Bacterial communities associated with the ctenophores *Mnemiopsis leidyi* and *Beroe ovata*

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**Abstract**

Residing in a phylum of their own, ctenophores are gelatinous zooplankton that drift through the ocean’s water column. Although ctenophores are known to be parasitized by a variety of eukaryotes, no studies have examined their bacterial associates. This study describes the bacterial communities associated with the lobate ctenophore *Mnemiopsis leidyi* and its natural predator *Beroe ovata* in Tampa Bay, Florida, USA. Investigations using terminal restriction fragment length polymorphism (T-RFLP) and cloning and sequencing of 16S rRNA genes demonstrated that ctenophore bacterial communities were distinct from the surrounding water. In addition, each ctenophore genus contained a unique microbiota. Ctenophore samples contained fewer bacterial operational taxonomic units (OTUs) by T-RFLP and lower diversity communities by 16S rRNA gene sequencing than the water column. Both ctenophore genera contained sequences related to bacteria previously described in marine invertebrates, and sequences similar to a sea anemone pathogen were abundant in *B. ovata*. Temporal sampling revealed that the ctenophore-associated bacterial communities varied over time, with no single OTU detected at all time points. This is the first report of distinct and dynamic bacterial communities associated with ctenophores, suggesting that these microbial consortia may play important roles in ctenophore ecology. Future work needs to elucidate the functional roles and mode of acquisition of these bacteria.

**Introduction**

Ctenophores are gelatinous zooplankton that are ubiquitous throughout the world’s oceans. Although they are often mistaken for jellyfish, ctenophores comprise a phylum of their own, and a recent reorganization within the animal tree of life places ctenophores as the earliest-diverging extant form of multicellular animals (Dunn et al., 2008). Composed of >95% water (Bailey et al., 1995; Scolardi et al., 2006), these carnivorous animals glide through the oceans via ciliary action and/or currents (Mills & Haddock, 2007). Their position in the oceanic food web was initially thought to be an ecological dead end because of their low biomass content (Vinogradov et al., 1992; Dumont et al., 2004), but reports have now shown that ctenophores can be consumed by various fish, turtles, jellyfish, and even other ctenophores (Arai, 2005).

Recent work has demonstrated that the overall effect of gelatinous organisms such as ctenophores is to generate dissolved organic carbon that promotes microbial respiration, further fueling the microbial loop rather than being incorporated into biomass, which can be transferred up the food chain (Condon et al., 2011). Because of the effects of global climate change and the exploitation of global fish stocks, ctenophore blooms have increased in number and intensity, and it has been suggested that these gelatinous animals may replace top predators in the world’s oceans (Richardson et al., 2009). This scenario is especially problematic for fisheries, as ctenophores consume pelagic fish eggs and larvae (Purcell & Arai, 2001), outcompete fish under low oxygen conditions (Decker et al., 2004; Seibel & Drazen, 2007), and can be introduced by ballast water as invasive species (Ivanov et al., 2000; Oguz et al., 2008). It has been hypothesized that ctenophores and jellyfish can serve as vectors for fish pathogens (Purcell & Arai, 2001), and recent findings revealed that bacteria associated with jellyfish served as the source of secondary infections in farmed salmon.
Ctenophore-associated bacterial communities

(Ferguson et al., 2010; Delannoy et al., 2011). Such a compounding set of factors puts ctenophores in a unique position to severely alter marine food webs (Lynam et al., 2006), as anthropogeniceutrophication of coastal waters promotes increasing numbers of dead zones (Diaz & Rosenberg, 2008) and the number of ctenophore blooms continues to increase globally (Richardson et al., 2009; Purcell, 2012).

Studies characterizing the bacteria associated with marine invertebrates have largely focused on benthic organisms such as corals, sponges, and hydrothermal vent worms. These aforementioned animals all contain microbiota that are distinct from the water they inhabit, with these bacteria playing critical roles for host ecology. Corals and sponges contain diverse, often species-specific bacterial communities that fill niches to support functional roles involving carbon, nitrogen, and sulfur cycling, as well as protection from potential pathogens (Webster et al., 2001; Hentschel et al., 2002; Rohwer et al., 2002; Ritchie & Smith, 2004; Ritchie, 2006; Taylor et al., 2007; Wegley et al., 2007). Conversely, other marine invertebrates harbor specific microbiota that are constrained to a limited number of bacterial groups. For example, specific chemosynthetic bacterial symbionts allow the annelids Riftia pachyptila and Alvinella pompejana to thrive in the extreme environments near hydrothermal vents (Cavanaugh et al., 1981; Haddad et al., 1995). In pelagic invertebrates, the best-studied bacterial symbiosis is the Hawaiian bobtail squid Euprymna scolopes, whose light organ is colonized exclusively by Vibrio fischeri (Nyholt & McFall-Ngai, 2004). Whether the microbiota is extremely specific (such as in the squid-Vibrio system) or a diverse community similar to that seen in corals and sponges, it is clear that bacterial associates play crucial roles in the ecology of marine invertebrates.

