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Research article

First record of *Styela clava* (Tunicata, Ascidiacea) in the Mediterranean region

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Abstract

The solitary ascidian *Styela clava* Herdman, 1882 is recorded for the first time in the Mediterranean region. It was found in the Bassin de Thau, near Sète; it has not yet been found in the Mediterranean Sea. The Bassin de Thau specimens are described and compared with Northern European specimens. The proximity of commercial shellfisheries to the discovered populations, and the absence of *S. clava* from other harbours and marinas along the coast, suggests that the species may have been introduced by shellfish transfer.

Key words: *Styela clava*, Bassin de Thau, first record

Introduction

The solitary ascidian *Styela clava* Herdman, 1882 is native to the north-west Pacific, particularly Japanese and Korean waters. It was first discovered in European waters in 1953 in the estuary of the Lynher River, near Plymouth (UK) and recorded as *Styela mammiculata* sp. nov. (Carlisle 1954); Millar (1960) demonstrated that this "new species" was synonymous with *Styela clava* Herdman, 1882. It is probable that it was introduced into Plymouth Sound by military craft returning from the northwest Pacific during the Korean War. Since its initial discovery, it has spread up the North Sea coast of Europe as far north as Denmark (Lützen 1999), and south along the Atlantic coast to Portugal (Davis and Davis 2004; 2005). A full account of the current distribution is presented in Davis et al. (2007).

S. clava possesses a firm, club-shaped body that can grow up to 220 mm in total length. It is attached to the substratum by a short stolon, the base of which forms an expanded membranous plate that adheres the organism to the substratum. A solitary ascidian resembling *S. clava* was first observed in the Bassin de Thau, near Sète in 2004 (D. Luquet, personal communication); a sample for identification was collected by D. Luquet on June 1st, 2005. Despite extensive searches of harbours and marinas along the adjacent Mediterranean coastline, no populations could be located in the Mediterranean Sea itself. Consequently, publication of the new record was delayed until it could be established that a sustainable population of the ascidians exists in the Bassin de Thau.

Materials and Methods

Sampling site

The Bassin (or Etang) de Thau is a 21 km long and 5 km wide lagoon in southern France (Figure 1). The mean depth of the lagoon is 4.5m, but the depth can exceed 30m in places. The tidal range is small, generally less than 30 cm in amplitude (La Jeunesse and Elliot 2004), and the tidal currents are weak (Souchu et al. 2001). The 7,500 hectares expanse of brackish water is separated from the Mediterranean Sea by a narrow sand bar with openings at Marseillan-Plage, the Pisse-Saumes canal, and at Sète, the Quilles canal (Figure 2). The Bassin de Thau is also connected to the Mediterranean Sea by the Canal de Sète, which flows through the eastern edge of the town of Sète.

Six specimens of the solitary ascidian were collected, by scuba diving, from rocks at ~5m depth in the southern end of the Bassin de Thau (approximately 43°25.6'N 03°39.5'W) by David Luquet (L'Observatoire Océanologique de Villefranche-sur-Mer) on June 1st, 2005 and preserved in 10% formaldehyde-seawater solution for subsequent examination (Figure 3).

Figure 1. Location of the Bassin de Thau and Mediterranean sampling sites. Asterisk - *Styela clava* present (Bassin de Thau), open cycles - *Styela clava* absent (adjacent sites are included in a single symbol), filled cycles - nearest recorded *Styela clava* populations

