

Short communication

First record of the non-native light bulb tunicate *Clavelina lepadiformis* (Müller, 1776) in the northwest Atlantic

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Abstract

We report the first record of the colonial tunicate *Clavelina lepadiformis* (Müller, 1776) in the northwest Atlantic. Populations were found along the eastern Connecticut shoreline in October 2009. At one site *C. lepadiformis* had a mean percent cover of 19.95% (± 4.16 S.E.). A regional survey suggests that the invasion is relatively localized. Genetic analysis confirms our morphological identification and places the introduced population in the previously described 'Atlantic clade'. While it appears *Clavelina lepadiformis* is currently in the incipient stage of introduction in eastern Connecticut waters, its spread to other areas in the region could lead to competition with resident members of shallow water epifaunal assemblages and shellfish species.

Key words: invasive species, *Clavelina lepadiformis*, Long Island Sound, tunicate, ascidian, nonindigenous

Clavelina lepadiformis (Müller, 1776) is a distinctive and conspicuous colonial tunicate, commonly called the light bulb tunicate (Figure 1). Its native range was initially reported to be from the Shetland Islands and Bergen, Norway in the north to the Bay of Biscay, the Mediterranean, and the Adriatic in the south (Berrill 1951). During the 1990s, *C. lepadiformis* was reported in the Azores and Madeira, Portugal (Wirtz and Martins 1993; Wirtz 1998). During the 2000s, *C. lepadiformis* invaded Knysna Estuary and Port Elizabeth, South Africa (Primo and Vazquez 2004; Robinson et al. 2005).

We report the first record of *Clavelina lepadiformis* in the northwest Atlantic. Relatively dense but localized populations were found in southeastern CT, USA: specifically, Stonington Harbor (October 16, 2009, sites 1 and 2; Annex 1) and the lower Thames River estuary (November 11, 2009, sites 15-16; Annex 1). Elsewhere colonies are reported to be limited to shallow littoral habitats (<50m) occupying natural or artificial hard substrates (e.g., rocky outcrops, wooden docks) and are known to dominate disturbed habitats (Naranjo et al. 1996; Picton and Morrow 2007). We found them to occur only on vertical man-made structures (e.g.,

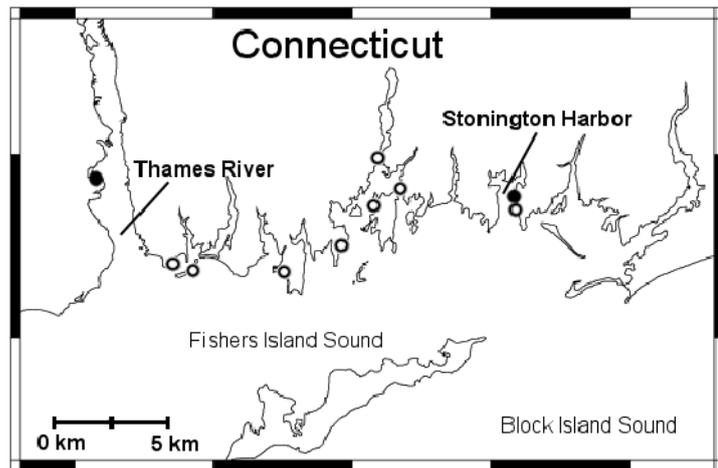
breakwaters, pilings) in relatively shallow water depths (to 3 m). The species can tolerate a relatively broad range of salinities (14 – 35 psu; Millar 1971). At both sites where we found *C. lepadiformis*, salinities were 29.5 psu, although near bottom salinities can be ~ 20 psu in the lower Thames River during the spring. Colonies typically consisted of a rosette of zooids showing connection only by a common basal test or stolons. Colonies were composed of a few, tens or hundreds of zooids, and fully developed zooids were 5-6 cm long. The thorax of *C. lepadiformis* is reported to be clear except for white, yellow, or pink bands around the oral siphon and along the dorsal lamina (Berrill 1951). All individuals found at Stonington Harbor and the Thames River had white pigmentation around the oral siphon (Figure 1).