Although ctenophores are known to be parasitized by a variety of eukaryotes including amoebae, dinoflagellates, and sea anemones (Moss et al., 2001; Hay, 2006; Reitzel et al., 2007; Smith et al., 2007), no published studies have described the bacterial communities associated with ctenophores. Given ctenophores’ potential impact on fisheries, ease of movement across the oceans, and the fact that environmental conditions are changing to favor their persistence, it is vital to determine whether ctenophores contain specific bacterial communities. This study examined the bacterial communities associated with two ctenophore genera: Mneiopsis leidyi, a lobate ctenophore widely distributed throughout the Gulf of Mexico, Caribbean, and along the eastern coasts of North and South America (Purcell et al., 2001), and its natural predator Beroe ovata. The aims of this study were to determine whether the bacterial communities associated with ctenophores are distinct from those in the surrounding water column, compare microbiota between the two ctenophore genera, and examine the temporal variability in ctenophore-associated bacterial communities.

Materials and methods

Sample collection

Ctenophore specimens (ranging in size from 2 to 30 mL total volume) were carefully collected from Tampa Bay using a dip net off the Bayboro Harbor seawall in St. Petersburg, Florida, USA (27.76°N, 82.63°W). Samples were collected on a single day of the following months: April 2009, February 2010, March 2010, May 2010, June 2010, August 2010, October 2010, and April 2011. Sampling dates were chosen based on the visual observation of ctenophores from the seawall and were sporadic due to the ephemeral nature of ctenophore blooms. Ctenophore blooms contained uneven distributions of the two ctenophore genera, and specimen recovery varied for each time point (numbers of samples collected from each genus on each sampling date are shown in Fig. 2). Intact ctenophores were immediately placed into separate sterile (100-kD-filtered) seawater baths to remove loosely associated microorganisms. To allow the ctenophores sufficient time to clear their guts, ctenophores were incubated in the sterile seawater baths for 4 h, with complete water changes performed every hour. Specimens were subsequently given a final rinse with sterile seawater, flash-frozen with liquid nitrogen, and stored at −80 °C. A bulk seawater sample (1 L) collected at the same time was filtered onto a 0.2-μm Sterivex filter (Millipore, Billerica, MA) to obtain bacteria from the water column for comparison with ctenophore-associated bacteria.

DNA extraction

Individual whole-ctenophore specimens were defrosted and then pelleted by centrifugation at 2000 g for 30 min at 4 °C. The supernatant was discarded, and 1 mL of the ctenophore pellet was transferred to a clean microcentrifuge tube. The Sterivex filter containing the water column bacteria was removed from its housing, cut with a sterile scalpel from its internal spool, and placed into a separate microcentrifuge tube with sterile forceps. DNA was extracted from ctenophore samples and Sterivex filters using the All Prep DNA/RNA kit (Qiagen, Valencia, CA), according to the manufacturer’s instructions, with an additional 1-min spin prior to elution into 50 μL of PCR-grade water. DNA was stored at −20 °C until further processing.

Species confirmation of ctenophore specimens

Visual identification of ctenophores can be difficult, given the resemblance of many groups and fragility of
ctenophore tissues. A molecular phylogenetic approach has been established for the Phylum Ctenophora (Podar et al., 2001), and others have since advocated the importance of combining morphological data with DNA-based identification (Gorokhova et al., 2009; McManus & Katz, 2009). Universal eukaryotic primers 18S-F (5′-ATGTTGATCCTGCA-3′) and 18S-R (5′-TGATCC TTCYGCGAGTTCAC-3′) were used to amplify ctenophore 18S rRNA genes to confirm visual species identifications (Medlin et al., 1988; Podar et al., 2001). PCRs (50 μL volume) contained final concentrations of 1 μM for each primer, 10 mg mL⁻¹ bovine serum albumin (ThermoFisher, Waltham, MA), 0.2 mM Apex dNTPs, 1× Apex Taq Buffer, 2.5 U Apex Taq polymerase (Genesee Scientific, San Diego, CA), and 5 μL of DNA template. PCR conditions were adjusted from Medlin et al. (1988) with denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 1 min, 55 °C for 2 min, 72 °C for 3 min, and a final extension at 72 °C for 10 min. The 18S PCR products were cleaned with the UltraClean PCR Kit (MO-BIO, Carlsbad, CA), sequenced bi-directionally, trimmed in Sequencher (Gene Codes, Ann Arbor, MI), and assembled in SeqMan (Lasergene, Madison, WI). A BLASTN (Altschul et al., 1990) query against the GenBank nonredundant database confirmed the identity of both M. leidyi and B. ovata (99% identity, Genbank accession numbers: JN653094 and JN653095).

