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Molecular evidence for the occurrence of ctenophore *Mertensia ovum* in the northern Baltic Sea and implications for the status of the *Mnemiopsis leidyi* invasion

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Abstract

Nucleotide sequence analysis of 18S ribosomal RNA gene (rRNA), internal transcribed spacer, and 5.8S rRNA was used for taxonomic identification of ctenophores collected in the northern Baltic Sea, where invasive *Mnemiopsis leidyi* and native *Pleurobrachia pileus* have been reported to occur. Contrary to previous reports, sequence analysis of 53 randomly selected specimens from seven stations revealed that none of them were *M. leidyi* or *P. pileus*. The 18S rRNA and 5.8S rRNA sequences were 100% identical to those of *Mertensia ovum*, a ctenophore with a broad Arctic and circumboreal distribution, which has never been reported to occur in the Baltic Sea. Polymerase chain reaction screening with primers designed to amplify all three species, and using ctenophores collected by vertically stratified sampling, confirmed that all ctenophores collected in this survey were *M. ovum*. The ctenophore abundance was high, up to 4500 individuals m^{-2}, positively correlating with salinity. Our findings emphasize the utility of applying molecular tools to biological surveys and the importance of rigorous species identification. They also indicate that *M. leidyi*, which is a threat to the southern Baltic ecosystem, does not occur in the northern part of the sea, and call for a pan-Baltic survey to establish current distributions of ctenophores, both native and invasive.

Blooms and invasions of jellyfish and ctenophores present problems in coastal waters worldwide, especially since the 1980s, and climatic and anthropogenic causes for changes in their populations have been suggested (reviewed in Purcell et al. 2007). When great abundances occur, jellyfish and ctenophores can consume large quantities of ichthyoplankton and zooplankton, interfere with fishing and aquaculture, clog water intakes, and cause health concerns for swimmers. Because of these negative effects, it is important to document long-term changes in abundance and distribution of their populations and to understand which environmental factors cause increases in jellyfish and ctenophore populations.

The invasive lobate ctenophore *Mnemiopsis leidyi*, which has caused serious ecological problems in the Black and Caspian Seas, has expanded its distribution to the European Atlantic coast (Faasse and Bayha 2006) and the Baltic Sea (Javidpour et al. 2006). As of the summer of 2007, it had been reported from virtually all basins of the sea, including its northern part (Lehtiniemi et al. 2007). The other comb jelly known to be native and present throughout the Baltic is *Pleurobrachia pileus*, belonging to the order Cydippida. This species is cosmopolitan, occurring in the marine waters of almost all of northern Europe (Mayer 1912; van der Veer and Sadée 1984), including the northern Baltic proper (Ackefors 1969), the Gulf of Finland, and the Bothnian Sea (Sandström and Sörlin 1981; Vuorinen 1987; Vuorinen and Vihersaari 1989). In the southern part of the Baltic, three other ctenophore species may also be found as a result of saline water inflows from the Kattegat into the western Baltic Sea. These are *Bolinopsis infundibulum*, belonging to the order Lobata, and *Beroe cucumis* and *Beroe gracilis*, belonging to the order Beroida (Greve 1975). Therefore, in view of the documented species distributions, the expectation would be that *M. leidyi* and *P. pileus* co-occur in the northern Baltic Sea.

Ctenophores are marine hermaphroditic invertebrates with direct development. Although morphologically quite diverse, ctenophores are unified by a characteristic developmental stage, the "cydippid larva" (Hyman 1940), which has very similar morphology in tentaculate (e.g., *P. pileus*) and lobate (e.g., *M. leidyi*) ctenophores. A newly hatched larva is 0.3–0.4 mm in diameter and possesses two tentacles that are used to capture prey. In lobate ctenophores, this tentaculate-stage larva grows into an ovoid transition-stage larva (~5 mm) characterized by tentacles and also small oral lobes that allow them to feed using a combination of mechanisms for prey capture. This transition-stage larva develops into a fully lobate individual (6–15 mm), in which the tentacles are resorbed, and food capture occurs either on the inner surface of the oral lobes or by prey entrainment in feeding currents generated by cilia-lined auricles (Sullivan and Gifford 2004; Haddock 2007). By contrast, in growing cydippid ctenophores, no transition occurs and tentacles remain in older stages. Although species identification is challenging for adult ctenophores (Podar et al. 2001), it is even more difficult for specimens in early life stages, for which there are often no easily identifiable morphological characters (Mayer 1912). In such cases, molecular methods could greatly improve the accuracy of identification.

