

First report of the planktonic copepod *Oithona davisae* in the northern Wadden Sea (North Sea) – evidence for recent invasion?

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Abstract

In October 2010 specimens of *Oithona* were taken from the List Tidal Basin in the northern Wadden Sea (North Sea) for a biogeographic study on *Oithona similis*. These specimens could not be assigned to *O. similis* or any of the other *Oithona* species known from the North Sea genetically. These specimens were identified as *Oithona davisae* Ferrari and Orsi 1984, a Northwest Pacific species, known as an invasive species from the Black Sea and the northwestern Mediterranean Sea. Recent sampling provided evidence that *O. davisae* is still present in the northern Wadden Sea and may thus now be a permanent plankton species.

Key words: *Oithona davisae*, non-indigenous species, copepoda, North Sea, Wadden Sea

Introduction

In the North Sea four species of *Oithona* are found regularly (Razouls et al. 2014): *Oithona similis* Claus, 1866, *Oithona nana* Giesbrecht, 1892, *Oithona plumifera* Baird, 1843 and *Oithona atlantica* Farran, 1908. Among these species *O. similis* is the most common species in the North Sea (reviewed in Fransz et al. 1991), which is also true for the northern Wadden Sea (Martens, 1980) where it occurs mainly in autumn. *O. nana* is found also in the Wadden Sea but only in low numbers (Franz 1983). *O. plumifera*, and *O. atlantica* are considered Atlantic species that can be found in the northern North Sea, drifted with a branch of the North-East Atlantic Current (Fransz et al. 1991). The authors also state that „several less common species are typical indicators for temperate-oceanic waters“, but no species names are provided. Thus, only *O. similis* and *O. nana* would be expected in Wadden Sea samples.

In the North Sea six alien zooplankton species have been found opposed to 83 zoobenthic species (review in Gollasch et al. 2009). Within the six species only two

copepods were recognized: the calanoids *Acartia tonsa* and *Eurytemora americana*. Apart from these records two other calanoid copepods have been identified from the southwestern North Sea: *Acartia omorii* (Seuront 2005) and *Pseudodiaptomus marinus* (Brylinski et al. 2012, Jha et al. 2013). It is assumed that the introduction of these species is most likely related to ballast water discharge (e.g. Gollasch et al. 2009, Brylinski et al. 2012).

During the course of a biogeography study on *Oithona similis*, we encountered a small (< 1 mm) species of *Oithona* in the northern Wadden Sea that could not be identified as any of the known species from the North Sea. Here we describe the characteristics of this species, compare them with those of other *Oithona* species, and identify the species with notes to its distribution. Possible causes for its introduction to the North Sea are also discussed.

Material and Methods

The List Tidal Basin is a semi-enclosed bight in the northern German Wadden Sea, surrounded by the islands Sylt and Rømø and connected with the North Sea only by a tidal inlet (Fig. 1A). It is the location of a zooplankton time series (55°01.30'N, 08°27.10'E; Martens and Beusekom 2008). At this station plankton samples (Apstein net, 80µm mesh size) were taken in October 2010 and 6 August 2014. *Oithona* specimens were preserved in 96% ethanol to allow also molecular analyses.

Total length (measured dorsally) and prosome:urosoma ratio of 20 females were measured under a stereo microscope (Leica MZ 16). The length of the prosoma was measured from the tip of the forehead to the end of the thoracic segment 4 (Th4), the urosoma length included Th5 and the urosoma segments to the end of the caudal rami. For a detailed inspection of morphological characters 10 female specimens were soaked in Lactic acid and stained with chlorazol black. Subsequently, the specimens were dissected to obtain mouthparts and swimming legs. Specimens were identified according to an identification guide for *Oithona* (Nishida 1985), the original description of *O. davisae* (Ferrari and Orsi 1984), and a description of *O. davisae* from the Black Sea (Temnykh and Nishida 2012). DNA of the *Oithona* species occurring in the North Sea was extracted using the QIAGEN DNeasy Mini kit. The marker gene large-subunit (28S) rRNA was amplified and sequenced using the primer set 28SF1 and 28SR1 (Ortman 2008; Cepeda et al. 2012). This gene has