**Bacterial clone library construction and sequencing**

16S rRNA gene clone libraries were constructed and sequenced to analyze the composition of the bacterial communities associated with selected ctenophore specimens and compare the ctenophore-associated bacteria with those in the surrounding water column. PCR mixtures (50 μL total volume) contained 5 μL of template DNA for each ctenophore or water sample and final concentrations of 1 μM of each primer (27F: 5′-AGAGTTTGATCMTGCGCTCAG-3′ and 1492R: 5′-TACGAGTACCTTGGTCGACTT-3′) (Lane, 1991), 1.5 mM MgCl₂, 0.2 mM dNTPs, 1 U Apex Red Taq, and 1× Apex Red Taq Buffer (Genesee Scientific). Touchdown PCR was performed by denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 65 °C (−0.5 °C each cycle) for 1 min, 72 °C for 3 min, and a final extension at 72 °C for 10 min. Amplicons were confirmed on a 1% agarose gel containing ethidium bromide. TOPO TA cloning (Invitrogen, Carlsbad, CA) was used to ligate PCR products into the pCR4-TOPO vector, which was transformed into chemically competent Escherichia coli, and grown on LB-AMP (50 μg mL⁻¹) plates containing 20 mg mL⁻¹ X-Gal. Clones were subsequently screened by PCR with M13 primers to confirm inserts and then sequenced by Beckman Genomics (Danvers, MA). Clone library dereplication and diversity index values were obtained using FastGroupII (Yu et al., 2006). Each clone library was analyzed separately based on 97% similarity with gaps, which considers single-base insertions or deletions that might occur during sequencing. Dereplicated sequences were queried by BLASTN (Altschul et al., 1990), and the CLASSIFIER module in the Ribosomal Database Project (RDP) was used to provide robust taxonomic identifications of the bacterial 16S rRNA gene sequences (Cole et al., 2009). Sequences were subsequently deposited in NCBI’s GenBank database (JN653096–JN653274).

**Bacterial community profiling (terminal restriction fragment length polymorphism)**

Terminal restriction fragment length polymorphism (T-RFLP) is a DNA community profiling technique that has been used to characterize microbial communities from a wide range of environments (Liu et al., 1997; Schutte et al., 2008). T-RFLP was selected for this study based on its reproducibility, ability to discriminate taxa in mixed communities, and the availability of robust databases for sequence comparison. The PCR composition was identical to that used for 16S rRNA gene sequencing, except that the primers were each labeled with different fluorophores (27F-TAM and 1492-HEX). PCR conditions consisted of initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 3 min, and a final extension at 72 °C for 10 min (Danovaro et al., 2006). Products were pooled from triplicate reactions performed on each sample and cleaned using the DNA Cleaner/Concentrator Kit-5 (Zymo Research, Irvine, CA). Cleaned PCR products from each ctenophore and water sample were digested with 10 U of either HhaI, HaeIII, or MspI (New England BioLabs, Ipswitch, MA) for 3 h at 37 °C in three separate 20-μL reactions, followed by incubation at 65 °C for 20 min to stop the digest. Digests were cleaned with the Zymo DNA Cleaner/Concentrator Kit-5, quantified with Pico Green (Invitrogen), and normalized to 5 mg μL⁻¹. Capillary runs on a DNA 3730xl genetic analyzer (Applied Biosystems, Foster City, CA) at the University of Illinois Urbana-Champaign Core Facility separated fragments by size (operational taxonomic units; OTUs) and detected fluorescence values (relative abundance) for each OTU in the community profiles. Instrument precision was validated by a series of control runs that included samples ranging from a monoculture to complex bacterial communities (Daniels et al., 2011).

**T-RFLP data analysis**

The Local Southern algorithm (Southern, 1979) in PEAKS-CANNER v 1.0 software (Applied Biosystems) was used to
confirm OTU sizes in the raw T-RFLP profiles for each enzyme and fluorophore. Data were filtered to remove shoulder peaks and only retain peaks > 5 times the baseline height (Schutte et al., 2008). An R script binner (Ramette, 2009) was subsequently employed to place the T-RFLP data into 2-base-pair (bp) bins to account for instrument error (±1 bp) and normalize the data to reflect the percent contribution of each OTU (peak area) to the total profile fluorescence. Visual confirmation of proper binning was performed prior to importing the binned T-RFLP profiles into Microsoft Excel, and OTUs ≥ 0.16% of the total fluorescence were retained for downstream analysis (Danovaro et al., 2006; Luna et al., 2006).