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The objective of our study was to assess species composition and distribution of ctenophores in the northern Baltic Sea; they were expected to be native *P. pileus* and invasive *M. leidyi*, in accordance with previous studies (Ackefors 1969; Vuorinen and Vihersaari 1989; Lehtiniemi et al. 2007). Species identification was based on sequencing 18S ribosomal RNA gene (rRNA), internal transcribed spacer (ITS1), and 5.8S rRNA gene using ctenophores collected by vertically stratified sampling. However, contrary to our expectations, the only ctenophore species that was present was a cydippid ctenophore, *Mertensia ovum*. Here we report the occurrence, identification strategy, and vertical distribution of *M. ovum*, previously unknown in the Baltic Sea.

Methods

Sampling methods and locations—Distribution and abundance of ctenophores were surveyed in September 2008 in the Gulf of Finland, the Åland Sea, and the Bothnian Sea (Table 1; Fig. 1). The samples were collected on a research cruise on board the R/V *Aranda*. Ctenophores were sampled using a 500-μm mesh WP-2 plankton net with a cod end (100-μm mesh size). At each station, net tows were taken at a rate of 0.5 m s^{-1} from the bottom to the halocline, then from the halocline to the thermocline, and finally from the thermocline to the surface. In addition, at some stations one or two tows were taken from the bottom to the surface to estimate total abundance. From one station (US5B), higher-resolution vertical profile samples were taken every 20 m, with two replicates for each depth stratum; the replicates were pooled for the analyses. All ctenophores were counted live using a dissecting microscope with 10–60× magnification within 1–2 h after collection. On each station, a subsample of 36–107 specimens was used to measure individual diameter (aboral–oral dimension); the size classes were set to the closest 1 mm. The counts per tow were converted to abundances per bottom area (individuals [ind.] m^{-2}) and per volume (ind. m^{-3}). From the same subsamples, ctenophores of different size classes were randomly selected for molecular analyses.

In addition, ctenophore specimens obtained in August 2007 in the Landsort Deep (Sta. BY31) within the Swedish National Monitoring Program were used for molecular analyses; samples were taken using a WP-2 net (mesh size 90 μm) by vertical hauls from 100 to 60 m (one specimen, 2 mm) and from 60 to 30 m (two specimens, <1 mm).

Deoxyribonucleic acid (DNA) extraction—Ctenophores collected at each station (Table 1) were placed individually or in groups of up to five individuals (those ≤1 mm) into Eppendorf tubes containing 400 μL of 4 mol L^{-1} urea, 1% Tween 20, 1% Nonidet P-40, 10% Chelex 100, and 0.005 mg proteinase K in sterile water (Aranishi and Okimoto 2006) within 2 h after collection. The tubes were incubated at 55°C for 20 min, and then at 105°C for 8 min. At this point, the samples were frozen at −20°C and stored for 1–4 months (samples collected in 2008) or 16 months (samples collected in 2007) until analyses. The extract was thawed and then centrifuged at 14,000 × *g* for 15 min, and the precipitate containing the chelating resin was discarded. The supernatant was purified using QIAamp Mini spin columns (QIAGEN®), eluted with 100 μL of sterile water, and used in polymerase chain reaction (PCR). The total DNA yields from the samples were 0.2–138 μg $sample^{-1}$.

Molecular species identification—The ribosomal DNA gene complex includes the 18S, 5.8S, and 28S genes, which code for rRNA and have relatively conserved nucleotide sequences. It also includes the variable DNA sequence areas of the intervening internal transcribed spacer regions, ITS1 and ITS2. Both rRNA genes and spacers are very useful in delineating species and genera in a variety of organisms, including ctenophores (Podar et al. 2001). To identify the species in question, we amplified and sequenced the 18S rRNA, ITS1, and 5.8S rRNA from the ctenophore DNA samples. We used 50 randomly selected samples from the collection made in 2008 and all 3 specimens available from 2007 to amplify 18S rRNA, ~1800 base pairs (bp). The amplifications were performed on an MJ Research MiniCycler using universal eukaryotic primers (Kober and Nichols 2007) and a ~600-bp fragment covering the 3′ terminus of 18S rRNA, ITS1, and 5.8S rRNA using the primers located at the 3′ terminus of the 18S rRNA (Podar et al. 2001) and the 3′ terminus of the 5.8S rRNA (Table 2). PCR of 25 μL contained 12.5 μL of Promega PCR Master Mix, 2 μL of each primer (final concentration 0.2 μmol L^{-1}), 3 μL of DNA template, and 5.5 μL nuclease-free water (Gibco). The cycling regime was as follows: initial denaturing period of 8 min at 95°C followed by 30 cycles of 95°C (30 s), 64°C (30 s), 72°C (15 s), with a final extension for 7 min at 72°C. Completed PCRs were separated in 1.5% (w/v) agarose gel with a 100-bp ladder at 70 V in Tris-borate-ethylenediaminetetraacetic acid buffer (pH 8.6), and visualized by staining with ethidium bromide. PCR products were purified using the Nucleo-Spin® Extract Kit (Macherey-Nagel) according to the manufacturer's instructions and subsequently cloned into the TOPO TA vector (Invitrogen). Plasmid DNAs from 3–10 individual clones were sequenced in both directions with an ABI 373 automated sequencers at Bioneer (Chungwon) and ABI 3730 PRISM® DNA Analyzer at KIGene (Karolinska Institute). The resulting nucleotide sequences were assembled and aligned using BioEdit software (Hall 1999) and electropherograms were checked by eye for poor base calls and sequence quality, yielding a sequence for 18S rRNA gene, ITS1, and 5.8S rRNA gene that was identical for all individuals analyzed. The sequence obtained was deposited in GenBank (FJ668937). Sequence identity was evaluated by performing BLASTN searches against GenBank and aligned against Atlantic *M. leidyi* (NCBI accession number AF293700, originated from Woods Hole, Massachusetts, Podar et al. 2001; L10826, unknown origin, Wainright et al. 1993; and EF175463, coastal waters of the Netherlands, Faasse and Bayha 2006), *P. pileus* (AF293678; Woods Hole, Massachusetts, Podar et al. 2001), *M. ovum* (AF293679; Newfoundland coastal waters, Canada) and two undescribed mertensiids

