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The clubbed tunicate *Styela clava* has arrived in the Lagoon of Venice

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Abstract

Individuals of the solitary ascidian *Styela clava* Herdman, 1881 have been recorded for the first time in Italian waters, in Chioggia, within the Lagoon of Venice. This finding represents the third record of the species in the Mediterranean Sea. The Lagoon of Venice is a well-known hotspot of introduction of Non-Indigenous Species (NIS) along Italian coasts. It hosts several important vectors of introduction such as commercial shipping, recreational boating, aquaculture facilities and the live seafood trade. Monitoring surveys are a crucial step for the early identification of NIS, especially those characterized by a high invasive potential such as *S. clava*.

Keywords: *Styela clava*, *Tunicata*, non-indigenous species, Lagoon of Venice, Mediterranean Sea

Introduction

Styela clava Herdman, 1881 is a solitary ascidian, originally from the Northwestern Pacific, along the coasts of Japan, Korea and China (Lützen 1998). It was first described in Kobe (Japan) during the Challenger expedition by Herdman (1881), who first reported the “remarkable” shape and the abundance of this species in Japanese seas.

Styela clava is commonly known as “clubbed tunicate”, “stalked sea squirt”, “Asian tunicate”, “leathery sea squirt” or “rough sea squirt”. These common names refer to its characteristic leathery tunic, club-shaped body or its provenance.

The individuals can measure up to 16 cm including a long stalk for attaching to the substrate; this stalk is usually about one-third of the whole length. They are characterized by a tough, wrinkled tunic; larger specimens may have a light brown body and a darker brown stalk, while smaller ones are yellow-brown, and they may grow in very dense clusters (Lützen 1998).

The clubbed-tunicate was first recorded in Europe, along the coasts of Plymouth by Carlisle (1954), who considered it a new species, *Styela mammiculata* Carlisle, 1954. Later, Millar (1960)

demonstrated that *S. mammiculata* and *S. clava* were the same species.

The species was accidentally introduced into England, probably attached to vessel hulls from Korea or to oysters imported from Japan during the early 1950s (Lützen 1998; Izquierdo-Muñoz et al. 2009).

Styela clava is one of the most impacting invasive alien species in the world, and it is considered a real pest (Clarke & Therriault 2007). Indeed, individuals of *S. clava* can colonize any kind of abiotic or biotic substrata, establishing populations in temperate waters worldwide. *Styela clava* has been reported as a major pest in oyster and mussel farms in Japan, Denmark and Canada, competing over the cultured organisms and forming dense aggregations on fishing gears, moorings and ropes (Clarke & Therriault 2007).

In the Mediterranean Sea, *S. clava* was reported for the first time within the Bassin de Thau (Sète, Marseille) in the early 2000s, where the population appeared to be expanding, on the nearby Mediterranean French coasts (Davis & Davis 2008), in Port Said, along Egyptian coasts in 1991 (Ghobashy & Abdel Messeih 1991) (even if not included in the list compiled for the region by

Halim & Messeih 2016) and in the Sea of Marmara (Çinar 2016).

This study reports a new record of the highly invasive species *S. clava* within the Mediterranean Sea, adding a further expansion to the list of NIS (Non-Indigenous Species) ascidian species within this basin (Mastrototaro et al. 2019; Ragkousis et al. 2020; Montesanto et al. 2021a, 2021b; Orfanidis et al. 2021) as well as a further “alien” to the list of the several NIS recorded within the Lagoon of Venice, which represents the first Italian hotspot of species introduction (Occhipinti-Ambrogi et al. 2011). In addition, the Lagoon of Venice is not only a well-known area of high risk of introduction of NIS but also several NIS firstly recorded in this area have later further spread elsewhere (Marchini et al. 2015; Servello et al. 2019). We provide a detailed morphological description of the collected specimens of *S. clava* together with a review of its ecological characteristics as well as potential pathways of introduction and future spread within the Mediterranean basin.

Material and methods

Description of the study area

The Lagoon of Venice is the largest coastal lagoon in the Mediterranean region. It is located in the Northern Adriatic Sea (Italy) (Figure 1), and it covers an area of about 550 km², with an average depth of 1.5 m (Brambati et al. 2003; Semprucci et al. 2019). In detail, the total area of approximately 550 km² includes approximately 390 km² of open

lagoon. This area also covers 40 km² of channels, 70 km² of salt marshes, and 90 km² dedicated to aquaculture activities (Madricardo et al. 2019).