been successfully applied to differentiate between *Oithona* species (Cepeda et al. 2012). Mitochondrial cytochrome c oxidase subunit I (COI) was sequenced using the primer pair LCO1394 and HCO2198 (Folmer et al. 1994) to obtain DNA barcodes for this species to compare with future sequences of *O. davisae* from other regions. PCR amplifications and sequencing were performed according to the protocols of Cepeda et al. (2012) and Wend-Heckmann (2013). In CodonCode Aligner Vers. 3.7.1.1 (CodonCode Corporation) both strands of 28S were assembled into consensus sequences and checked for sequencing errors. They were aligned with MAFFT v7.157b under the L-INS-I option (Kato and Standley 2013). New sequences of 28S and COI were deposited in GenBank (KP033160 – KP033188). To enlighten the position of *O. davisae* in comparison to the other *Oithona* species from the North Sea and from GenBank (for accession numbers see Fig. 2) a maximum likelihood phylogenetic analysis of 28S was carried out with RAxML 7.2.8 (Stamatakis 2006). RAxML was run under the option GTRGAMMA and a complete random starting tree of 10,000 bootstrap replicates. Best-known likelihood tree search was performed under GTRMIX and a completely random starting tree. The final tree topology was evaluated under GTRGAMMA to yield stable likelihood values.

Results and Discussion

The female specimens (n=20) had a total length ranging between 0.54 and 0.57 mm (Fig. 1B). They possessed a strong and ventrally curved, sharply pointed rostrum (Fig. 1D). The caudal rami were nearly 1,2x longer than wide. The distal spines of the basipodid 2 (B2) of the mandible (Md) were thick, slightly curved and blunt (Fig. 1C). At the maxillule (Mx1) the distal spine of the inner lobe (Li1) was longer than the other spines (Fig. 1F) and the endopod (End) carried only one seta. The outer margins of the exopod segments 1 to 3 (Exp1, Exp2, Exp3) of the swimming legs (P1-P4) have the following number of spines 1,1,3; 1,1,3; 1,1,3; 1,1,2 (Fig. 1E). The combination of a strong ventrally pointed rostrum and the same number of spines of the outer margins of the P1–P4 exopods is only known for five *Oithona* species (*O. robusta*, *O. brevicornis*, *O. aruensis*, *O. wellershausi* and *O. davisae*; Ferrari and Orsi 1984). However, *O. davisae* has only one seta on the endopod of Mx1, while the other four species have three setae (Nishida 1985). Thus, we conclude that the

specimens found in the List Tidal Basin equal *Oithona davisae* Ferrari and Orsi, 1984, which was confirmed by S. Nishida (pers. comm.).

O. davisae specimens can easily be differentiated from the other *Oithona* species occurring in the North Sea (Table 1): In *O. plumifera* and *O. atlantica* the rostrum is pointed anteroventrally and as such is visible from a dorsal view. In *O. similis* the rostrum is also pointed ventrally, but much smaller. *O. nana* does not have a rostrum. In all four species the distal spines of the mandibles are pointed and not blunt as in *O. davisae*.

But not only the morphological characters revealed that the *Oithona* species found in the northern Wadden Sea was different from the other species known from the North Sea, the evidence can also be seen in the maximum likelihood tree of 28S sequences. Interesting to note is also that three species from the northern Atlantic seemed to be closely related in 28S and were divided from the Pacific species *O. dissimilis*, *O. brevicornis* from GenBank (Accession numbers are included in Figure 2), and *O. davisae* provided by this study. So far, no *O. davisae* sequences have been submitted to GenBank to compare our data with. But the sequences we submitted may help other researchers to determine the geographic origin of *O. davisae* in the Wadden Sea.

In the List Tidal Basin *O. davisae* was first observed in October 2010 while conducting sampling for *Oithona* specimens. However, most of the specimens were used for genetic purposes and thus not available for morphological analysis. In June 2014 the remaining three female specimens were first identified as *Oithona davisae*. To investigate if the occurrence of *O. davisae* in the North Sea had been only a single event in 2010, samples of the time-series (2007 – 2012, August - September) in the List Tidal Basin were checked (Martens and Beusekom 2008) and a new non-quantitative sample was taken in August 2014 at the same station. This sample contained many copepodites, males and females with or without egg sacks. Next to *Acartia* sp. *O. davisae* was the most common zooplankton species in this sample. From the time series only the presence or absence of *Oithona* species was noted. *O. davisae* was present in all of the samples inspected dating back to 2008. In 2007 it could not be found, and we did not have access to older samples. These findings provide evidence that *O. davisae* not only is present in the Wadden Sea since at least 2008, but that it has also successfully reproduced and settled. Furthermore, in

all samples we looked at neither *O. similis* nor *O. nana* was found.

