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A "Rosetta Stone" for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean)

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ABSTRACT

Species diversity of the metazoan holozooplankton assemblage of the Sargasso Sea, Northwest Atlantic Ocean, was examined through coordinated morphological taxonomic identification of species and DNA sequencing of a ~650 base-pair region of mitochondrial cytochrome oxidase I (mtCOI) as a DNA barcode (i.e., short sequence for species recognition and discrimination). Zooplankton collections were made from the surface to 5,000 meters during April, 2006 on the R/V *R.H. Brown*. Samples were examined by a ship-board team of morphological taxonomists; DNA barcoding was carried out in both ship-board and land-based DNA sequencing laboratories. DNA barcodes were determined for a total of 297 individuals of 175 holozooplankton species in four phyla, including: Cnidaria (Hydromedusae, 4 species; Siphonophora, 47); Arthropoda (Amphipoda, 10; Copepoda, 34; Decapoda, 9; Euphausiacea, 10; Mysidacea, 1; Ostracoda, 27); and Mollusca (Cephalopoda, 8; Heteropoda, 6; Pteropoda, 15); and Chaetognatha (4). Thirty species of fish (Teleostei) were also barcoded. For all seven zooplankton groups for which sufficient data were available, Kimura-2-Parameter genetic distances were significantly lower between individuals of the same species (mean=0.0114; S.D. 0.0117) than between individuals of different species within the same group (mean=0.3166; S.D. 0.0378). This difference, known as the barcode gap, ensures that mtCOI sequences are reliable characters for species identification for the oceanic holozooplankton assemblage. In addition, DNA barcodes allow recognition of new or undescribed species, reveal cryptic species within known taxa, and inform phylogeographic and population genetic studies of geographic variation. The growing database of "gold standard" DNA barcodes serves as a Rosetta Stone for marine zooplankton, providing the key for decoding species diversity by linking species names, morphology, and DNA sequence variation. In light of the pivotal position of zooplankton in ocean food webs, their usefulness as rapid responders to environmental change, and the increasing scarcity of taxonomists, the use of DNA barcodes is an important and useful approach for rapid analysis of species diversity and distribution in the pelagic community.

1. Introduction

1.1. DNA barcoding

The Rosetta Stone unlocked the mysteries of ancient Egypt by providing keys to decipher the hieroglyphics (Parkinson, 2005). The term is now idiomatic for a critical key used to translate encoded information or unlock a mystery. In this sense, we may consider that species diversity of the metazoan holozooplankton (i.e., higher animals that drift freely with currents throughout their lives) remains a mystery, locked behind the detailed morphological characters that define and discriminate the estimated 6,000 described species in 11 metazoan phyla.

DNA barcodes (i.e., short DNA sequences used for species recognition and discrimination) are ancillary – and logically independent – characters that allow identification of an unknown specimen in terms of a known classification (Schindel and Miller, 2005). The use of barcodes to translate or decipher the complex array of morphological characters that are used to describe and discriminate species by traditional taxonomists is serving to revive traditional morphological taxonomy but not replace it (DeSalle et al., 2005; Miller, 2007; Stoeckle and Hebert, 2008). In this sense, the growing database of DNA barcodes linked to species names and morphological characters for marine zooplankton may be said to a Rosetta Stone for decoding patterns of species diversity in the pelagic realm. DNA barcodes are also useful to discover new species, reveal cryptic species, and assess

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taxonomically-significant variation within species with broad or disjunct distributions (DeSalle, 2006; Bucklin et al., 2011).

The usual DNA barcode region for animals is a 708 base-pair region of mitochondrial cytochrome oxidase I (mtCOI), which exhibits favorable levels of divergence within and between species of most metazoan groups to allow accurate species identification (Hebert et al., 2003; Meyer and Paulay, 2005). DNA barcodes serve as ancillary taxonomic characters for identification and delimitation of known species, allow recognition of new or undescribed species, and reveal cryptic species within known taxa (Knowlton, 2000; Wiens, 2007; Stoeckle and Hebert, 2008).

The 11 metazoan phyla represented in holozooplankton assemblage provide an excellent opportunity to examine the broad taxonomic utility of DNA barcodes and to evaluate the feasibility of using the mtCOI barcode region to assess species diversity in complex environments and communities. In fact, a number of invertebrate groups have been shown to exhibit patterns of variation of mtCOI that are useful for DNA barcoding (Bucklin et al., 2003, 2007, 2010a; Schander and Willassen, 2005; Costa et al., 2007; Machida et al., 2006; Shearer and Coffroth, 2008; Moura et al., 2008; Ortman, 2008; Ortman et al., 2010 Jennings et al., 2010, 2010).

1.2. The Sargasso Sea

The Sargasso Sea, the only sea without coastal boundaries, is defined by four currents (the Gulf Stream, North Atlantic Current, Canary Current, and North Atlantic Equatorial Current) that form the western North Atlantic Subtropical Gyre. This region has an extensive historical record of oceanographic observations, including a comprehensive time-series study from the Bermuda Atlantic Time-series Study (BATS; Steinberg et al., 2001). The Sargasso Sea zooplankton assemblage has been examined over many decades from many perspectives, including biophysical interactions, climate change, biogeochemical cycling and carbon sequestration, among others (e.g., Ortner et al., 1978; Madin et al., 2001; Goldthwait and Steinberg, 2008; Rosa et al., 2008; Edena et al., 2009). Yet despite years of study, few efforts have sampled the deep layers of the mesopelagic (200–1,000 meters), bathypelagic (1,000–4,000 meters), and abyssopelagic (4,000–7,000 meters) of this region. Deep-sea fishes have received greater attention in this region for many years, including recent studies of biodiversity (Miller and McCleave, 2007) and trophic dynamics (Sutton, 2005). In 2006, Wiebe et al. (2010) set out to sample Sargasso Sea zooplankton from the surface to bottom (~5,000 meters) using uniquely-designed sampling gear to ensure capture of rare species.

1.3. Zooplankton diversity

A persistent challenge for understanding and predicting ocean ecosystem function, health, and overall dynamics is to characterize the zooplankton assemblage at the species level. Eleven metazoan phyla are represented among holozooplankton, and many species are locally rare but have wide – even circumglobal – distributions. Moreover, for some taxonomic groups local diversity may comprise a significant fraction of the known global species diversity (Angel et al., 1997). For the Copepoda (the most species-rich zooplankton group with ~2,200 described species), one net sample from oceanic waters may contain hundreds of species or ~10% of the global total; the mechanism allowing such high local diversity is unclear (Kuriyama and Nishida, 2006).

The deep-sea zooplankton assemblage in particular is hypothesized to be characterized by high species diversity with low abundances of each species. Given the huge volume of this habitat, even rare species may have very large population sizes and play a critical role in the dynamics of deep-sea environments. Such patterns of diversity, distribution, and abundance contribute to the time-consuming nature of analyzing zooplankton samples by traditional morphological taxonomic approaches, and undoubtedly there remain many opportunities for species discovery.

This study is one component of a larger effort by Wiebe et al. (2010) to characterize diversity in the mesopelagic and bathypelagic zones of the Sargasso Sea, Northwest Atlantic Ocean by large-volume sampling to depths of 5,000 meters, while ensuring that samples collected were subjected to both traditional morphological taxonomic analysis and molecular systematic (i.e., DNA barcoding) approaches, with the taxonomists and barcoders working in close collaboration to assess the species diversity of the region (Wiebe et al., 2010). A primary goal of this study is the creation of a DNA barcode database for identified specimens of metazoan holozooplankton, which can serve as a Rosetta Stone to decipher patterns of species diversity in the open ocean pelagic realm. DNA barcodes will provide a new method for recognizing known species and discovering new or cyptic species of zooplankton, and may thus ensure timely recognition of shifts in species composition, richness, and biogeographical distributions associated with environmental variability and climate change.

2. Methods

2.1. Collection and preservation of zooplankton samples

A biodiversity survey of zooplankton and fish from deep waters of the Sargasso Sea, Northwest Atlantic Ocean, was carried out during an oceanographic research on the R/V *Ronald H. Brown* from 10–30 April 2006, as described by Wiebe et al. (2010). Zooplankton and micronekton were quantitatively sampled at five stations (Fig. 1) throughout the water column using a 1/4 m, 1 m, and 10 m MOCNESS (Multiple Opening/Closing Net and Environmental Sensing System; Wiebe et al., 1985). The MOC-10 carried five separate nets; the trawl was deployed with the first net (3 mm mesh) open from the surface to the deepest depth desired (~5000 m), where it was closed. The subsequent nets (335 μm)

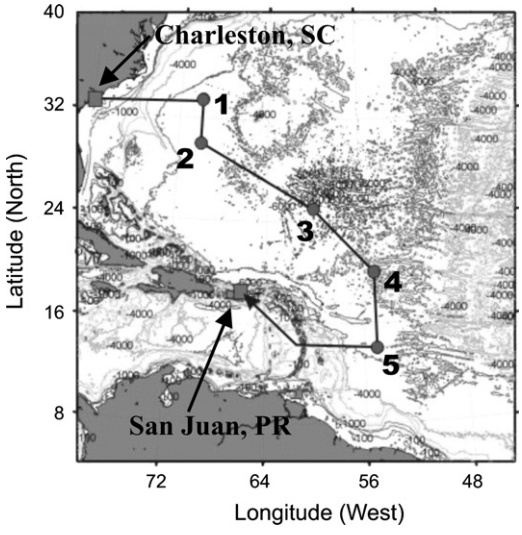

Fig. 1. Sampling locations and cruise track of the R/V *Ronald H. Brown* through the Sargasso Sea during April 2006. Specimens for barcoding were collected at all sampling stations shown. Stations are numbered sequentially 1–5.

were opened to sample specified depth strata (usually 1,000 m) as the net was hauled to the surface. The large volumes of water sampled by the larger nets (tens of thousands of cubic meters) compensated for the very low abundance of species that occur at bathy- and abyssopelagic depths. Sampling above 1,000 m was done using a 1 m MOCNESS equipped with nine nets with 335 μm mesh. Several other nets were used for surface or near-surface zooplankton collections. A Reeve Net consisting of a ½ m ring net attached to a large-volume cod-end was used to collect fragile gelatinous animals and microzooplankton in the upper few hundred meters (Wiebe and Benfield, 2003).