Statistical tests were performed in PRIMER-E v.6.1 (Clarke, 1993) and MATLAB v. 7.8 (MathWorks, Natick, MA) software packages to determine variation among sample types and across time. Bray–Curtis distances (Bray & Curtis, 1957), which reflect similarity based on OTU composition and abundance, were calculated from square-root-transformed profile data. Similarity matrices were produced via pairwise sample comparisons and used to generate multidimensional scaling (MDS) plots. Analysis of similarity (ANOSIM, ranks of distances) and permutational analysis of variance (PERMANOVA, means of distances, which is less prone to type I errors) were performed to determine whether sample types were significantly different. Non-parametric dispersion analyses were conducted (Anderson, 2006) to confirm that the T-RFLP data did not violate the PERMANOVA assumption of equal variances (Anderson, 2001). All statistical tests were run with five thousand permutations, and a P-value ≤ 0.05 was considered significant. OTUs responsible for driving differences among samples or across time were identified using the similarity percentages (SIMPER) module in PRIMER.

**Linking clone libraries to T-RFLP profiles**

To link T-RFLP OTUs to ctenophore bacterial community 16S rRNA gene sequences, selected clones were sequenced bidirectionally with the M13 primers to identify dominant bacterial ribotypes and those that influenced community variation. After vector trimming, separate in silico digests of these sequences were performed in Geneious (Biomatters, Auckland, New Zealand) with the same enzymes used for T-RFLP to match sequences to specific T-RFLP OTUs.

**Results**

**Comparison of bacterial communities from ctenophores and the water column**

In March 2010, specimens from both *M. leidyi* (*n* = 7) and its predator *B. ovata* (*n* = 2) were simultaneously collected along with a sample of the surrounding water for bacterial community analyses. Significant differences were observed between T-RFLP profiles from the ctenophores and water samples for all three enzymes employed (average ANOSIM *R* = 0.91, *P*-value = 0.02; average PERMANOVA *F* = 4.81, *P*-value = 0.01, Fig. 1a). Bray–Curtis values revealed that the bacterial community profile in the water was on average 71% dissimilar to the *M. leidyi* bacterial profiles and 86% dissimilar to the *B. ovata* profiles. The presence of distinct bacterial communities in the ctenophores vs. the water column was confirmed by 16S rRNA gene sequencing, which revealed very few shared sequences between the ctenophore and water column samples (Table 1). Figure 1a further illustrates that the two ctenophore genera contained different bacterial communities, with *M. leidyi* and *B. ovata* specimens clearly separated from each other, regardless of the restriction enzyme chosen (average ANOSIM *R* = 0.94, *P*-value = 0.07; average PERMANOVA *F* = 6.3, *P*-value = 0.07). The *M. leidyi* bacterial profiles were on average 82% dissimilar to the *B. ovata* profiles. In contrast to the distinct communities observed in the two ctenophore genera, comparisons revealed that bacterial communities from individual *M. leidyi* specimens were only 44% dissimilar to each other, and bacterial communities from *B. ovata* specimens were 32% dissimilar to each other.

The average number of OTUs detected in the *M. leidyi* profiles was 16, while *B. ovata* profiles contained an average of 10 OTUs, and the water samples contained 30 OTUs on average. The lower number of OTUs in the ctenophore T-RFLP profiles compared with the water column was supported by results obtained from 16S rRNA gene sequencing of clone libraries. As measured by the Shannon–Weiner index, bacterial community diversity was lower in *M. leidyi* and *B. ovata* specimens (*H*׳ = 1.9 and *H*׳ = 2.4, respectively) compared with the water column sample (*H*׳ = 3.8). Rarefaction analysis indicated that further sequencing is necessary to capture the diversity in all samples, but the water column curve is much steeper than the ctenophore curves (Fig. 1b).

