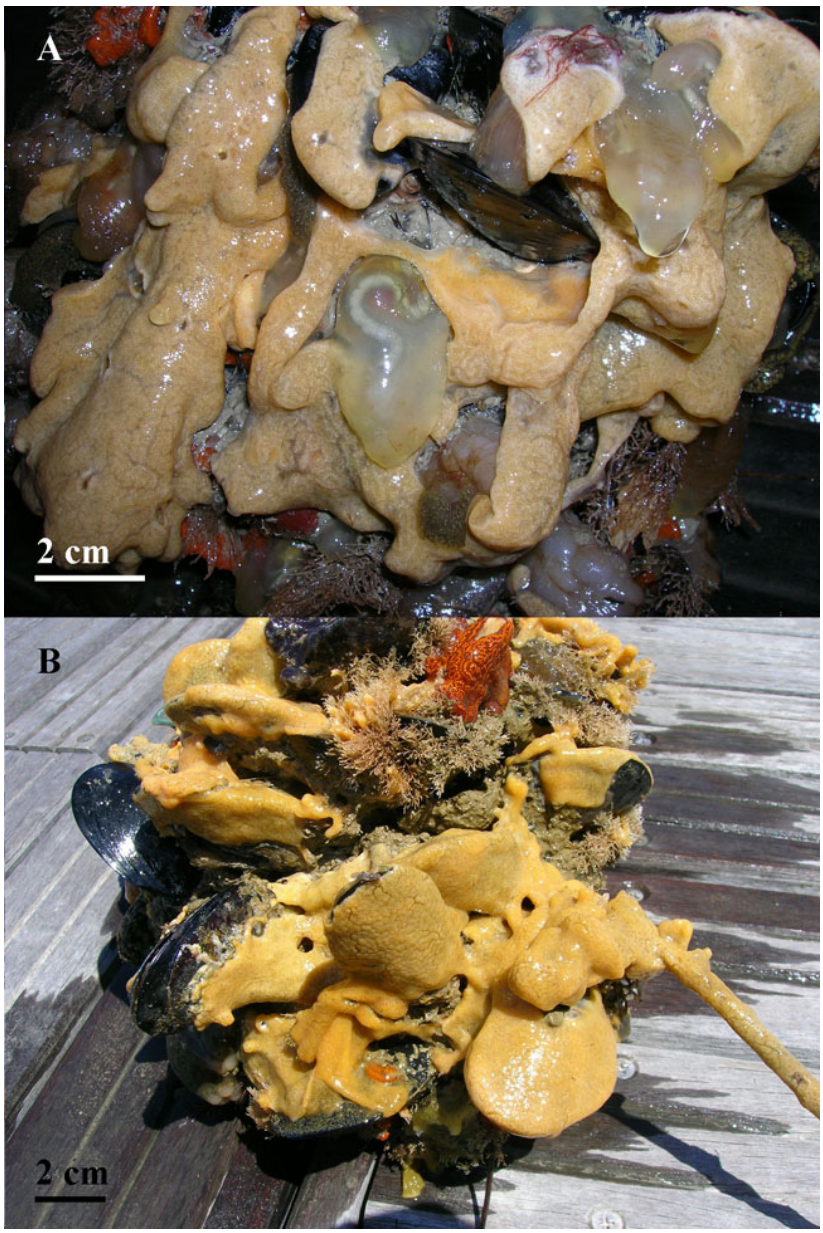

Figure 1. *Didemnum vexillum* overgrowing fouling organisms at the Arsenal harbour. **A**) January 2012; **B**) June 2012. Photographs by Davide Tagliapietra.

the overall shape and size of the larvae were the same as for *D. vexillum* (see Lambert 2009). The zooids had a single round testis, covered by a spiral sperm duct with about 8 coils (9 in a few).

Annual growth of the colonies

In January, the colonies at the Arsenal were small, becoming smaller (a few tens of square centimetres) in February. During the winter 2011-2012 there were episodes of surface ice in the lagoon, even in the basin of Arsenal. With the arrival of spring, colonies recovered rapidly (a few hundreds of square centimetres), developing from May to June the characteristic lobing. May-June was, therefore the period of maximum growth observed in the sampling site, which is a very sheltered harbour with a salinity level close to sea water. Brooded larvae in the June 12 and June 22 samples appeared to be mature and ready to be released; it is possible that some had already been released in late May.