The lagoon is characterized by a variety of shallow habitats, such as salt marshes, seagrass beds, intertidal and subtidal mudflats. These areas are crossed by a network of channels with a maximum depth of about 15 m. The channels link three inlets that connect the lagoon to the Adriatic Sea: Lido, Malamocco and Chioggia (Rova et al. 2019; Semprucci et al. 2019).

The tidal excursion is usually less than 1 m, with a salinity of 34.4–34.9‰ at high tide and 32.8–33.6‰ at low tide (Semprucci et al. 2019). The annual average temperature in 2008 was about 24.5°C, with the highest temperature occurring during the summer (up to 29°C) and the minimum during the winter (10°C), sometimes also reaching lower temperature in areas characterized by a minimum tidal flow (Coccioni et al. 2009; Bertolini et al. 2021).

The lagoon is affected by several human activities, such as municipal wastewater discharges, agricultural drainage, dredging, fishing activities, aquaculture activities and maritime traffics (Madricardo et al. 2019). Moreover, several recreational marinas and two main harbours have been built in Venice and Chioggia. In detail, the Marghera harbour is mainly devoted to commercial and international traffic, while the Chioggia one is mainly devoted to regional commercial traffic (Marchini et al. 2015). Furthermore, the whole lagoon hosts one of the most important areas in Europe for clam production (Marchini et al. 2015).



Figure 1. (a) Study area, boxes encircling the sampling site, previous occurrences of *Styela clava* in the Mediterranean Sea are numbered in temporal order; (b) alive specimens of *S. clava* collected in Lagoon of Venice.

The benthic communities of the lagoon are mainly dominated by seagrass meadows represented by *Cymodocea nodosa* (Ucria) Asch., *Zostera noltei* Hornemann, 1832 and *Zostera marina* Linnaeus, 1753 (Solidoro et al. 2010) and by algal associations comprising 300 species of macroalgae (Sfriso & Curiel 2007). From the 1990s, some introduced species of macroalgae, such as *Sargassum muticum* (Yendo) Fensholt and *Undaria pinnatifida* (Harvey) Suringar, have replaced several autochthonous species (Curiel & Marzocchi 2010). Macrofauna is mostly dominated by filter-feeder species of bivalves and polychaetes, with a high biomass of invasive species such as *Magallana gigas* (Thunberg, 1793) and opportunistic/detritivore species of polychaetes of the genus *Owenia* Delle Chiaje, 1844 that are commonly found in the estuarine areas (Solidoro et al. 2010). As already stated, the Lagoon of Venice is the most important hotspot of NIS introduction in Italy; indeed, 50 alien species were first spotted in this area (Occhipinti-Ambrogi et al. 2011; Servello et al. 2019). Among them, the ascidians, *Botrylloides violaceus* Oka, 1927 (Zaniolo et al. 1998) and the highly invasive species *Didemnum vexillum* Kott, 2002 (Tagliapietra et al. 2012) were recorded in this area for the first time within the Mediterranean Sea (Servello et al. 2019).

Sampling

Several specimens of *S. clava* were observed, photographed and then six of them were collected during April 2021 at about 0.5 m depth on ropes and floating docks in the south-west of Chioggia, Venice lagoon (Adriatic Sea, Italy) (45°12'47.9" N 12°16'24.3" E) (Figure 1). In the same area, other ascidian species were detected such as colonial species belonging to the genus *Botryllus* and *Botrylloides*. These observations were not the result of any systematic survey of the lagoon environment but a casual observation during sampling of other target species.

The salinity recorded was about 30 psu, while the temperature was about 14–15°C.

All collected specimens were relaxed with menthol crystals in sea-water for about 3 h until no reaction was detected and then preserved in 4% formalin solution in seawater. Individuals were observed, dissected and then treated with Mayer's haemalum solution in order to identify the inner morphological characteristics. In detail, the dissected specimens were rinsed in distilled water overnight and then stained with Mayer's haemalum solution for about 60 s. After being stained, the specimens were again rinsed with distilled water.

