Journal of Experimental Marine Biology and Ecology 475 (2016) 154–162

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

First time DNA barcoding of the common shipworm *Teredo navalis* Linnaeus 1758 (Mollusca: Bivalvia: Teredinidae): Molecular-taxonomic investigation and identification of a widespread wood-borer

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ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 9 November 2015
Accepted 12 November 2015
Available online 10 December 2015

Keywords:
Baltic Sea
COI barcoding
Genetic diversity
Teredinidae
Teredo navalis

ABSTRACT

The common shipworm *Teredo navalis* is one of the most widespread marine wood-boring bivalves of the world and probably one of the most wood destructive and cost-incurring marine invertebrates. First reports on *T. navalis* for Europe date back to 1731 for the North Sea (The Netherlands) and to 1835 for the Baltic Sea (Germany). It is still unclear, however, where this species originates from. Therefore, *T. navalis* is considered cryptogenic for European waters, including the Baltic Sea.

In this study, 181 specimens of *Teredinidae* from six different sampling areas all over Europe and North America were molecular-taxonomically investigated using several molecular markers, two nuclear (18S/28S) as well as one mitochondrial marker (cytochrome c oxidase subunit I, hereafter COI). For the COI gene amplification, a new specific primer pair (Ter fw II/Ter rev I) for *T. navalis* was developed, which allowed sequencing of a 675 bp COI gene fragment for the first time. For amplifying the COI gene fragment of other examined teredinids than *T. navalis*, a third primer (Ter fw III) was designed. These three new primers are valuable tools to identify teredinid species with the DNA barcoding approach.

Classification of *T. navalis* into the system of wood-boring bivalves using a combined 18S/28S dataset showed no differentiation between specimens from Europe and the North American East Coast. The results of the COI dataset analyses showed high haplotype diversity in combination with a low nucleotide diversity and a star-shaped network with a predominant haplotype occurring in all investigated regions. Moreover, no indications have been found on a sibling species in the Baltic Sea. The data indicate a recent population expansion for the examined sampling sites whereas the origin of the assumed worldwide distributed species *T. navalis* remains open.

1. Introduction

Marine wood-boring Teredinidae (commonly known as shipworms or teredinids) occur in almost all aquatic ecosystems from tropical to cold-temperate waters (Turner, 1966). At least nine wood-boring bivalves have been reported so far in European coastal waters (Borges et al., 2014) of which four have been examined in the present study. Most of these species occur predominantly in fully marine conditions instead of brackish waters (Borges et al., 2012). The monophyletic group of wood-boring molluscs (Distel et al., 2011) inhabits all types of wood including mangrove roots and drift wood but also anthropogenic structures like docks or groynes which are used in coastal protection (Roch, 1937; Nakel, 1954; Hahn, 1956). These xylotrophic organisms perform an important ecosystem function by decomposing wood faster than the decay caused by marine fungi and bacteria (Borges, 2007). On the other hand, these bivalves cause enormous economic costs by damaging wooden coastal protection structures and harbour infrastructure. This is leading to substantial maintenance costs worldwide on an annual scale (Distel et al., 2011). The German Federal Maritime and Hydrographic Agency, for example, estimated economic damages up to 25 million Euro between 1993 and 2004 (BSH, 2004) for the Baltic Sea. In addition, these bivalves are also threatening invaluable underwater cultural heritage because the Baltic Sea hosts up to 100,000 well-preserved shipwrecks and other maritime related constructions of high archaeological value (Björdal et al., 2012).

