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ORIGINAL PAPER

The broadcast spawning Caribbean shipworm, *Teredothyra dominicensis* (Bivalvia, Teredinidae), has invaded and become established in the eastern Mediterranean Sea

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Abstract Teredinids, commonly referred to as shipworms, are wood-boring bivalves estimated to cause over one billion dollars' worth of damage to submerged wooden structures per annum. This paper reports the detection and establishment of the Caribbean shipworm *Teredothyra dominicensis* (Bivalvia, Teredinidae) in the eastern Mediterranean Sea. Identification was confirmed using an integrative taxonomical approach combining morphology, morphometry and molecular markers (COI-5P and 18S), thus improving both the taxonomic resolution and tractability of this invasive species. Sequence comparisons between indigenous Caribbean and Mediterranean specimens were at least 99 % identical. Wooden panels placed at the site of discovery were infested exclusively by *T. dominicensis* with specimens of varying size and age, indicating multiple settlement events and the presence of breeding populations in the region. Anatomical and behavioural observations confirm the species as a broadcast spawner with larvae undergoing planktotrophic development, thus distribution range is potentially extensive. Of the possible introduction vectors, transport via ballast water is proposed as the most likely. The establishment of breeding populations of a tropical teredinid in the Mediterranean is of considerable concern as tropical species are particularly destructive and degrade wood more rapidly than the species currently found in the region. This threat is likely to increase in severity due to global warming, as increases in temperature and salinity may lead to an increase in the distribution range, development rate and boring activity of teredinids.

Keywords Distribution · Invasive · Teredinid · Shipworm · Life History strategies · COI-5P and 18S sequences

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Introduction

Xylotrophy has evolved in a range of invertebrates to exploit the wood entering the sea via rivers and mangroves. More recently, human activity has provided new niches for xylotrophs, including boats, piers, and sea defences. Teredinids, commonly referred to as shipworms, are the principal consumers of wood in the marine environment. These highly specialised bivalves are characterised by elongated bodies and a greatly reduced shell, adaptations which facilitate their wood-boring life style. Adult shipworms are confined to the timber into which they burrowed as newly metamorphosed larvae: continued burrowing gradually

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destroys this habitat, thus there are strong selection pressures for the evolution of life history strategies that maximise dispersal.

Teredinids readily colonise driftwood and are thus pre-adapted for dispersal by rafting (Thiel and Gutow 2005). The rafting voyages of floating timber may extend beyond regional scales *i.e.*, >1,000 km (Donlan and Nelson 2003), and the extensive distribution of some teredinids have resulted from rafting (Edmondson 1962). Dispersal may also be achieved during the larval stage; generally, species with a longer planktotrophic stage have a larger dispersal potential. Species with planktonic larvae may be carried along the coasts of continents and even trans-oceanically (Scheltema 1971) and the adults and larvae of several species are thought to be highly adapted to an oceanic existence, which greatly extends the distribution range (Edmondson 1962).

Human maritime activity is also believed to have contributed towards the cosmopolitan distribution of certain species. Wooden sailing vessels can become regularly colonised by shipworm (Woods Hole Oceanographic Institution 1952; Turner 1966) which are then transported beyond their natural distribution range (Carlton 1999). Furthermore, tropical species have been known to survive freezing temperatures during transit (Turner 1966). The decline in traffic of seafaring wooden vessels has limited this means of dispersal, though the established distributions of teredinids may still reflect the influence of this former means of dispersal. Modern shipping, particularly the transportation of ballast water, has provided a new vector by which they may now spread. Larvae of the shipworm *Teredo navalis* have been discovered in ballast water and it is likely that larvae from other teredinids are present in ship ballast water across the globe (Gollasch 2002; Carlton 1999).

Teredinids display one of three developmental modes: broadcast spawners with planktotrophic larvae and short-term brooders and long-term brooders. Broadcast spawners release large quantities of eggs in a single spawning (Sigerfoos 1908), yet parental investment is limited and larvae spend an extended period of time (20–25 days) developing in the water column (Turner 1966). Broadcast spawners have an extensive distribution range, yet only a fraction of the spawned eggs will survive to become successful larvae (Scheltema 1971). Both short-term and long-term brooders fertilize internally with larvae developing in specialised brood pouches on the parental gill. The former release larvae at the straight-hinged veliger stage and development continues in the water column for between 10 and 15 days (Calloway and Turner 1988), whilst the latter release competent pediveligers which are capable of settling immediately (Cragg et al. 2009). The long-term brooders have a higher likelihood of geographical larval retention and re-recruitment and are therefore often localised (Strathmann et al. 2002). Indeed, successive generations may remain in the same piece of wood. Short-term brooders are believed to represent the optimum strategy for dispersal and are geographically widespread (MacIntosh et al. 2012). However, species representing all strategies have become successfully distributed across the globe (Turner 1966).

Reproductive mode may be determined by examination of teredinid spermatozoa, as the ratio between the axial rod length and sperm body length is higher in sperm of external fertilizers than internal fertilizers (Popham 1974). Furthermore, brooding species may be identified by the presence of larvae in the gill (Turner 1966). Morphologically identical (cryptic) species may sometimes be distinguished when the developmental mode is examined. For example, *Lyrodus floridanus* is morphologically identical to *Lyrodus pedicellatus*, but can be differentiated on the basis that larvae are brooded to the D-shaped veliger stage, whereas *L. pedicellatus* releases advanced pediveligers (Calloway and Turner 1983).