Immediately after net recovery, samples were examined and large gelatinous forms, fishes, and macrozooplankton/nekton were removed. Samples were split, with ½ preserved in formalin and ½ split again, with ¼ used to identify live specimens for photography and DNA barcoding, and ¼ preserved immediately in undenatured 95% ethanol for molecular analysis, using protocols described by Bucklin (2000). Specimens and sample splits designated for barcoding were usually examined under a dissecting microscope very soon following collection. Specimens were identified to species by taxonomists for each group using diagnostic morphological characters. Identified specimens for molecular analysis were placed in labeled vials, preserved immediately in 95% ethyl alcohol, and placed in the queue for barcoding onboard the ship (Fig. 2). Most specimens were analyzed within a few days, but those not analyzed immediately were archived for longer-term storage, and the alcohol was changed 24 hr after collection. For species smaller than ~25 mm, at least one intact individual was retained from at least one collection as a physical voucher. Up to three individuals from the remaining collections were removed and the entire organism extracted. For species larger than ~25 mm, an intact individual from one collection was retained where possible, as long as three other individuals were present from which to remove a small portion for extraction (i.e., at least 4 total individuals). If fewer than four individuals were collected, the smallest portion allowable for DNA extraction was removed from each from a non-taxonomically important region of the specimen.

2.2. Molecular analysis

DNA extraction was performed with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) using standard protocols, except that elution volumes varied to reflect individual size (usually 100–200 μL). A 708 base-pair region of mtCOI was amplified in a GeneAmp 9600 PCR machine (Applied Biosystems, Inc.) using consensus PCR primers (Folmer et al., 1994). For specimens or species that did not amplify with consensus primers, different group-specific forward and/or reverse primers and PCR protocols were designed and used to obtain barcode sequences (Table 1). PCR amplifications were also done in a Thermal Cycler Model 480 (Perkin Elmer). Perhaps because of their low-stringency protocols with very long ramp times, these legacy PCR machines yield DNA sequences suitable for primer design – if not definitive barcodes – from otherwise recalcitrant species (Bucklin, 2000).

PCR products were cleaned with QIAquick PCR Purification Kit (Qiagen, Valencia, CA). DNA sequences were determined directly from PCR amplification products using the forward and reverse primers. The sequencing reactions were carried out using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Carlsbad, CA) at ¼ standard volume in an ABI GeneAmp 9600 PCR machine. DNA templates were purified and suspended in ABI Hi-Di Formamide for sequencing.

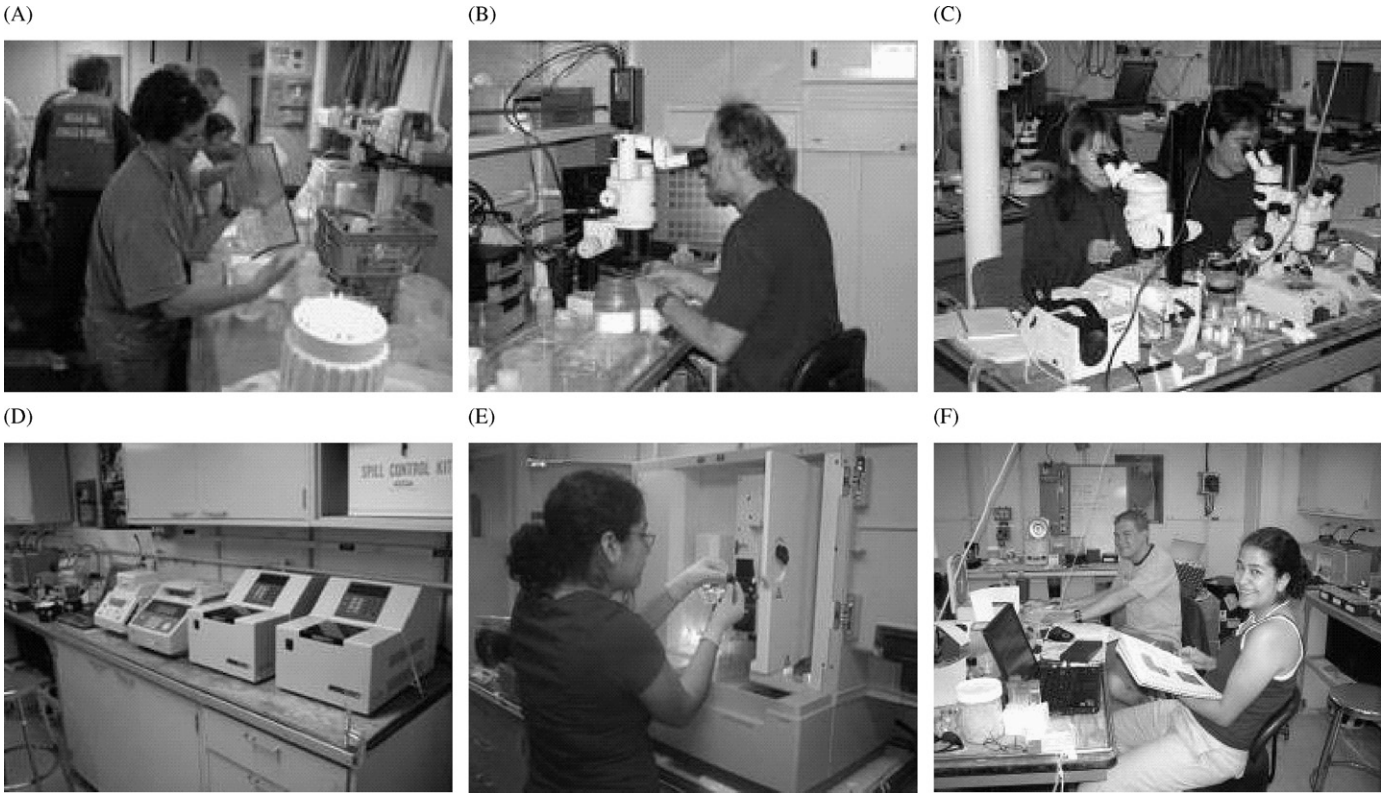

Fig. 2. DNA barcoding of zooplankton was carried out during the cruise in a sequencing laboratory onboard the R/V *Ronald. H. Brown*. An analytical assembly line operated around the clock and included (A) sample splitting; (B) photography of living plankton; (C) morphological species identification; (D) PCR amplification using ABI GeneAmp 9600 PCR machines and legacy Perkin Elmer Model 480 Thermal Cyclers; (E) DNA sequencing at sea, using an unmodified ABI 3130 Genetic Analyzer 4-capillary automated DNA sequencer; and (F) sequence data analysis and validation.

Table 1
PCR primers and protocols used for analysis of Sargasso Sea zooplankton and fish. For specimens that did not amplify using the consensus primers from Folmer et al. (1994), a second or third PCR reaction was done using other consensus or group-specific forward and/or reverse primers among those listed below. The names and sequences of the PCR primers used to amplify the barcode region for each specimen are included in the GenBank record, which can be accessed using the Accession Number given in Table 2. The non-standard DNA base designations (beyond A, C, G, and T) indicate mixtures of two or more nucleotide bases at that site. PCR protocol numbers refer to the following: #1) 94 °C for 1 min, 45 °C for 2 min, and 72 °C for 3 min, for 40 cycles; #2) 95 °C for 3 min; then 94 °C for 45 sec, 45 °C for 1 min, and 72 °C for 1.5 min, for 35 cycles; 72 °C for 3 min.

Phyulm and Group	Primer Name	Primer Sequence	PCR Protocol	Citation	Author
Consensus	LCO-1490F	GGTCAACAAATCATAAAGATATTGG	#1	Folmer et al. (1994)	
	HCO 2198R	TAAACTTCAGGGTGACCAAAAAATCA	#1	Folmer et al. (1994)	
Cnidaria					
Medusozoa	Con-COI-2607R	ACATAGTGGAAATGTGCTACAACATA	#1	Hill et al. (2001)	R.S. Hill
	Med-COI-2414R	GGAACTGCTATAATCATAGTTGC	#1	Ortman et al. (2010)	B.D. Ortman
Arthropoda					
Ostracoda	Ost-COI-1535F	GGDGCHTGAAGWGCWATGYTAGG	#2	This paper	L.M. Nigro
Copepoda	Con-COI-2607R	ACATAGTGGAAATGTGCTACAACATA	#1	Hill et al. (2001)	R.S. Hill
	Cop-COI-1498F	AAYCATAAAGAYATYGGDAC	#1	This paper	L.M. Nigro
	Cop-COI-2105R	CGRTCHGTHARNARYATDGTAATDGC	#1	This paper	L. Blanco Bercial
	Cop-COI-2189R	GGGTGACCAAAAAATCARAA	#1	This paper	L. Blanco Bercial
	Crus-COI-2198R	CCHACDGTAAAYATRTGRTG	#1	This paper	L.M. Nigro
	Crus-COI-2428R	TTAATHCCHGTDGGNACVGCAAT	#1	This paper	L.M. Nigro
Euphausiacea	Eup-COI-2000R	CADACAAAYARWGGDATTCGGTCTAT	#2	This paper	L.M. Nigro
Teleosti	Fish-F1	TCAACCAACCACAAAGACATTGGCAC	#1	Ward et al. (2005)	
	Fish-R2	ACTTCAGGGTGACCGAAGAATCAGA	#1	Ward et al. (2005)	

The automated DNA sequencer used onboard the R/V *R.H. Brown* and in the lab was an ABI 3130 Genetic Analyzer, with an array of four 50-cm capillaries, which was operated using standard conditions. A one-hour electrophoresis time on the 3130 produced approximately 500–700 base-pair reads in one direction, providing complete or almost complete bi-directional coverage of the mtCOI barcode region. Three individuals were sequenced for most species; one or two individuals were sequenced for rare species for which no additional specimens were collected; additional individuals were sequenced when problems with taxonomic identification were suspected or large differences among the sequences for nonspecific individuals were found. All sequences were manually checked for accurate machine reading using the Molecular Evolutionary Genetics Analysis (MEGA, Version 4) software package (Tamura et al., 2007) and the DNA sequence assembly program, Sequencher Ver. 4.10.1. (Gene Codes Corp., Ann Arbor, MI).

Establishing a DNA sequencing laboratory onboard an oceanographic research vessel required very few special arrangements. The effect of the ship's motion was minimized by locating the lab amidships (as close as possible to the center of moment of the ship). The sequencer was lashed securely to a stable bench oriented along the ship's axis. The sequencer buffer chamber was sealed to prevent spillage during the cruise. Air quality was a primary concern; the sequencing lab opened onto an internal passageway and the air conditioning was kept on to reduce humidity. After the first sequencing runs, reduced NaCl concentration in the cleanup protocol remedied precipitation in the ethanol and EDTA solutions, likely caused by high humidity and salty air.