Based on morphological treatments, the dominant wood-borer in the Baltic Sea was identified as the worldwide distributed common shipworm (also referred to naval or great shipworm) *Teredo navalis*

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http://dx.doi.org/10.1016/j.jembe.2015.11.008

(Mollusca: Bivalvia: Teredinidae) Linnaeus 1758 (Sordyl et al., 1998; Appelqvist et al., 2014). According to several authors, *T. navalis* reaches a maximum length up to 30 cm and a lifespan up to three years in the Baltic Sea (Nakel, 1954; Hahn, 1956; Schütz, 1961). This species is a protandric hermaphrodite species with rapid sexual maturity 7–8 weeks after metamorphosis (Grave, 1942). A single female adult can release up to five million larvae per year into the water column (Kaestner, 1982), and therefore has a very high reproductive potential. The planktonic veliger larvae remain in the water column up to 34 days before settlement (Nair and Saraswathy, 1971). In the Baltic Sea, *T. navalis* tolerates salinities as low as 7 (Blum, 1922; Nair and Saraswathy, 1971; Hoagland, 1986; Strömberg and Spicer, 2003, Borges et al., 2014). The optimum temperature for *T. navalis* is between 15 and 25 °C. At temperatures less than 10 °C metabolic activities decelerate and stop at temperatures below 5 °C (Roch, 1932). Furthermore, the average winter water temperatures in the Baltic Sea do not have lethal effects for adults (Sordyl et al., 1998).

Based on the data of Turner (1966), Nair and Saraswathy (1971) have specified the worldwide distribution of *T. navalis* for six regions: Australia/New Zealand, Southeast Asia, Japan, West Coast of North America, East of North America/Greenland and Europe/Atlantic Coast. Trustworthy proofs supporting this distribution are rare. Nevertheless, there are references of the occurrence of *T. navalis* for Australia (Ibrahim, 1981), Japan and India in Asia (Tsunoda, 1979; Ibrahim, 1981; Kasyanov et al., 1998), Brazil in South America (Barreto et al., 2000), the West (Ibrahim, 1981; Cohen and Carlton, 1995) and East Coasts (Ibrahim, 1981, Culliney, 1975) of North America and the Atlantic, Baltic Sea, North Sea, Black Sea and Mediterranean Sea in Europe (see e.g. Tuente et al., 2002; Culha, 2010; Appelqvist et al., 2014; Borges et al., 2014).

While for most European waters like the Mediterranean Sea or the North Sea *T. navalis* has been reported long before 1800 (Streftaris et al., 2005), the earliest known report of this species for the Baltic Sea dates back to 1835 in the Bay of Kiel (Meyer and Möbius, 1865). Afterwards, *T. navalis* had been reported on the southern coast of the Baltic Sea in 1875 and in the 1920s (Sordyl et al., 1998). Subsequently several periodic mass outbreaks with increased abundances have been documented in the Baltic Sea (Germany, southern Sweden and Denmark) during the 1930s and 1950s lasting each for 2–3 years only (Bavendamm and Schmidt, 1948; Nakel, 1954; Hahn, 1956; Schütz, 1961). These periodic outbreaks between the early 20th century and the 1990s seem to be a special characteristic in the Baltic Sea, probably due to the strong salinity gradient from West to East in the Baltic Sea (Leppäranta and Myrberg, 2009).

Nevertheless, it is unclear whether *T. navalis* occurred between these outbreaks in the Baltic Sea, for example, as population with low specimen numbers or as stable, but simply undetected population in those years. Between these mass outbreaks, *T. navalis* is not mentioned in the literature. One of the problems related to ‘invisible’ wood-boring species is, that they will only be registered if occurring in great masses or causing great damages. In addition, there is also a lack of scientific investigations or regular monitoring programmes dealing with *T. navalis* at those years in the Baltic Sea. Therefore, reliable data about the abundances of *T. navalis* are missing for the Baltic Sea.

But this has greatly changed with the last mass outbreak in 1993 and the observed reproducing adult animals at the coast of Mecklenburg–Western Pomerania (Sordyl et al., 1998). The latest outbreak is well documented because of the establishment of a more or less regular monitoring of groynes over the last 20 years by the local State Agency for Agriculture and Environment of Central Mecklenburg (StALU MM). In contrast to earlier times, it seems that *T. navalis* established a stable, reproductive population present in the brackish south-western part of the Baltic Sea (Sordyl et al., 1998; Lippert, unpublished results). Sordyl et al. (1998) observed for the first time all stages of gonad development as well as free living larvae in the water column. In addition, for several years, there is evidence that *T. navalis* has been spreading into lower salinity brackish waters of various coastal regions in Europe which were formerly not inhabited by this species (Sordyl et al., 1998; Tuente et al., 2002; Paalvast and van der Velde, 2011; Borges et al., 2014). These observations might be related to global change phenomena such as e.g. increasing water temperatures (Neumann, 2010; Störmer, 2011).