Typically, bivalve identification is based on shell characteristics; yet the shells of teredinids are taxonomically uninformative (Turner 1966). Instead, interspecific variations in pallet features, the calcareous structures which plug the entrance to the burrow, are used (Turner 1971). Recently, morphological identification has been integrated with molecular techniques (16S rRNA, COI-5P subunit and 18S ribosomal markers) to improve the taxonomic resolution within the Teredinidae (Santos et al. 2005; Borges et al. 2012).

This paper reports the establishment of a substantial population of a Caribbean teredinid, *Teredothyra dominicensis*, in the eastern Mediterranean Sea. Integrative taxonomy, including DNA barcoding, nuclear locus sequencing, morphological and morphometric examination, was used to confirm the identity and increase the taxonomic resolution of this species. As previous descriptions of the reproductive mode and

larval development type of *T. dominicensis* were based on a small number of poorly preserved specimens (Turner 1966), investigations were undertaken to clarify these characteristics in order to predict the ability of this invasive species to establish and disperse throughout the region.

Teredinids are estimated to cause over one billion dollars' worth of damage to submerged wooden structures per annum and the introduction of shipworms into new areas is often followed by rapid and extensive destruction (Turner 1966; Cohen and Carlton 1995; Distel et al. 2011). This can be further compounded by costly restoration and the interruption of business (Fernandes and Costa 1967 cited in Filho et al. 2008). As the most effective means for wood protection such as treatment with chromated copper arsenate (CCA) are now heavily restricted, the spread of shipworms is still a major economic issue.

The appearance of *T. dominicensis* in the Mediterranean is of particular concern as tropical teredinid species are more destructive than their temperate counterparts (Castagna 1961; Southwell and Bultman 1971). As the Mediterranean is gradually increasing in both temperature and salinity due to global warming (Giorgi and Lionello 2008; Giannakopoulos et al. 2009; Coma et al. 2009)—factors which are known to increase distribution range (Borges et al. 2010), boring activity (Paalvast and van der Velde 2011) and speed at which teredinids reach sexual maturity (Ibrahim 1981), their distribution range will continue to increase.

Materials and methods

Sample collection and rearing

Sample collection was carried out in Kaş in southern Turkey, during August 2010 and June 2011. Infested wood was recovered from the shipwreck, Uluburun III, located approximately 36 m below mean sea level. Wooden panels of *Pinus sylvestris* (2.5 cm × 10 cm × 20 cm) were also attached to the mast of the wreck during August 2010 for retrieval and analysis the following year. Caribbean specimens were collected in Carlisle Bay, Barbados, from wooden panels exposed over a 6 month period at 15 m below mean sea level. Aegean sampling was conducted during SCUBA dive surveys.

Specimens of *T. dominicensis* were reared in the aquaria at the Institute of Marine Sciences, University of Portsmouth, for 1 year. The seawater was maintained on a flow-through system at 25 °C and a salinity of 33 Practical Salinity Units (PSU). A larval capture device (Raskoff et al. 2003) was installed to monitor gamete and larval release.

Identification and morphometry

Calcareous structures were photographed using a stereo microscope. Images were captured using the JVC KY-F 1030U digital camera with accompanying KY0Link image capture program. Pallets and shells were measured using the image analysis programme, ImageJ. Pallet dimensions were measured from the base of the stalk to the tip of the blade to give the length, and across the broadest section of the blade to give width. Identification was based on species descriptions and pallet characteristics provided by Turner (1971).

Molecular identification

DNA was extracted from siphonal tissue and associated musculature. Total genomic DNA was extracted using DNeasy Blood & Tissue kit (Qiagen), deviating from the manufacturer's protocol as follows: tissue was lysed overnight at 56 °C and DNA was eluted from the spin column using molecular grade water (2 × 70 µL washes) as opposed to elution buffer. Concentration, yield and purity of DNA were determined by UV spectrophotometry, with 1 absorption unit at 260 nm approximately equal to 50 ng/µL dsDNA). When necessary, DNA template was diluted using molecular grade water to a concentration of 10–20 ng/µL.

A 658 bp fragment from the 5′ end of the cytochrome oxidase subunit I (COI-5P) was amplified using the primer pair LCO1490 (forward 5′ GGT CAA CAA ATC ATA AAG ATA TTG G 3′) and HCO2198 (reverse 5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′) (Folmer et al. 1994). Amplifications were performed in 25 µL reactions, with each reaction containing (stock concentrations in parentheses): 2.5 µL 10 × PCR buffer, 2.5 µL $MgCl_2$ (25 mM), 0.25 µL dNTP mixture (10 mM each), 0.5 µL of each primer (10 mM), 0.25 µL Taq Polymerase (5 U/µL), 1–2 µL DNA template (10–20 ng/µL) and brought to

volume with molecular grade water. The PCR protocol was as follows: an initial cycle of 94 °C for 90 s, 45 °C for 90 s and 72 °C for 60 s, followed by 35 cycles of 94 °C for 30 s, 51 °C for 90 s and 72 °C for 60 s, with a final extension of 72 °C for 5 min.

