First report on *Beroe ovata* in an unusual mixture of ctenophores in the Great Belt (Denmark)

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Abstract

Between mid-December 2011 and mid-January 2012 an unusual mixture of ctenophores was observed and collected at Kerteminde harbor (Great Belt, Denmark). In addition to native zooplanktivorous species *Pleurobrachia pileus* (O.F. Müller, 1776) and *Bolinopsis infundibulum* (O.F. Müller, 1776), non-native zooplanktivorous *Mnemiopsis leidyi* Agassiz, 1865 and their predator *Beroe cucumis* Fabricius, 1780 had earlier been recorded in the area, two more predators on zooplanktivorous ctenophores were recorded in inner Danish waters: *Beroe ovata* sensu Mayer and *Beroe gracilis* Künne, 1939. The identity of *B. ovata* was confirmed using nuclear marker 18S. Identification of *B. gracilis* was based on morphology. The presence of the observed mixture of ctenophores in Great Belt may predict future faunal changes in Danish and perhaps other temperate coastal waters.

Key words: genetic analyses, morphology, non-native species, Danish waters

Introduction

The occurrence of the invasive ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 in the inner Danish waters has been monitored with varying intensity since it was first recorded in large numbers in 2007, especially in Limfjorden, in Great Belt, and in the western and central Baltic Sea (Javidpour et al. 2006; Riisgård et al. 2007; Tendal et al. 2007; Huwer et al. 2008). Native ctenophore species zooplanktivorous *Pleurobrachia pileus* (O.F. Müller, 1776) and *Bolinopsis infundibulum* (O.F. Müller, 1776) and its predator *Beroe cucumis* Fabricius, 1780 have not been monitored frequently in the inner Danish waters, in contrast to the adjacent and more intensively monitored Baltic Sea (e.g. Gorokova et al. 2009; Jaspers et al. 2012, 2013; Lehtiniemi et al. 2013). It was unexpected when several additional species to the area appeared in large numbers in Great Belt during December 2011 and January 2012, possibly due a change in currents and water exchange in this area.

The Great Belt is one of the Danish Straits that form the transition between the tidal North Sea and the non-tidal Baltic Sea (Figure 1). The freshwater supply to the Baltic Sea generates an outgoing brackish-water surface current of less density than the more saline water from the Kattegat; therefore, the Great Belt is permanently stratified (halocline at about 15 m depth). Shifting winds changes flow conditions such that outflow of water from the Baltic Sea decreases salinities to less than 10 whereas inflow to the Baltic Sea increases salinities to about 27 in the upper layer of the Great Belt (Kullenberg and Jakobsen 1981; Møller 1996). This study describes the unusual mixture of ctenophores observed in the Great Belt, and suggests possible future faunal changes in Danish and other temperate coastal waters.
Materials and methods

Sampling and photography

Salinity and temperature were measured at 1 m water depth in the northern Great Belt (55°30.46 N, 10°51.72 E) between 25 January 2011 and 2 July 2012 by the Environmental Centre Odense (Danish Ministry of the Environment; Figure 2). During the period 15 December 2011 to 18 January 2012, ctenophores were frequently collected individually by means of a small hand-held net from the wharf in Kerteminde harbor and gently transferred to a bucket with water from the site of collection. Within 20 minutes, the specimens were brought to the nearby Marine Biological Research Centre (University of Southern Denmark) to be photographed (JVC digital still camera GC-X3) in an observation aquarium (height×width×depth = 20×20×10 cm) with black-painted backside, using strong light from a cold lamp. For higher magnification detail images of the ctenophores, a stereo-microscope (Leica MZ 125) fitted with a video camera (Leica IC80HD) was used for taking pictures of an individual transferred to a petri dish with seawater.

Genetic analyses

Three individuals of *Beroe sp.* were sampled and stored separately in 95% ethanol. The genomic DNA was extracted using DNeasy Blood and Tissue Kit from Qiagen Inc., Canada. We used universal 18s primers, 18S-F (5′ ACCTGGTTGAT CCTGCCA- 3′) and 18S-R (5′-TGATCCTTC YGCAGGTTCAC-3′) to amplify 18s rDNA gene(Moon-van der Staay et al. 2000; Daniels and Breitbart 2012). PCR amplifications were carried out in a 40-µl reaction volume, with 1X PCR buffer, 2.5 mM of MgCl₂, 0.2 mM of dNTPs, 0.4 µM of each primer, about 50 ng of genomic DNA, and 1 unit of *Taq* DNA Polymerase (Qiagen). PCR was performed with an initial denaturing step at 95°C for 4 min, followed by 30 amplification cycles (95°C for 30 s, 55°C for 60 s, 72°C for 2 min), and a final elongation step at 72°C for 7 min. PCR products were purified using Agencourt® Clean SEQ from Beckman Coulter®. Cleaned PCR products were sequenced using 18S-F and 18S-R. BigDye terminator sequencing chemistry with an ABI 3130XL genetic analyzer (Applied Biosystems) was used to obtain sequences. The identity of these sequences was confirmed after BLASTN query against GenBank.

