Multigene phylogeny of the scyphozoan jellyfish family Pelagiidae reveals that the common U.S. Atlantic sea nettle comprises two distinct species (*Chrysaora quinquecirrha* and *C. chesapeakei*) (#17919)

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Robert Toonen / 29 Jun 2017

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Multigene phylogeny of the scyphozoan jellyfish family Pelagiidae reveals that the common U.S. Atlantic sea nettle comprises two distinct species (*Chrysaora quinquecirrha* and *C. chesapeakei*)

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Background. Species of the scyphozoan family Pelagiidae (e.g. *Pelagia noctiluca*, *Chrysaora quinquecirrha*) are well-known for impacting fisheries, aquaculture and tourism, especially for the painful sting they can inflict on swimmers. However, historical taxonomic uncertainty at the genus (e.g. new genus *Mawia*) and species levels hinder studies of their biology and evolutionary adaptations that make them nuisance species, as well as the ability to understand and/or mitigate their ecological and economic impacts. **Methods**. We collected nuclear (*28S* rDNA) and mitochondrial (cytochrome c oxidase I [*COI*] and *16S* rDNA) sequence data from individuals representing all four pelagiid genera, including eleven of thirteen currently recognized species of *Chrysaora*. To finely examine species boundaries in the U.S. Atlantic sea nettle *Chrysaora quinquecirrha*, specimens were included from its entire range along the U.S. Atlantic and Gulf of Mexico coasts, with representatives also examined morphologically (macromorphology and cnidome). **Results**. Phylogenetic analyses show that the genus *Chrysaora* is paraphyletic with respect to other pelagiid genera. In combined analyses, *Mawia*, sampled from the coast of Senegal, is most closely related to *Sanderia malayensis,* and *Pelagia* forms a close relationship to a clade of Pacific *Chrysaora* species (*C. achlyos, C. colorata, C. fuscescens* and *C. melanaster*). *C. quinquecirrha* is polyphyletic, with one clade occurring in the U.S. coastal Atlantic and another in U.S. Atlantic estuaries and Gulf of Mexico. These genetic differences are reflected in morphology, e.g., tentacle and lappet number, oral arm length and nematocyst dimensions. Caribbean sea nettles (Jamaica and Panama) are genetically similar to the U.S. Atlantic estuaries and Gulf of Mexico clade of *C. quinquecirrha*. **Discussion**. Our phylogenetic hypothesis for Pelagiidae contradicts current generic

definitions, revealing major disagreements between DNA-based and morphology-based phylogenies. A paraphyletic *Chrysaora* raises systematic questions at the genus level for Pelagiidae; accepting the validity of the recently erected genus *Mawia*, as well as past genera, will require the creation of additional pelagiid genera. Historical review of the species-delineating genetic and morphological differences indicate that *Chrysaora quinquecirrha* Desor 1848 should be used for the U.S. Coastal Atlantic *Chrysaora* species, while the name *C. chesapeakei* Papenfuss 1936 should apply to the U.S. Atlantic estuarine and Gulf of Mexico *Chrysaora* species. We provide a detailed redescription, with designation of a neotype for *C. chesapeakei*, and clarify the description of *C. quinquecirrha*. Since Caribbean sea nettles are genetically similar to *C. chesapeakei*, we provisionally term them *Chrysaora* c.f. *chesapeakei*. The presence of *M. benovici* off the coast of western Africa provides a potential source region for jellyfish that were introduced into the Adriatic Sea in 2013.

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Short Title: U.S. Atlantic sea nettle is two species

ABSTRACT

Background. Species of the scyphozoan family Pelagiidae (e.g. *Pelagia noctiluca*, *Chrysaora quinquecirrha*) are well-known for impacting fisheries, aquaculture and tourism, especially for the painful sting they can inflict on swimmers. However, historical taxonomic uncertainty at the genus (e.g. new genus *Mawia*) and species levels hinder progress in understanding their biology, and evolutionary adaptations that make them nuisance species, as well as our ability to understand and/or mitigate their ecological and economic impacts.

Methods. We collected nuclear (*28S* rDNA) and mitochondrial (cytochrome c oxidase I [*COI*] and *16S* rDNA) sequence data from individuals representing all four pelagiid genera, including eleven of thirteen currently recognized species of *Chrysaora*. To finely examine species boundaries in the U.S. Atlantic sea nettle *Chrysaora quinquecirrha*, specimens were included from its entire range along the U.S. Atlantic and Gulf of Mexico coasts, with representatives also examined morphologically (macromorphology and cnidome).