2.3. DNA sequence analysis

The mtCOI barcode sequences were aligned using CLUSTAL X (Thompson et al., 1997). The complete alignment was trimmed to a length of 650 base-pairs for preliminary analysis to confirm the accuracy and validity of the sequences. Pairwise differences were calculated among all species to identify sequences of the same species that differed by $>2\%$. When possible, specimens with aberrant sequences were rechecked for correct species identification. The nucleotide sequences were further checked by aligning the translated protein (amino acid) sequences with consensus protein sequences for this gene region, which facilitated detection of artifacts (e.g., insertion or deletion of one or more nucleotide bases) and pseudogenes (i.e., non-functional copies of genes), which can confound barcoding analysis (e.g., Song et al., 2008). DNA sequences that could not be verified and validated, including aberrant or highly divergent sequences, were omitted from the dataset. The verified mtCOI sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank database using the BARCODE submission portal. The designated GenBank Accession Numbers (Table 2) can be used to access the GenBank record, which includes data and metadata for each specimen: nucleotide sequences in text format, conceptual translations to protein (amino acid) sequences, specimen voucher number, collection date, geospatial coordinates of the collection site, PCR primer names and sequences, and names of the persons collecting and identifying the specimens.

Specimens not consumed in the molecular analysis were assigned a voucher number. Voucher numbers for small organisms that were used entirely for analysis were assigned to specimens that were collected in the same sample and identified by the same person. Specimen vouchers are stored permanently in the lead author's laboratory in 95% ethyl alcohol at -20 °C. The person responsible for identifying and providing the specimen for barcoding also frequently retains additional specimens preserved in formalin. DNA vouchers are kept for all barcoded specimens, assigned the same voucher number, and stored in an ultracold (-80 °C) freezer in the lead author's laboratory. Data and metadata associated with each barcode is maintained in a specimen-tracking database created using Microsoft ACCESS software.

Kimura-2-Parameter (K2P) genetic distances (Kimura, 1980) were calculated between barcodes for individuals of the same species and between individuals of different species within each of nine groups of zooplankton using MEGA, Ver. 4 (Tamura et al., 2007). The mean and standard deviation of within- and between-species K2P distances were calculated for each zooplankton group and for the dataset as a whole.

The mtCOI sequences were analyzed using the Neighbor Joining (NJ) algorithm and K2P distances of MEGA Ver. 4 (Tamura et al., 2007) and the resultant tree was bootstrapped using 1,000 subreplicates. Separate analysis using the NJ algorithm and K2P distance was done for 69 barcodes for 34 species of Copepoda.

Table 2
Sargasso Sea zooplankton and fish specimens for which DNA barcodes were analyzed for this study. Specimen information includes: phylum and group, species name, voucher number (V. No.), collection location given as Latitude (Lat °N), Longitude (Long °W), date collected (Coll. Date), and GenBank Accession Number (Acc. No.). The DNA sequence data and additional specimen information can be accessed from the NCBI GenBank BARCODE database using the Accession Number.

PHYLUM							
Group	**Sp. No.**	**Genus and Species**	**V. No.**	**Lat °N**	**Long °W**	**Coll. Date**	**Acc. No.**
CNIDARIA							
Siphonophora	1	*Abylopsis tetragona*	Hy06.2	33.597	69.410	14-Apr-2006	GQ119939
		Abylopsis tetragona	Hy06.5	19.820	54.726	23-Apr-2006	GQ119938
	2	*Agalma elegans*	Hy63.1.1	24.839	60.136	19-Apr-2006	GQ119940
		Agalma elegans	Hy63.1.2	24.839	60.136	19-Apr-2006	GQ119941
		Agalma elegans	Hy63.1.3	24.839	60.136	19-Apr-2006	GQ119942
	3	*Agalma okeni*	Hy73.3	19.785	54.594	23-Apr-2006	GQ119945
		Agalma okeni	Hy73.4	14.003	55.000	25-Apr-2006	GQ119946
		Agalma okeni	Hy73.5	14.042	54.891	25-Apr-2006	GQ119947
		Agalma okeni	Hy73.1.2	14.042	54.891	25-Apr-2006	GQ119948
	4	*Amphicaryon acaula*	Hy23.2	29.995	70.027	15-Apr-2006	GQ119950
	5	*Amphicaryon earnesti*	Hy90.1	14.003	55.000	25-Apr-2006	GQ119951
		Amphicaryon earnesti	Hy90.2	14.003	55.000	25-Apr-2006	GQ119952
	6	*Amphicaryon polifera*	Hy28.1.1	14.042	54.891	16-Apr-2006	GQ119953
	7	*Apolemia* sp	Hy100.1	14.097	54.780	25-Apr-2006	GQ119955
	8	*Athorybia rosacea*	Hy71.1.1	24.959	60.678	21-Apr-2006	GQ119956
	9	*Bargmannia* sp. 2	Hy40.1	29.830	70.238	16-Apr-2006	GQ119958
	10	*Bassia bassensis*	HY24.1	33.524	69.961	13-Apr-2006	GQ119960
	11	*Ceratocymba sagittata*	Hy78.1	24.959	60.678	21-Apr-2006	GQ119962
	12	*Chuniphyes multidentata*	Hy93.1	14.042	54.891	25-Apr-2006	GQ119964
		Chuniphyes multidentata	Hy93.2	14.042	54.891	25-Apr-2006	GQ119965
	13	*Dimophyes arctica*	Hy92.1	14.042	54.891	25-Apr-2006	GQ119966
	14	*Diphyes bojani*	Hy38.2	14.003	55.000	25-Apr-2006	GQ119969
		Diphyes bojani	Hy38.3	14.003	55.000	25-Apr-2006	GQ119970
	15	*Diphyes dispar*	Hy87.1	14.003	55.000	25-Apr-2006	GQ119973
	16	*Erenna* sp	Hy99.1	14.097	54.780	25-Apr-2006	GQ119976
	17	*Eudoxoides mitra*	Hy17.2.1	29.995	70.027	15-Apr-2006	GQ119979
		Eudoxoides mitra	Hy17.3.1	33.597	69.410	14-Apr-2006	GQ119977
		Eudoxoides mitra	Hy17.3.2	33.597	69.410	14-Apr-2006	GQ119978
		Eudoxoides mitra	Hy17.4.1	29.995	70.027	15-Apr-2006	GQ119980
	18	*Eudoxoides spiralis*	Hy13.1	33.524	69.961	13-Apr-2006	GQ119981
		Eudoxoides spiralis	Hy13.2	29.995	70.027	15-Apr-2006	GQ119982
		Eudoxoides spiralis	Hy13.3	25.000	59.945	16-Apr-2006	GQ119983
	19	*Forskalia contorta*	Hy60.1	24.869	60.487	20-Apr-2006	GQ119984
	20	*Forskalia tholoides*	Hy89.1	14.018	54.911	25-Apr-2006	GQ119985
	21	*Frillagalma* sp	Hy35.5	55.000	55.000	25-Apr-2006	GQ119986
		Frillagalma sp	Hy35.6	55.000	55.000	25-Apr-2006	GQ119987
	22	*Halistemma amphitridis*	Hy48.6	33.642	69.795	13-Apr-2006	GQ119990
		Halistemma amphitridis	Hy48.8	14.097	54.780	25-Apr-2006	GQ119992
	23	*Halistemma* sp	Hy48.5	24.839	60.136	19-Apr-2006	GQ119989
		Halistemma sp	Hy48.7	14.042	54.891	25-Apr-2006	GQ119991
	24	*Hippopodius hippopus*	Hy05.1	33.524	69.961	13-Apr-2006	GQ119995
		Hippopodius hippopus	Hy27.1.1	29.995	70.027	15-Apr-2006	GQ119996
		Hippopodius hippopus	Hy27.2	25.000	59.945	19-Apr-2006	GQ119997
		Hippopodius hippopus	Hy27.6	14.042	54.891	25-Apr-2006	GQ119998
	25	*Kephyes ovata*	Hy86.1	33.524	69.961	13-Apr-2006	GQ119999
	26	*Lensia achilles*	Hy56.1	24.869	60.487	20-Apr-2006	GQ120000
	27	*Lensia campanella*	Hy30.2	29.995	70.027	15-Apr-2006	GQ120066
	28	*Lensia exeter*	Hy58.1	24.869	60.487	20-Apr-2006	GQ120003
	29	*Lensia fowleri*	Hy25.1.1	33.597	69.410	14-Apr-2006	GQ120004
		Lensia fowleri	Hy25.1.3	33.597	69.410	14-Apr-2006	GQ120006
		Lensia fowleri	Hy25.4	33.524	69.961	13-Apr-2006	GQ120007
	30	*Lensia grimaldii*	Hy85.1	33.524	69.961	13-Apr-2006	GQ120008
	31	*Lensia hospur*	Hy65.1	24.869	60.487	20-Apr-2006	GQ120009
	32	*Lensia multicristata* (Type 1)	Hy64.1	24.869	60.487	20-Apr-2006	GQ120011
		Lensia multicristata (Type 1)	Hy64.3	14.042	54.891	25-Apr-2006	GQ120013
	33	*Lensia multicristata* (Type 2)	Hy64.2	25.549	60.593	20-Apr-2006	GQ120012
		Lensia multicristata (Type 2)	Hy64.4	14.042	54.891	25-Apr-2006	GQ120014
	34	*Lilyopsis fluoracantha*	Hy88.1	14.003	55.000	25-Apr-2006	GQ120015
	35	*Lilyopsis rosea*	Hy46.1	33.556	69.669	14-Apr-2006	GQ120016
		Lilyopsis rosea	Hy46.2	33.556	69.669	14-Apr-2006	GQ120017
	36	*Maresearsia praeclara*	Hy57.2	14.003	55.000	25-Apr-2006	GQ120018
	37	*Nanomia bijuga*	Hy48.2	33.556	69.669	14-Apr-2006	GQ120022
	38	*Nectopyramis natans*	Hy44.1	29.830	70.238	16-Apr-2006	GQ120031
	39	*Physalia* sp	Hy82.1.1	19.764	54.612	23-Apr-2006	GQ120034
	40	*Praya reticulata*	Hy67.1	24.869	60.487	20-Apr-2006	GQ120037
	41	*Rhizophysa eysenhardti*	HY11.1	33.045	75.033	11-Apr-2006	GQ120038
		Rhizophysa eysenhardti	Hy11.2	29.995	70.027	15-Apr-2006	GQ120039
		Rhizophysa eysenhardti	Hy11.3	19.823	54.477	24-Apr-2006	GQ120040
	42	*Rhizophysa filiformis*	Hy66.1	24.839	60.136	19-Apr-2006	GQ120041
	43	*Rosacea cymbiformis*	Hy77.1	24.959	60.678	21-Apr-2006	GQ120042

Table 2 (*continued*)