A small fragment of the 18S rRNA gene, approximately 345 bp, was amplified using the primer pair SSU_FO4 (5′-GCTTGTCTCAAAGATTAAGCC-3′) and SSU_R22 (5′-GCCTGCTGCCTTCCTTGGA-3′) (Blaxter et al. 1998). Amplifications were performed in 25 µL reactions, with each reaction containing (stock concentrations in parentheses): 2.5 µL 10 × PCR buffer, 2.5 µL $MgCl_2$ (25 mM), 0.25 µL dNTP mixture (10 mM each), 0.5 µL of each primer (10 mM), 0.25 µL Taq Polymerase (5 U/µL), 1–2 µL DNA template (10–20 ng/µL) and bought to volume with molecular grade water. The PCR protocol was as follows: an initial cycle of 2 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 45 s at 57 °C, 3 min at 72 °C and a final extension of 10 min at 72 °C.

A 6 µL aliquot of PCR product was then electrophoresed in a 2 % agarose gel. Amplified products were purified using a NucleoSpin Gel and PCR Clean-up kit (Macherey–Nagel, Duren, Germany) according to the manufacturer's guidelines. All PCR products (COI-5P and 18S) products were sequenced by Source Bioscience.

Data analysis

COI-5P and 18S sequences were edited and aligned using MEGA 5.1 (Tamura el al. 2011). Sequences were uploaded onto GenBank (accession numbers will be provided upon acceptance of paper). Edited sequences were compared with those on the GenBank database to confirm species identity and ensure that endosymbiont bacteria or other contaminant had not been co-amplified in error. Sequences were aligned using Clustal W (Tamura et al. 2011) and COI sequences were translated to check for the presence of frameshift mutations, stop codons or unusually divergent amino acid profiles. Sequences consisting of 658 and 345 bp for COI and 18S, respectively, were used for phylogenetic inference using Neighbour-joining (NJ). Neighbour joining trees for both COI and 18S sequences were constructed using the Kimura 2-parameter model (K2P) with the programme MEGA 5.1. Selected GenBank sequences were used to compare with our data set and to be used as outgroups.

Electron microscopy

Samples for observation under the electron microscope were fixed in 4 % v/v glutaraldehyde in a cacodylate buffer (0.2 M sodium cacodylate, 0.3 M sodium chloride, 2 mM calcium chloride) for 1 h at room temperature. Samples were then rinsed three times in buffer for 10 min each. All samples were then post-fixed in 1 % w/v osmium tetroxide for 1 h and rinsed three times in seawater for 10 min each. Samples were then taken immediately through an ethanol dehydration series, evaporation dried via hexamethyldisilazane (HMDS) and then mounted on aluminium stubs. Sputter coating was carried out under an argon atmosphere using a gold and palladium target, at a voltage of 1.4 kV using a current of approximately 18 mA for 3 min. Specimens were examined using a JEOL 6060LV Scanning Electron Microscope operating in secondary electron mode using an accelerating voltage of 18 kV.

Results

Identification

Three species of teredinid, *Lyrodus pedicellatus* (Quatrefages), *Nototeredo norvagica* (Spengler) and *Teredothyra dominicensis* (Bartsch), were identified from sampling sites in the Mediterranean. Specimens were predominantly acquired from a shipwreck off the coast of Kaş, in which *T. dominicensis* was the dominant species present (93 out of 104 specimens). Wooden panels placed at the wreck site and recovered the following year were colonised exclusively by *T. dominicensis*. A single specimen of this species was also found in a piece of driftwood recovered off the coast of Fourni Island (Greece). *T. dominicensis* was the only species found in wooden panels placed off the Barbadian coast.

The pallets were identified as *T. dominicensis* (Fig. 1a) using both the original description of pallet features by Bartsch (1921) and the key in Turner (1971). Pallets collected from Caribbean specimens were indistinguishable from those collected in the