Table 1. Details on sampling locations, depth, sample sizes, and environmental factors; n/a, not available.

Sta.	Location		Date	Bottom depth (m)	Depth strata (m)	Temperature (°C)	Salinity	Number of specimens (ind. tow^{-1})	Average abundance (ind. m^{-2})	Number of samples for DNA analysis		
	Latitude	Longitude								Individual	Pooled	Sequenced
BY31	58°35.90	18°14.21	21 Aug 07	454	60–30	4.9	7.4	n/a	n/a	2	0	2
					100–60	5.2	9.8	n/a	n/a	1	0	1
LL7	59°50.79	24°50.27	08 Sep 08	100	20–0	15.1	5.4	2	1532	1	0	0
					50–20	8.9	6.4	32		12	0	1
					98–50	4.3	7.9	349		20	15	3
F64	60°11.34	19°08.55	09 Sep 08	285	20–0	14.0	5.3	0	1164	0	0	0
					60–20	7.4	6.3	196		22	4	3
					281–60	4.7	7.0	95		27	6	4
US5B	62°35.17	19°58.13	10 Sep 08	211	20–0	10.2	5.2	0	352	0	0	0
					40–20	3.5	5.5	0		0	0	0
					60–40	3.2	5.7	0.5		1	0	1
					80–60	3.0	5.9	7		3	0	1
					100–80	3.4	6.1	4.5		3	0	0
					120–100	3.5	6.1	20.5		2	8	1
					140–120	3.6	6.2	8.5		2	2	0
					160–140	3.7	6.2	14		10	0	3
					180–160	3.7	6.2	20.5		16	5	4
					200–180	3.7	6.2	12.5		7	2	3
F26	61°59.01	20°03.78	11 Sep 08	143	15–0	13.0	5.2	0	125	0	0	0
					80–15	4.0	5.7	3		1	0	1
					131–80	3.6	6.3	27		15	0	3
					131–0			32		18	3	3
SR5	61°05.00	19°35.00	11 Sep 08	130	20–0	13.9	5.5	0	60	0	0	0
					70–20	5.4	5.7	0		0	0	0
					120–70	3.6	6.3	20		5	2	1
					120–0			10		3	0	1
F67	59°56.00	19°49.80	15 Sep 08	210	30–0	13.5	5.6	2	4488	0	0	0
					70–30	8.1	6.5	943		34	22	8
					200–70	5.3	7.1	177		22	15	9