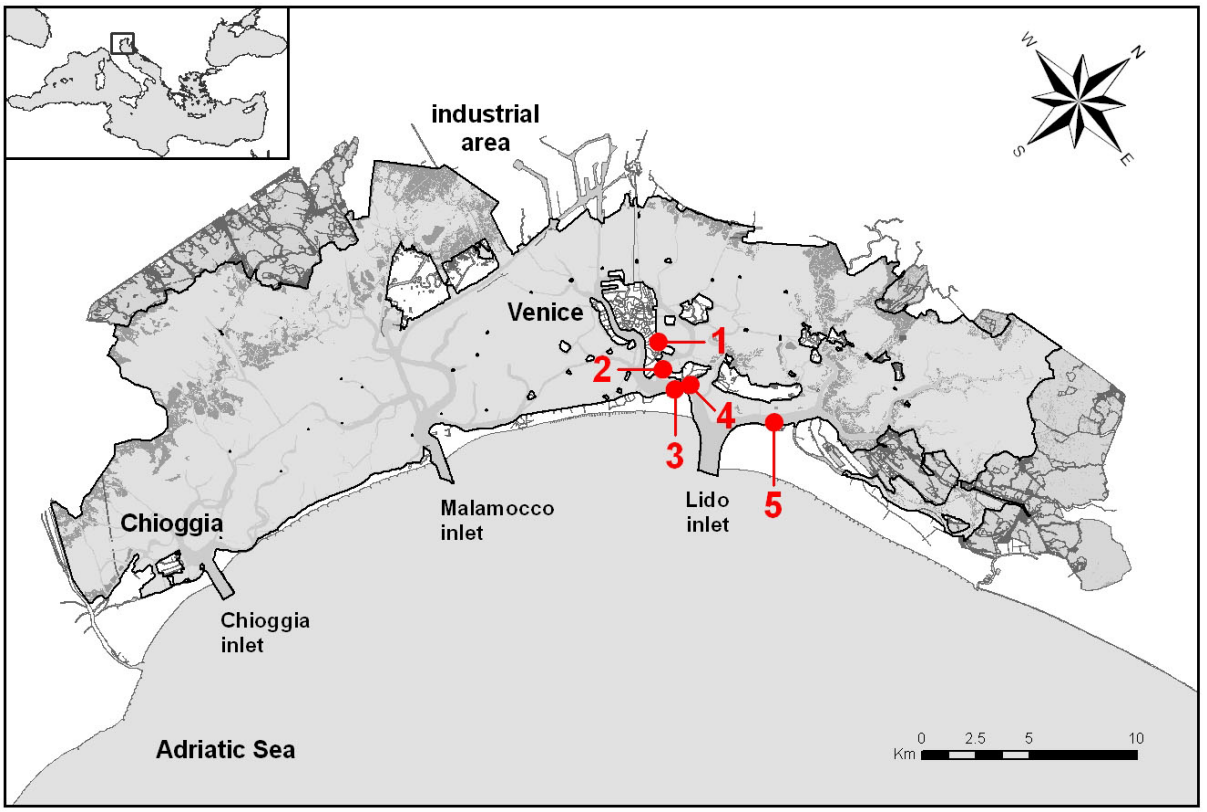

Figure 2. Verified distribution of *Didemnum vexillum* in the Lagoon of Venice, 1: Arsenal of Venice; 2: Certosa marina; 3: San Nicolò; 4: Sant'Andrea beacon tower; 5: Lio Grando marina.

During the month of July, with water temperature approaching 30°C at the surface, the colonies underwent an intense regression until they almost disappeared.

Distribution of Didemnum vexillum in the Lagoon of Venice

After the first record at the Arsenal of Venice (# 1 in Figure 2), *Didemnum vexillum* was collected at Certosa marina floating pontoon (45°25'53" N, 012°22'04" E) (# 2), a mooring at San Nicolò (45°25'47" N, 012°22'56" E) (# 3), Sant'Andrea beacon tower (45°26'11" N, 012°23'06" E) (# 4), Lio Grando marina (45°27'15" N, 012°26'01" E) (# 5).