Results

Systematics

Phylum Chordata
Subphylum Tunicata
Class Ascidiacea
Order Stolidobranchia
Family Styelidae
Genus *Styela*
Styela clava Herdman, 1881

New record. 2021, Chioggia, Lagoon of Venice, Adriatic Sea, North Mediterranean Sea, 0.5 m depth. Individuals preserved in 4% formalin (Code: MUZAC-6667) have been deposited in the collection of the Zoological Museum of the University of Bari.

Previous records in the Mediterranean Sea. 2005, Bassin de Thau, 5 m depth (Davis & Davis 2008) 1991, Egyptian coasts (Port Said), depth not reported (Ghobashy & Abdel Messeih 1991)

2016, Sea of Marmara, 10 m depth (Çinar 2016)

Description. Individuals of *S. clava* collected from the Venice lagoon (Chioggia, Italy) range from 9 to 13 cm in size, with a very long stalk, almost half the whole-body length (Figure 2(a)). The external appearance of the tunic is dark brown with the typical leathery consistence, wrinkled and mamillated (Figure 2(a)). The siphons are close to each other (Figure 2(a)) and externally marked by stripes (Figure 2(b)).

About 5–6 gonads lie on the left side, while about 6–8 gonads are placed on the right (Figure 2(b)–(d)). The testicular follicles consist of small white lobes separated from the long pinkish female gland (Figure 2(b)–(d)). The stomach is elongated and has about 40 stomach folds (Figure 3(f)). Endocarps are visible on the body wall between the gonads (Figure 2(e)).

The prebranchial area is smooth (Figure 3(b)). The oral siphon is characterized by the presence of about 30 long thick tentacles (Figure 3(b)), while the atrial siphon does not have any tentacles (Figure 3(c)). The dorsal tubercle has a spiral shape (Figure 3(d)).

The branchial sac is characterized by 12 to 44 longitudinal vessels on the 4 branchial folds and from 4 to 20 in the interspaces (Figure 3(e)), numerous transverse vessels of two orders of size (the thicker ones are about 0.1 mm, while the thinner ones are about 0.05 mm) and no parastigmatic vessels (Figure 3(g)). Four to six stigmata per branchial mesh are counted (Figure 3(g)–(h)).