PHYLUM							
Group	**Sp. No.**	**Genus and Species**	**V. No.**	**Lat °N**	**Long °W**	**Coll. Date**	**Acc. No.**
	44	*Rosacea* sp. 1	Hy18.1	33.621	69.865	12-Apr-2006	GQ120043
		Rosacea sp. 1	Hy19.2	29.995	70.027	15-Apr-2006	GQ120044
	45	*Rosacea* sp. 2	Hy18.3	14.018	54.911	25-Apr-2006	GQ120045
	46	*Sphaeronectes gracilis*	HY20.1	33.563	69.493	14-Apr-2006	GQ120046
	47	*Sulculeolaria quadrivalvis*	Hy04.1	33.524	69.961	13-Apr-2006	GQ120049
		Sulculeolaria quadrivalvis	Hy04.2	14.003	55.000	25-Apr-2006	GQ120050
Hydromedusae	1	*Colobonema sericeum*	Hy52.1	25.000	59.945	19-Apr-2006	GQ120076
	2	*Geryonia proboscidalis*	Hy68.1.1	24.978	60.542	20-Apr-2006	GQ120078
	3	*Porpita porpita*	HY19.1	33.597	69.410	14-Apr-2006	GQ120060
	4	*Rhopalonema velatum*	Hy39.2	29.875	70.074	16-Apr-2006	GQ120080
ARTHROPODA							
Amphipoda	1	*Brachyscelus crusculum*	Am05.1.1	33.631	69.526	14-Apr-2006	GU145038
	2	*Brachyscelus* sp	Am16.1.1	24.978	60.542	20-Apr-2006	GU145047
	4	*Eupronoe maculata*	Am03.1.1	33.524	69.961	13-Apr-2006	GU145036
	5	*Eupronoe minuta*	Am04.1.1	33.524	69.961	13-Apr-2006	GU145037
	6	*Phronima sedentaria*	Am08.2.1	29.995	70.027	15-Apr-2006	GU145043
	7	*Phronimella elongata*	Am07.1.1	33.524	69.961	13-Apr-2006	GU145040
		Phronimella elongata	Am07.2.1	29.995	70.027	15-Apr-2006	GU145041
	8	*Phrosina semilunata*	Am12.1.1	29.830	70.238	16-Apr-2006	GU145046
	9	*Primno latreillei*	Am06.1.1	33.524	69.961	13-Apr-2006	GU145039
	10	*Streetsia challengeri*	Am11.1.2	29.995	70.027	15-Apr-2006	GU145044
		Streetsia challengeri	Am11.1.3	29.995	70.027	15-Apr-2006	GU145045
Copepoda	1	*Centropages violaceus*	Co010.1.1	33.524	69.961	13-Apr-2006	GU171310
		Centropages violaceus	Co010.1.2	33.524	69.961	13-Apr-2006	GU171311
	2	*Clausocalanus arcuicornis*	Co009.1.2	33.524	69.961	13-Apr-2006	GU171291
		Clausocalanus arcuicornis	Co009.2.2	33.524	69.961	13-Apr-2006	GU171292
	3	*Clausocalanus lividus*	Co016.1.1	33.524	69.961	13-Apr-2006	GU171293
		Clausocalanus lividus	Co016.1.2	33.524	69.961	13-Apr-2006	GU171294
		Clausocalanus lividus	Co016.1.3	33.524	69.961	13-Apr-2006	GU171295
	4	*Clausocalanus mastigophorus*	Co012.1.1	33.524	69.961	13-Apr-2006	GU171296
	5	*Clausocalanus pergens*	Co015.1.1	33.654	69.196	14-Apr-2006	GU171297
		Clausocalanus pergens	Co015.1.2	33.654	69.196	14-Apr-2006	GU171298
		Clausocalanus pergens	Co015.1.3	33.654	69.196	14-Apr-2006	GU171299
		Clausocalanus pergens	Co015.1.5	33.654	69.196	14-Apr-2006	GU171300
	6	*Ctenocalanus vanus*	Co007.2.1	33.524	69.961	13-Apr-2006	GU171288
		Ctenocalanus vanus	Co007.2.2	33.524	69.961	13-Apr-2006	GU171289
		Ctenocalanus vanus	Co007.3.1	33.524	69.961	13-Apr-2006	GU171290
	7	*Euaugaptilus affinis*	Co134.3.1	20.000	54.997	23-Apr-2006	GU171336
	8	*Euaugaptilus angustus*	Co065.1.2	24.791	60.364	20-Apr-2003	GU171337
	9	*Euaugaptilus gracilis*	Co092.2.1	20.000	54.997	23-Apr-2006	GU171338
		Euaugaptilus gracilis	Co092.2.2	20.000	54.997	23-Apr-2006	GU171339
		Euaugaptilus gracilis	Co092.2.3	20.000	54.997	23-Apr-2006	GU171340
	10	*Euaugaptilus laticeps*	Co100.2.1	14.097	54.780	25-Apr-2006	GU171341
	11	*Euaugaptilus magnus*	Co046.1.1	29.830	70.238	16-Apr-2006	GU171342
		Euaugaptilus magnus	Co046.3.2	29.868	70.076	16-Apr-2006	GU171343
		Euaugaptilus magnus	Co046.3.4	29.868	70.076	16-Apr-2006	GU171344
	12	*Euaugaptilus maxillaris*	Co067.1.1	24.791	60.364	20-Apr-2003	GU171345
	13	*Euchaeta media*	Co026.1.1	33.524	69.961	13-Apr-2006	GU171301
		Euchaeta media	Co026.1.4	33.524	69.961	13-Apr-2006	GU171302
		Euchaeta media	Co026.1.5	33.524	69.961	13-Apr-2006	GU171303
	14	*Euchirella messinensis*	Co040.2.3	29.868	70.076	16-Apr-2006	GU171306
		Euchirella messinensis	Co040.3.1	29.868	70.076	16-Apr-2006	GU171307
		Euchirella messinensis	Co040.3.2	29.868	70.076	16-Apr-2006	GU171308
	15	*Gaetanus miles*	Co036.2.1	29.995	70.027	15-Apr-2006	GU171321
	16	*Heterorhabdus spinifrons*	Co031.2.1	33.524	69.961	13-Apr-2006	GU171320
	17	*Lophothrix humilifrons*	Co075.2.1	24.791	60.364	20-Apr-2003	GU171346
		Lophothrix humilifrons	Co075.3.1	20.000	54.997	23-Apr-2006	GU171347
	18	*Lucicutia aurita*	Co048.1.1	29.830	70.238	16-Apr-2006	GU171331
	19	*Lucicutia intermedia*	Co283.1.1	33.642	69.795	13-Apr-2006	GU171352
	20	*Lucicutia wolfendeni*	Co229.1.1	33.642	69.795	13-Apr-2006	GU171351
	21	*Miracia efferata*	Co087.1.1	24.946	60.531	20-Apr-2006	GU171349
		Miracia efferata	Co087.1.2	24.946	60.531	20-Apr-2006	GU171350
	22	*Nannocalanus minor*	Co006.3.1	33.524	69.961	13-Apr-2006	GU171285
		Nannocalanus minor	Co006.3.2	33.524	69.961	13-Apr-2006	GU171286
		Nannocalanus minor	Co006.3.3	33.524	69.961	13-Apr-2006	GU171287
	23	*Phyllopus impar*	Co060.2.1	25.549	60.593	20-Apr-2006	GU171353
	24	*Pleuromamma abdominalis*	Co029.1.1	33.524	69.961	13-Apr-2006	GU171314
	25	*Pleuromamma gracilis*	Co027.1.1	33.524	69.961	13-Apr-2006	GU171312
		Pleuromamma gracilis	Co027.1.2	33.524	69.961	13-Apr-2006	GU171313
	26	*Pleuromamma piseki*	Co028.1.1	33.524	69.961	13-Apr-2006	GU171304
		Pleuromamma piseki	Co028.1.2	33.524	69.961	13-Apr-2006	GU171305
	27	*Pleuromamma xiphias*	Co030.1.1	33.524	69.961	13-Apr-2006	GU171315
		Pleuromamma xiphias	Co030.1.2	33.524	69.961	13-Apr-2006	GU171316

Table 2 (*continued*)