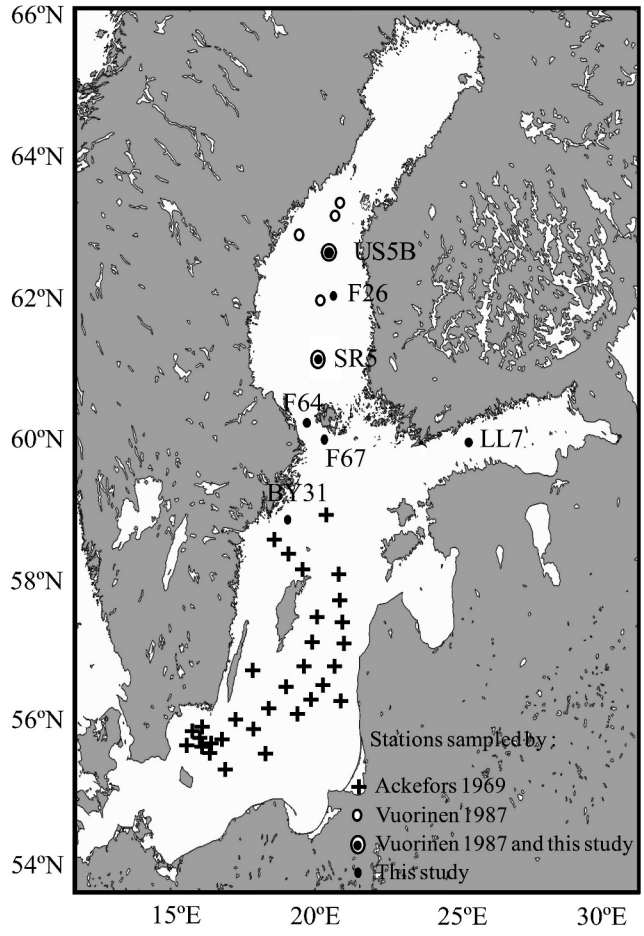

Fig. 1. Stations where ctenophores were collected for this study (2007 and 2008) as well as those visited in earlier surveys of *Pleurobrachia pileus* abundance and distribution in the Baltic proper and the northern Baltic Sea (Ackefors 1969; Vuorinen 1987).

(AF293680 and AF293681; Bahamas and Santa Barbara, California, Podar et al. 2001). In addition to the reference specimen of *M. ovum* collected from Newfoundland, a second sequence covering the 18S rRNA, ITS1, and 5.8S rRNA region was obtained from an adult *M. ovum* specimen collected in the Arctic Ocean, north of Alaska, and compared to that in the Baltic specimens.

The phylogenetic tree based on sequence analyses of the ITS1 was generated using maximum-parsimony methods using command-line PAUP (Swofford 2003). The alignment was generated using MUSCLE (Edgar 2004), with minor adjustments when default gap insertion was inconsistent between taxa. Trees were examined and visualized using FigTree 1.2.2 (Rambaut 2007).

Following the original sequencing, species-specific primers were designed for the sequence obtained for the Baltic specimens of *M. ovum* (M1F-M1R and M2F-M2R) as well as for the available sequences for *M. leidyi* (ML1F-ML1R and ML2F-ML2R) and *P. pileus* (PP1F-PP1R and PP2F-PP2R), based on a multiple alignment and using Primer3 software and BLASTN (Table 2). The primers were used in PCR reactions using the same protocol as above. Completed PCRs were analyzed by electrophoresis with a 50-bp ladder; a sample was considered successful when a band was observed in the expected size range (250–590 bp); band intensity was not evaluated. The primers were tested using the Baltic *Mertensia* and a DNA sample of *M. leidyi* (lobate, fully grown individual originated from the Caspian Sea) donated by Dr. M. Orlova (Zoological Institute, Russian Academy of Science, St. Petersburg, Russia) as positive controls for *Mertensia* sp. and *M. leidyi*, respectively. No DNA sample from *P. pileus* was available; therefore, we relied on extensive in silico testing and stringent primer design. Thereafter, the remaining DNA samples of the Baltic ctenophores (224 samples containing individual specimens and 84 pooled samples; 612 individuals in total) were used in separate PCR reactions with all pairs of the species-specific primers; all samples were run in duplicates.

Results

Species identification—The sequence analysis of 53 randomly selected specimens from seven sampling sites

Table 2. Oligonucleotide primers used for PCR.

Primer	Sequence (5′ to 3′)	Priming site	Main target region	Reference
18SF	CTGGTTGATCCTGCCAGTAGT	5′ terminus of 18S rRNA	18S rRNA	Kober and Nichols 2007
18SR	GCAGGTTCACCTACAGAAACC	3′ terminus of 18S rRNA		Kober and Nichols 2007
1400F	TGYACACACCGCCCGTC	3′ terminus of 18S rRNA	ITS1+5.8S rRNA	Podar et al. 2001
5.8SR	GTTTGCTGCGTTCTTCATCG	3′ terminus of 5.8S rRNA		this study
M1F	CGCCGAAAACTTGCTCAAAC	3′ terminus of 18S rRNA	ITS1	this study
M1R	CCGAGCGACAGATCGGATAC	ITS1		this study
M2F	GTGCTGATTACGTCCCTGCC	3′ terminus of 18S rRNA	ITS1	this study
M2R	CCCACGGACGATTTAACGAA	ITS1		this study
PP1F	CGTAGGTGAACCTGCGGAAG	3′ terminus of 18S rRNA	ITS1+5.8S rRNA	this study
PP1R	GCTCGGGGATCGCTCTACTT	ITS2		this study
PP2F	AGACTTCATCGTGCTGGGGA	18S rRNA	18S rRNA+ITS1	this study
PP2R	GTTAGGCCAACCCCGAAGAC	ITS1		this study
ML1F	TCGATGAAGAACGCAGCAAA	ITS1	ITS1+5.8S rRNA	this study
ML1R	GAACCCTTTCCAGTCGTCCC	ITS2		this study
ML2F	TAGGTGAACCTGCGGAAGGA	3′ terminus of 18S rRNA	ITS1+5.8S rRNA	this study
ML2R	CTTCGGACATCCTGCAAAGC	ITS2		this study