The tunicate was found mainly overgrowing fouling invertebrates, mostly on mussels (*Mytilus galloprovincialis*), solitary ascidians and other fouling organisms, growing on maritime structures. Other sites sharing the same environmental features were inspected but *D. vexillum* was not found, e.g. Chioggia harbour (45°13'29" N, 012°16'33" E) and San Felice marina (45°13'26" N, 012°17'39" E) in the southern sub-basin. The zones of Porto Marghera (Canale Vittorio Emanuele, 45°27'14" N, 012°15'58" E) and Porto San Leonardo (45°21'28" N, 012°15'01" E) in the Industrial Zone, and Fusina (45°25'20" N 012°15'24" E; 45°25'33" N 012°15'31" E), all characterized by high commercial shipping traffic and connected to the sea by a deep channel, were also inspected with no positive results. It was absent in areas of lower salinity, such as the examined estuarine tracts and at the zones surrounding the saltmarshes.

On the basis of these observations *D. vexillum* can be considered well established in the euhaline and tidally well flushed zones of the lagoon. Figure 2 shows the confirmed locations of *D. vexillum* in the LoVe.

Discussion

The discovery of *D. vexillum* in the LoVe is the first record not only for Italy but for the entire Mediterranean Sea. Brunetti (1978-79) first detected *Polyandrocarpa zorritensis* (Van Name, 1931) in 1974 from the Gulf of La Spezia (Italy), and and Zaniolo et al. (1998) described *Botrylloides violaceus* Oka, 1927, from the LoVe, detected in 1993; both were also first records for the Mediterranean. Brunetti (1979) listed 12 species of ascidians for the LoVe, including a didemnid, *D. granulosum*, which demonstrated some characters similar to *D. vexillum* such as a reticulate tunic pattern; however, the larvae were not described and the spicules appear to be different. Izquierdo-Muñoz et al. (2009) listed 14 non indigenous species (NIS) of ascidians from the Mediterranean Sea during the last 50 years but did not include any didemnids. Mastrototaro and Tursi (2010) recorded 21 species of Didemnidae in Italian waters but *D. vexillum* was not included, nor has it been recorded elsewhere in the Mediterranean (Galil 2007; Zenetos et al. 2010). The list of alien species along the Italian coast by Occhipinti-Ambrogi et al. (2011), which consider the LoVe "the main hotspot of introduction" in Italy, includes only four ascidians, none of them didemnids. *B. violaceus* remains the only alien ascidian reported for the LoVe before the present record (Occhipinti-Ambrogi 2000; Occhipinti-Ambrogi et al. 2011).

Although the present paper reports specimens noticed in 2010 but not collected until 2012, the species had been seen by the authors since at least November 2007, who observed colonies with characteristic tan to orange lobes growing on a wooden navigation piling (Pellestrina 45°17'10" N, 012°18'08" E). It is possible that the species could have been present even earlier and described as *Didemnum* sp. (A. Occhipinti-Ambrogi and R. Brunetti pers. communication).

An established, or naturalized, population has been defined as a free-living, self-maintaining and self-perpetuating population (European Commission 2004). Different sighting criteria have been proposed to classify an established population, with the CIESM atlas series (2012) requesting at least two distinct records of the species. By these criteria *D. vexillum* can be considered as established in the Lagoon of Venice.

According to the Köppen–Geiger–Pohl Climatic Classification (Geiger and Pohl 1953), temperate Northern Europe coasts have a *Cfb* climate (temperate maritime climate, with cool summers and mild winters, abundant precipitation distributed throughout year) whereas the main climatic type for the Mediterranean is *Csa* (hot summer Mediterranean climate with dry season and precipitation during the winter). The Northern Adriatic, where the LoVe is located, and the Black Sea experience a less common climate *Cfa* (humid subtropical climate, hot summers and precipitation distributed throughout year). A *Cf* is climate similar to the Atlantic European coast but with warmer summers. Among *Cf* climates, *Cfa* and *Cfb* differ in the temperature of the warmest month, above (*Cfa*) or below (*Cfb*) 22°C. The Northern Adriatic is, within the Mediterranean context, the only region experiencing both non-negligible tides and a *Cfa* climate (Tagliapietra and Volpi Ghirardini 2006). The environment of the LoVe combines Atlantic and Mediterranean traits; these peculiar (hydro)climatic features allow the presence of species with boreal affinity that have disjunct geographic distributions split between the northwest Atlantic and a few Mediterranean areas: the Adriatic (mostly the northern end), Gulf of Lion in France and the North Aegean Sea in Greece. All these areas have similar hydro-climatic conditions, as they correspond to the major sources of dense waters regulating the thermohaline circulation in the Mediterranean Sea (Boero et al. 2008). Species showing Atlantic-Mediterranean disjunction include, for example, the anthozoan *Diadumene cincta* Stephenson, 1925 and the cord grass *Spartina maritima* (Curtis) Fernald. The tunicates *Polycarpa discoidea* Heller, 1877 was detected, in the Mediterranean, only in the Northern Adriatic (Monniot and Monniot 1971; Mastrototaro and Tursi 2010).