To our knowledge the cyclopoid copepod *O. davisae* is the fifth non-indigenous planktonic copepod species found in the coastal waters of the North Sea. However, the other four species were all calanoid copepods. In the Atlantic and its adjacent seas *O. davisae* has only been reported from the western Mediterranean and the Black Sea (Saiz et al. 2003, Temnykh and Nishida 2012). Originally *O. davisae* is considered to be endemic to the temperate coastal waters of East Asia (review in Temnykh and Nishida 2012). However, it was first described from the St. Joaquin estuary in northern California (Ferrari and Orsi 1984). The authors speculated due to its close morphological relationship to the Indo-Pacific species *O. wellershausi* and *O. aruensis* that this species might have been introduced to the estuary by ballast water from ships coming from Asian harbours. Later it has been observed in ballast water tanks arriving mainly from Japan in the San Francisco Bay area (e.g. Choi et al. 2005). All other records in the Pacific and the Atlantic Ocean are considered to be evidence of newly introductions probably transferred with ballast water tanks. Thus, we suggest that *O. davisae* was most likely transported via ballast water tanks into the North Sea. This is also congruent with the findings for the other four introduced planktonic copepod species (Brylinski 1981, Seuront 2005, Brylinski et al. 2012). However, all other four species were first found in coastal waters of the southwestern North Sea near big harbours, while *O. davisae* was found in the northeastern Wadden Sea. The nearest harbour would be Hamburg. Thus, *O. davisae* might have been arrived near the harbour, established itself there and then extended its distribution further to the north. However, to our knowledge the station at the List Tidal Basin is the only regularly sampled zooplankton station in the eastern Wadden Sea. To investigate how far *O. davisae* has spread within the Wadden Sea further sampling is needed at different locations.

The Wadden Sea may be a favourable habitat for *O. davisae*, a unique coastal environment subjected to a pronounced seasonality in temperature, phytoplankton and zooplankton (e.g. Martens and Beusekom 2008), which is also true for its original habitat, the coastal waters of southwestern Japan (Nishida 1985, Uye and Sano 1998). There temperatures range between 8.9 and 28.2 °C (Uye and Sano 1998), which is generally higher than in the studied Wadden Sea station (between -1.8 and more than 20°C (Martens and Beusekom 2008)). The warming of the Wadden Sea

(Martens and Beusekom 2008) may have provided the right environment for *O. davisae* to settle. Reise and Beusekom (2008) revealed that global warming is an important factor for species invasions in the Wadden Sea, since most of the introduced species originate from warmer regions than the North Sea. Martens and Beusekom (2008) published results from the time series in the List Tidal Basin between 1984 and 2004 and recorded only *O. similis*, which occurred in low numbers throughout the year until 1996 when suddenly the abundances increased in autumn and have stayed high ever since. As stated above we did not observe any *O. similis* specimens in the time-series autumn samples between 2007 and 2012. In the eutrophic embayments of southwestern Japan, the natural habitat, *O. davisae* is often the most abundant copepod (review in Uye and Sano 1998), even more abundant than *O. similis*, especially in the inner part of inlets. In the Black Sea, especially at Sevastopol Bay, *O. davisae* has become one of the most common copepod species with abundance peaks in autumn since its first appearance in 2001 (Altukhov et al. 2014). Thus, within less than 15 years this species seems to be able to establish itself successfully in a new environment. So the question arises if *O. davisae* has replaced *O. similis* in the List Tidal basin. A comparison between the List Tidal Basin and a reference station outside of the bight may give further insight in this matter.

Most probably *O. davisae* is now a permanent immigrant in the northern Wadden Sea, documented by its presence in 2008 - 2012, and 2014. It also seems to be able to reproduce as copepodite stages, males and females with egg sacks were found in 2014. However, it remains unknown when *O. davisae* was introduced to the North Sea, from where and how. Some answers to these questions may be given by revisiting the older samples of the zooplankton time series in the List Tidal Basin, by genetic population studies and investigations of ballast water tanks arriving to the harbours connected to the North Sea. The most plausible explanation how this species arrived in the Wadden Sea would be the introduction by ballast water from ships traveling through the North Sea. It also remains unknown how far *O. davisae* has spread in the Wadden Sea. Thus, it might be worth to investigate the extent of the success of *O. davisae* in coastal North Sea waters.

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Figures

Figure 1: Map of the sampling location (A) and photographs of *Oithona davisae* from the Wadden Sea. (B) female, habitus, (C) mandible with terminal setae (arrow), (D) rostrum (arrow), (E) Swimming leg 3 (arrows indicate the outer spines of the exopod), (F) maxillule inner lobe 1 (arrow points at long distal seta).

Figure 2: 28S maximum likelihood tree for the North Sea species *Oithona similis*, *Oithona plumifera*, *Oithona atlantica*, *Oithona nana* and *Oithona davisae* (NS: North Sea, TEA: Tropical Eastern Atlantic, NEA: Northeastern Atlantic, ARK: Arctic Ocean) and other *Oithona* species (taken from GenBank with their accession numbers).