The species name *T. navalis* is often used as a synonym for all kinds of wood-boring bivalves mainly due to lack of taxonomic knowledge about most other species of this group/family and the difficulties to identify them morphologically (Turner, 1966; Borges et al., 2012). Consequently, the place of origin and the bio(phylo)geography of *T. navalis* remain still unsolved. Therefore, this taxon is considered to be cryptogenic (Hoppe, 2002; Gollasch et al., 2009; Borges et al., 2014). Even less is known about the pathways of introduction of *T. navalis* into the Baltic Sea. To address any type of range expansion or bioinvasion, an unambiguous taxonomic identification of the species of interest is absolutely essential. Nevertheless, morphological identification of bivalve wood-boring species often fails. This may be partly related to their way of life as they live hidden in a calcified tube drilled into the wood. Therefore, there are associated difficulties to extract the animals unharmed in one piece out of the tubes. The only known morphological features for accurate taxonomic identification are exclusively represented by two calcareous pallets, located at the distal end of the elongated body (Turner, 1966; Borges et al., 2012), which, however, are easily lost or destroyed during preparation. Molecular taxonomy such as barcoding is thus an appropriate approach to solve these taxonomic difficulties within wood-boring bivalves and has already been successfully used for the identification of other teredinids (Borges et al., 2012).

Molecular taxonomic identification of animals by using the DNA barcoding approach started in 2003 (Hebert et al., 2003). Although there are some challenges with this DNA barcoding (see Collins and Cruickshank and references therein, 2013) it is now accepted as a reliable, standardized, cost-effective and widely-used method for biodiversity investigations on all life stages (Hebert et al., 2003; Radulovici et al., 2010). It includes sequences from the 5′ region of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene as marker for species-level identification. Although the BOLD system (barcode of life data system; www.boldsystems.org; Ratnasingham and Hebert, 2007) contains actually more than 4×10^6 (15.05.2015) data entries, appropriate data on *T. navalis* are missing. The closest related species with COI sequences within GenBank and the BOLD system are *Lyrodus pedicellatus* and *Bankia carinata*. In addition, until now there are only three sequences of *T. navalis* deposited in the NCBI GenBank (18S/28S and actin gene). No COI sequences are available yet (15.05.2015), probably because the universal COI primers of Folmer et al. (1994) fail to amplify this mitochondrial marker in this species (Borges, pers. com.).

Therefore, one of the aims of the present study was to develop specific COI primers for *T. navalis* as a new molecular tool for reliable taxonomic identification of this species. Afterwards, the hypothesis was tested whether occurring Teredinidae in the Baltic Sea are exclusively represented by *T. navalis*, as commonly accepted, or by better adapted sibling species with respect to now well established and self-reproducing populations in contrast to previous years. The occurrence of sibling species has ecological and evolutionary implications and they are common in all major marine groups and habitats (Knowlton, 1993). The phenomenon of sibling species which occurs in the same regions has been documented for related teredinid species (Calloway and Turner, 1983; Borges et al., 2012) and in other marine invertebrates (Bastrop and Blank, 2006).

Furthermore, also the hypothesis that *T. navalis* is a species with an amphi-Atlantic distribution was verified. Hence, this study tried to clarify the phylogenetic relationship of the European *T. navalis* in the general phylogeny of wood-boring molluscs by using two nuclear markers (18S and 28S) as used in the study of Distel et al. (2011). To outline whether there are differences between other teredinid species from Europe and from North America, available samples from three of