In the Mediterranean, some alien species have been recorded exclusively from these areas. The mytilid *Xenostrobus securis* (Lamarck, 1819) was recorded in both the northern Adriatic lagoons and the Palavasian lagoon complex (Gulf of Lion), and the gastropod *Rapana venosa* (Valenciennes, 1846), in the Adriatic and the Black Sea (CIESM 2012). All of the LoVe ascidian non indigenous species that have been recorded are temperate species that fit the environmental pattern for this area. On this basis we are not surprised to find the arrival of *D. vexillum* in this Mediterranean area.

The habitat of *D. vexillum* is reported to be essentially sheltered marine, particularly harbors and marinas (Bullard et al. 2007), though it has

successfully invaded large subtidal areas (Valentine et al. 2007a, 2007b). Experimental laboratory studies have demonstrated an elevated mortality and cessation of growth at salinity <27 psu, with a discrete resistance to short-term (about two hours) exposure to water of 10 psu (Gröner et al. 2011). The distribution in the LoVe, as verified in July, confirms the previously reported species' preferences in terms of environmental conditions.

From seasonal observations, it appears that the optimum period of growth of *D. vexillum* in the LoVe is limited by both winter and summer temperatures. The colonies found in September 2011 were in good vegetative state, although not fully developed. These observations may suggest a recovery in autumn similar to other northern Mediterranean colonial ascidians (Turon 1992; Turon and Becerro 1992) after the summer reduction. In LoVe *D. vexillum* could then have two periods of stasis, a winter one and a summer one probably due to temperature. This species dies back partially and undergoes a winter period of hibernation in the NE U.S. (Valentine et al. 2007a). Similar behaviour is also found, in LoVe, in two alien species of algae, the Wakame, *Undaria pinnatifida* (Harvey) Suringar and the Japanese wireweed, *Sargassum muticum* (Yendo) Fensholt (Gargiulo et al. 1992; Curiel et al. 1998). Venice attracts vessels coming from all over the world; the LoVe has a high maritime activity hosting one of the main industrial ports of the Mediterranean and attracting visits from numerous cruise ships. Leisure yachts sail to Venice continuously and local boat traffic is very high. Climatic, physiographic and saline traits, together with high opportunity for maritime transportation, make the LoVe a perfect destination for temperate non-native species and their subsequent dispersal to other nearby ports.

The invasiveness of *D. vexillum* is regarded as a threat for native species that can be outcompeted, dominated and overgrown and as a significant nuisance for the maintenance of maritime structures. *D. vexillum* has also been found to grow massively on seagrass blades, threatening this important habitat (Carman and Grunden 2010). Considering the fast-growing ability and the *habitus* of this colonial sea squirt, there is also concern about the potential impacts on shellfish aquaculture (Coutts and Forrest 2007; Carman et al. 2010). Encapsulating mussels (Figure 1B) as it does in New Zealand, *D. vexillum* represents a major economic threat to the mussel farms traditionally present along the northern Adriatic shores and in the lagoons. The impact of this invasive species prompted extensive (and very expensive) programs for eradication in countries such as New Zealand (Pannell and Coutts 2007; Coutts and Forrest 2007), Wales (http://www.ccw.gov.uk/about-ccw/newsroom/press-releases/ccw-tackles-invasive-sea-squir.aspx?lang=en), and the NW U.S. (Pleus et al. 2008). However, because abatement programs were incomplete, and did not begin until *D. vexillum* was well established, the eradication was only temporary and did not have any long term effect on the population size and distribution.

The summer high and winter low temperatures of the LoVe and its probable further warming during this century (Tagliapietra et al. 2011), and the species' intolerance to very low salinity might limit the spread of this species in the LoVe and prevent establishment in warmer regions of the Adriatic and elsewhere in the Mediterranean Sea. Careful monitoring is necessary, with immediate eradication of new populations to prevent further spreading.

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