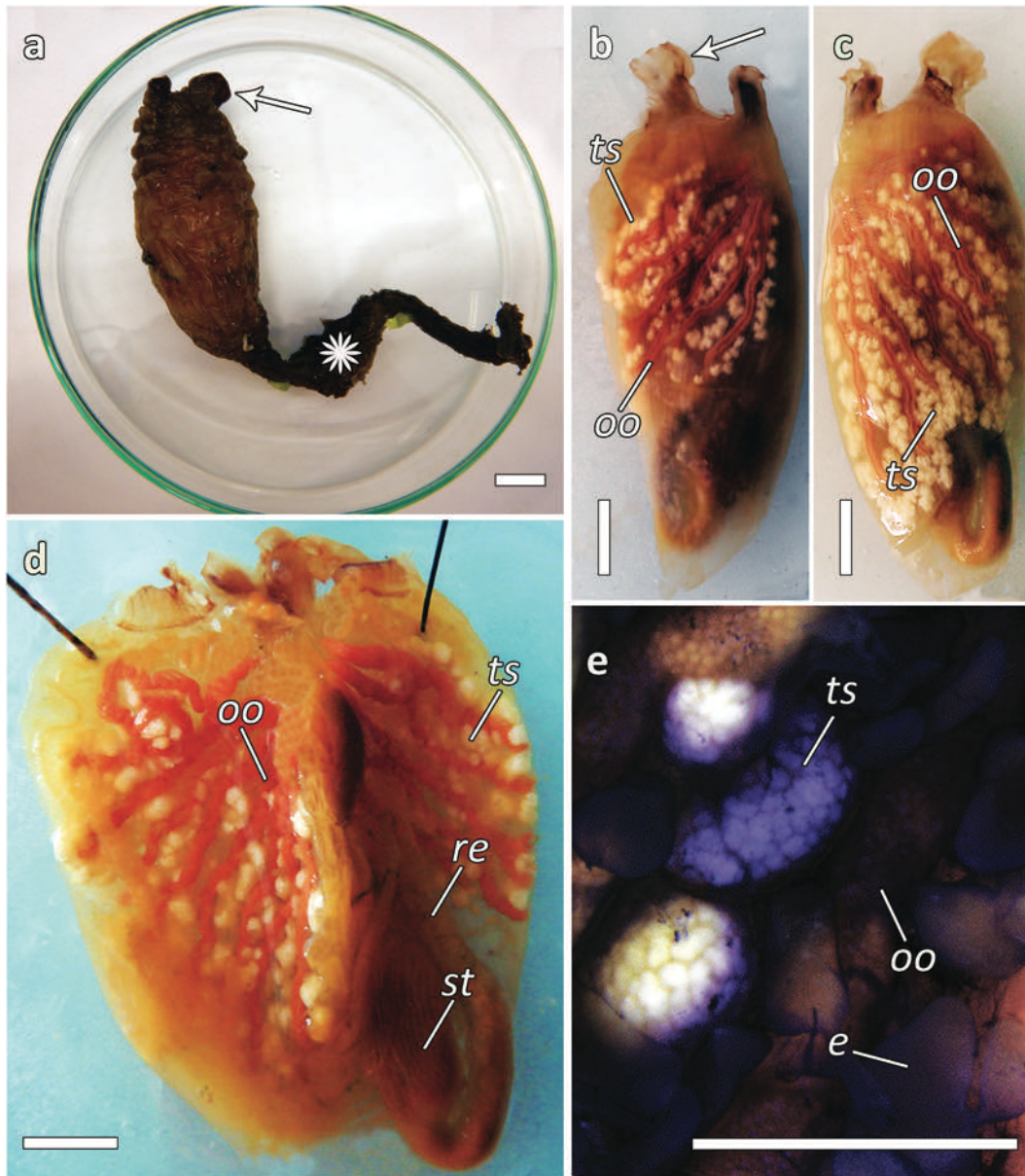


Figure 2. *Styela clava*. (a) Individual of *S. clava* with the characteristic leathery and mammillated brown tunic and a long dark brown stalk (asterisk) and the siphons lying close to each other (arrow); (b–c) specimens without tunic showing the gonads; (b) the left side, testicular follicles constituted by small white lobes (*ts*) separated from the long pinkish female gland (*oo*), siphons marked with brown stripes (arrow); (c) right side; (d) dissected specimens without branchial sac showing gonads on both sides; (e) magnification of the gonads showing the endocarps (*e*), testis (*ts*) and ovaries (*oo*). Scale bars: 1 cm.

A lobed anus opens close to apertures of the siphons (Figure 3(a, i)).

Remarks

Considering the macroscopic features of the species and based on the comparison with previous observations (Herdman 1881; Carlisle 1954; Millar 1960; Ghobashy & Abdel Messeih 1991; Davis & Davis

2008; Çinar 2016), it was possible to identify all the specimens collected in the Venice Lagoon as *S. clava*. Although this species can be “easily” recognized by its external peculiar shape and the characteristic “leathery” tunic, morphological analysis of anesthetized samples confirmed the specific identification.

Slight differences in the number of gonads of the specimens were found, with respect to previous descriptions made by other authors, who report a lower number of gonads on the right side,