Results. Phylogenetic analyses show that the genus *Chrysaora* is paraphyletic with respect to other pelagiid genera. In combined analyses, *Mawia*, sampled from the coast of Senegal, is most closely related to *Sanderia malayensis,* and *Pelagia* forms a close relationship to a clade of Pacific *Chrysaora* species (*C. achlyos, C. colorata, C. fuscescens* and *C. melanaster*). *C. quinquecirrha* is polyphyletic, with one clade occurring in the U.S. coastal Atlantic and another in U.S. Atlantic estuaries and Gulf of Mexico. These genetic differences are reflected in morphology, e.g., tentacle and lappet number, oral arm length and nematocyst dimensions. Caribbean sea nettles (Jamaica and Panama) are genetically similar to the U.S. Atlantic estuaries and Gulf of Mexico clade of *C. quinquecirrha*.

Discussion. Our phylogenetic hypothesis for Pelagiidae contradicts current generic definitions, revealing major disagreements between DNA-based and morphology-based phylogenies. A paraphyletic *Chrysaora* raises systematic questions at the genus level for Pelagiidae; accepting the validity of the recently erected genus *Mawia*, as well as past genera, will require the creation of additional pelagiid genera. Historical review of the species-delineating genetic and morphological differences indicate that *Chrysaora quinquecirrha* Desor 1848 should be used for the U.S. Coastal Atlantic *Chrysaora* species (U.S. Atlantic sea nettle), while the name *C. chesapeakei* Papenfuss 1936 should apply to the U.S. Atlantic estuarine and Gulf of Mexico *Chrysaora* species (U.S. Atlantic bay nettle). We provide a detailed redescription, with designation of a neotype for *C. chesapeakei*, and clarify the description of *C. quinquecirrha*. Since Caribbean *Chrysaora* are genetically similar to *C. chesapeakei*, we provisionally term them *Chrysaora* c.f. *chesapeakei*. The presence of *M. benovici* off the coast of western Africa provides a potential source region for jellyfish that were introduced into the Adriatic Sea in 2013.

INTRODUCTION

Scyphozoan jellyfishes (Cnidaria, Class Scyphozoa), which include the conspicuous moon, lion's mane and sea nettle jellyfishes, exhibit significant and widespread economic and ecological impacts on a wide array of marine and estuarine communities. Jellyfish aggregations, blooms and swarms damage economically important fisheries, close tourist beaches by stinging swimmers, clog intakes of coastal power and desalination plants, invade ecosystems and can affect oxygen levels when mass numbers of carcasses are deposited (Arai 1997; Purcell, Uye & Lo 2007; Richardson et al. 2009; Bayha & Graham 2014; Qu et al. 2015). On the other hand, jellyfish serve important roles as major prey items for some fish and sea turtles, in carbon capture and advection to the Deep Ocean, as important microhabitat for fish, invertebrates and symbiotic algae, and as economic resources for humans (as food and therapeutic compounds) (Omori & Nakano 2001; Castro, Santiago & Santana-Ortega 2002; Arai 2005; Houghton et al. 2006; Lynam & Brierley 2007; Ohta et al. 2009; Lebrato et al. 2012; Briz et al. 2016). Recent attention given to large medusae blooms has led to speculation that anthropogenic events are driving global increases in jellyfish bloom magnitudes, though long term data sets are still equivocal on this point (Richardson et al. 2009; Brotz & Pauly 2012; Condon et al. 2013).

Despite their importance, evolutionary and taxonomic relationships of even some of the most recognizable scyphozoan species remain unsettled, which can impede our abilities to effectively study, predict and mitigate the ecological and economic effects of these nuisance species. Recent systematics studies have directly challenged taxonomic relationships at all levels. A mitogenomic analysis recently challenged the placement of the order Coronatae, such as *Periphylla*, within Scyphozoa (Kayal et al. 2013) and the new family Drymonematidae was created based on morphological, molecular and life history data (Bayha & Dawson 2010; Bayha

et al. 2010). Studies employing molecular and/or morphological data have revealed novel species in multiple scyphozoan genera, including the moon jellyfish *Aurelia* (Dawson & Jacobs 2001; Schroth et al. 2002; Dawson 2003), the genus *Drymonema* (Bayha & Dawson 2010), the upside down jellyfish *Cassiopea* (Holland et al. 2004), and the lion's mane jellyfish *Cyanea* (Dawson 2005a; Kolbasova et al. 2015). Many of these studies have uncovered unrecognized jellyfish invasions and clarified evolutionary relationships in the group (from order to species level) vital to understanding their ecological and economic impacts, and elucidating the evolution of traits that permit these impacts.