Table 3. Identities (%) between the consensus nucleotide sequence for *Mertensia ovum* collected in the northern Baltic Sea (accession number FJ668937) and sequences for relevant ctenophore species: *Mnemiopsis leidyi*, *Pleurobrachia pileus*, *Mertensia ovum*, and two undescribed mertensiids. GenBank accession numbers are in parentheses. n/a, not available.

Species	18S rRNA	ITS1	5.8S rRNA
M. ovum (North Atlantic; AF293679)	100	n/a	n/a
M. ovum (High Arctic; AF293679)	100	97	100
Undescribed mertensiid 1 (AF293680)	99	n/a	n/a
Undescribed mertensiid 2 (AF293681)	97	n/a	n/a
M. leidyi (AF293700)	96	58	95
M. leidyi (L10826)	96	n/a	n/a
M. leidyi (EF175463)	n/a	58	n/a
P. pileus (AF293678)	95	55	96

(Fig. 1; Table 1) revealed that a single species was present in our collections, and this was neither *P. pileus* nor *M. leidyi*. The BLASTN search in the NCBI GenBank database revealed that the perfect match for the 18S rRNA of the species in question was *M. ovum* (AF293679), a ctenophore with a broad Arctic and circumboreal distribution, which has never been reported to occur in the Baltic Sea.

Sequences were obtained for a 2252-bp-long region covering the 18S rRNA (1807 bp), ITS1 (275 bp), and 5.8S rRNA genes (170 bp) from the Baltic ctenophores. The consensus sequence was aligned with published sequences for *M. leidyi*, *P. pileus*, *M. ovum*, and two undescribed mertensiid species (Podar et al. 2001). We then amplified and sequenced the ITS1 and 5.8S rRNA from the Arctic *M. ovum* DNA. Therefore, the identity of the ctenophore in question was inferred from the combination of 18S rRNA and 5.8S rRNA sequences, which had 100% identity with the Atlantic and Arctic *M. ovum* sequences, and the ITS1 region, which was 97% identical with the Arctic *M. ovum* sequences (Table 3). The entire sequence was invariant in all Baltic specimens examined, whereas there was some variability between ITS1 rRNA clones in the Arctic *M. ovum* (Fig. 2). Both *M. leidyi* and *P. pileus* were ruled out as possible matches because of low sequence identity in the ITS1 region, 58% and 55%, respectively (Table 3). For 18S and 5.8S rRNA genes, which are exceptionally conserved in ctenophores (Podar et al. 2001), sequence identities between the consensus sequence and those for *M. leidyi* and *P. pileus* were also lower than for the mertensiids (95–96% vs. 97–100%; Table 3).

All Baltic ctenophore samples analyzed produced positive amplifications with the expected fragment sizes when primers designed as species-specific for *M. ovum* were used (~250 and 420 bp for M1F-M1R and M2F-M2R, respectively), whereas there were no positive amplifications for either *Mnemiopsis*- or *Pleurobrachia*-specific primers. All samples used for sequencing produced positive amplifications with the *Mertensia*-specific primers but not with any of the *Mnemiopsis*- or *Pleurobrachia*-specific primers.

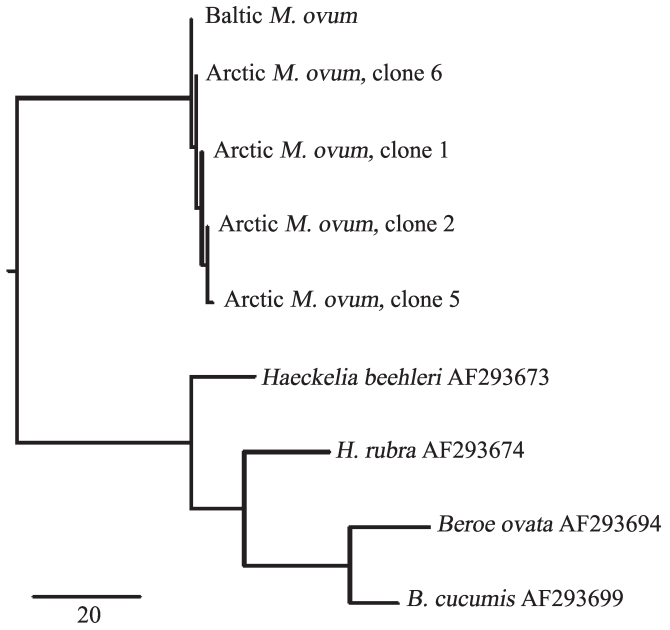

Fig. 2. Maximum parsimony tree for ITS1 (214–278 bp) from selected ctenophores, showing the variability between even closely related species (*Beroe* spp. and *Haeckelia* spp.; sequences were obtained from GenBank, *see* accession numbers), in contrast with the near identity of Baltic and Arctic specimens of *Mertensia ovum*. Bootstrap values are 100% for (*Beroe* + *Haeckelia*) and 93–98% for the two *Beroe* species. The scale bar on the tree corresponds to 20 changes.