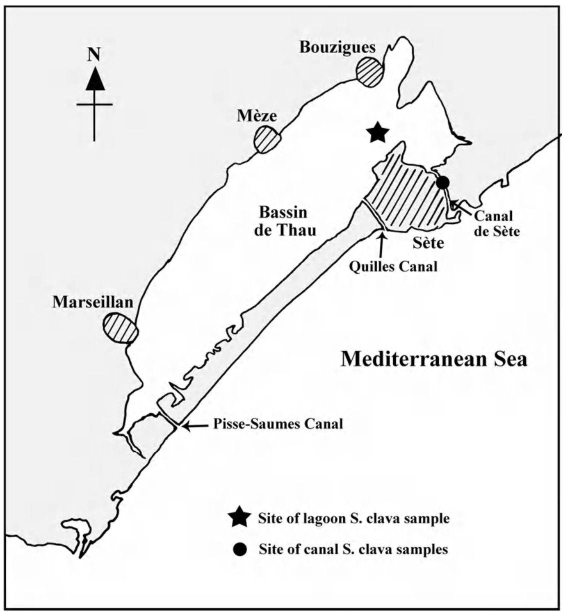

Figure 2. The Bassin de Thau showing the sites where *Styela clava* was found

Description of the specimens

The specimens exhibited the same general morphology as *Styela clava* that we have found in Northern European waters. The individuals collected ranged from 65 mm to 145 mm total length. Each had a firm club-shaped body, a short stem-like stolon and a membranous basal plate. The orange-brown tunic on the body and stolon had longitudinal grooves; the anterior tunic also had lateral grooves, which produced a mammillated pattern. The siphons were anterior facing, close together, raised and tapered; they were distally marked with four dark brown stripes alternating with narrower pale stripes. The oral siphon was terminal and the atrial siphon was set close by on the dorsal surface.

Internal examination of three of the specimens (100 mm, 125 mm and 130 mm total length) revealed the stomach was in the descending portion of the intestinal tract and the intestine curved upwards towards the atrial siphon. There were more sinuous ovaries on the right side (5, 7 and 7) than on the left (3, 3 and 3), and the numerous testicular follicles were clustered together along the ovaries to form white lobes. The gut and gonad descriptions are characteristic of *Styela clava*; comparison with specimens collected from Hornet Marina, Portsmouth Harbour (50°47.31′N 001°07.18′W) and with descriptions in the literature (Table 1) indicated that these specimens were indeed *S. clava*.

Figure 3. *Styela clava* collected from the Bassin de Thau on June 1st, 2005 (Photograph by Mary E. Davis)

Table 1. Summary of the distinguishing features of *Styela clava* specimens

Feature	Literature descriptions	Portsmouth specimens	Bassin de Thau specimens
Stolon	Longitudinal grooves (1)	Longitudinal grooves	Longitudinal grooves
Tunic	Brown, with paler mammillations (1)	Dark yellow-brown; mammillated pattern on anterior tunic.	Orange-brown; mammillated pattern on anterior tunic
Siphons	Siphons marked with longitudinal stripes of almost white and a rich chocolate brown, four of each (1).	Anterior facing, raised and tapered; with four dark brown stripes alternating with narrower, pale stripes.	Anterior facing, raised and tapered; with four dark brown stripes alternating with narrower, pale stripes
Ovaries	Left 3 or 4	Left 2 or 3	Left 3, 3 & 3
	Right 5 - 8 (2)	Right 5 - 7	Right 5, 7 & 7
Testes	A mass of small tubular testis follicles (brilliant opaque white) opens into each vas efferens (1)	Clustered into compact white lobes.	Clustered into compact white lobes.

1 Carlisle (1954)

2 Millar (1960)

Sustainability of the population

The specimens of *S. clava* were collected from the southern end of the Bassin de Thau, between the Quilles Canal and the Canal de Sète. A survey of accessible sites around the edge of the lagoon carried out in June 2006 failed to locate any specimens. The survey consisted of inspecting the near-surface areas of floating objects either visually, if water visibility was good, or feeling the fouling community for the characteristic stolon (Davis et al. 2007); ropes that had been submerged at several meters depth in the water column for a long time were also examined. Where there were no floating objects, accessible walls and rocks around the lagoon were examined; a scraper and hand net were used to search less accessible areas. Each site was searched for a minimum of one hour. However, in June 2007 we found a few specimens in the Canal de Sète (Figure 2) at 43°24.53'N, 03°41.70'E and a few more in November (at 43°24.31'N, 03°41.82'E); the ascidians were attached to rope at approximately 0.5-1 m depth. Thus the population appears to be sustainable and expanding. We have not yet found any specimens in Sète Marina or the Mediterranean Sea.

Potential introduction routes

Styela clava is oviparous; the eggs hatch after approximately 12 hours. The pelagic lecithotrophic larvae, which are approximately 0.85 mm in length, rarely travel more than a few centimetres in sustained swimming activity, and are only active for approximately 12 h (Davis 1997). The nearest recorded populations are in Portugal (Davis and Davis 2005), approximately 1200 miles away by sea (Figure 1). Given the short planktonic period, colonization by the natural dispersal of larvae is unrealistic. Therefore, introduced sessile adults must have established the Bassin de Thau population by spawning. Although natural dispersal of settled animals attached to floating debris is feasible, the narrow entries into the Bassin de Thau from the Mediterranean Sea render this vector unlikely. Long-distance spread of sessile organisms is generally attributable to the inadvertent transport by man. The two most likely mechanisms of ascidian introduction are: as juveniles transported attached to shellfish that are subsequently grown to marketable size, e.g. re-laid oysters (Minchin and Duggan 1988), or attached to ships' hulls (Gollasch 2002) including the hulls of pleasure craft, or in sea chests (Davis and Davis 2004; Coutts and Dodgshun 2007).