PHYLUM							
Group	**Sp. No.**	**Genus and Species**	**V. No.**	**Lat °N**	**Long °W**	**Coll. Date**	**Acc. No.**
		Pleuromamma xiphias	Co030.1.4	33.524	69.961	13-Apr-2006	GU171317
		Pleuromamma xiphias	Co030.1.5	33.524	69.961	13-Apr-2006	GU171318
		Pleuromamma xiphias	Co030.1.6	33.524	69.961	13-Apr-2006	GU171319
	28	*Pontellina plumata*	Co037.1.1	29.995	70.027	15-Apr-2006	GU171322
		Pontellina plumata	Co037.1.2	29.995	70.027	15-Apr-2006	GU171323
		Pontellina plumata	Co037.3.1	20.003	55.002	23-Apr-2006	GU171324
	29	*Rhincalanus cornutus*	Co004.2.1	33.524	69.961	13-Apr-2006	GU171309
	30	*Sapphirina angusta*	Co041.1.1	33.524	69.961	13-Apr-2006	GU171328
	31	*Sapphirina metallina*	Co047.2.1	29.482	70.506	17-Apr-2006	GU171329
		Sapphirina metallina	Co047.3.1	24.996	59.999	19-Apr-2006	GU171330
	32	*Scaphocalanus brevirostris*	Co079.1.1	29.868	70.076	16-Apr-2006	GU171348
	33	*Undeuchaeta major*	Co043.2.1	29.868	70.076	16-Apr-2006	GU171325
		Undeuchaeta major	Co043.2.2	29.868	70.076	16-Apr-2006	GU171326
		Undeuchaeta major	Co043.2.3	29.868	70.076	16-Apr-2006	GU171327
	34	*Undinula vulgaris*	Co063.1.2	24.833	60.447	20-Apr-2006	GU171332
		Undinula vulgaris	Co063.1.3	24.833	60.447	20-Apr-2006	GU171333
		Undinula vulgaris	Co063.1.4	24.833	60.447	20-Apr-2006	GU171334
		Undinula vulgaris	Co063.1.6	24.833	60.447	20-Apr-2006	GU171335
Mysidacea	1	*Siriella thompsonii*	Cr20.1.3	25.549	60.593	20-Apr-2006	GU183794
Decapoda	1	*Acanthephyra brevirostris*	Cr06.1.1	24.869	60.487	20-Apr-2006	GU183784
	2	*Acanthephyra curtirostris*	Cr16.1.1	14.097	54.780	25-Apr-2006	GU183785
	3	*Acanthephyra microphthalma*	Cr18.1.1	14.282	54.366	26-Apr-2006	GU183786
	4	*Acanthephyra purpurea*	Cr01.1.1	33.524	69.961	13-Apr-2006	GU183787
		Acanthephyra purpurea	Cr01.1.2	33.524	69.961	13-Apr-2006	GU183788
		Acanthephyra purpurea	Cr01.1.3	33.524	69.961	13-Apr-2006	GU183789
	5	*Acanthephyra stylorostratis*	Cr10.2.1	20.000	54.997	23-Apr-2006	GU183790
	6	*Eucopia grimaldii*	Cr21.1.1	25.056	60.626	21-Apr-2006	GU183795
	7	*Lucifer typus*	Cr03.1.1	29.999	69.915	15-Apr-2006	GU183792
	8	*Meningodora compsa*	Cr13.1.1	14.097	54.780	25-Apr-2006	GU183791
	9	*Systellaspis debilis*	Cr04.1.1	24.869	60.487	20-Apr-2006	GU183793
Euphausiacea	1	*Bentheuphausia amblyops*	Eu05.1.1	33.642	69.795	13-Apr-2006	GU183771
	2	*Nematobrachion sexspinosus*	Eu07.1.1	24.996	59.999	19-Apr-2006	GU183772
	3	*Nematoscelis atlantica*	Eu14.1.1	14.097	54.780	25-Apr-2006	GU183774
		Nematoscelis atlantica	Eu06.1.1	29.995	70.027	15-Apr-2006	GU183773
	4	*Stylocheiron abbreviatum*	Eu11.1.1	25.056	60.626	21-Apr-2006	EF467301
		Stylocheiron abbreviatum	Eu11.4.1	25.056	60.626	21-Apr-2006	GU183775
	5	*Stylocheiron carinatum*	Eu12.1.1	24.869	60.487	20-Apr-2006	GU183776
		Stylocheiron carinatum	Eu12.1.2	24.869	60.487	20-Apr-2006	GU183777
	6	*Stylocheiron elongatum*	Eu04.1.1	29.995	70.027	15-Apr-2006	GU183778
	7	*Stylocheiron suhmii*	Eu08.1.1	24.996	59.999	19-Apr-2006	GU183779
	8	*Thysanoessa gregaria*	Eu03.1.1	33.642	69.795	13-Apr-2006	GU183781
	9	*Thysanopoda aequalis*	Eu52.3.1	14.097	54.780	25-Apr-2006	GU183780
	10	*Thysanopoda obtusifrons*	Eu02.2.1	24.869	60.487	20-Apr-2006	GU183782
		Thysanopoda obtusifrons	Eu02.3.1	33.642	69.795	13-Apr-2006	GU183783
Ostracoda	1	*Conchoecetta acuminata*	Os050.1.1	14.003	54.999	25-Apr-2006	GU073366
		Conchoecetta acuminata	Os050.1.2	14.003	54.999	25-Apr-2006	GU073367
		Conchoecetta acuminata	Os050.1.3	14.003	54.999	25-Apr-2006	GU073368
	2	*Conchoecia hyalophyllum*	Os005.1.1	33.524	69.961	13-Apr-2006	GU073325
		Conchoecia hyalophyllum	Os005.1.2	33.524	69.961	13-Apr-2006	GU073326
		Conchoecia hyalophyllum	Os005.2.1	33.524	69.961	13-Apr-2006	GU073327
	3	*Conchoecilla daphnoides*	Os012.1.1	33.524	69.961	13-Apr-2006	GU073334
		Conchoecilla daphnoides	Os012.1.2	33.524	69.961	13-Apr-2006	GU073335
		Conchoecilla daphnoides	Os012.2.1	33.524	69.961	13-Apr-2006	GU073336
	4	*Conchoecissa ametra*	Os020.1.1	33.524	69.961	13-Apr-2006	GU073342
	5	*Conchoecissa imbricata*	Os011.1.1	33.524	69.961	13-Apr-2006	GU073331
		Conchoecissa imbricata	Os011.1.2	33.524	69.961	13-Apr-2006	GU073332
		Conchoecissa imbricata	Os011.2.1	33.524	69.961	13-Apr-2006	GU073333
	6	*Discoconchoecia elegans*	Os001.1.1	33.045	75.033	11-Apr-2006	GU073321
	7	*Euconchoecia chierchiae*	Os026.1.1	33.524	69.961	13-Apr-2006	GU073347
	8	*Gigantocypris dracontovalis*	Os041.1.1	14.097	54.780	25-Apr-2006	GU073364
		Gigantocypris dracontovalis	Os041.3.1	20.000	54.997	23-Apr-2006	GU073365
	9	*Macroconchoecia macroreticulata*	Os023.1.2	33.642	69.795	13-Apr-2006	GU073345
	10	*Macroconchoecia spinireticulata*	Os024.1.2	33.642	69.795	13-Apr-2006	GU073346
	11	*Metaconchoecia acuta*	Os015.1.1	33.524	69.961	13-Apr-2006	GU073339
	12	*Metaconchoecia arcuata*	Os032.1.2	33.642	69.795	13-Apr-2006	GU073355
		Metaconchoecia arcuata (small)	Os032.2.1	24.791	60.364	20-Apr-2003	GU073356
		Metaconchoecia arcuata (small)	Os032.2.2	24.791	60.364	20-Apr-2003	GU073357
		Metaconchoecia arcuata (small)	Os032.2.3	24.791	60.364	20-Apr-2003	GU073358
	13	*Mikroconchoecia curta*	Os017.1.1	33.524	69.961	13-Apr-2006	GU073341
	14	*Mikroconchoecia echinulata*	Os027.2.1	33.631	69.526	14-Apr-2006	GU073348
		Mikroconchoecia echinulata	Os027.2.2	33.631	69.526	14-Apr-2006	GU073349
	15	*Mollicia tyloda*	Os035.1.1	33.642	69.795	13-Apr-2006	GU073362

Table 2 (*continued*)

PHYLUM							
Group	**Sp. No.**	**Genus and Species**	**V. No.**	**Lat °N**	**Long °W**	**Coll. Date**	**Acc. No.**
	16	*Orthoconchoecia atlantica*	Os031.1.1	29.868	70.076	16-Apr-2006	GU073352
		Orthoconchoecia atlantica	Os031.2.1	14.042	54.891	25-Apr-2006	GU073353
		Orthoconchoecia atlantica	Os031.2.3	14.042	54.891	25-Apr-2006	GU073354
	17	*Orthoconchoecia secernenda*	Os016.1.2	33.524	69.961	13-Apr-2006	GU073340
	18	*Paraconchoecia aequiseta*	Os021.1.1	33.524	69.961	13-Apr-2006	GU073343
	19	*Paraconchoecia dasyophthalma*	Os036.1.1	29.830	70.238	16-Apr-2006	GU073363
	20	*Paraconchoecia dorsotuberculata*	Os033.1.1	33.642	69.795	13-Apr-2006	GU073359
		Paraconchoecia dorsotuberculata	Os033.1.2	33.642	69.795	13-Apr-2006	GU073360
	21	*Paraconchoecia mamillata*	Os022.1.1	33.524	69.961	13-Apr-2006	GU073344
	22	*Paraconchoecia oblonga* B	Os007.1.2	33.524	69.961	13-Apr-2006	GU073328
		Paraconchoecia oblonga B	Os007.1.3	33.524	69.961	13-Apr-2006	GU073329
	23	*Paramollicia dichotoma*	Os034.1.1	33.642	69.795	13-Apr-2006	GU073361
	24	*Porroecia parthenoda*	Os013.1.1	33.524	69.961	13-Apr-2006	GU073337
		Porroecia parthenoda	Os013.1.2	33.524	69.961	13-Apr-2006	GU073338
	25	*Porroecia spinirostris*	Os004.1.1	33.045	75.033	11-Apr-2006	GU073322
		Porroecia spinirostris	Os004.1.2	33.045	75.033	11-Apr-2006	GU073323
		Porroecia spinirostris	Os004.1.3	33.045	75.033	11-Apr-2006	GU073324
	26	*Proceroecia brachyaskos*	Os009.1.1	33.524	69.961	13-Apr-2006	GU073330
	27	*Proceroecia procera*	Os030.1.1	29.868	70.076	16-Apr-2006	GU073350
		Proceroecia procera	Os030.1.2	29.868	70.076	16-Apr-2006	GU073351
MOLLUSCA							
Heteropoda	1	*Atlanta gaudichaudi*	Ga30.1.1	24.950	60.530	20-Apr-2006	FJ876837
		Atlanta gaudichaudi	Ga30.1.2	24.950	60.530	20-Apr-2006	FJ876838
		Atlanta gaudichaudi	Ga30.1.3	24.950	60.530	20-Apr-2006	FJ876839
	2	*Atlanta* sp	Ga24.4.2	25.060	60.620	21-Apr-2006	FJ876843
		Atlanta sp	Ga24.4.3	25.060	60.620	21-Apr-2006	FJ876844
	3	*Atlanta inclinata*	Ga24.5.1	25.060	60.620	21-Apr-2006	FJ876845
	4	*Atlanta peronii*	Ga03.2.1	33.570	69.650	14-Apr-2006	FJ876846
	5	*Firoloida demarestia*	Ga41.2.1	14.000	55.000	25-Apr-2006	FJ876850
		Firoloida demarestia	Ga41.4.1	14.000	55.000	25-Apr-2006	FJ876851
	6	*Pterotrachea hippocampus*	Ga20.1.2	24.950	60.530	20-Apr-2006	FJ876854
		Pterotrachea hippocampus	Ga20.2.1	24.950	60.530	20-Apr-2006	FJ876855
Pteropoda	1	*Cavolinia gibbosa*	Ga05.1.1	33.520	69.960	13-Apr-2006	FJ876856
	2	*Cavolinia globulosa*	Ga43.3.1	14.000	55.000	25-Apr-2006	FJ876857
	3	*Cavolinia longirostris*	Ga32.1.1	24.950	60.530	20-Apr-2006	FJ876859
		Cavolinia longirostris	Ga32.1.2	24.950	60.530	20-Apr-2006	FJ876860
	4	*Cavolinia uncinata uncinata*	Ga29.1.1	24.950	60.530	20-Apr-2006	FJ876862
		Cavolinia uncinata uncinata	Ga29.9.2	14.000	55.000	25-Apr-2006	FJ876863
	5	*Clio pyramidata lanceolata*	Ga01.1.2	33.520	69.960	13-Apr-2006	FJ876872
		Clio pyramidata lanceolata	Ga01.1.3	33.520	69.960	13-Apr-2006	FJ876873
	6	*Creseis virgula virgula*	Ga53.1.1	19.760	54.610	23-Apr-2006	FJ876889
		Creseis virgula virgula	Ga14.1.1	29.880	70.070	16-Apr-2006	FJ876890
	7	*Cuvierina columnella*	Ga06.1.1	30.000	70.030	15-Apr-2006	FJ876893
		Cuvierina columnella	Ga06.2.1	29.480	70.510	17-Apr-2006	FJ876894
		Cuvierina columnella	Ga06.4.1	24.990	59.990	19-Apr-2006	FJ876895
	8	*Diacria quadridentata*	Ga07.2.1	24.990	59.990	19-Apr-2006	FJ876901
		Diacria quadridentata	Ga07.2.2	24.990	59.990	19-Apr-2006	FJ876902
	9	*Diacria major*	Ga02.1.1	33.520	69.960	13-Apr-2006	FJ876908
		Diacria major	Ga02.2.1	33.520	69.960	13-Apr-2006	FJ876914
	10	*Hyalocylis striata*	Ga09.2.1	24.990	59.990	19-Apr-2006	FJ876919
	11	*Limacina inflata*	Ga11.1.1	29.880	70.070	16-Apr-2006	FJ876927
		Limacina inflata	Ga11.1.2	29.880	70.070	16-Apr-2006	FJ876928
		Limacina inflata	Ga11.1.3	29.880	70.070	16-Apr-2006	FJ876929
	12	*Gleba cordata*	Ga27.1.1	25.000	59.950	19-Apr-2006	FJ876933
		Gleba cordata	Ga27.2.1	24.990	59.990	19-Apr-2006	FJ876934
	13	*Thliptodon diaphanus*	Ga28.1.1	24.830	60.450	20-Apr-2006	FJ876950
		Thliptodon diaphanus	Ga28.2.1	24.790	60.360	20-Apr-2006	FJ876951
	14	*Clione limacina*	Ga04.1.1	33.520	69.960	13-Apr-2006	FJ876941
	15	*Pneumodermopsis macrochira*	Ga16.1.1	29.870	70.080	16-Apr-2006	FJ876946
		Pneumodermopsis macrochira	Ga16.2.1	29.830	70.240	16-Apr-2006	FJ876947
		Pneumodermopsis macrochira	Ga16.3.1	24.790	60.360	20-Apr-2006	FJ876948
Cephalopoda	1	*Bathyteuthis* sp A	Mo26.1.1	14.280	54.370	25-Apr-2002	GU145068
	2	*Cirrothauma* sp	Mo18.1.1	20.000	55.000	22-Apr-2002	GU145063
	3	*Helicocranchia* sp	Mo16.1.1	20.000	55.000	22-Apr-2002	GU145061
	4	*Histioteuthis* sp	Mo04.1.1	30.000	70.030	14-Apr-2002	GU145057
	5	*Leachia* sp	Mo24.1.1	14.040	54.890	24-Apr-2002	GU145067
	6	*Pyroteuthis* sp	Mo17.1.1	20.000	55.000	22-Apr-2002	GU145062
	7	*Selenoteuthis scintilans*	Mo23.1.1	19.790	54.590	22-Apr-2002	GU145066
	8	*Vampyroteuthis infernalis*	Mo15.1.1	25.060	60.630	20-Apr-2002	GU145058
		Vampyroteuthis infernalis	Mo15.2.1	14.100	54.780	24-Apr-2002	GU145059
CHAETOGNATHA	1	*Sagitta bipunctata*	Ch22.1.1	14.000	55.000	24-Apr-2002	GQ368396
		Sagitta bipunctata	Ch22.1.2	14.000	55.000	24-Apr-2002	GQ368397
		Sagitta bipunctata	Ch22.2.1	14.000	55.000	24-Apr-2002	GQ368398