In addition four *Mnemiopsis* samples were collected and stored separately in 95% ethanol. Analyses showed that they were similar to previously examined individuals from the Baltic Sea; all ITS alleles are similar to ones recovered from *Mnemiopsis leidyi* by Ghabooli et al. (2011).

Results

During the period between 15 November 2011 and 9 January 2012, the salinity in Great Belt increased from 10.7 to 22.2 indicating replacement of Baltic Sea water with Kattegat water, and subsequently there was a reduction in salinity to 18 on 23 January and a further decline to 17 on 6 February (Figure 2). The ctenophores collected...
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**Figure 3.** *Bolinopsis infundibulum* and *Mnemiopsis leidyi*. Note termination of oral-lobe (2) and location of statocyst (1). (Photography by Hans Ulrik Riisgård).

**Figure 4.** (A) *Beroe ovata* (about 5 cm long); (B) *Beroe cucumis* (about 5 cm) (Photography by Hans Ulrik Riisgård).

**Figure 5.** *Beroe gracilis* (A) 2.5 cm long individual, (B) aboral pole fringed with a row of papillae, (C) view of meridional canal structures. (Photography by Hans Ulrik Riisgård).

in the harbor of Kerteminde (Denmark) between 15 December 2011 and 18 January 2012 comprised 6 species: *Pleurobrachia pileus* of the order Cydippida, *Mnemiopsis leidyi* and *Bolinopsis infundibulum* of the order Lobata (Figure 3), and *Beroe cucumis*, *B. ovata* sensu Mayer 1912 (Figure 4), and *B. gracilis* (Künne, 1939) (Figure 5) of the order Beroida. After the 18 January, no ctenophores were observed at Kerteminde, which indicated the water from the Kattegat (containing ctenophores) had been replaced by brackish Baltic Sea water (without ctenophores).

Of the three analyzed sequences, two of them (*B. ovata*) had 99% identity with Genbank accession numbers JN653095 (Daniels and Breitbart 2012, collected from Tampa Bay, FL, USA) and AF293694 (Podar et al. 2001, collected from Woods Hole, MA, USA). The closest match for the third sequence (*B. cucumis*), with 99% correspondence, were AF293699 and AF293695 (Podar et al. 2001, collected from Santa Barbara, CA and Gulf Stream, FL, USA).

The identification of *B. gracilis* was based on morphology alone.

The ITS alleles recovered from four *Mnemiopsis* samples were similar to previously examined individuals from the Baltic Sea (by Ghabooli et al. 2011).
Discussion

*Beroe ovata* is a new species for Danish waters. In Europe, it was first found in the Black Sea, probably brought there with ballast water. It was identified based on both morphological (Seravin et al. 2002) and genetic characteristics (Bayha et al. 2004). *B. ovata* is native to the Atlantic coasts of North and South America (Purcell et al. 2001). The identification *B. ovata* is complicated because the same name has been used for specimens from the Mediterranean (*B. ovata* sensu Chun) and specimens from the western Atlantic and Caribbean (*B. ovata* sensu Mayer) (Bayha et al. 2004). ITS-1 length variation, sequence divergence and molecular phylogenetic analysis indicates these are two well-differentiated groups (Bayha et al. 2004) with *Beroe ovata* sensu Chun from the Mediterranean and *B. cucumis* sensu Mayer from the Atlantic and Pacific forming a common group. Consequently Bayha et al. (2004) suggests the name *B. ovata* sensu Mayer be used for *B. ovata* from the northern Atlantic and Black Sea, and the name *B. cucumis* sensu Mayer (=*B. ovata* sensu Chun) be used for those found in the Mediterranean, western Atlantic, and eastern Pacific. Clearly a thorough systematic revision of the genus *Beroe* is warranted. Shiganova and Malej (2009) recently performed morphological examination of the two Mediterranean *Beroe* species in the northern Adriatic and of *B. ovata* that arrived in the Adriatic from the Black Sea, and this work supports the conclusions of Bayha et al. (2004). The species now found in Danish waters is *B. ovata* sensu Mayer 1912, and it is known from the Black Sea and parts of the eastern and central Mediterranean (Shiganova et al. 2007; Shiganova and Malej 2009; Galil et al. 2011).

Fully developed specimens of *Beroe ovata* have mitten-shaped body, widest at the oral end and not tapered at the aboral end, up to 16 cm in length (Figure 4A). They typically have pink tint but can be more brownish in the largest indivi-duals. The lateral compression of the body is obvious: the large diameter of the ‘cross-section ellipse’ is 3 or more times greater than the small diameter. Ratio of length to width (l/w) does not exceed 1.5. The 8 meridional canals are inter-connected by numerous branching and anastomosing diverticulae forming a wide, characteristic, meshwork. There are two lateral aboral canals, and the aboral pole is smooth (not fringed with papillae) (Mayer 1912).