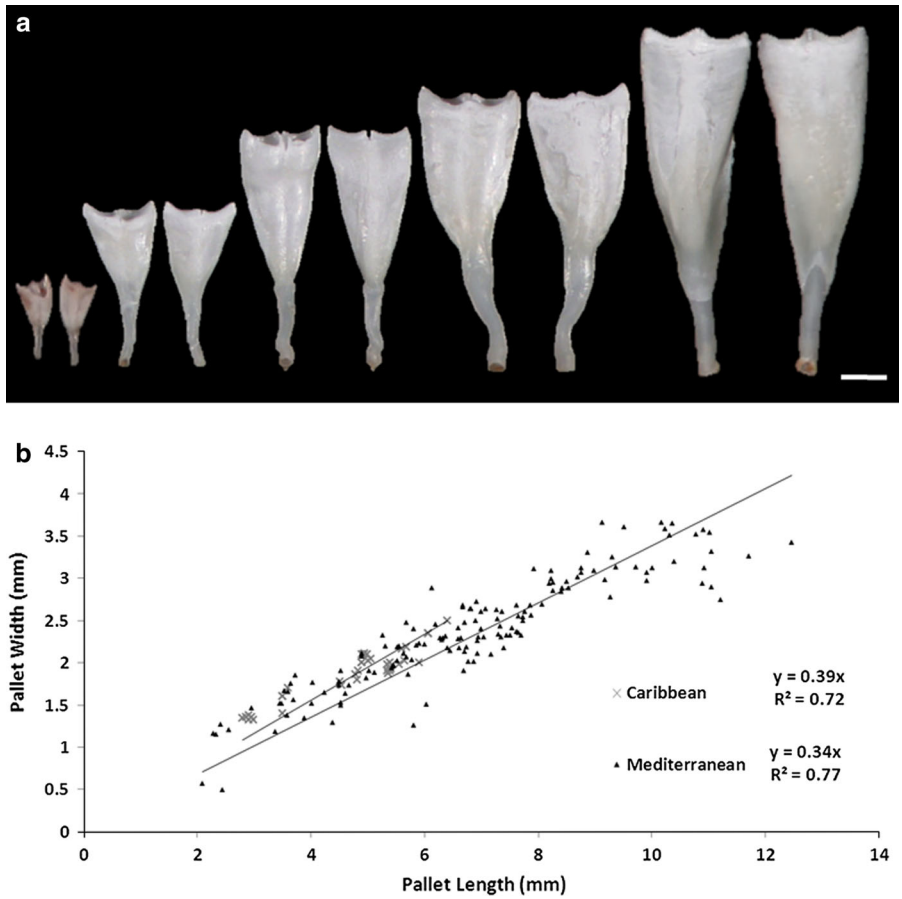

Fig. 1 Variation in pallet characteristics of *Teredothyra dominicensis*. **a** Inner and outer pallet face of pallet pairs taken from specimens extracted from panels placed at the Uluburun III wreck site. *Scale bar* 1 mm. **b** Variation in pallet proportions of Caribbean and Mediterranean specimens of *Teredothyra dominicensis* during growth

Mediterranean and Aegean Seas and the shifts in pallet proportions during ontogeny between the Mediterranean and Caribbean specimens (Fig. 1b) were also indistinguishable (GLM $P = 0.954$).

COI-5P and 18S sequences of sampled teredinids

A total of nine COI-5P sequences were obtained for *T. dominicensis* (seven Mediterranean specimens, one Caribbean and one Aegean specimen) all of which were free from stop codons or indels. A BLAST comparison of all sequences with those already published for *T. dominicensis* in GenBank revealed a 99 % or more maximum identity (Borges et al. 2012). Both nucleotide and amino acid sequences aligned unambiguously with those from other teredinids. An average intraspecific divergence of 0.78 % was observed between specimens of *T. dominicensis* obtained in this survey. These diverged by an average of 0.43 % with COI-5P sequences from *T. dominicensis* uploaded on GenBank (Borges et al. 2012). The Caribbean specimen diverged by an average of 0.43 % with specimens obtained from the Mediterranean, 0 % with specimens on GenBank (Borges et al. 2012) and 1.7 % with the Aegean specimen. The *T. dominicensis* specimen from the Aegean Sea matched the specimens located in the Mediterranean from this survey (average pairwise distance of 1.81 %) and also with other *T. dominicensis* specimens uploaded onto GenBank (Borges et al. 2012). The average pairwise distances between *T. dominicensis* and *L. pedicellatus*, *N. norvagica* and *Bankia carinata* were 29.6, 26.2 and 33.7 %, respectively.

Thirteen 18S sequences, all free from indels, were obtained for *T. dominicensis* (nine of which were from Mediterranean specimens, three from Caribbean specimens and one from the Aegean Sea). A BLAST comparison of the sequences in GenBank from the