Table 2 (*continued*)

Group	Sp. No.	Genus and Species	V. No.	Lat °N	Long °W	Coll. Date	Acc. No.
PHYLUM							
	2	*Sagitta enflata*	Ch15.1.1	14.000	55.000	24-Apr-2002	GQ368399
		Sagitta enflata	Ch15.1.2	14.000	55.000	24-Apr-2002	GQ368400
	3	*Sagitta helenae*	Ch16.1.1	14.000	55.000	24-Apr-2002	GQ368402
		Sagitta helenae	Ch16.2.1	14.040	54.890	24-Apr-2002	GQ368403
		Sagitta helenae	Ch16.3.1	14.040	54.890	24-Apr-2002	GQ368404
	4	*Sagitta sibogae*	Ch21.1.1	14.040	54.890	24-Apr-2002	GQ368418
		Sagitta sibogae	Ch21.1.2	14.040	54.890	24-Apr-2002	GQ368419
		Sagitta sibogae	Ch21.1.3	14.040	54.890	24-Apr-2002	GQ368420
		Sagitta sibogae	Ch21.2.1	14.000	55.000	24-Apr-2002	GQ368421
VERTEBRATA							
Teleostei	1	*Argyropelecus hemigymnus*	Ve37.1	29.868	70.076	16-Apr-2006	GU071749
	2	*Astronesthes similus*	Ve33.1	14.282	54.366	26-Apr-2006	GU071747
	3	*Benthalbella infans*	Ve15.1	24.791	60.364	20-Apr-2003	GU071731
	4	*Benthosema glaciale*	Ve36.1	25.000	59.945	19-Apr-2006	GU071748
	5	*Bolinichthys distofax*	Ve40.1	19.785	54.594	23-Apr-2006	GU071750
	6	*Ceratoscopelus warmingii*	Ve07.1	29.830	70.238	16-Apr-2006	GU071724
	7	Ceratiidae sp	Ve20.1	25.056	60.626	21-Apr-2006	GU071736
	8	*Cetostoma regani*	Ve11.1	25.000	59.945	19-Apr-2006	GU071727
	9	*Chauliodus danae*	Ve19.1	25.549	60.593	20-Apr-2006	GU071735
	10	*Cyclothone acclinidens*	Ve25.1	14.003	54.999	25-Apr-2006	GU071741
	11	*Cyclothone braueri*	Ve24.1	14.003	54.999	25-Apr-2006	GU071740
	12	*Cyclothone pallida*	Ve12.1	25.000	59.945	19-Apr-2006	GU071728
		Cyclothone pallida	Ve12.2	14.003	54.999	25-Apr-2006	GU071729
	13	*Cyclothone pseudopallida*	Ve04.1	29.995	70.027	15-Apr-2006	GU071722
	14	*Diaphus brachycephalus*	Ve17.1	24.869	60.487	20-Apr-2006	GU071733
	15	*Diaphus subtilis*	Ve31.1	14.282	54.366	26-Apr-2006	GU071745
	16	*Gigantactinidae* sp	Ve29.1	14.097	54.780	25-Apr-2006	GU071743
	17	*Idiacanthus fasciola*	Ve10.2	19.820	54.726	23-Apr-2006	GU071726
	18	*Idiacanthus* sp	Ve27.1	14.097	54.780	25-Apr-2006	GU071742
	19	*Lampanyctus alatus*	Ve22.1	14.003	54.999	25-Apr-2006	GU071738
	20	*Lampanyctus photonotus*	Ve16.1	24.869	60.487	20-Apr-2006	GU071732
	21	*Lepidophanes guentheri*	Ve23.1	14.003	54.999	25-Apr-2006	GU071739
	22	*Leptostomias* sp	Ve32.1	14.282	54.366	26-Apr-2006	GU071746
	23	*Lestidiops ringens*	Ve47.1	19.820	54.726	23-Apr-2006	GU071752
	24	*Maulisia* sp	Ve30.1	14.097	54.780	25-Apr-2006	GU071744
	25	*Notolychnus valdiviae*	Ve18.1	24.869	60.487	20-Apr-2006	GU071734
	26	*Photonectes dinema*	Ve05.1	29.995	70.027	15-Apr-2006	GU071723
	27	*Photostomias goodyeari*	Ve08.1	29.830	70.238	16-Apr-2006	GU071725
	28	*Rhynchactis* sp	Ve14.1	24.791	60.364	20-Apr-2003	GU071730
	29	*Taaningichthys minimus*	Ve42.1	25.000	59.945	19-Apr-2006	GU071751
	30	*Vinciguerria poweriae*	Ve21.1	14.003	54.999	25-Apr-2006	GU071737

3. Results

Considering both at-sea and subsequent land-based analysis, a ~650 base-pair region of mitochondrial cytochrome oxidase I (mtCOI) was sequenced for 298 identified specimens of 176 species of 12 groups in four phyla of metazoan holozooplankton collected from the Sargasso Sea, Northwest Atlantic Ocean (Table 2). The mean number of barcodes per species was 1.69 (s.d.=0.908; median=1). The number of species of each group was: Cnidaria (Hydromedusae, 4; Siphonophora, 47); Arthropoda (Amphipoda, 10; Copepoda, 34; Decapoda, 9; Euphausiacea, 10; Mysidacea, 1; Ostracoda, 27); and Mollusca (Cephalopoda, 8; Heteropoda, 6; Pteropoda, 15); and Chaetognatha (4; Table 3). A total of 31 specimens of 30 species of fish (Teleostei) was also barcoded. Despite the presence of a large team of expert taxonomists (Table 3), not all specimens could be identified to species due to lack of time and specialists for every group.

Kimura-2-Parameter distances between DNA barcodes of the same and different species showed a characteristic "barcode gap", with marked differences between within-species versus between-species distances for eight zooplankton groups for which sufficient data were available for analysis (Fig. 3). Omitted from these analyses were the Hydromedusae and Mysidacea, for which fewer than five species were analyzed, and the Cephalopoda, for which all but one species was analyzed for one individual (Table 4). Within-species distances varied among the eight groups and ranged from the Ostracoda (mean=0.0070; S.D. 0.0165) to the Chaetognatha (mean=0.0388, S.D. 0.0208). Between-species distances also varied among groups, with the Ostracoda the smallest (mean=0.2785; S.D. 0.0812) and the Siphonophora the largest (mean=0.4767; S.D. 0.1153; Table 4). Considering all groups together, distances between individuals of the same species (mean=0.0114; S.D. 0.0117) were much smaller than those between individuals of different species within the same group (mean=0.3166; S.D. 0.0378; Table 4).

Analysis of all DNA sequence data using the NJ algorithm and K2P distances yielded a tree that resolved barcodes for five major groups of zooplankton (Copepoda, Ostracoda, Heteropoda, Cephalopoda, and Chaetognatha) and the Teleostei with bootstrap values of 97–99% (Fig. 4). Most, but not all, species of the Amphipoda and Pteropoda formed monophyletic clusters with significant ($>95\%$) bootstrap support; other groups, including the Cnidaria, were not well-resolved by this analysis of mtCOI sequence variation (Fig. 4).