As with all colonial tunicates, *C. lepadiformis* reproduces sexually, and brooding larvae reside at the base of the zooid atrial chamber. Once released, the free swimming period of the tadpoles is ~3 hr. Following settlement, formation of oozoids is completed in ~2 to 3 days (Berrill 1951). Colonies collected from Stonington Harbor contained numerous brooded larvae and embryos.

Figure 1. Photograph of *Clavelina lepadiformis* taken October 16, 2009 in Stonington Harbor, Connecticut. Scale bar ~ 1 cm. (Photograph by J. F. Reinhardt).



Figure 2. Map of eastern Connecticut shoreline. Closed circles represent sites where *C. lepadiformis* is present and open circles where it is absent. Circles correspond to survey sites listed in Annex 1. Not all surveyed sites are shown.



Following its discovery, we conducted preliminary surveys to estimate percent cover of *C. lepadiformis* on the dock where it was initially found in Stonington Harbor (site 2, Annex 1) as well as qualitatively examining other natural and artificial surfaces of the harbor and adjacent coves and embayments within ~ 16 km to assess its current distribution (Figure 2). Two 20 m transects were run at 1 m and 2 m below MLW along the dock using SCUBA. Underwater photographs were taken at random locations along each transect ($n = 8$) and percent cover of *C. lepadiformis* was estimated using 100 grid counts placed over the images. Colonies occupied on average $19.95\% \pm 4.16$ S.E. (range = 36.59% to 6.12%) of space on the dock. Other invasive ascidians can have percent cover on this order (Osman and Whitlatch 2004; Dijkstra et al.

2007). Our search for the species at nearby docks and other structures in the harbor revealed the species was only present on the pier immediately adjacent to the primary dock (within 65 m) but at much lower densities (5 colonies; $\sim < 1\%$ cover). No colonies were observed in surveys of sites in the adjacent Mystic and Poquonnock Rivers. A recreational boating and fishing pier in the lower Thames River (~16km distance from Stonington Harbor; site 15/16, Annex 1) was found to have low densities ($\sim 1\%$ cover) of *C. lepadiformis*. Underwater photographs taken December 2008 at this pier also revealed the presence of the species, indicating that the population appeared to have over-wintered. During 2008, other sites in the lower Thames River had been surveyed, none of which had *C. lepadiformis* present (Annex 1).

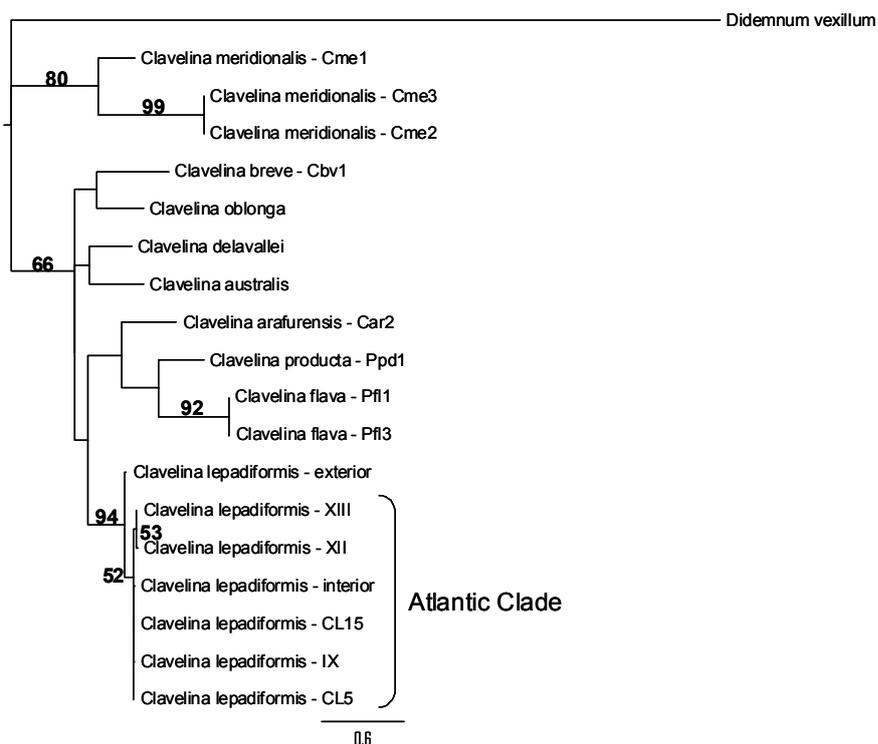


Figure 3. Maximum likelihood tree of CO1 (369 bp). All branches with greater than 50 percent bootstrap support are labeled. Sequences of *C. lepadiformis* from the Atlantic clade (Turon et al. 2003) are indicated with a bracket. Scale bar indicates number of substitutions/site.