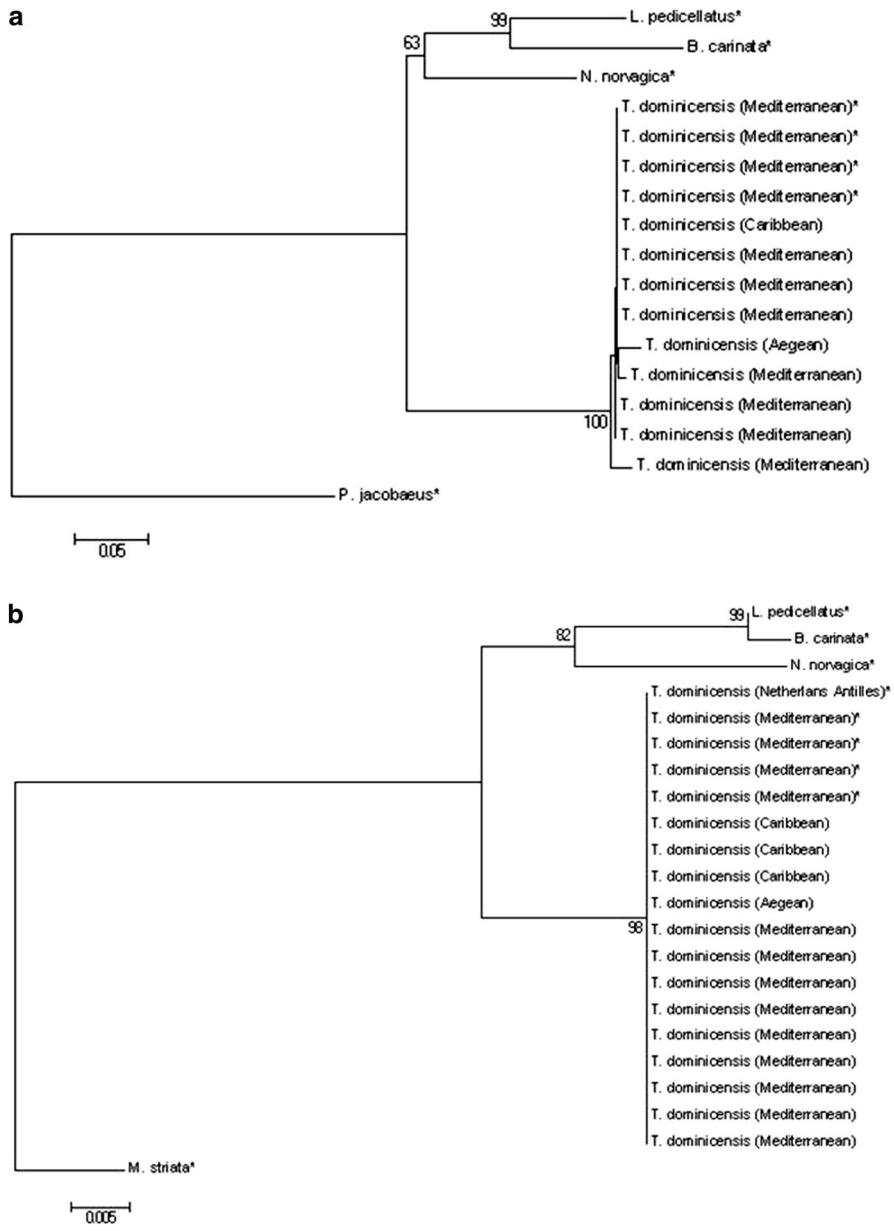

Fig. 2 Molecular identification of *Teredothyra dominicensis* based on COI-5P and 18S sequences. **a** Neighbour-joining nucleotide tree based on the partial sequences of the cytochrome c subunit I gene (COI-5P). *Asterisks* indicate sequences obtained from GenBank. **b** Neighbour-joining tree based on the partial sequences of the 18S rRNA gene obtained from Mediterranean, Aegean and Caribbean specimens of *T. dominicensis*. Asterisks indicate sequences obtained from GenBank. Phylogenetic trees include the teredinids *Lyrodus pedicellatus*, *Nototeredo norvagica* and *Bankia carinata*. The bivalves *Pecten jacobaeus* and *Martesia striata* are used as outgroups in the COI-5P and 18S tree, respectively

Mediterranean, Aegean and Caribbean specimens revealed a 99–100 % maximum identity with sequences previously published for *T. dominicensis* (Distel et al. 2011; Borges et al. 2012). Pairwise comparison revealed 0 % divergence between all *T. dominicensis* specimens from this study (Mediterranean, Aegean and Caribbean) and these showed 0 % divergence with published sequences of *T. dominicensis* from both the Mediterranean (Borges et al. 2012) and Netherlands Antilles (Distel et al. 2011). The pairwise distances between *T. dominicensis* and *L. pedicellatus*, *N. norvagica* and *B. carinata* were 3.6, 4.0 and 4.0 %, respectively. Although the 18S alignment was more conserved than the COI, the phylogenetic tree topologies were analogous. As tree topologies of COI-5P sequences of nucleotides and amino acid were identical, only the nucleotide tree is shown here. The tree building method used for COI-5P

Table 1 Pairwise COI-5P nucleotide (black) and 18S rRNA sequence (blue) divergence for teredinid groups using K2P distances (%)

Taxon	Within species	Average pairwise distance (%)				
		Between species				
		1	2	3	4	5
1 T. dominicensis Mediterranean	0.78/0.0					
2 J. dominicensis Mediterranean[a]	0.0/0.0	0.43/0.0				
3 T. dominicensis Caribbean	N/A	0.43/0.0	0.0/0.0			
4 Lyrodus pedicellatus[a]	N/A	29.6/3.6	26.0/3.6	29.2/3.6		
5 Nototeredo norvagica[a]	N/A	26.2/4.0	29.2/4.0	26.0/4.0	23.5/3.6	
6 Bankia carinata[a]	N/A	33.7/4.0	32.2/4.0	33.1/4.0	28.6/3.3	19.6/3.4

N/A not applicable, denotes group with only a single specimen

[a] Sequences obtained from GenBank

Table 2 Sequence details

Species (no. of specimens)	Location	GenBank accession details		
		Accession no. COI accession no. 18 Source		
Teredothyra dominicensis (9)	Kaş, Turkey			This study
Teredothyra dominicensis (4)	Kaş, Turkey	KC157940–KC157943	KC158219–KC158222	Borges et al. (2012)
Teredothyra dominicensis (1)	Fourni, Greece			This study
Teredothyra dominicensis (1)	Carlisle Bay, Barbados			This study
Teredothyra dominicensis (1)	Netherlands Antilles		JF899225	This study
Nototeredo norvagica (1)	Mersin Bay, Turkey	KC157926	KC158207	Borges et al. (2012)
Lyrodus pedicellatus (I)	Berder, France	KC157937	KC158216	Borges et al. (2012)
Bankia carinata (1)	Mersin Bay, Turkey			Borges et al. (2012)
Martesia striata (1)	Indonesia		JF899213	Distel et al. (2011)
Pecten jacobaeus (1)	Ankara, Turkey	JQ623969		Keskin, unpublished

and 18S sequences was Neighbour-Joining (NJ), the most common method used, to allow comparison with other studies. The neighbour-joining phylogenetic trees for COI and 18S sequences are shown in Fig. 2a–b and all pairwise comparisons are shown in Table 1. GenBank accession numbers and species location are given in Table 2.