Similarly, both pairs of primers designed to amplify *Mnemiopsis* tested positively for *M. leidyi* in the reference sample, producing fragments of ~330 and 590 bp for ML1F-ML1R and ML2F-ML2R, respectively, whereas both *Mertensia*- and *Pleurobrachia*-specific primers tested negatively.

Total ctenophore abundance and distribution—Ctenophores were found from all six stations sampled in the Gulf of Finland, the Åland Sea, and the Bothnian Sea in September 2008 (Table 1). They were most abundant (4.5×10^3 ind. m^{-2}) at Sta. F67, south of Åland Island. High abundances were also observed in the Gulf of Finland (Sta. LL7) and in the Åland Sea (Sta. F64), with 1.5×10^3 and 1.1×10^3 ind. m^{-2}, respectively. Ctenophores occurred predominantly in the deeper water layers, with >99% of the population situated below 15–30 m and 72% below 50–80 m. This indicates a possible avoidance of the upper part of the water column above the thermocline, which was situated at 25 ± 8 m (mean ± SD, $n = 6$). In contrast with this pattern of increasing ctenophore abundance with depth, at two stations (F64 and F67) the highest densities (20 and 94 ind. m^{-3}, respectively) were observed in a middle water layer, 20–70 m. Generally, salinity started to increase at a depth of 20 m, but there was no sharp halocline at any of the stations. Ctenophore abundance correlated positively with salinity (Spearman rank correlation $\rho = 0.87$, $n = 25$, $p < 0.001$), whereas no correlation was found between abundance and temperature ($\rho = -0.11$, $n = 25$, $p > 0.6$).

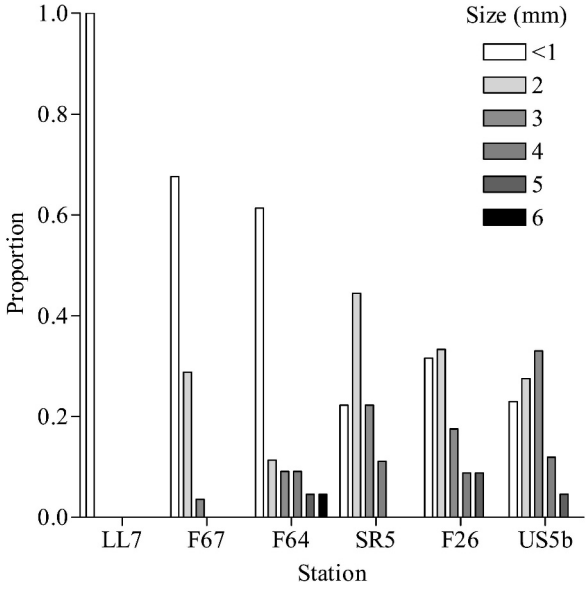

Fig. 3. Size distributions of *Mertensia ovum* collected at different sites (*see* Fig. 1 and Table 1 for details on sampling locations and codes). Individual diameter (aboral–oral dimension) was measured in subsamples of 36–107 specimens; the size classes were set to the closest 1 mm. Stations are ordered from south to north.

Size distribution—Length distribution of the ctenophores differed between the stations (Fig. 3). At the southernmost Sta. (LL7 and F67), most individuals were ≤1 mm, whereas body size spanned a wider range in the more northern stations, with average body size significantly increasing from south to north, as revealed by linear regression on log-transformed ctenophore body size and latitude ($R^2 = 0.5$, $p < 0.001$; Fig. 3). The largest individuals observed at the southernmost stations (LL7 and F67) were 3 mm, whereas specimens up to 6.5 mm were found in the Bothnian Sea.