Hydrodynamic analysis indicates that hull transport is unlikely because any *S. clava* larvae that do settle and develop on the external hull would be unlikely to survive the sustained high-velocity movement through water when the ship is in service. Fouling organisms may be transported on ships' hulls if they are attached in areas of reduced flow such as sea-chests, bow-thruster tubes, stabiliser pockets and the cavities around the stern tubes and propellers, but large commercial ships do not enter the Bassin de Thau. Therefore, if *S. clava* arrived in the area as hull fouling, the most likely points of entry would be the military port of Toulon (which has shipping links with Brest and Cherbourg where *S. clava* populations flourish) and the commercial port of Marseilles. No specimens of *S. clava* could be found in marinas adjacent to these ports; marinas were surveyed because they provide easy access to permanently submerged hard substrate in a sheltered environment. Despite exhaustive searches, using the methods described above, no specimens of *S. clava* have been found in any northern Mediterranean ports or marinas (Figure 1).

Long distance dispersal of *S. clava* could occur if juvenile animals were transported attached to oyster shells when the oysters were re-laid. It is probable that this was the vector by which *S. clava* was introduced into the Limfjord (Lützen 1999) and Prince Edward Island (Locke et al. 2007). The Bassin de Thau has a thriving commercial shellfishery, the most important product being oysters - mainly *Crassostrea gigas* (Thunberg, 1793). There are approximately 750 oyster farmers in the lagoon, producing approximately 13,000 tonnes of oysters annually. *C. gigas* was imported from Japan to the Bassin de Thau from the late 1960s until at least the mid 1970s; however, it is possible that some juvenile oysters have been imported during the last few years and grown to marketable size.

Significance of the new record

In Prince Edward Island, Canada, *Styela clava* is a major pest to the mussel farming industry

(Thompson and MacNair 2004). It settles on the mussel ropes and competes with the mussels for food, reducing mussel production by up to 40% and costing the mussel industry several million C$ per year. The recent discovery of *S. clava* in New Zealand (Davis and Davis 2006) caused considerable alarm; the green lipped mussel industry in New Zealand is worth an estimated NZ$ 150 million per year and is now considered to be at risk.

Many species of shellfish are farmed commercially in the Bassin de Thau, the most important being oysters. There are many similarities between oyster farming in the Bassin de Thau and mussel farming in Prince Edward Island, so the threat from *S. clava* should be similar. However, the two sites differ in the availability of suitable settlement substrate, a major resource limitation for growth of *S. clava* populations. The natural substrate available in Prince Edward Island is mainly soft, typically mud (Bourque et al. 2007), so *S. clava* exploits the mussel farming equipment. The abundant hard substrate in the Bassin de Thau may mitigate the threat of *S. clava* to oyster production.

Potential for further spread of *Styela clava*

The most important prerequisite for *Styela clava* to spread from one site to another is that the donor and receiving ecosystems are connected by a dispersal vector. Within the lagoon, *S. clava* will spread by natural larvae dispersion; the animals are free spawners so planktonic eggs and larvae can be carried to new sites by horizontal currents. In addition, drifting flotsam can carry attached juveniles and adults to new sites. Spread outside the lagoon is likely to be the result of larvae dispersion through the canals at Marseillan-Plage and Sète, possibly with sublittoral "stepping stone" populations established on suitable bottom substrate, although flotsam and man-aided transport of adult animals into the Mediterranean are possible. In either case, successful establishment depends on the receiving habitat being suitable; the key criteria appear to be salinity between 22 and 34.5 psu and summer water temperature in excess of 15°C for spawning (Bourque et al. 2007).

Spot salinities varied from 28 to 30 psu in the Bassin de Thau, and from 27 to 34.5 psu in some adjacent harbours (Annex 1). Therefore, it is possible that *S. clava* could become established in the Mediterranean in the Languedoc-Roussillon region. However, extensive range expansion is unlikely as salinity exceeded 34.5 psu in many of the Mediterranean harbours and marinas surveyed (Annex 2). In fact, 63 Mediterranean sites were inspected (Figure 2) but no specimens of *S. clava* were found at any. Most of the sites with salinity greater than 22 psu supported populations of other solitary ascidians, mainly *Styela plicata* (Lesueur, 1823) with occasional *Ciona intestinalis* (L.), *Molgula manhattensis* (De Kay, 1843) and *Ascidiella aspersa* (Müller, 1776). However, it should be remembered that the inability to find specimens at a site does not necessarily mean that there are no populations present in the area.