The scyphozoan family Pelagiidae (~~Gegenbauer~~, 1856), currently made up of four genera (*Pelagia, Chrysaora*, *Sanderia* and *Mawia*), contains some of the world's most notorious blooming jellyfish. The geographically widespread mauve stinger (*Pelagia noctiluca*) forms dense aggregations that heavily impact aquaculture, fisheries and tourism along the North Sea and Mediterranean Sea (Canepa et al. 2014). Recently, an ~~introduced~~ species found for the first time in the Mediterranean was described and assigned first to the genus *Pelagia* (Piraino et al. 2014), but later to the novel genus *Mawia*, based on molecular and morphological data (Avian et al. 2016). Blooms of the jellyfish *Chrysaora fulgida* (previously identified as *C. hysoscella*) have increased over past decades in the Northern Benguela current on the west coast of Africa, coinciding with decreased fish catches and general breakdown of beneficial trophic interactions as compared to nearby ecosystems not jellyfish-dominated (Lynam et al. 2006; Flynn et al. 2012; Roux et al. 2013). Likewise, blooms of very large *Chrysaora plocamia* medusae form off the coast of Peru, interfering with fisheries, aquaculture and power plants by clogging nets, seines and water intakes (Mianzan et al. 2014).

A species of special note is the U.S. Atlantic sea nettle *Chrysaora quinquecirrha* (Desor 1848), one of the most recognizable, well-studied and ecologically important jellyfish along the U.S. Atlantic and Gulf of Mexico coasts (Mayer 1910; Hedgpeth 1954; Larson 1976). Because its predation pressure shows ecosystem-wide, controlling influence on zooplankton dynamics (Feigenbaum & Kelly 1984; Purcell 1992; Purcell & Decker 2005), *C. quinquecirrha* has been termed a keystone predator for the Chesapeake Bay ecosystem (Purcell & Decker 2005). The jellyfish negatively impacts economically important fisheries by feeding on eggs and larvae (Duffy, Epifanio & Fuiman 1997; Purcell 1997) and blooms impact tourism by stinging swimmers (Cargo & Schultz 1966; Schultz & Cargo 1969; Cargo & King 1990). As a result, a program was developed to predict both real-time occurrences of sea nettle blooms (Decker et al. 2007) and year-to-year bloom magnitudes using past data on environmental conditions (salinity, temperature, etc.) that favor jellyfish populations (Purcell et al. 1999; Purcell & Decker 2005).

Generic definitions within what is currently accepted as Family Pelagiidae (~~Gegenbauer~~ 1856) have been historically vague and genera have traditionally been differentiated, to a great extent, on a single morphological character (tentacle number). The generic names *Pelagia* and *Chrysaora* were originated by ~~Peron~~ & Lesueur (~~1809~~), though both included species not recognized today as pelagiids. ~~Gegenbauer~~ (1856) was the first to create a higher taxon, the family Pelagiidae, including all pelagiids known at the time, but which also included some jellyfish currently classified as coronates. Noting differences based on tentacle number between *Chrysaora* and *Pelagia*, Agassiz (1862) erected a new genus, *Dactylometra*, within the family. Among other characters, Agassiz (1862) classified genera based on tentacle and lappet numbers: *Pelagia* (8 tentacles, 16 marginal lappets), *Chrysaora* (24 tentacles, 32 marginal lappets) and *Dactylometra* (40 tentacles, 48 marginal lappets). Kishinouye (1902) subsequently described the

genus *Kuragea* (56 tentacles, 64 marginal lappets) and Goette (1886) described Sanderia (16 tentacles, 32 lappets and 16 rhopalia). To the genus *Dactylometra*, Agassiz (1862) added *Pelagia quinquecirrha* (Desor 1848) from Nantucket Bay (MA) and *Chrysaora lactea* (Eschscholtz 1829) from Rio de ~~Janiero.~~ Based on established generic definitions, Piraino et al. (2014) placed an undescribed, invasive Mediterranean pelagiid, *Pelagia benovici*, in the genus *Pelagia*. However, Avian et al. (2016) created the novel genus *Mawia* for this new species (*Mawia benovici*) based on fine-scale morphological characters (tentacle, gonad and basal pillar morphology) and molecular differences from other pelagiid genera included in a lightly sampled phylogenetic analysis of Pelagiidae.