**Proteobacteria**, a bacterial supergroup composed of many taxa with diverse metabolisms, dominated all 16S rRNA gene libraries regardless of sample type, comprising 50–83% of the clones (Fig. 1c). Overall, *Alphaproteobacteria*, *Gammaprotobacteria*, and *Bacteroidetes* were the most abundant groups detected (Table 1 and Fig. 1c). Further resolution of the *Alphaproteobacteria* revealed that the water column contained the Order Rickettsiales and Rhodobacterales (Supporting Information, Table S1), while the ctenophore-associated *Alphaproteobacteria* primarily consisted of the Order Rhodospirillales (Table 1). An uncultured *Marinomonas* sp. (*Gammaproteobacteria* belonging to the Order Oceanospirillales) dominated the *M. leidyi* library (52% of sequences), but was not detected in the water column or the *B. ovata* specimen. When linked to T-RFLP pro-
files through in silico digests of clones, the Marinomonas OTU was responsible for 9% of the variation between the *M. leidyi* and water column profiles. The *B. ovata* sample was dominated by an uncultured *Alphaproteobacteria* from the *Rhodospirillaceae* family, which accounted for 22% of the clones in the library. Interestingly, a sequence 98% identical to *Tenacibaculum aiptasiae a5*, which was previously isolated from diseased sea anemones (Wang et al., 2008), accounted for the second highest number of clones (21%) in the *B. ovata* sample and was exclusive to this ctenophore. Both ctenophore libraries contained sequences similar to cultured representatives of the *Alphaproteobacteria* capable of hydrocarbon degradation (*Thalassospira* and/or *Alcanivorax*). In addition, the *M. leidyi* library also contained sequences similar to *Nisaea* (Table 1), a bacterial genus known to be involved in nitrogen metabolism (Urios et al., 2008).

**Temporal variation of ctenophore-associated bacteria**

Because of the episodic nature of ctenophore blooms, March 2010 was the only time point at which *M. leidyi* and *B. ovata* were found simultaneously, allowing for direct comparison between the two genera. However, to expand our understanding of ctenophore-associated bacterial communities, specimens of *M. leidyi* were collected at six time points (April 2009, February 2010, March 2010, June 2010, August 2010, April 2011), and *B. ovata* specimens were collected at three time points (March 2010, May 2010, October 2010). Despite high levels of variability in the *M. leidyi* bacterial communities over time, these ctenophores always contained a microbiota distinct from that of the water column (Fig. 2a). Consistent bacterial T-RFLP patterns were not found across all *M. leidyi* temporal samples; however, most T-RFLP profiles from specimens collected in a specific month grouped together (average ANOSIM *R* = 0.69, *P*-value < 0.001; average PERMANOVA *F* = 12.5, *P*-value < 0.001, Fig. 2a). Bray–Curtis values demonstrated that the *M. leidyi* specimens collected closest to each other in time (i.e. February/March 2010 and June/August 2010) had the most similar bacterial communities, while samples collected further apart had more dissimilar bacterial communities (Fig. 2a). For example, the *M. leidyi* February 2010 vs. March 2010 bacterial community comparison...
produced an average dissimilarity value of 59.6% (average \textit{ANOSIM} R = 0.28), while the February 2010 samples were 78% dissimilar to those from June 2010 (average \textit{ANOSIM} R value = 0.85). \textit{Beroe ovata} bacterial profiles were also distinct from the water column at all sampling time points. Although \textit{B. ovata} samples from May and October 2010 collections consistently clustered together and displayed greater overlap (63% average dissimilarity) in bacterial community profiles than the March 2010 vs. October 2010 \textit{B. ovata} profile comparisons (88% average