Water temperature may be an important factor controlling population growth. In 2006, a maximum surface water temperature of 29.1°C was recorded between Bouzigues and Sète in July (Ifremer 2007), although it only reached 24.3°C in 2007. The maximum recorded bottom water temperatures at the site were 26.6°C in July 2006 and 22.9°C in August 2007 (Ifremer 2007), temperatures that occur at other sites where *S. clava* has been found, so *S. clava* may be able to survive at depth. However the effect of temperature may be exacerbated by the development of anoxic conditions in the bottom waters of the lagoon during the summer months. Mazouni et al. (1996) noted anoxia in the lagoon in July which they thought could be responsible for a massive mortality of the benthos. Indeed, Mazouni et al. (2001) reported that only young ascidians (*Ciona intestinalis*, *Phallusia mammillata* and *Botryllus* sp.) were present five weeks after a bottom anoxia event in 1992, although the maximum water temperature recorded was only 26°C. Incidentally, although Mazouni et al. (2001) monitored the composition of biofouling communities in the Bassin de Thau during 1992, they did not record the presence of *S. clava*.

Conclusions

The solitary ascidian collected in the Bassin de Thau, near Sète, on June 1st, 2005 was identified as *Styela clava*. This is the first record of its presence in the Mediterranean region. The lagoon population appears to be sustainable. However, extensive searches of harbours and marinas along the adjacent Mediterranean coastline did not reveal any populations of *S. clava* in the Mediterranean Sea itself.

The proximity of commercial shellfisheries to the discovered populations, the lack of commercial shipping routes into the lagoon and the absence of *S. clava* from other harbours and marinas along the coast, suggest that the species may have been introduced by shellfish transfer.

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Annex 1

Sites examined along the French Mediterranean coast during this study

Site	Country	Record coordinates		Date	Salinity (psu)
		Latitude	Longitude		
Marsielle (Old Port)	France	43° 17.76'N	05° 22.36'E	26-Feb-04	34.8
La Pointe-Rouge	France	43° 14.58'N	05° 21.89'E	26-Feb-04	37.2
La Seyne	France	43° 06.25'N	05° 53.05'E	27-Feb-04	37.6
Toulon (Darse du Mourillon)	France	43° 06.88'N	05° 55.65'E	27-Feb-04	36.2
Toulon (Darse viéille)	France	43° 07.21'N	05° 55.96'E	27-Feb-04	37.4
Frejus	France	43° 25.25'N	06° 44.86'E	28-Feb-04	37.6
St Raphael (Port Santa Lucia)	France	43° 24.72'N	06° 46.95'E	28-Feb-04	37.4
St Maxime	France	43° 18.36'N	06° 38.43'E	28-Feb-04	36.2
Port Grimaud	France	43° 16.39'N	06° 34.52'E	29-Feb-04	36.4
Cavalaire-sur-Mer	France	43° 10.27'N	06° 32.15'E	29-Feb-04	37.8
Le Lavandou	France	43° 08.26'N	06° 22.34'E	29-Feb-04	37.8
Port-de-Miramar	France	43° 06.98'N	06° 14.85'E	29-Feb-04	37.5
Bandol	France	43° 07.95'N	05° 45.15'E	29-Feb-04	37.9
La Ciotat	France	43° 10.55'N	05° 36.70'E	29-Feb-04	37.8
La Ciotat (Old Port)	France	43° 10.45'N	05° 36.41'E	01-Mar-04	37.8
Cassis	France	43° 12.82'N	05° 32.17'E	01-Mar-04	27.3
Marseille	France	43° 17.76'N	05° 22.36'E	01-Mar-04	37.8
Martigues	France	43° 24.37'N	05° 03.00'E	02-Mar-04	10.6
Port-St-Louis	France	43° 23.27'N	04° 48.57'E	02-Mar-04	37.7
Villefranche	France	43° 41.87'N	07° 18.43'E	31-May-05	33.1
Menton (Old Port)	France	43° 46.54'N	07° 30.64'E	31-May-05	35.1
Menton (Garavan)	France	43° 47.05'N	07° 31.18'E	01-Jun-05	35.4
Cap D'Ail	France	43° 43.50'N	07° 25.02'E	01-Jun-05	35.9
Beaulieu sur Mer	France	43° 42.54'N	07° 20.15'E	01-Jun-05	34.8
Nice	France	43° 41.74'N	07° 17.02'E	01-Jun-05	22.6
Martigues	France	43° 24.10'N	05° 03.59'E	24-Feb-06	20.8
Port du Bouc	France	43° 24.17'N	04° 59.01'E	24-Feb-06	25.3
Saint-Gervais	France	43° 25.71'N	04° 56.48'E	24-Feb-06	34.9
Port-St-Louis du Rhone	France	43° 23.28'N	04° 48.56'E	24-Feb-06	27.0
Méze, Bassin de Thau	France	43° 25.29'N	03° 36.33'E	25-Feb-06	30.3
Marseillan, Bassin de Thau	France	43° 21.13'N	03° 32.06'E	25-Feb-06	28.3
Sete Marina	France	43° 23.75'N	03° 41.96'E	25-Feb-06	33.9
Le Barrou	France	43° 24.72'N	03° 39.61'E	25-Feb-06	29.8
Cap D'Agde	France	43° 17.03'N	03° 30.76'E	25-Feb-06	34.5
Palavas les Flots	France	43° 31.57'N	03° 55.88'E	26-Feb-06	32.9
Grande Motte	France	43° 33.40'N	04° 05.02'E	26-Feb-06	33.6
Port Camargue	France	43° 31.13'N	04° 07.83'E	26-Feb-06	34.4