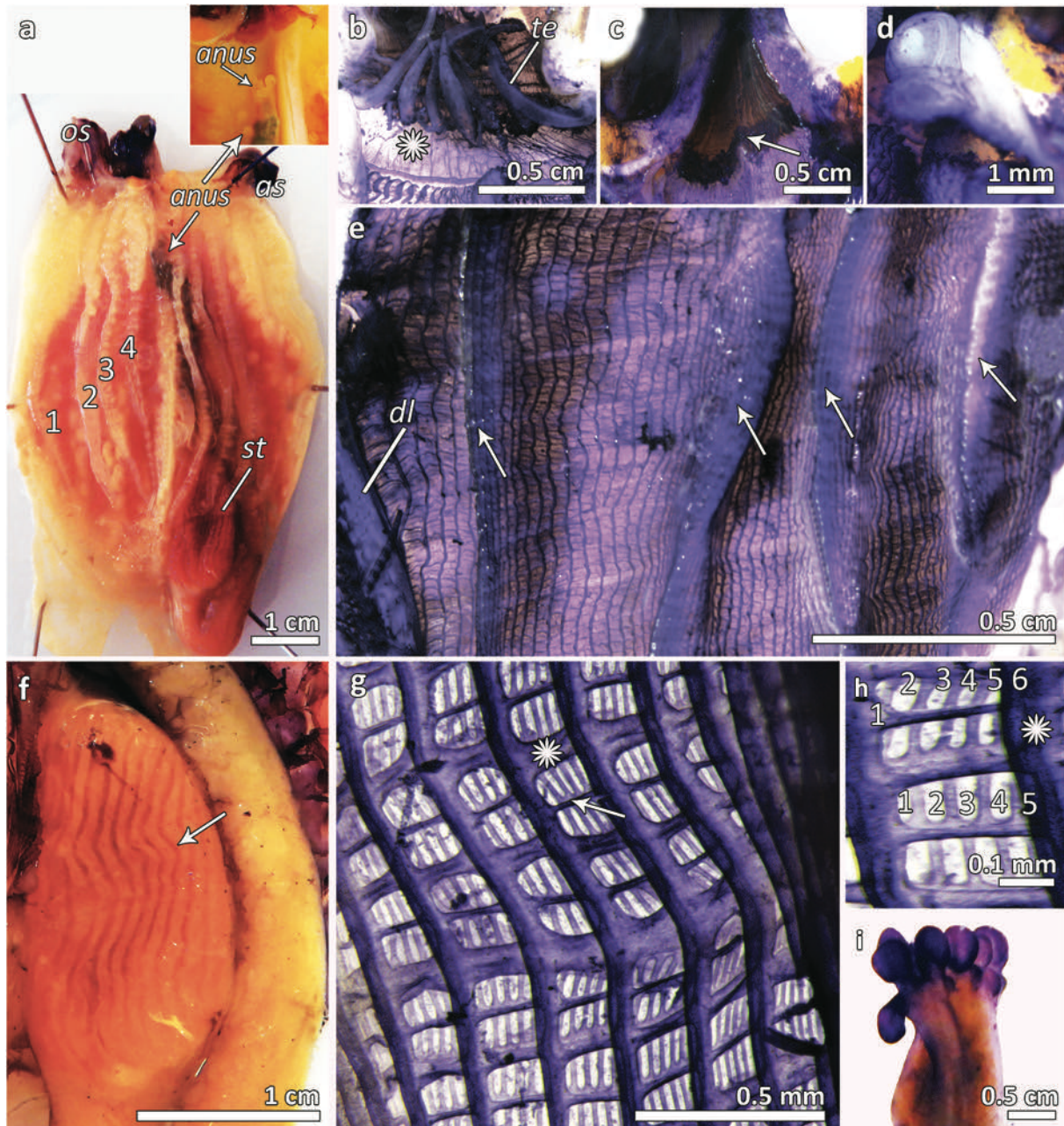


Figure 3. (a) Dissected specimen of *S. clava* showing the branchial sac with four folds per side, the oral siphon (*os*) and the atrial one (*as*), the stomach (*st*) and the anus opening (*anus*), with magnification of the anus (*anus*); (b) oral siphon with long thick oral tentacles (*te*) and smooth prebranchial area (asterisk); (c) atrial siphon without tentacles (arrow); (d) dorsal tubercle spiral-shaped; (e) branchial sac showing four folds from the dorsal lamina (*dl*), and 12 to 44 longitudinal vessels on the 4 branchial folds and from 4 to 20 in the interspace; (f) stomach with about 20 folds per side (arrow); (g) branchial sac with transverse vessels of two different size (asterisk points out a thicker one) (h); branchial mesh with about 5–6 stigmata, the asterisk points out the longitudinal vessel; (i) lobed anus.

generally around three (Carlisle 1954; Millar 1960; Davis & Davis 2008) and sometimes a higher number of oral tentacles (see Millar 1960). Furthermore, the specimens collected from the Bassin de Thau were characterized by a slightly different orange-brown coloration (Davis & Davis 2008).