Not long after Agassiz erected *Dactylometra*, *Dactylometra quinquecirrha* served to cast doubt on pelagiid generic discrimination. Bigelow (1880) recognized that some brackish water (e.g. Chesapeake Bay) *D. quinquecirrha* matured at 24 tentacles (a character of *Chrysaora*) rather than 40 (a character of *Dactylometra*), something Mayer (1910), saw as the "*Chrysaora*" stage in their development to the "*Dactylometra*" stage. Stiasny (1930) also cast doubt on the ability to effectively differentiate *Chrysaora* and *Dactylometra*. As a result, Kramp (1955) reasoned *Dactylometra* and *Kuragea* to be merely developmental stages and subsumed both within the genus *Chrysaora* (Eschscholtz 1829), since it has taxonomic priority. Calder (1972) determined that *C. quinquecirrha* went through stages of one to more than seven tentacles per octant, often in the same geographic region, supporting the contentions of Mayer (1910) and Kramp (1955). A morphology-based phylogeny of the Pelagiidae (Gershwin & Collins 2002) indicated two groups coinciding with the previous genera *Dactylometra* and *Chrysaora*, but noted that the weak phylogenetic support would make resurrecting the genus *Dactylometra* premature. Another morphology-based phylogeny (Morandini & Marques 2010) found support

for a *Dactylometra* clade based on tentacle and lappet number, but noted that this would require many *Chrysaora* species to have their own genera. A robust phylogenetic hypothesis of relationships within Pelagiidae based on comprehensive taxon sampling is an important step toward removing taxonomic confusion at the genus and species-levels, including assessing the taxonomic status of the new genus *Mawia* (Avian et al. 2016) and clarifying taxonomic questions related to *C. quinquecirrha*.

In order to examine evolutionary relationships and taxonomic boundaries in the family Pelagiidae, with special focus on the genus *Chrysaora* and the species *C. quinquecirrha*, we collected nuclear (large subunit ribosomal rDNA) and mitochondrial (cytochrome c oxidase I and large subunit ribosomal rDNA) sequence data from individuals representing all four extant genera (*Chrysaora, Mawia, Pelagia* and *Sanderia*), including eleven currently recognized species of *Chrysaora* and one species each of *Mawia* (*M. benovici*), *Pelagia* (*Pelagia noctiluca*) and *Sanderia* (*S. malayensis*). To further examine the taxonomy of the U.S. Atlantic sea nettle *Chrysaora quinquecirrha*, specimens were included from its entire range along the U.S. Atlantic and Gulf of Mexico coasts (estuarine and coastal), taking care to sample all recognized morphotypes, with representatives also examined morphologically (macromorphology and cnidome).

MATERIALS AND METHODS

Sample Collection

Specimens were collected in the field or at public aquaria husbandry facilities, either by the authors or others with extensive knowledge of Scyphozoa, in an effort to collect as many species of *Chrysaora* as possible, as well as representative species of *Pelagia* and *Sanderia*

(Table 1; Figure 1). An unknown and unidentified pelagiid specimen was collected from Dakar, Senegal and was accompanied by a photograph that did not allow for specific identification. For *Chrysaora quinquecirrha*, samples were collected from 10 different sites along the Atlantic and Gulf of Mexico coasts (Table 1; Figure 2), covering both coastal and estuarine environments, with the intention of capturing as many structural and color morphotypes as possible (Figure 3). Both white (Table 1: NF1-NF3) and red-striped (Table 1: NF4-NF5) color morphs (Figure 3C, D) were collected from Norfolk, VA (NF). In all cases, a small piece of gonad, tentacle or oral arm tissue was excised and preserved in 80—99% ethanol or DMSO-NaCl solution (Dawson, Raskoff & Jacobs 1998). Where possible for some sites (Table S1), individuals were also preserved in 4% buffered formalin and seawater for later morphological analyses. Additional published pelagiid sequences were included in the final data set (Table 2).