Annex 2
Other sites examined in the Mediterranean during this study

Site	Country	Record coordinates		Date	Salinity (psu)
		Latitude	Longitude		
Monte Carlo (Port de Monaco)	Monaco	43° 44.16'N	07° 25.34'E	31-May-05	35.4
Monte Carlo (Port Fortville)	Monaco	43° 43.81'N	07° 25.15'E	01-Jun-05	35.8
Altea	Spain	38° 35.49'N	00° 03.30'W	03-Feb-06	34.8
Mascarat	Spain	38° 37.80'N	00° 00.17'W	03-Feb-06	22.5
Calpe	Spain	38° 38.34'N	00° 04.27'E	03-Feb-06	34.2
Moraira	Spain	38° 41.18'N	00° 08.15'E	03-Feb-06	34.0
Denia	Spain	38° 50.72'N	00° 06.57'E	03-Feb-06	28.4
Alicante	Spain	38° 35.49'N	00° 03.30'W	04-Feb-06	35.0
Santa Pola	Spain	38° 11.42'N	00° 33.83'W	04-Feb-06	35.3
Torrevieja	Spain	37° 58.48'N	00° 41.00'W	04-Feb-06	35.5
Benidorm	Spain	38° 32.07'N	00° 07.99'W	04-Feb-06	35.5
Gandia	Spain	38° 59.83'N	00° 09.38'W	05-Feb-06	33.1
Cagliari Port	Sardinia	39° 01.39'N	09° 06.91'E	12-Apr-06	27.9
Cagliari Marina	Sardinia	39° 12.07'N	09° 07.62'E	12-Apr-06	16.7
Porto di La Caletta	Sardinia	40° 36.77'N	09° 45.14'E	14-Apr-06	35.9
Arbatax	Sardinia	39° 56.63'N	09° 42.09'E	14-Apr-06	35.0
Palau	Sardinia	41° 10.81'N	09° 23.11'E	15-Apr-06	36.0
Porto Rotundo	Sardinia	41° 01.71'N	09° 32.76'E	15-Apr-06	35.8
Porto Torres (Marina)	Sardinia	40° 50.37'N	08° 24.19'E	16-Apr-06	35.4
Alghemar Marina	Sardinia	40° 03.67'N	08° 18.89'E	16-Apr-06	35.6
Porto Torre Grande (Marina)	Sardinia	39° 54.31'N	08° 29.49'E	17-Apr-06	35.3
Portoscuso	Sardinia	39° 12.18'N	08° 22.77'E	17-Apr-06	35.2
Calasetta	Sardinia	39° 06.73'N	08° 22.16'E	18-Apr-06	36.2
Buggerru	Sardinia	39° 24.06'N	08° 23.95'E	18-Apr-06	31.2
Genoa (Porto Antico)	Italy	44° 24.43'N	08° 55.48'E	19-Jul-04	36.9
Napoli	Italy	40° 50.33'N	14° 15.27'W	10-Aug-06	32.1
Reggio di Calabria	Italy	38° 07.71'N	15° 09.12'E	21-Aug-06	35.8
Salerno	Italy	40° 40.64'N	14° 44.87'E	24-Aug-06	31.2