*Beroe cucumis* (Figure 4B) has an oval egg-shaped body, much less flattened in the paragastral plane (large diameter of the ellipse is twice or less greater than small diameter) (Figure 4B). Its lateral canals have numerous diverticulae, which may branch out in adult ctenophores, but they do not anostomose with each other, and do not connect with paragastral canals. The polar-plate is surrounding the sense organ at the aboral pole, which is fringed with a row of long branched papillae. This native ctenophore has ratio of length to width (l/w) more than 2.0 (Mayer 1912; Seravin et al. 2002).

In 2006, *Mnemiopsis leidyi* was first recorded in the Baltic Sea and later in the North Sea where it was brought from the northwestern Atlantic (Javidpour et al. 2006; Faasse and Bayha 2006; Ghabooli et al. 2011; Reusch et al. 2010). During the following years *M. leidyi* continued dispersing widely in both southern and northern European waters (Galil et al. 2009; Fuentes et al. 2010). *M. leidyi* was recorded in large numbers all over the inner Danish waters from February 2007 onwards, but scattered observations and underwater photographs of the species were traced back to August 2005 (Tendal et al. 2007; Riisgård et al. 2007, 2012). The earliest observation of *M. leidyi* in 2011 was made in Limfjorden 20 September (Riisgård et al. 2012). Later, between 15 December 2011 and 18 January 2012, it occurred in Great Belt at the inlet to Kerteminde harbor, and during the same period it was observed also in Øresund (Cornelia Jaspers, pers. comm.) and in Kattegat (Lene Friis Møller, pers. comm.). These records support the hypothesis that the eutrophic Limfjorden may act as an incubator for ctenophores which subsequently disperse into Kattegat and other inner Danish waters (Riisgård et al. 2012). Although the abundance of the species is fluctuating from year to year it is now considered a permanent element of the Danish fauna.

*Pleurobrachia pileus, Bolinopsis infundibulum* and *Beroe cucumis* have been known in the Danish marine fauna for more than 100 years, commonly occurring throughout the area (Kramp 1913, 1915, 1937; Mortensen 1912), but *B. gracilis* is new to the inner Danish waters. It may, however, has been present for years, misidentified as young stages of *B. cucumis*, because it is difficult to distinguish larval and juvenile stages of species within the same order of ctenophores.

*B. gracilis* is known from the North Sea and Skagerrak (Kűnne 1939; Hansson 2006; Granhag et al. 2012). Fully developed specimens have slender cylindrical body up to 3 cm in length (Figure 5A) with milky or slightly pink color. The body is only moderately compressed. The 8 meridional canals have very few diverticulae and they extend inwards toward the stomodeum.
rather than in the plane of the body surface (Figure 5C). The aboral pole is fringed with a row of papillae (Künne 1939; Greve 1975) (Figure 5B).

Before Mnemiopsis leidyi became widely distributed in western European waters, the two lobate species Bolinopsis infundibulum and M. leidyi did not overlap in distribution. Co-occurrence is now a fact, and it seems to be a widespread phenomenon in the region as the two species were also observed in Kattegat in December-January 2011/2012 (L. F. Møller, pers. comm.), and even earlier, in Kerteminde harbor 4 February 2008 along with Møller, pers. observation). Morphologically they resemble each other but they can be distinguished based on the position of the oral lobes. In M. leidyi, these originate near the level of infundibulum, whereas in B. infundibulum the position is lower, approximately half-way between the mouth and the infundibulum (Mayer 1912) (Figure 3).

The recent increase from 3 to 6 species of ctenophores found in inner Danish waters may have been caused by a combination of changes in the hydrographic patterns (MacKenzie et al. 2007), the rise in sea temperature allowing southern species to penetrate northern waters (Lynam et al. 2010; Galil et al. 2011), and a lack of systematic monitoring of the gelatinous plankton. Two other species occur in adjacent waters and should be looked for: the cydippid Mertensia ovum (Fabricius, 1780), which has been found in the northern part of the Baltic Sea (Gorokhova et al. 2009) and recently also in Skagerrak and Kattegat along the Swedish coast (Jaspers et al. 2013); and Euplokamis sp. found in the Gullmar Fjord on the Swedish west coast (Granhag et al. 2012).

Due to absence of predators, the Mnemiopsis leidyi invasion around 1982 had dramatic effects on the planktonic ecosystems of the Ponto-Caspian basin and the Sea of Marmara (Shiganova et al. 2004 a, b). In 1997 its natural predator, Beroe ovata (sensu Mayer, 1912), was introduced in the Black Sea (Konsulov and Kamburska 1998), and it now controls the population of M. leidyi in the area (Shiganova et al., in press). So far, B. ovata has followed its prey and is spreading into adjacent parts of the Mediterranean where established M. leidyi populations are found (Shiganova et al. 2007; Galil et al. 2011). Accordingly, the presence of the unusual mixture of ctenophore species in Great Belt may predict future changes in Danish and other temperate coastal waters.

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