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from preserved tissue samples by CTAB (cetyltrimethylammonium bromide) methods (Ausubel et al. 1989) and stored at -20°C. Polymerase chain reaction (PCR) amplifications targeted three genetic regions: mitochondrial large subunit ribosomal DNA (*16S*) and cytochrome c oxidase subunit I (*COI*) and nuclear large subunit ribosomal DNA (*28S*) using primers shown in Table S2. We chose genetic regions that have been useful in examining species boundaries and/or examining genus and family level relationships in the Scyphozoa (Dawson & Jacobs 2001; Schroth et al. 2002; Holland et al. 2004; Dawson 2005a; Dawson, Gupta & England 2005; Bayha & Dawson 2010). Reaction conditions for *16S* consisted of one cycle of 94°C for 180 seconds (s), then 38 cycles of 94°C for 45 s, 50°C for 60 s and 72°C for 75 s, followed by a final step of 72°C for 600 s and storage at 4°C.

Reaction conditions for *COI* consisted of one cycle of 94°C for 180 s, followed by two cycles of 94°C for 45 s, 46°C for 60 s and 72°C for 75 s, two cycles of 94°C for 45 s, 47°C for 60 s and 72°C for 75 s and 35 cycles of 94°C for 45 s, 48°C for 60 s and 72°C for 75 s, followed by a final step of 72°C for 600 s and storage at 4°C. Lastly, reactions conditions for *28S* consisted of 94°C for 180 s, then 38 cycles of 94°C for 45 s, 48°C for 60 s and 72°C for 90 s, followed by 72°C for 600s then storage at 4°C. Successful amplification was evaluated by running the PCR products on a 2% agarose gel. PCR amplicons were directly sequenced using a combination of sequencing primers (Table S2). DNA sequencing was performed by University of Washington High Throughput Genomics Unit (Seattle, WA) or Beckman-Coulter Genomics (Danvers, MA). Sequences were assembled using Lasergene SeqMan Pro v. 8.1.5 (DNAStar, Inc.) and then compared to the GENBANK nucleotide database using BLASTn or BLASTx (Altschul et al. 1997) to confirm identity of sequenced region and ensure no sequencing errors that affected amino acid reading frames (*COI*). All DNA sequences were submitted to NCBI Genbank (MF141552-MF141718; MF167556-MF167568).

Phylogenetic Reconstruction

For all analyses, *Cyanea capillata* was used as the outgroup because it was shown to be among those scyphozoans least diverged from Pelagiidae (Bayha et al. 2010). *COI* sequences were aligned using CLUSTALX v2.1 (Larkin et al. 2007) under default parameters, and checked by eye using their amino acid translations as a guide. *16S* and *28S* sequences were aligned using MAAFT v7.245 employing the E-INS-I strategy (Katoh & Standley 2013), since this strategy has been demonstrated to be effective for loci containing conserved motifs embedded within hypervariable regions (Katoh & Toh 2008). Hypervariable regions of questionable alignment

were removed from the MAAFT alignments using GBlocks v0.91b (Castresana 2000) under default parameters, except that gapped positions were set to half.

Phylogenetic analyses were run under Maximum Likelihood (ML) and Bayesian Inference (BI) frameworks for *COI, 16S, 28S* and a combined dataset. Maximum Likelihood phylogenetic trees were constructed using PhyML v3.0 (Guindon et al. 2010), employing the best-fit substitution models assessed using jMODELTEST v2.1.7 (Darriba et al. 2012) under Akaike (AIC) and Bayesian (BIC) Information Criteria, as well as Decision Theory Performance-Based Selection (DT). For *COI* (TPMμf+I+G), *16S* (TIM2+I+G), and combined (GTR+I+G) datasets, selection criteria were unanimous, while BIC and DT chose TrNef+I+G for *28S*. A 1000 bootstrap replicate analysis was performed in PhyML to obtain node support values. Bayesian Inference (BI) of gene phylogenies was carried out using MrBayes v3.2.6 (Ronquist et al. 2012). The same model of nucleotide evolution (GTR+I+G, with gamma distribution approximated by four discrete categories) was assumed for all analyses, since it is not possible to implement the less complicated models used in the ML tree searches (in the cases of *16S* and *COI*). For each dataset, two independent MCMC runs were conducted until the standard deviation of split frequencies decreased to less than 0.01 (*16S*: 6,481,000; *COI*: 19,608,000; *28S*: 1,390,000; combined: 1,002,000) generations, sampling every 1,000. The number of generations was determined by assessment of convergence using the minimum Estimated Sample Size and Potential Scale Reduction Factor, as implemented in MrBayes. Posterior probabilities were calculated using all trees other than the first 25%, which were discarded as "burnin". All trees were visualized using Figtree v1.4.2 (Rambaut 2014) and redrawn for presentation using Adobe Illustrator CC v19.1.0 (Adobe Systems, Inc.). Mean interclade and intraclade, as well as minimum interclade sequence divergence values (Kimura 2-