Evidence of life history strategies

Spermatozoa were located in a mass aggregation, or spermatozeugmata. All spermatozoa had an elongated pyramidal acrosome, approximately 1.7 μm long, with a distinct and prominent axial rod (Fig. 3a). This axial rod lies in a canal which is formed by invaginations of the acrosomal vesicle. The mid-piece (measuring 1.3 μm in length) is typical of bivalve sperm morphology and at its base consists of four spherical mitochondria and a flagellum. Dimensions of the axial rod to sperm body length are shown in Fig. 4a.

The larval capture device installed in the aquaria did not detect any larvae during the entire period in which *T. dominicensis* was cultured. Following extraction from wooden panels reared specimens began to spawn, releasing a continuous and uninterrupted stream of unfertilized eggs which ranged from 48 to 52 μm in diameter (Fig. 3b). Egg dimensions for *T. dominicensis* are comparable with other known broadcast spawning teredinids and pholads (Fig. 4b). No brooded larvae or associated brooding structures were found in any of the 96 dissected specimens. Specimens obtained from wooden panels showed large variation in total body length (Fig. 5), with individuals as small as 1.3 cm and as large as 31.2 cm.

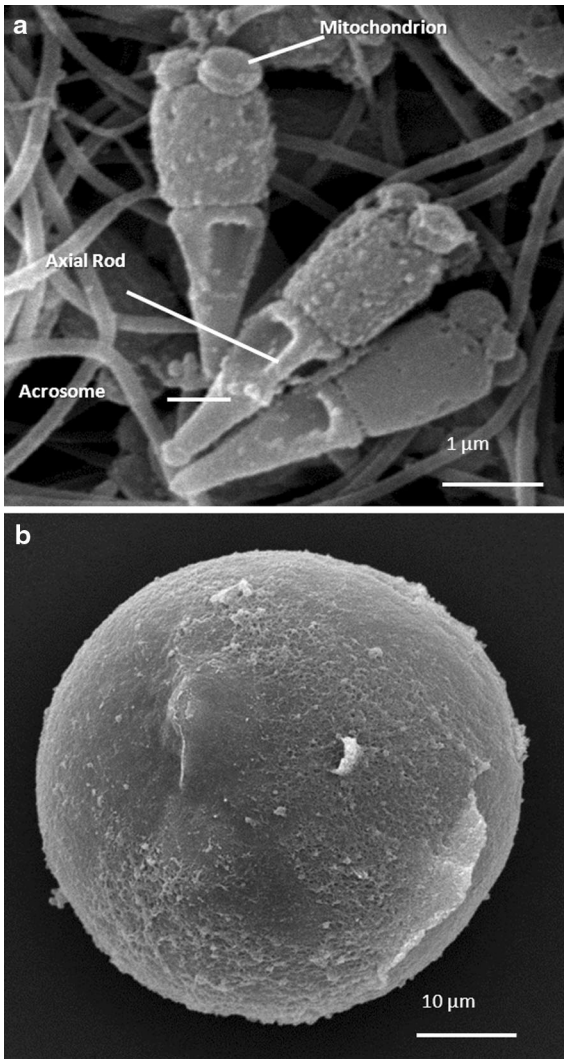

Fig. 3 The gametes of *Teredothyra dominicensis*. **a** Spermatozoa features. **b** a free spawned egg. *Scale bars* represent 1 and 10 µm respectively

Discussion

Prior to molecular based identification, teredinids were categorised based on pallet morphology. As pallets vary throughout ontogeny and are also influenced by ecological factors, identification based on these features is more assured if a profile of natural pallet variation is measured (Tan et al. 1993; Turner 1966; Cragg et al. 2009). The profile of pallet variation between the invasive population and the native population of *T. dominicensis* indicate no morphological difference between the two sites. As cryptic species morphologically indistinguishable from 'true' species exist among the Teredinidae (Calloway and Turner 1983), further means of identification are necessary. Molecular analysis using both COI-5P and 18s markers showed low interspecific genetic divergence between *T. dominicensis* specimens from the Caribbean, Mediterranean and Aegean. Furthermore, sequences were matched with those of *T. dominicensis* in GenBank, including a specimen from the Netherlands Antilles (Distel et al. 2011). Thus, both morphological and molecular data confirm the invasive species identity as *T. dominicensis* and rule out the possibility of the Mediterranean population representing a cryptic species. Both sequence and morphological data provided herein help to improve the traceability of this poorly-studied invasive species.

Originally discovered off the coast of Dominica, *T. dominicensis* was thought to be exclusively confined to the Gulf of Mexico and Caribbean Sea (Bartsch 1921; Turner 1966). This species has never been documented in the Mediterranean, despite numerous and extensive surveys of the region (Roch 1940; Turner 1966; Sen et al. 2010). Due to the striking differences between the pallets of this species with the known teredinid fauna of the Mediterranean, misidentification is unlikely and, whilst there is a slim chance these surveys failed to find *T. dominicensis*, the pair-wise distance between specimens from the Caribbean and Mediterranean suggests a recent invasion. Specimens were obtained from the timber of a wreck which was deliberately capsized in 2006 to promote dive tourism in the region. Infestation was first recorded the following year, thus proving the first known reference point for larval colonisation of *T. dominicensis* in the Mediterranean.