In order to confirm our morphological identification, a 586 base pair fragment of the mitochondrial gene CO1 was isolated from two colonies of *C. lepadiformis*, one from either side of the pier in Stonington Harbor where the species was initially found (site 2, Annex 1). DNA was isolated from the branchial sacs of individual zooids using a CTAB based method, PCR amplified with tunicate-specific primers, and sequenced from plasmids (Stefaniak et al. 2009 for further details and primer sequences). The resultant sequences were aligned in Clustal X (v1.83, Chenna et al. 2003) with sixteen sequences from nine species of *Clavelina* obtained from GenBank as well as a sequence from *Didemnum vexillum* as an outgroup (Annex 2). The final alignment was 369 bases in length with no gaps. A maximum likelihood analysis was implemented in PAUP* (Swofford 2003) using the GTR+G substitution model (e.g. Lavane et al. 1984). The substitution model was chosen based on the AIC scores of several models, and the model parameters values

Table 1. Model parameters used in Maximum Likelihood analysis.

Model Parameter	Parameter Value
Base Frequencies	
A	0.339645
C	0.072419
G	0.097182
T	0.490754
Substitution Rate Matrix	
AC	0.23400
AG	9.73780
AT	0.13165
CG	2.19251
CT	7.13916
GT	1.00000
Number of Substitution Rate Categories	
	5
Shape	0.185483

estimated during model testing were used for the subsequent maximum likelihood analysis with 100 bootstrap pseudoreplicates (Table 1). Branch lengths on the consensus tree (Figure 3) were optimized in PAUP* using the same model.

Bootstrap support was weak over much of the tree (Figure 3), most likely due to the short length of the alignment, however, samples from Stonington Harbor morphologically identified as *C. lepadiformis* group strongly (94% support) with other samples of *C. lepadiformis* and weakly (52% support) with samples identified as belonging to the Atlantic clade of the species by Turon et al. (2003). The two samples from Connecticut were identical to haplotype IX from Turon et al. (2003) which was the most common haplotype sampled in that study and was found throughout the native range of *C. lepadiformis* as well as in the Azores which is considered to be a non-native location (Wirtz and Martins 1993; Turon et al. 2003).

It is likely that the introduction of *C. lepadiformis* to the northwest Atlantic was human-mediated given its short non-feeding larval life span and the past history of human-mediated dispersal of other tunicate species (Lambert and Lambert 1998; Lambert 2001). Turon et al. (2003) found only colonies of *C. lepadiformis* belonging to the Atlantic clade exclusively inside harbors in the Mediterranean and not on sites with natural substrates suggesting that the Mediterranean harbor populations are the result of ship-mediated invasion from the Atlantic. The introduction likely resulted from hull fouling, fouling of sea chests, or (less likely) larvae transported in ballast water (Carlton and Geller 1993; Coutts and Dodgshun 2007). It is presently uncertain whether populations in Stonington Harbor and the Thames River were established from individuals from European populations or are the result of a secondary invasion within the NW Atlantic region. The Thames River has a variety of maritime operations including commercial shipping operating from the New London State Pier, private boating, fishing vessels, and berthing for cruise ships. The Thames River is also home to a U.S. Coast Guard station and a U.S. Navy submarine base. Stonington Harbor has a commercial fishing fleet and private recreational boating. Locke (2009) predicted *C. lepadiformis* as one of the most likely species to become established in Atlantic Canada, because of its broad native and invaded distributional range. While potential ecologic and economic impacts of this new invader are presently unknown, the species is known to form relatively dense aggregations (de Caralt et al. 2002 per. obs.) and may have the potential of over-growing and out competing resident fouling

species and economically important shellfish. As the distribution of *C. lepadiformis* appears to be localized in southeastern Connecticut, we recommend an increase in surveying efforts along the southern New England coastline to better understand its current distribution and suggest that the species may be a good candidate for undertaking an immediate eradication program in order to prevent its spread.