parameter) were determined using MEGAv7.0.14 (Kumar, Stecher & Tamura 2016) and nucleotide statistics calculated in Seaview v4.6 (Gouy, Guindon & Gascuel 2010).

Morphological Analysis of *Chrysaora quinquecirrha*

While our study did not include a family-wide morphological analysis, we did perform morphological analyses on jellyfish identified as *Chrysaora quinquecirrha* from the U.S. Atlantic and Gulf of Mexico coasts. We examined a total of 57 formalin-preserved samples we collected from Charlestown Pond (RI), Cape Henlopen (DE), Rehoboth Bay (DE), York River (VA), Charleston (SC) and Dauphin Island (AL) (Table S1). In addition, we examined a total of 63 individuals housed at the Smithsonian Institution National Museum of Natural History (NMNH) that were collected from the U.S. Atlantic and Gulf of Mexico coasts and identified as *Chrysaora quinquecirrha* or *Chrysaora* sp. (Table S1). We examined morphological characters (and their states) previously employed for Pelagiidae (Gershwin & Collins 2002) that pertained to the medusa stage, with the addition of maximum oral arm length (Table 3). In addition, a total of 35 individuals that were examined morphologically, but not included in the phylogenetic analyses, were assigned to molecular species/clades using mitochondrial *16S* sequence data collected using the established procedure described above (Table S1).

Cnidome of *Chrysaora quinquecirrha*

Lastly, we examined the cnidome of multiple specimens originally identified as *Chrysaora quinquecirrha* to determine if species could be delineated based on nematocyst measurements (of each type) and/or nematocyst diversity (counts of nematocyst types). Nematocyst terminology followed convention used in previous studies (Weill 1934; Calder

1971; Calder 1974a; ~~Ostman~~ & Hydman 1997; Morandini & Marques 2010) in defining four different nematocyst types: holotrichous A-isorhiza, holotrichous a-isorhiza, holotrichous O-isorhiza and heterotrichous microbasic rhopaloid. In agreement with Morandini & Marques (2010), we use the term heterotrichous microbasic rhopaloid, recognizing that there are likely at least two nematocysts that cannot be effectively delineated based on basic light microscopy, as shown in other previous work (Sutton & Burnett 1969).

In all cases, tentacle tissue was homogenized in distilled water in 1.5 mL microcentrifuge tubes and nematocysts were examined using differential interference contrast microscopy (DIC). A small piece of formalin-fixed tentacle tissue was homogenized in 100 uL of distilled water in a 1.5 uL tube using a plastic microcentrifuge pestle until little visible intact tissue remained. A small drop was then placed on a slide under cover slip and examined at 60X in DIC using an Olympus BX63 microscope, with photographs taken using an Olympus DP80 camera run by CellSens Dimension 1.13 (Olympus Life Science, Inc.).

A total of 15 individuals were examined for nematocyst size measurements (Table S1). In all cases, 10 samples of each nematocyst type were photographed and later measured using CellSens Dimension 1.13 computer program for length and width. Linear Discrimination Analysis (LDA) was used to determine whether species could be distinguished on the basis of nematocyst measurements using the lda routine in the R package MASS (Venables & Ripley 2002).

A total of 10 individuals were examined for nematocyst diversity (Table S1). Since initial estimates indicated that nematocyst diversity varied by tentacle region, nematocyst counts were done from three tentacle regions for each individual: proximal (near the base of the tentacle), medial (in the middle of the tentacle) and distal (at the end of the tentacle). For each

region, the first 200 nematocysts were photographed and categorized according to nematocyst type. Only lone nematocysts were enumerated, with any nematocysts still adhering to epithelial tissue ignored, since smaller nematocysts (e.g. a-isorhizas) could be obscured. In order to examine any differences in nematocyst diversity between different tentacle regions (distal, medial, proximal), a mosaic plot showing the relative proportions of nematocyst types in the various regions was made using the R package *vcd* version 1.4-3 (Meyer, Zeileis & Hornik 2016). In order to visualize differences in proportions of nematocyst types (four types, three regions) between the two species we conducted non-metric multidimensional scaling of the Euclidean distance matrix using the isoMDS routine in the R package MASS (Venables & Ripley 2002).