Teredothyra dominicensis has been recorded outside of its natural distribution range once before, when small numbers (37 specimens, representing 0.2 % of the 17,812 teredinids collected) were discovered during an extensive survey of Papua New Guinea (Rayner 1983). The fact this species has never been found in surrounding regions, including major surveys of teredinid distribution around the Australian coast and Hawaiian waters (Edmondson 1942; Ibrahim 1981; MacIntosh et al. 2012) and that specimens from Papua New Guinea were all found in deep water ports, suggests this species colonised from shipping ballast water. Given the pattern of ocean currents and the distance between the

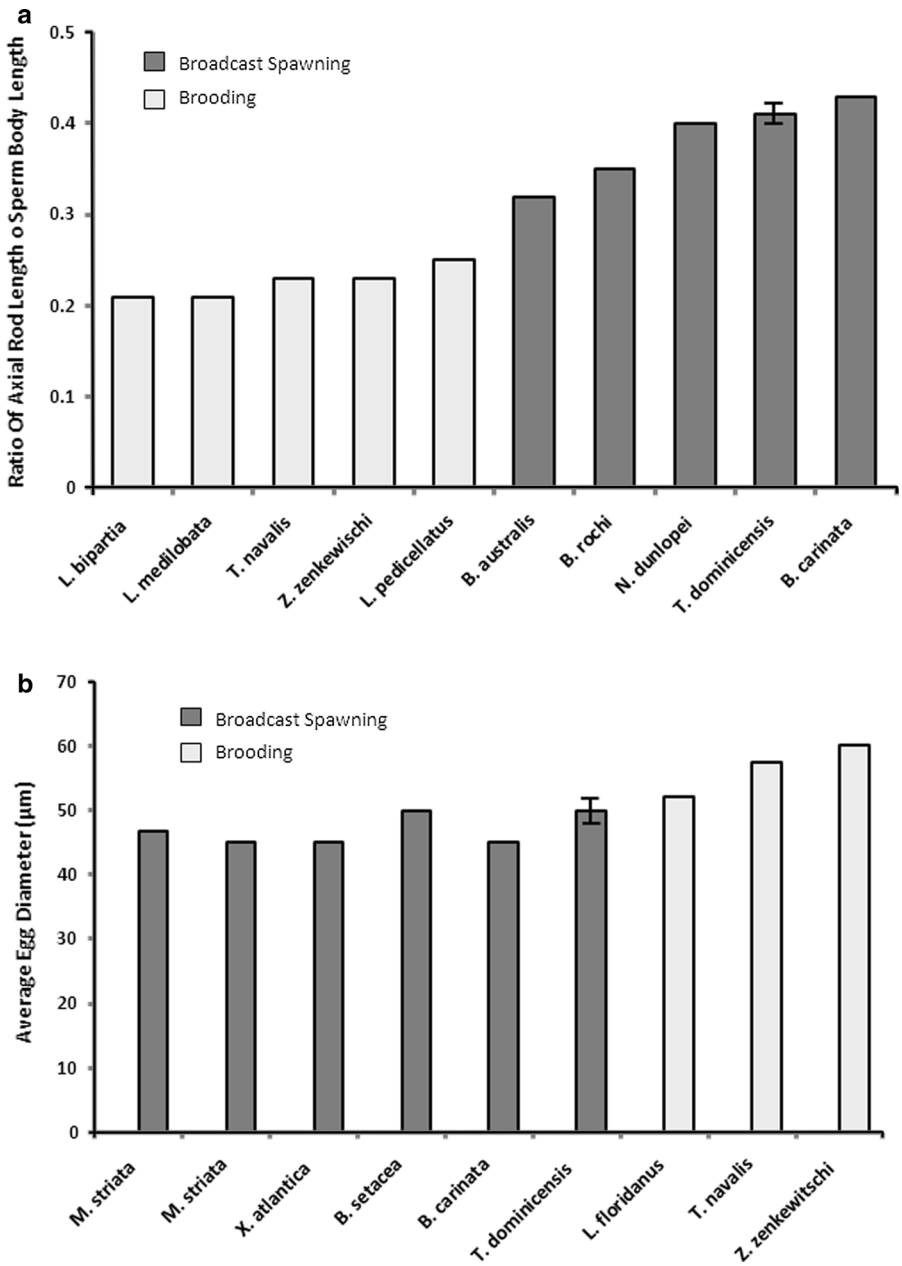

Fig.. 4 Correlation of gamete features with life history characteristics in the Teredinidae. **a** Ratio of axial rod length to sperm body length (mean ± SE) of internally and externally fertilizing teredinids. Data for *Lyrodus bipartita, L. medilobata, L. pedicellatus, Teredo navalis, Zachsia zenkewitschi, Bankia australis, B. rochi, B. carinata* and *Nausitora dunlopei* were taken from Popham (1974). **b** Egg size (mean ± SE) of broadcast spawning and brooding teredinids and pholads. Data for *Martesia striata* (Boyle and Turner, 1976), *Xylophagia atlantica* (Culliney and Turner 1976), *Bankia setacea* (Townsley and Lee 1967), *Bankia carinata* (Nair and Saraswathy 1956), *Lyrodus floridanus* (Calloway and Turner 1983) *Teredo navalis* (Costello et al. 1957) and *Zachsia zenkewitschi* (Yakovlev et al. 1998)

Mediterranean and Caribbean Seas, invasion of *T. dominicensis* via drift wood or by direct larval recruitment seems highly unlikely. Thus, we propose that the *T. dominicensis* was introduced to the Mediterranean via ballast water. This is believed to represent the world's largest invasion vector in Marine environments (Ruiz et al. 1997), with thousands of species being transported around the globe in billions of gallons of seawater per day (Carlton 2011). Furthermore, this mode of dispersal has been found to affect teredinid distribution (Gollasch 2002).