Species of all groups for which multiple individuals were analyzed yielded consistent results: with very few exceptions, multiple barcodes for a given species were resolved with bootstrap values of 99–100%. Separate analysis of 69 barcodes for 34 species of the Copepoda, including 18 species analyzed for more than one individual, was done to visualize the species-level

Table 3
Summary of the number of species and individuals (Barcodes) by phylum and taxonomic group for which DNA barcodes were determined from the Sargasso Sea, Northwest Atlantic Ocean, with names of the taxonomists responsible for species identifications within each group (Taxonomic Identification).

Phylum	CMarZ Group	Species	Barcodes	Taxonomic Identification
Cnidaria	Siphonophora	47	76	D. Lindsay, F. Pages
	Hydromedusae	4	4	D. Lindsay, L.P. Madin
Arthropoda	Amphipoda	10	12	R.R. Hopcroft, D. Lindsay
	Copepoda	34	70	L. Blanco-Bercial, A. Cornils, J. Bradford-Grieve, M. Kuriyama, H. Matsuura
	Mysidacea	1	1	S. Panampunnayil
	Decapoda	9	11	H.Ø. Hansen
	Euphausiidae	10	14	N.J. Copley, P.H. Wiebe
	Ostracoda	27	48	M.V. Angel
Mollusca	Heteropoda	6	11	R.R. Hopcroft, D. Lindsay
	Pteropoda	15	29	R.R. Hopcroft, D. Lindsay
	Cephalopoda	8	9	D. Lindsay
Chaetognatha	Chaetognatha	4	12	V. Nair, A. Pierrot-Bults (both after the cruise)
Vertebrata	Teleostei	30	31	T. Sutton, C.J. Sweetman
TOTAL		205	328	

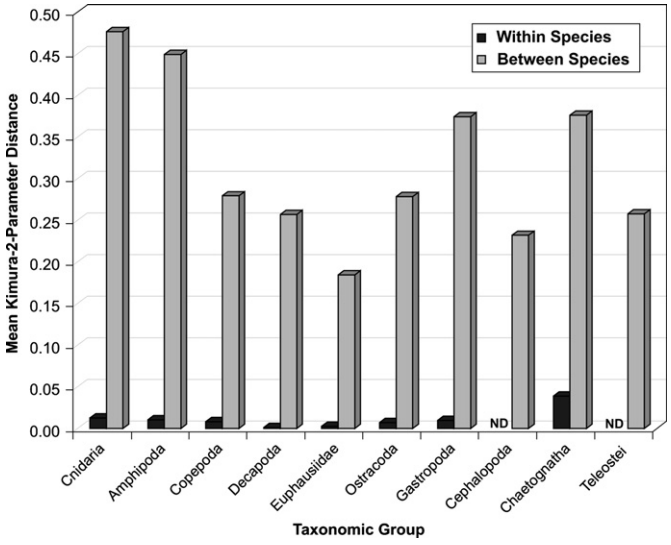

Fig. 3. Kimura-2-Parameter (K2P) genetic distances for zooplankton and fish collected from the Sargasso Sea. Bars show mean distances between individuals of the same and different species within each group. Groups for which fewer than five species were analyzed were not included. No data (ND) are available for within-species differences for Cephalopoda or Teleostei. See Table 4 for number of pairwise comparisons, mean, and standard deviation for each group.

Table 4
Sequence divergences shown as mean and standard deviation (St Dev) for Kimura-2-Parameter (K2P) distances for mtCOI barcodes between individual of the same (Within Species) and different species (Between Species) within each group of metazoan holozooplankton and fishes of the Sargasso Sea, Northwest Atlantic Ocean. Number of pairwise comparisons (N); standard deviation (St Dev).

Group	Within Species			Between Species		
	N	Mean	St Dev	N	Mean	St Dev
Siphonophora	42	0.0126	0.0165	3,656	0.4767	0.1153
Amphipoda	2	0.0105	0.0148	52	0.4493	0.1418
Copepoda	54	0.0083	0.0088	2,292	0.2795	0.0615
Decapoda	3	0.0013	0.0012	51	0.2571	0.0835
Euphausiidae	4	0.0028	0.0043	86	0.1845	0.0352
Ostracoda	27	0.0070	0.0165	1,149	0.2785	0.0812
Gastropoda	21	0.0097	0.0101	759	0.3745	0.1018
Cephalopoda	N/A	N/A	N/A	35	0.2320	0.0270
Chaetognatha	13	0.0388	0.0208	53	0.3763	0.0521
Teleostei	N/A	N/A	N/A	464	0.2579	0.0383
Overall	166	0.0114	0.0117	8,597	0.3166	0.0378

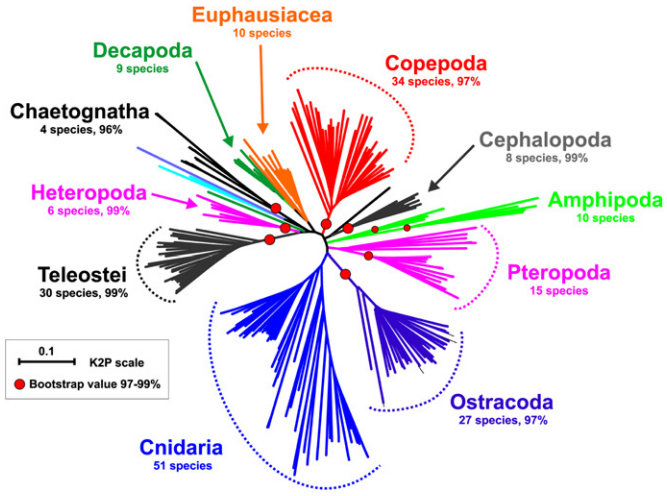

Fig. 4. Unrooted Neighbor Joining tree of mtCOI barcodes for 328 individuals of 205 species of Sargasso Sea zooplankton and fishes. Four major groups of zooplankton and also the fish were clearly resolved with bootstrap values of 97–99%. Many, but not all, species of the Amphipoda and Pteropoda formed monophyletic clusters with significant ($\geq$95%) bootstrap support. Analysis was based on Kimura-2-Parameter (K2P) distances and 1,000X bootstrapping.

resolution of barcodes in an NJ tree using K2P distances (Fig. 5). Multiple barcodes were resolved with 100% bootstrap values for 15 of 18 species; two species, *Pleuromamma gracilis* and *P. piseki*, were resolved with 99% and 89% bootstrap values, respectively, and were grouped by a 100% value. As demonstrated by the Copepoda, mtCOI accurately resolves barcode clusters for some, but not all, species with 100% bootstrap values; one exception was *Nannocalanus minor*, which showed exceptional levels of within-species divergence (Fig. 5).

4. Discussion

4.1. Analysis of species-level diversity using barcodes

Species-level analysis of the zooplankton assemblage is critically needed to accurately assess and understand ocean ecosystems. This information is necessary as a baseline for analysis and prediction of changes in ocean environments in response to climate change and global warming (Beaugrand et al., 2002; Edwards and Richardson, 2004). Since many commercially-harvested species are selective

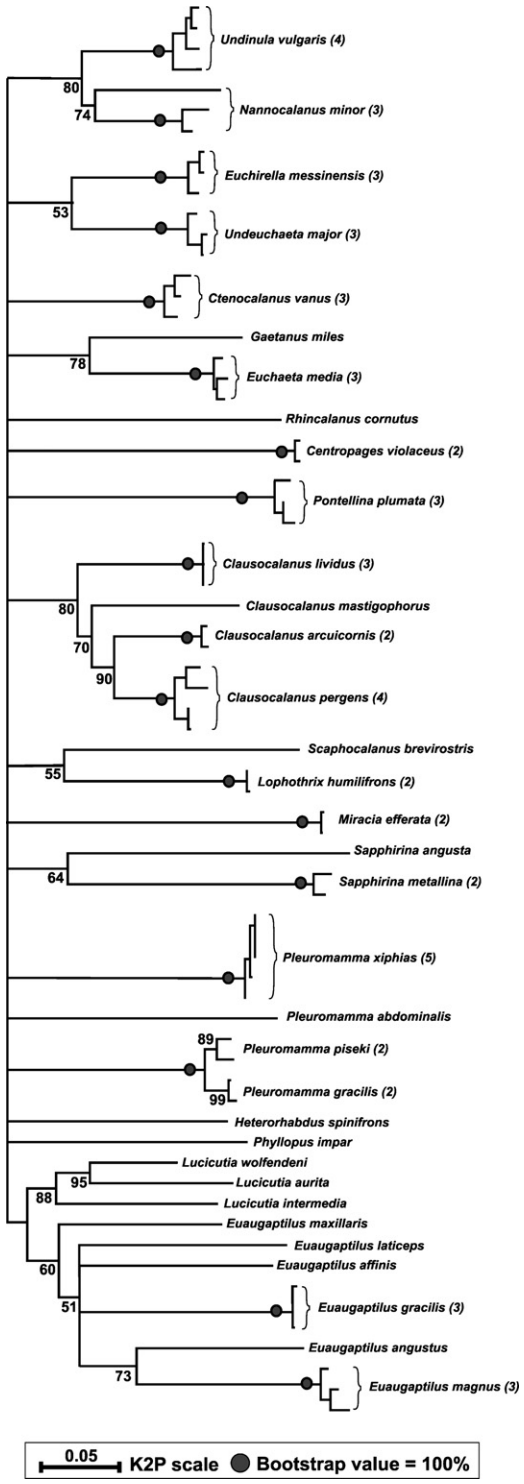

Fig. 5. Unrooted Neighbor Joining tree of mtCOI barcodes for 69 individuals of 34 species of the Copepoda. Multiple barcodes were resolved with 100% bootstrap values for 16 of 18 species; two species, *Pleuromamma gracilis* and *P. piseki*, were resolved with 99% and 89% bootstrap values, respectively, and were grouped by a 100% value. Barcodes for one species, *Nannocalanus minor*, showed exceptional levels of intraspecific divergence likely associated with subspecies differentiation (Bucklin et al., 1996). Analysis was based on Kimura-2-Parameter (K2P) distances and 1,000X bootstrapping. Filled circles indicate 100% bootstrap values; numbers at nodes are bootstrap percentages.

predators on zooplankton species, analysis of zooplankton species diversity, distribution, and abundance are needed for ecosystem approaches to fisheries management and as indicators of ocean ecosystem health (Link et al., 2002).). Importantly, short-lived, small-bodied zooplankton serve as rapid-responders for climate change (Greene and Pershing, 2007).