\begin{table}
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\begin{tabular}{lllll}
\hline
AN & RDP CLASSIFIER (80% threshold) & Identity (%) & Sample source (GenBank top hit) & \# of clones represented \\
\hline
\textit{M. leidyi} (n = 92) & & & & \\
JN653096 & Gammaproteobacteria, Oceanospirillales & 100 & Sapelo Island Microbial Observatory salt marsh & 48 \\
JN653097 & Alphaproteobacteria, Rhodospirillales & 99 & Xiaomaidao Island seawater (China) & 10 \\
JN653098 & Alphaproteobacteria, Rhodospirillales* & 100 & Yellow Sea water & 7 \\
JN653099 & Alphaproteobacteria, Rickettsiales & 100 & Seawater (North Carolina, USA) & 5 \\
JN653100 & Bacteroidetes, Flavobacteria & 100 & Desalination reverse osmosis membrane & 4 \\
JN653101 & Bacteroidetes, Flavobacteria & 99 & Monterey Bay Station MO plankton extracts & 3 \\
JN653102 & Actinobacteria & 100 & Seawater (Chesapeake Bay, USA) & 2 \\
JN653103 & Cyanobacteria & 99 & N.E. Pacific Ocean (10 m) & 2 \\
JN653104 & Tenericutes, Mollicutes & 98 & \textit{Mnemiopsis leidyi} (Baltic Sea) & 2 \\
JN653105 & Alphaproteobacteria, Rhodobacterales & 99 & \textit{Gymnodinium catenatum} (Dinoflagellate) & 1 \\
JN653106 & Bacteroidetes, Flavobacteria & 100 & NW Mediterranean Sea water & 1 \\
JN653107 & Bacteroidetes, Flavobacteria & 97 & Gose island seawater (South Korea) & 1 \\
JN653108 & Betaproteobacteria, Methylophilales & 100 & \textit{Mnemiopsis leidyi} Microbial Observatory salt marsh & 1 \\
JN653109 & Deltaproteobacteria & 100 & Saanich Inlet, 10 m depth & 1 \\
JN653110 & Gammaproteobacteria & 89 & Zebra mussel Loon Lake (Michigan) & 1 \\
JN653111 & Gammaproteobacteria & 94 & Bantou freshwater reservoir & 1 \\
JN653112 & Gammaproteobacteria & 99 & \textit{Mnemiopsis leidyi} Microbial Observatory salt marsh & 1 \\
JN653113 & Bacteria & 91 & Hadopelagic sediments (Japan) & 1 \\
\textit{B. ovata} (n = 91) & & & & \\
JN653114 & Alphaproteobacteria, Rhodospirillales & 99 & Xiaomaidao Island seawater (China) & 22 \\
JN653115 & Bacteroidetes, Flavobacteria & 98 & \textit{Aiptasia pulchella} (Sea anemone) & 19 \\
JN653116 & Alphaproteobacteria, Rhodospirillales & 100 & Station Aloha seawater (HOTS, Hawaii) & 12 \\
JN653117 & Alphaproteobacteria, Rhodospirillales* & 100 & Nabet Bay seawater (Japan) & 11 \\
JN653118 & Bacteria & 90 & \textit{Erythropsidium caribaeorum} (Octocoral) & 4 \\
JN653119 & Alphaproteobacteria, Rickettsiales & 100 & Beaufort Inlet seawater (NC, USA) & 3 \\
JN653120 & Gammaproteobacteria, Chromatiales & 97 & Hydrothermal vent (SW Indian Ocean Ridge) & 3 \\
JN653121 & Gammaproteobacteria, Oceanospirillales & 100 & Enrichment culture with heavy fuel oil & 3 \\
JN653122 & Alphaproteobacteria, Rhodobacterales & 100 & South China Sea water & 2 \\
JN653123 & Bacteroidetes, Flavobacteria & 100 & Newport Harbour seawater (RI, USA) & 2 \\
JN653124 & Actinobacteria & 99 & Urban watershed (Brazil) & 1 \\
JN653125 & Alphaproteobacteria, Rhodospirillales & 98 & \textit{Emiliania huxleyi} culture & 1 \\
JN653126 & Bacillariophyta & 99 & Anoxic fjord sediment & 1 \\
JN653127 & Bacteroidetes, Flavobacteria & 100 & \textit{Oura Bay seawater} (Japan) & 1 \\
JN653128 & Bacteroidetes, Flavobacteria & 100 & Monterey Bay Station MO plankton extracts & 1 \\
JN653129 & Firmicutes & 91 & \textit{Nematostella vectensis} (Sea anemone) & 1 \\
JN653130 & Gammaproteobacteria, Enterobacteriales & 100 & Drinking water & 1 \\
JN653131 & Gammaproteobacteria, Pseudomonadales & 100 & Jiaozhou Bay seawater (China) & 1 \\
JN653132 & Gammaproteobacteria, Xanthomonadales & 100 & Catheter biofilm & 1 \\
JN653133 & Tenericutes, Mollicutes & 90 & \textit{Penaeus vannamei} (Pacific white shrimp) & 1 \\
\hline
\end{tabular}
\caption{Bacterial 16S rDNA sequences from \textit{Mnemiopsis leidyi} and \textit{Beroe ovata} specimens collected during a single sampling effort in March 2010}
\end{table}
dissimilarity, Fig. 2b), no temporal trends can be inferred, given the small sample size.

16S rRNA gene sequencing of representative individual *M. leidyi* specimens over time verified the temporal variability observed from the T-RFLP profiles (Fig. 3). Although *Proteobacteria* consistently represented > 48% of the bacterial community, the types of *Proteobacteria* varied greatly between months. For example, *Gammapro-
teobacteria dominated the M. leidyi bacterial community in March 2010, whereas this group was not detected in June 2010. In addition, Epsilonproteobacteria made up 18% of the bacterial community associated with M. leidyi in April 2009, but were not detected at any other time points. Other bacterial groups also displayed temporal variation, such as the Bacteroidetes, which comprised 45% of the sequences in February 2010, but were not recovered in April 2009. Representatives from the Tenericutes phylum were only present at half of the time points (February 2010, March 2010, August 2010 libraries).