Discussion

All specimens collected from different locations in the northern Baltic Sea in August 2007 and September 2008 shared a single 18S rRNA sequence that was 100% identical to those in two taxonomically validated *M. ovum* specimens sampled in the North Atlantic and high Arctic. Moreover, a complete identity was observed for 5.8S rRNA between the Baltic ctenophores and *M. ovum* sampled in the Arctic. *M. ovum* is the only described species in the *Mertensia* genus (order Cydippida, family Mertensiidae). It has a broad Arctic and circumboreal distribution, contributing greatly to gelatinous zooplankton in Arctic waters and marginal seas (Mayer 1912; Percy 1989; Swanberg and Båmstedt 1991), but has never been reported from the Baltic Sea. The complete identity of 18S rRNA and 5.8S rRNA suggests that the species in question is *M. ovum*. Moreover, comparison of the more variable gene ITS1 showed that the sequences from the Baltic ctenophores were 97% identical to those of the two *M. ovum* specimens. The wide range over which the specimens were collected makes it even more convincing that they are all the same species. Based on these sequences, we identified the Baltic ctenophore as *M. ovum*; its phylogenetic relationships with Arctic and Atlantic populations have to be further analyzed using other molecular and morphological characters.

It is not that surprising that *M. ovum* is present in the biogeographically heterogeneous Baltic Sea as a member of the North Atlantic boreal marine complex or an Arctic relict. In the Baltic Basin, temporally continuous brackish-water conditions have existed for only the past 7000 yr. It is generally agreed that the marine flora and fauna of the present-day Baltic Sea derive from species that invaded the area during the Littorina Sea period, 8000–4000 yr B.P. (Ignatius et al. 1981); however, for some species, repeated trans-Arctic invasions have been suggested (Väinölä 2003). The distributions of these marine species often extend all the way to the oligohaline northern Baltic (Segerstråle 1957); however, to the best of our knowledge *M. ovum* has never been reported to be euryhaline in other regions. Yet, because anthropogenic introductions of marine species into the Baltic Sea are frequent (Leppäkoski et al. 2002), the possibility could not be ruled out that the occurrence of *M. ovum* in the Baltic is due to recent human activity. Clearly, comprehensive studies including genetics, morphology, physiology, and ecology of this previously unnoticed species are urgently needed to investigate evolutionary ancestry and gene flow between geographical populations, distribution, and ecological role in the Baltic Sea.

Assessing the ecological role of a species requires knowledge of its energy utilization and diet. Mertensiids have a feeding biology similar to that of other cydippid ctenophores, catching micro- and mesozooplankton with a pair of tentacles (Haddock 2007). Feeding experiments (Swanberg and Båmstedt 1991) and lipid composition (Lundberg et al. 2006; Graeve et al. 2008) suggest that calanoid copepods are a main food source of *M. ovum* in the Arctic seas. However, when compared to other zooplanktivores in the same food webs (e.g., chaetognaths), field-collected *M. ovum* had substantially lower trophic level as revealed by stable isotope analysis (Hobson et al. 2002), indicating possible importance of microplankton in the diet. Unfortunately, extrapolating diets inferred for Arctic *M. ovum* to the Baltic populations is not possible because of the great difference in average body size (*see* below). In this sense, it seems more relevant to assume that the diet of Baltic *M. ovum* would be closer to that of tentaculate-stage larval *M. leidyi*, which feeds largely on microplankton, such as phototrophic and heterotrophic dinoflagellates, ciliates, and euglenoid flagellates (Sullivan and Gifford 2004). In Baltic pelagic food webs, predation on a combination of heterotrophic and autotrophic microplankton by *M. ovum* could potentially imply competitive interactions with herbivorous crustaceans. On the other hand, similar to ctenophores in other regions (Mianzan et al. 1996), *M. ovum* could serve as a prey for zooplanktivores, such as mysids, herring, and sprat—a prey that has never been accounted for in food web models. The assessment of *M. ovum*'s ecological significance in the Baltic Sea requires coherent experimental and field studies as well as data on its distribution and population dynamics.

The abundances of the ctenophores recorded in our study (60–4500 ind. m^{-2}; Table 1) were much higher than maximal abundances reported for *M. ovum* from the Arctic (e.g., Frobisher Bay, Canada, 25 ind. m^{-2}, at stations of 30–600 m depth, Percy 1989; Barents Sea, 44 ind. m^{-2}, 100–300 m depth, Swanberg and Båmstedt 1991; Kongsfjorden, Svalbard, 140 ind. m^{-2}, <300 m depth, Lundberg et al. 2006), but similar to those in the Resolute Passage area of the Canadian High Arctic, where up to 900 ind. m^{-2} was observed (Siferd and Conover 1992). The body size range in the Baltic ctenophores, on the other hand, was 0.5–6.5 mm, which is much lower than sizes reported for the Arctic *M. ovum* (Barents Sea, 3–57 mm, Swanberg and Båmstedt 1991; the Resolute Passage, 3–55 mm, Siferd and Conover 1992; Kongsfjorden, 1–48 mm, Lundberg et al. 2006). The large differences in abundance and body size distribution may in part be explained by the fact that nets with greater mesh size (300–1000 μm) were commonly used to sample ctenophores in high latitudes, including the studies mentioned above, whereas the cod end of 100 μm used in our study made it possible to collect individuals <1 mm, which comprised 22–58% of the total ctenophore abundance (Fig. 3). Studies of the vertical distribution of *M. ovum* have given varying results, perhaps depending on water depth and degree of vertical stratification. In our study, the major distribution zone was below 50–80 m and abundances correlated positively with salinity, with only solitary individuals found above the thermocline. However, at some stations the bulk of the population was situated between 20 and 70 m, which is similar to the major distribution zone of 20–50 m reported for *M. ovum* in the Barents Sea (Swanberg and Båmstedt 1991). The large variability in ctenophore abundance and distribution due to variations in temperature and salinity as well as patchy distribution of these animals is the most probable causes of the discrepancies in their abundance estimates (Vuorinen 1987; Lundberg et al. 2006). Because there are no records of *M. ovum* in low salinity environments, the Baltic population of *M. ovum* is extremely interesting from the ecological plasticity perspective of this species and its evolution.