Although species diversity of the metazoan holozooplankton assemblage is not particularly high (~6,000 species), the taxonomic complexity (11 phyla) presents a persistent challenge and genuine impediment to rapid species-level analysis. Complete analysis of a single sample may require the attention of as many as 20 taxonomists, whose expertise is limited to one of >20 different taxonomic groups found in the pelagic realm.

Marine species diversity is a topic of fascination for both oceanographers and the general public, as well as a topic of some urgency for ocean management and regulation, and thus both curiosity and necessity have driven the search for new approaches and tools for decoding the mysteries of marine biodiversity. One of the promising recent approaches is DNA barcoding, which seeks to develop new characters for species recognition and discrimination (Hebert et al., 2003; Stoeckle and Hebert, 2008). There are currently ongoing comprehensive global-scale DNA barcoding campaigns for birds (Hebert et al., 2004), butterflies (Burns et al., 2008), fishes (Ward et al., 2009), and all marine life (http://www.marinebarcoding.org/). However, the invertebrate and fish assemblages of the open ocean pelagic realm – and especially the deep-sea meso- and bathypelagic zones below 1,000 meters – has remained a mystery, due primarily to logistical difficulties of collecting from the deepest regions of the ocean and the special challenges of collecting and identifying the fragile gelatinous forms that populate the ocean depths.

The impediments resulting from the increasing scarcity of taxonomic expertise and the difficulties of collecting intact, identifiable specimens of pelagic invertebrate groups can be addressed through DNA barcoding. The foundation of this approach is a "gold standard" DNA barcode database that links the species name, diagnostic morphological characters, specimen and DNA vouchers (or photo vouchers for fragile organisms), and a DNA sequence. The DNA barcode database is thus a Rosetta Stone that will allow new understanding of marine zooplankton species diversity in terms of information encoded in the DNA sequence of the barcode gene region.

4.2. DNA sequence variation of zooplankton barcodes

The DNA barcodes reported here for marine zooplankton and fishes were determined from specimens identified by taxonomic experts and have been submitted to the NCBI GenBank database that is specially tailored to barcode records (http://www.ncbi.nlm.nih.gov/Genbank/barcode.html). In addition to the DNA sequence data, the GenBank record includes metadata on specimen and data vouchers, time and place of collection, and names of the people who collected and identified the samples. Such "gold-standard" barcodes are the necessary foundation of Rosetta Stone approach. However, the usefulness of the Rosetta Stone or DNA barcode database for rapid analysis of species diversity and routine species identification lies in the patterns of DNA sequence variation of the barcode gene region.

Of primary importance is our finding of a marked barcode gap (i.e., significant differences between pairwise genetic distances between individuals of the same species versus those of different species) for all taxonomic groups represented in the Sargasso Sea pelagic assemblage (Fig. 3; Table 4). This result bears out earlier analyses of Cnidaria (Ortman, 2008); Copepoda (Bucklin et al., 2003; Bucklin and Frost, 2009); and Euphausiacea (Bucklin et al., 2007), and is consistent with concurrent detailed analysis of several groups (using some of the same data), including the Cnidaria (Ortman et al., 2010); Ostracoda (L.M. Nigro, unpubl.

data); Heteropoda and Pteropoda (Jennings et al., 2010); and Chaetognatha (Jennings et al., 2010).

The barcode gap is a consistent feature of the Sargasso Sea zooplankton assemblage despite observed differences among groups in levels of within- and between-species distances (Table 4). These differences are marked, but must be interpreted in light of large differences in the density of taxon sampling and the numbers of individuals and species analyzed, both of which may cause variation in the distance measure. Thus, the higher distances between species of Cnidaria compared to Copepoda (Table 4) may reflect the deeper evolutionary divergences among species and/or the sparser taxon sampling.

With only one, two, or three individuals analyzed per species, our conclusions about levels of variation within any species must remain speculative. For some species, these data can be compared with barcodes for individuals collected in other ocean regions or basins, as part of sampling carried out by the Census of Marine Zooplankton (CMarZ) throughout the global ocean (e.g., Bucklin et al., 2010b). For others, our Sargasso Sea collections provided the only individual available for molecular analysis, as for a number of the Siphonophora (Ortman, 2008; Ortman et al., 2010). In this case, the DNA barcode may be considered to be a tentative identifier of that species, pending further and more geographically-intensive sampling and analysis. However, patterns of DNA variation have been found to be quite consistent within the groups identified, thus providing reason for confidence that a DNA barcode from a single specimen is a useful and valid identifier of that species.

4.3. Analysis of barcode data

A usual approach to analysis of DNA barcode data includes determining a Neighbor Joining (NJ) tree using Kimura-2-Parameter (K2P) distances (Kimura, 1980) or other tree-building algorithms such as Maximum Likelihood (Stamatakis, 2006). Regardless of the algorithm, size of the dataset, or density of taxon sampling, multiple barcodes for many species of most taxonomic groups are resolved with high bootstrap values (Stoeckle and Hebert, 2008). Notable exceptions include cnidarian Anthozoa (Shearer and Coffroth, 2008). Also, barcodes are not resolved for species that exhibit unusual levels of intraspecific genetic diversity, as a result of wide distribution, divergence among geographic populations, and/or incipient speciation (Knowles and Carstens, 2007). An example of exceptional levels of variation within species is that of *Nannocalanus minor,* which had COI sequences differences of ~12%. Although careful morphological examination of the specimens was not done prior to barcoding, a possible explanation is the inclusion of two known morphological types or putative cryptic species (see Bucklin et al., 1996; Fig. 5).

Patterns of DNA sequence variation of the mtCOI barcode region have revealed taxonomically-significant genetic variation among geographic populations, as well cryptic speciation, among diverse marine organisms. Among zooplankton, mtCOI is a useful marker for large-scale population genetic differentiation and phylogeography (Peijnenburg et al., 2004; Govindarajan et al., 2005; Blanco-Bercial et al., 2009). Cryptic species have been found within zooplankton taxa with widespread or disjoint distributions (Knowlton, 2000; Bucklin et al., 2003, 2007; Moura et al., 2008).

Above the species level, NJ tree-based analysis appears to resolve some, but not all, taxonomic groups with high bootstrap values, including zooplankton (Machida et al., 2009; Fig. 4). For these groups, it may be possible to accurately classify both known and unknown barcodes into these higher-level groups, such as those typically used by marine ecologists and oceanographers to characterize the pelagic assemblage (Table 3). The extent to which NJ tree-based analyses will resolve higher taxonomic groups of zooplankton for barcode datasets with more individuals and species collected from a broader geographic range is unclear. Deeper phylogenetic analysis with DNA barcodes is useful for some groups, but should incorporate models of sequence evolution and use different algorithms than distance-based NJ for tree-building. MtCOI shows congruence with other molecular markers in multi-gene phylogenies for some groups (e.g., Bracken et al., 2009).

4.4. Species identification using barcodes

A variety of distance-based approaches are used to identify and classify species based on mtCOI sequences. Identification of species for which DNA barcodes are available can be done by adding the unknown barcode to the dataset and using a tree-based analytical approach, such as NJ, to identify the barcoded specimen based on "barcode clusters" (Hajibabaei et al., 2006). A more usual method of species identification using barcodes is to use BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990) to query the DNA sequence database NCBI GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) to find the closest match to an unknown barcode. Alternatively, subscribers may query the Barcode of Life Data Systems (BOLD, see http://www.boldsystems.org), which uses an ID engine that identifies the query sequence by comparison to a global alignment (Ratnasingham and Hebert, 2007). A challenge for these approaches is that rates of divergence vary among different taxonomic groups, making the classification of unknown or novel barcodes statistically and analytically challenging (Lefébure et al., 2006). Also, these methods rely upon the difference between within- and between-species sequence distances (i.e., the barcode gap).

Alternative approaches to analysis of barcode data include: character analysis (Rach et al., 2008; Sarkar et al., 2008); simulation (Ross et al., 2008); empirical determination of divergences (Pons et al., 2006; Vogler et al., 2008); and vector analysis (Sirovich et al., 2009, 2010); among others (Blaxter et al., 2005; Knowles and Carstens, 2007; Zhang et al., 2008; Bucklin et al., 2011). Most of these approaches have yet to be examined for the accuracy and reliability of identification of species or classification of novel barcodes. Regardless of the analytical approach to zooplankton species identification using barcodes, a partial solution is to continue to work toward completion of the barcode library, since the more densely populated the database, the more accurate will be the query results (Ekrem et al., 2007).

4.5. Future of marine barcoding

Once a taxonomically-comprehensive gold-standard DNA barcode database has been created for the zooplankton of a particular ocean region, such as the Sargasso Sea, morphological taxonomic analysis of new samples for known species may no longer be necessary for some studies – although new species descriptions will require expert taxonomists and studies of size, cohort, and stage structure will require microscopic examinations. In the near future, analysis of the diversity and distribution of known zooplankton species may be done by environmental barcoding (i.e., sequencing of the mtCOI barcode region from bulk environmental samples). Machida et al. (2009) were able to identify 189 species among 1,336 mtCOI sequences from a cDNA library constructed from a plankton net sample collected from the western equatorial Pacific Ocean. New advances in next-generation high-throughput sequencing (e.g., Richardson et al., 2007) may make such approaches both complete and cost-effective.

DNA barcodes may also be used to print DNA microarrays or "chips" (i.e., matrices with affixed detector DNA sequences or

probes that bind or hybridize to the test sample DNA). It may be possible to create microarrays to detect species, as has been done for fish (Kochzius et al., 2008), although whether mtCOI probes can distinguish species of the more diverse zooplankton assemblage is not certain.

5. Conclusions

A gold-standard DNA barcode library was created for described species of zooplankton occurring in the Sargasso Sea, North Atlantic Ocean. Coordinated at-sea taxonomic analysis and DNA sequencing was used to determine DNA sequences for a ~650 base-pair region of the mitochondrial cytochrome oxidase I (mtCOI) barcode gene for 328 identified specimens of 205 species of holozooplankton and fish. Despite variation in mtCOI divergence rates among the groups, there was a marked barcode gap (i.e., non-overlapping distributions of genetic distances within- and between-species) for each of eight zooplankton groups for which sufficient data were available. Without doubt, many more zooplankton species remain to be collected and discovered in the deep meso- and bathypelagic zones of the Sargasso Sea and elsewhere in the world ocean. The growing database of metazoan zooplankton and fish linked to species names, morphological characters, and DNA, photo, or specimen vouchers will serve as a Rosetta Stone or key to unlock the mysteries of species diversity in the open ocean pelagic realm.

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