Although the majority of the M. leidyi sequences were most similar to bacteria previously identified in seawater, temporal 16S rRNA gene libraries contained many sequences closely related to bacteria previously described in various marine invertebrates, including corals (healthy and diseased), sponges, anemones, and bivalves (Table S2). Bacteria with order- to species-level identity to sequences previously described from various corals (Acropora, Diploria, Montastraea, Porites, and Gorgonia) were found at multiple time points, peaking at 11% of the sequences in April 2009. Sponge-associated sequences represented a small number (<10%) of the clones at most time points, except March 2010, and were similar to bacteria previously described from Callyspongia, Cymbastela, Gelloides, or Tethys spp. (Table S2). In addition, 34% of the sequences from the August 2010 M. leidyi library were 99% identical to Gammaproteobacteria previously identified as gill symbionts of the bivalve Phacoides pectinatus (Table S2). Sequences related to parasites and intestinal bacteria from various bivalves constituted 4% of the clones in the April 2011 library (Table S2). All Betaproteobacteria clones (18%) from the August 2010 library were similar to Polynucleobacter sp., which have previously only been reported in freshwater environments (Jezebra et al., 2011) and were also detected in M. leidyi from June 2010 (Fig. 3, Table S2).

**Discussion**

Although microbial communities associated with several marine invertebrates have been characterized, this is the first published study to describe bacteria associated with representatives from Phylum Ctenophora. The high ANOSIM R values based on T-RFLP profiles indicate little to no overlap (Ramette, 2007) among bacterial communities from the ctenophores and the surrounding water column, or between the two ctenophore genera. Given that these gelatinous animals are composed primarily of water, it is striking that tissue samples of M. leidyi and B. ovata collected simultaneously contained bacterial communities different from the water column and distinct from each other (Fig. 1a). The ANOSIM and PERMANOVA P-values for both taxa were slightly greater than the significance cutoff; however, this is a function of sample size (only two B. ovata specimens were obtained during the March 2010 collection). Ctenophore samples contained fewer bacterial OTUs by T-RFLP and lower diversity communities by 16S rRNA gene sequencing, when compared with the water column. The low diversity of the ctenophore-associated bacterial communities is different than the trend previously observed in corals and sponges, where bacterial diversity is typically higher than that of the surrounding water column (Rohwer et al., 2002; Taylor et al., 2007; Webster et al., 2009; Lee et al., 2011).

Ctenophore and water 16S rRNA gene libraries were dominated by sequences similar to uncultured Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes (Fig. 1c). Unlike the water samples, which were primarily composed of free-living bacteria from the Rickettsiales (specifically Pelagibacter) and Rhodobacterales orders (Table S1), a majority of the Alphaproteobacteria in the M. leidyi and B. ovata samples from March 2010 were from the Order Rhodospirillales (Table 1), which are the purple nonsulfur, photosynthetic bacteria (Kersters et al., 2006; Gupta & Mok, 2007). These bacteria could potentially be involved in carbon cycling for ctenophores, providing an additional source of fixed carbon to the animal, especially when prey items are scarce. In addition, the detection of clones 99% identical to Thalassosira spp. in both M. leidyi and B. ovata samples from March 2010 is interesting, as these bacteria are known to be capable of chemotaxis to inorganic phosphate (Hutz et al., 2011).
Sequences similar to bacteria described from other marine invertebrates were present in both *M. leidyi* and *B. ovata* species. *Marinomonas* sp., *Gammaproteobacteria* from the Order *Oceanospirillales*, accounted for approximately half of the clones in the *M. leidyi* 16S rRNA gene library from March 2010 and has previously been described in corals and sponges (Cassler et al., 2008; Thompson et al., 2011). *Marinomonas* sp. contain genes for the breakdown of dimethylsulfiniopropionate, suggesting a potential functional role in the biogeochemical cycling of sulfur (Todd et al., 2007; Johnston et al., 2008). *Mycoplasma* and *Spiroplasma* sp., representatives from the Phylum *Tenericutes* appeared at low numbers in both ctenophore libraries from March 2010 (Table 1), but were not detected in the surrounding water. These bacteria are known to parasitize animal and plant tissues to obtain nutrition and compensate for their reduced genome size (Rottem, 2003; Pitcher & Nicholas, 2005). *Mycoplasma* sp. have previously been reported as associates of other marine invertebrates, such as deep-water corals (Penn et al., 2006; Kellogg et al., 2009; Neuling et al., 2009). Some of the *Mycoplasma* sequences identified in *M. leidyi* in this study were 98% identical to *Mycoplasma* sp. from *M. leidyi* specimens collected from the Baltic Sea (S. Hammann, unpublished GenBank record), suggesting that there may be a consistent association of this bacterial genus with *M. leidyi*.