The only previous report on the reproductive mode for *T. dominicensis* suggested the species represented a transition between broadcast spawning and brooding modes (Turner 1966). However, this was based on a small number of poorly preserved specimens. Cultures reared over a year-long period did not spawn larvae

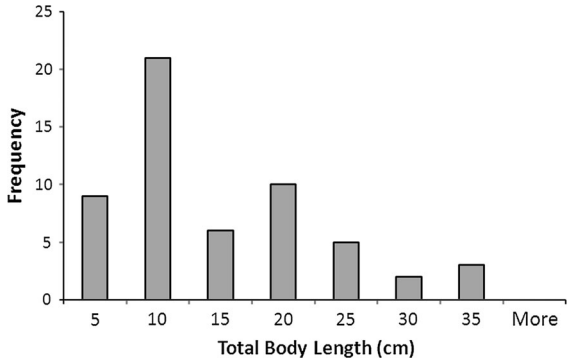

Fig. 5 Variation in total body length of *Teredothyra dominicensis* from Mediterranean sample sites

and the large numbers of examined specimens (Caribbean, Mediterranean and laboratory reared) failed to reveal brooding structures or internally developing larvae. A number of the laboratory cultures also released unfertilized eggs (averaging 50 µm in diameter). Free spawning suggests the eggs were ripe and their small size is comparable to other externally-fertilizing teredinids (Nair and Saraswathy 1971). *T. dominicensis* spermatozoa also showed structural characteristics of externally-fertilizing bivalve spermatozoa, with four mitochondria gathered at the base of the sperm head (Popham 1979), found in externally fertilizing teredinids (Popham 1974). Thus, mode of fertilization for this species is likely to be external with larvae undergoing planktotrophic development, which typically lasts 20–25 days in other broadcast spawning teredinids (Nair and Saraswathy 1971; Culliney 1975).

Panels placed at the wreck site were infested exclusively by *T. dominicensis* specimens and the marked size distribution (from small, recently settled juveniles, to large, mature adults) suggests at least three settlement events over the ten month exposure period. Recruitment is unlikely to have taken place directly from the wreck site itself: having spent a number of weeks developing in the water column, larvae from local parents would disperse away from the area. It is also unlikely that larval supply was maintained from Caribbean ballast water, so larval recruitment probably originated from other populations of *T. dominicensis* in the Mediterranean. With conditions in the region able to sustain breeding populations which produce viable larvae that can settle and metamorphose, *T. dominicensis* may now be considered as part of the established teredinid fauna in the Mediterranean.

Prior to its complete disintegration, the Uluburun III wreck supported a substantial population of this invasive species over a period of five years, providing ample time for dispersal. Teredinids are prodigiously fecund: adult *Psiloteredo megatora* are estimated to produce over 10^7 eggs in a single spawning (Sigerfoos 1908) and huge numbers of eggs are also produced by other broadcast-spawning teredinids (Turner 1966). It has been suggested that other members of the Teredothyra genus are highly adapted to an oceanic existence (Edmondson 1962). Thus, combined with the long planktotrophic larval period, it is likely that *T. dominicensis* has a huge dispersal potential. Over time, broadcast spawning teredinids are outcompeted by brooding species (MacIntosh et al. 2012); but *T. dominicensis* was the dominant species at the wreck site over a number of years and was the only species found infesting wooden panels placed at the wreck site. Furthermore, no brooding species were found in the wreck timber, even though the long-term brooder *L. pedicellatus* was found in driftwood close to the wreck site and other brooders, such as *T. navalis*, are known to exist in the region (Sen et al. 2010). This monopolisation and dominance of the wood resource suggests substantial quantities of larvae in the region and the ability of this species to locally outcompete brooding species.

The appearance and establishment of a Caribbean shipworm in the Mediterranean is of concern. Tropical borers can grow to sizes far surpassing European borers (Castagna 1961) and a number of specimens of *T. dominicensis* recovered from wooden panels showed growth of over 30 cm in a ten month period. Tropical borers are also known to be more destructive than their temperate counterparts (Edmondson 1942; Southwell and Bultman 1971), as demonstrated by the rapid destruction of the entire shipwreck, Uluburun III, in the space of just six years. The impact of global warming, particularly the rise in temperature of Mediterranean, also needs to be considered in relation to teredinid activity. Inceases in the temperature and salinity of the region has already been observed (Gibelin and Déqué 2003; Sanchez et al. 2004) and are expected to continue over the coming decades (Giorgi and Lionello 2008; Giannakopoulos et al. 2009). The increase of these factors is known to extend teredinid distribution ranges (Borges et al. 2010; Paalvast and van der Velde 2011). Furthermore, warmer temperatures are known to accelerate growth, increase boring

activity (Eckelbarger and Reish 1972), hasten maturity, reduce larval development time (Grave 1928) and increase settlement rates (Ibrahim 1981). Higher temperatures also prolong the breeding season, providing more opportunity for reproductive output thus increasing larval recruitment. Introduced tropical teredinids have also been shown to out-compete native species as they respond more favourably to environmental change (Hoagland 1986). Thus, the warming of the Mediterranean will increase the threat posed by all teredinids in the region. Yet as the temperature and salinity become more similar to those in tropical regions, they also become more suitable for *T. dominicensis*, thus amplifying the threat this Caribbean species poses to submerged wood structures in the region.

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