Despite this small morphological difference, diagnostic characteristics such as the absence of parastigmatic vessels and atrial tentacles, the number of stomach folds, the presence of endocarps and of a lobed anus allowed the identification of the specimens from the Lagoon of Venice as *S. clava*.

Ecology and distribution

The native range of distribution of the species *S. clava* is the Northwestern Pacific Ocean (Herdman 1881; Lützen 1998), but at the present time, *S. clava* is distributed in North America (Californian coasts), Canada (Atlantic and Pacific coasts), southern British Columbia (Pacific coast and along the Atlantic coasts of Prince Edward Island) and along Northeastern European coasts as well (from Denmark to Portugal, including Ireland) (Clarke & Therriault 2007).

Styela clava is a littoral species that can tolerate tidal emersion and it inhabits artificial and natural substrata down to a depth of 40 m (Lützen 1998; Clarke & Therriault 2007).

It is considered a major pest for shellfish aquaculture, smothering target species (such as bivalves) and fouling gear and equipment. *Styela clava* tolerates a wide range of temperature, from 2°C to 23°C (Clarke & Therriault 2007). It has been demonstrated that *S. clava* is intolerant of low salinity; the limit of a successful establishment depends on the temperature ranging from 15°C to 23°C for spawning and values of salinity between 22 and 34.5 psu (Davis & Davis 2008), although this species can also survive with salinity close to the known tolerance limit of the species, such as in the Sea of Marmara, where the salinity recorded was around 23 psu (Çinar 2016).

Styela clava, like many solitary ascidian species, is characterized by a short larval planktonic phase of about 24–48 hours at 20°C (Holmes 1969). This leads to a limited larval dispersal; therefore, a different dispersal vector of introduction of the species, such as the transport of adults on ship hulls or on the shells of the bivalves used in aquaculture, plays a major role in its dispersal and expansion.

Discussion

The occurrence of *S. clava* in the Lagoon of Venice represents the third record of this NIS in the Mediterranean Sea and the first along Italian coasts.

Styela clava is known to have a high invasion potential and its successful establishment mainly depends on the suitability of the new habitat, especially in terms of salinity and water temperature. The average temperature in the Lagoon of Venice reaches 29°C in the summer, and the salinity values range from 25 to 32 ‰ (Bertolini et al. 2021); these conditions are favourable for its establishment. The Mediterranean Sea mean temperature in littoral waters ranges from 11°C to 28°C with salinity

between 36.2 and 39‰ (Gačić et al. 2013; Pastor et al. 2020). These conditions may represent a high risk of invasion by *S. clava* in further areas within the Mediterranean basin.

To date, *S. clava* occurrence is restricted to confined areas in the Mediterranean basin and the Black Sea (Sea of Marmara), such as the Bassin de Thau and the Lagoon of Venice, but the presence of several vectors of introduction/spread in the Lagoon of Venice may play an important role in a possible spread of the species. In fact, this area is characterized by the presence of marinas influenced by recreational boating, commercial maritime traffic and aquaculture farms. Indeed, since the larval dispersal is limited for this species, the transport of adult individuals attached to vessel hulls or on aquaculture gear are the most likely pathways of introduction and future spread of this species inside the lagoon and in further areas of the Mediterranean Sea.

The tidal excursion in the Lagoon of Venice is about 1 m (Bertolini et al. 2021), but the tolerance of large individuals of *S. clava* to air exposure has been estimated to be about 2 weeks (Hillock & Costello 2013), so they can easily resist being exposed to the air at low tide. Therefore, air exposure for a possible control of *S. clava* can be applied during ordinary cleaning of dry-docking of boats, moorings and aquaculture gears, but 2 weeks of air exposure is needed to ensure the mortality of all individuals (Hillock & Costello 2013).

However, since the eradication of a NIS is almost impossible, early identification and efficient monitoring surveys performed by specialised staff are needed to allow the setting up of appropriate management strategies able to control and prevent the further spread of this highly invasive species in the area. Only in this way, it will be possible to predict future spreading trends and vectors not only within the Lagoon of Venice but also throughout the Mediterranean basin.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Geolocation information

The study area is located within the Lagoon of Venice (Italy): 45°12'47.9" N; 12°16'24.3" E.

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