A large component of the *B. ovata* 16S rRNA gene library was 99% identical to *T. aiptasia* a5, a member of the *Bacteroidetes* that was previously isolated from diseased sea anemones (Wang et al., 2008). Ctenophores are known to harbor eukaryotic parasites and are used as a refuge by other animals (Gasca et al., 2007); however, this study also suggests that ctenophores can potentially serve as vectors for bacteria that are pathogenic to other marine organisms. The genus *Tenacibaculum* also contains other pathogenic species that have been detected on jellyfish and implicated in fish gill disease (Ferguson et al., 2010; Delannoy et al., 2011). Sequences related to the freshwater-restricted *Betaproteobacteria* genus, *Polynucleobacter* sp., were found in the June and August 2010 *M. leidyi* libraries and may be indicative of a freshwater input into Bayboro Harbor, where these ctenophores were collected.

Although the ctenophores in this study always contained bacterial communities that were distinct from the surrounding water column, the ctenophore-associated bacterial communities varied significantly over time (Figs 2 and 3). This type of community flux has been described by Littman et al. (2009) in juvenile acroporid corals; however, adult corals and most sponges ultimately contain conserved bacterial communities (Hentschel et al., 2006; Taylor et al., 2007; Littman et al., 2009). Unlike these sessile invertebrates, the ctenophores analyzed in this study are pelagic animals, and the changing bacterial community may represent a lifestyle strategy to take advantage of the bacteria available in the environment at any given time, rather than spending resources for maintenance and protection of a conserved consortia. Although the individual community members (i.e. OTUs or ribotypes) varied over time, it is possible that the different bacterial communities contained members fulfilling a given set of metabolic functions for the ctenophore. However, further work is needed to assess the functional roles of the ctenophore-associated microbiota. The distribution of *M. leidyi* populations is linked to temperature and food availability, and their life span is on the order of months (Ghabooli et al., 2011; Shiganova & Dumont, 2011); therefore, it is likely that several distinct populations were sampled during this study. If the bacterial communities are dependent on factors in early life stages (e.g. the bacterial community present in the water column at the time of initial colonization), the sampling of different ctenophore populations may explain the temporal variation observed in their bacterial communities. Additionally, the bacterial community associated with individual ctenophores may change over time. If this is the case, then perhaps the composition of the surrounding bacterial community in the water column or food availability may influence ctenophore-associated microbiota. Cydippid larvae of *M. leidyi* primarily feed on protistan prey; however, an ontogenetic shift occurs such that the diet of lobate adults is dominated by metazoan prey including copepods, cladocera, and larvae of fish and mollusks (Rapoz et al., 2005). Although dietary diversity is similar among *M. leidyi* > 3 cm in length, the composition of the diet varies based on prey availability. Total food availability as well as dietary composition may affect the ctenophores’ bacterial community. Future studies should consider analyzing zooplankton prey community composition while examining the ctenophore-associated bacterial community to determine whether diet is driving the temporal variability reported here.

Condon et al. (2011) recently demonstrated that the release of dissolved organic matter by jellyfish could alter food web dynamics and restructure planktonic microbial communities, and ctenophores are known to adversely impact marine food webs (Purcell et al., 2001; Oguz et al., 2008). The apparent increase in frequency and intensity of ctenophore blooms, combined with recent invasions of ctenophores into new areas, enforces the need for a detailed study of factors influencing ctenophore ecology. This is the first report of distinct and dynamic bacterial communities associated with ctenophores, suggesting that these microbial consortia may play important roles in ctenophore biology. While the 16S rRNA gene data in this study provide...
some clues on the potential metabolic capability of ctenophore-associated bacteria, future work needs to be performed to elucidate the functional roles of microorganisms associated with ctenophores (e.g. through a metagenomic survey). Future studies should also examine the location of the bacteria within the ctenophore body plan, assess the mode of acquisition of bacteria by the ctenophores, and attempt to determine the factors driving the temporal variability in ctenophore-associated bacterial communities observed in this study.

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References


Ctenophore-associated bacterial communities


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Community composition of bacterial 16S rDNA libraries sequenced from M. leidyi individuals from the same collections in April 2009 and February 2010.

Table S1. Bacterial 16S rDNA sequences from the water sample collected during the March 2010 sampling effort.

Table S2. Bacterial 16S rDNA sequences from the April 2009, February 2010, June 2010, August 2010, and April 2011 M. leidyi samples.

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