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Annex 1. Location and dates surveyed along the southeastern Connecticut shoreline.

Location	Survey date	Location coordinates		Presence/ Absence	Habitat type	Site number
		Latitude °N	Longitude °W			
Stonington Harbor	Oct 2009	41,334542	71,910481	Present	State fixed pier	1
	Oct 2009	41,333889	71,910400	Present	Private wooden pier	2
	Oct 2009	41,333622	71,910378	Present	Wood wave attenuator	3
	Oct 2009	41,331747	71,909525	Absent	Private wooden pier	4
	Sep 2008	41,340917	71,911044	Absent	Private wooden pier	5
Mystic River	Sep 2008	41,338375	71,974281	Absent	Private floating dock	6
	Sep 2008	41,338375	71,959539	Absent	Private floating dock	7
	Oct 2009	41,351408	71,971528	Absent	Private floating dock	8
	Oct 2009	41,324531	71,984778	Absent	Commercial pier	9
Groton Long Point	Sep 2008	41,312567	72,010692	Absent	Private floating dock	10
	Sep 2008	41,320653	72,014883	Absent	Private floating dock	11
Poquonnock River	Sep 2009	41,315358	72,053147	Absent	Natural rock	12
	Sep 2009	41,316317	72,060664	Absent	University floating dock	13
	Sep 2009	41,313456	72,060547	Absent	Stone pier	14
Thames River	Dec 2008	41,351153	72,094583	Present	Public pier	15
	Nov 2009	41,351153	72,094583	Present	Public pier	16
Niantic River	Aug 2009	41,317003	72,195250	Absent	Natural rock	17
	Aug 2009	41,315383	72,178883	Absent	Natural rock	18
	Sep 2008	41,326706	72,175739	Absent	Town floating dock	19
	Sep 2008	41,311047	72,201489	Absent	Private floating dock	20
Branford, CT	Oct 2008	41,258808	72,818797	Absent	Private floating dock	21

Annex 2. Accession numbers of CO1 sequences.

Species name	Accession number	Haplotype/ sequence name	Reference
<i>Clavelina arafurensis</i>	AM706463.1	Car2	Pérez-Portela and Turon 2007
<i>Clavelina australis</i>	AM706464.1	-	Pérez-Portela and Turon 2007
<i>Clavelina breve</i>	AM706465.1	Cbv1	Pérez-Portela and Turon 2007
<i>Clavelina delavallei</i>	AY603105.1	-	Turon and López-Legentil 2004
<i>Clavelina flava</i>	AM706475.1	Pfl1	Pérez-Portela and Turon 2007
<i>Clavelina flava</i>	AM706477.1	Pfl3	Pérez-Portela and Turon 2007
<i>Clavelina lepadiformis</i>	AF368352.1	interior	Tarjuelo et al. 2001
<i>Clavelina lepadiformis</i>	AF368353.1	exterior	Tarjuelo et al. 2001
<i>Clavelina lepadiformis</i>	AY211529.1	IX	Turon et al. 2003
<i>Clavelina lepadiformis</i>	AY211530.1	XII	Turon et al. 2003
<i>Clavelina lepadiformis</i>	AY211531.1	XIII	Turon et al. 2003
<i>Clavelina lepadiformis</i>	HM012482	CL5	This study
<i>Clavelina lepadiformis</i>	HM012483	CL15	This study
<i>Clavelina meridionalis</i>	AM706468.1	Cme1	Pérez-Portela and Turon 2007
<i>Clavelina meridionalis</i>	AM706469.1	Cme2	Pérez-Portela and Turon 2007
<i>Clavelina meridionalis</i>	AM706470.1	Cme3	Pérez-Portela and Turon 2007
<i>Clavelina oblonga</i>	AY603106.1	-	Turon and López-Legentil 2004
<i>Clavelina producta</i>	AM706482.1	Ppd1	Pérez-Portela and Turon 2007
<i>Didemnum vexillum</i>	EU419401.1	-	Stefaniak et al. 2009