Neither *M. leidyi* nor *P. pileus* was present in our collections, as evidenced by low identity in the ITS1 region between sequences obtained in this study and GenBank sequences (Table 3). Although the ITS1 sequence in *M. leidyi* from the coastal waters of the Netherlands (EF175463) differed from that in *M. leidyi* from the U.S. Atlantic waters (AF293700) by a single base (i.e., >99% identity; Faasse and Bayha 2006), only 58% identity in this region was observed between the Baltic *M. ovum* and either of the *M. leidyi* sequences held in GenBank. This identity percentage is of the same magnitude or even lower than between ctenophores from different orders (*M. leidyi* vs. *P. pileus* is 59%, for example). Similarly, comparison for the conserved regions, 18S and 5.8S genes, revealed relatively low percentage sequence identities (Table 3), keeping in mind that these regions have extraordinary low variability in ctenophores (Podar et al. 2001) and that 18S rRNA of *M. leidyi* from the coastal waters of the Netherlands has 99.8% identity with that of *M. leidyi* from U.S. Atlantic waters.

Our findings call for a thorough survey covering different parts of the Baltic Sea and seasons, to revise species composition of ctenophores and their distribution, including invasive *M. leidyi*. During our field studies in the northern Baltic Sea, we have taken hundreds of ctenophore samples and examined tens of thousands of individuals; however, no lobate forms have ever been observed, with individuals ≤2 mm clearly dominating (Viitasalo et al. 2008) and the largest ones being 10–12 mm. Although *Mnemiopsis* occurrence reported from the North Sea, Kattegat, and Belt Sea and Bornholm areas is well supported by observations of lobate forms (Faasse and Bayha 2006; Javidpour et al. 2006; Haslob et al. 2007), to the best of our knowledge, they have never been collected north of Gotland. Similar to our observations in the northern Baltic Sea, most of the *Mnemiopsis* populations in the southwestern part of the sea consist of individuals <1 mm (Kube et al. 2007), for which taxonomic identification is far from reliable (Mayer 1912). Because *Mertensia* abundance correlates positively with salinity, it is likely to be present also in the southern parts of the Baltic. Moreover, *P. pileus* has been found to occur throughout the sea (Ackefors 1969; Vuorinen 1987; Vuorinen and Vihersaari 1989; Fig. 1), yet its presence is never reported in studies describing distribution of *M. leidyi*. This raises a question—could *Mertensia* be systematically misidentified as *P. pileus* and/or juvenile *M. leidyi* because of the superficial similarity between these species at a young age? Previously published abundance data should therefore be reexamined where populations were largely dominated by small-sized individuals. It might be possible that the spread of *M. leidyi* is less dramatic than is currently believed. Considering the ability of *M. leidyi* to modify the trophic structure of invaded ecosystems and its rapid expansion (Purcell et al. 2001; Haslob et al. 2007), it is crucial to reliably assess the species distribution and abundance, which underlines the urgency for undoubted species discrimination.

In many invertebrate species, including jellyfish, morphological identification of larval stages is challenging, which makes the DNA-based approach particularly useful. Moreover, a critical aspect of invasive species monitoring is the ability to accurately identify any intercepted specimen to the species level. At early stages of invasion, only a few specimens might be available for the analysis, and these might include morphologically indistinct immature life stages and damaged specimens. This is especially true for invasive ctenophores that are small, fragile organisms, poorly preserving with standard plankton fixation techniques (i.e., formalin or ethanol solutions). Molecular methods and, particularly, DNA bar coding are essential for associating different developmental stages in order to identify invasive species (Armstrong and Bell 2005). Therefore, the development of simple standardized PCR-based assays, which could be used to discriminate between morphologically similar ctenophore species and associated adult and juvenile stages, is essential for field surveys of ctenophores collected from different geographic locations by different scientists not familiar with these unique taxa.

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