RESULTS

Sequence Data Characteristics and Phylogenetic Inference

The *COI* dataset consisted of 73 sequences, 59 of which are new. All sequences were 616 bp in length. The *16S* data set was made up of 67 sequences, including 60 new sequences and 7 published sequences. New complete sequences varied in length from 598 base pairs (bp) for *C. lactea* to 608 bp (*C. chinensis*). The MAAFT-aligned data set (included published sequences) was 628 bp, but the dataset was truncated to 582 bp (95.7%) after treatment with GBlocks. The *28S* dataset included 35 sequences, including 24 new sequences and 11 published sequences. New sequences ranged in size from 998 (*C. chinensis*) to 1018 bp (*C. africana*). The MAFFT alignment (which included published sequences) was 1027 bp, but the final data set was 1015 bp (98.8%) after removal of regions via GBlocks.

All phylogenetic analyses (*COI, 16S, 28S* and combined) revealed similar terminal clades, but they differed in the resolution of relationships among them. The combined analysis provided the best resolution (smallest proportion of polytomous nodes) and highest support values for evolutionary relationships (Figures 4-7). In all analyses, *Chrysaora* is revealed as paraphyletic with respect to species of *Sanderia*, *Pelagia,* and *Mawia*. In the combined analyses, *Mawia benovici* is most closely related to *Sanderia malayensis* (Bayesian support 100 / maximum likelihood support 100), with these two species forming a close relationship with *C. africana* and *C. pacifica* in the combined (88/67) and *28S* trees (100/61) (Figures 6-7). Except for the *COI* tree, *Pelagia noctiluca* formed a close relationship with a clade of Pacific jellies (*C. achlyos, C. colorata, C. fuscescens and C. melanaster*) with high support values (combined: 100/99; *16S*: 100/92; *28S*: 82/58) (Figures 5-7). For the combined analyses (100/100) and *28S* (100/100), a highly supported clade was composed of Atlantic species, including *C. quinquecirrha, C. lactea, C. plocamia, C. fulgida, C. hysoscella, C. chesapeakei* [see Discussion] and the Caribbean *Chrysaora*, while this clade was less supported for *COI* (100/61) and *16S* (75/60) (Figures 4-7). *Chrysaora fulgida* (NAM), *C. plocamia* (PMA) and *C. hysoscella* (IRE) formed a closely related group in all analyses with high support values (combined: 100/100; *28S*: 100/99; *COI*: 100/94; *16S*: 100/83). For sequences taken from Piraino et al. (2014) only, nuclear *28S* sequences for *M. benovici* from the Mediterranean (ADR) occurred in the distantly related clade for *P. noctiluca* from the Atlantic (OVA), and a *P. noctiluca* from the Mediterranean (TYR) occurred in the distantly related clade for *M. benovici* from the Mediterranean (ADR) (Figure 6).

At the species level, our analyses highlighted multiple species boundaries, and showed the samples identified as *C. quinquecirrha* to be polyphyletic. In all analyses, *C. quinquecirrha*

328 sequences fell into two distinct clades that were highly diverged (Figures 4-7; Tables S3-S5),
329 with one clade (*C. chesapeakei* – see Discussion and Systematics) made up of animals from U.S.
330 Atlantic estuaries and the Gulf of Mexico animals and another (*C. quinquecirrha* – see
331 Discussion and Systematics) made up of U.S. coastal Atlantic animals. Caribbean *Chrysaora*
332 (Jamaica and Panama) formed a clade closely related to *C. chesapeakei* in all analyses (Figures
333 4-7). Aquarium animals previously identified as *C. melanaster* (AQA) were genetically distinct
334 from *C. melanaster* collected from the Bering Sea (BER) in all analyses where both were
335 included (Figs 4-6) and formed a clade with *Chrysaora pacifica* collected from South Korea
336 (KOR) and Japan (KYO) for *COI* and/or *16S*. While aquarium collected *C. chinensis* formed a
337 highly-supported clade with field collected *C. chinensis* (MAL), analyses differed in where this
338 species fell out in the trees (Figures 4-7). The unknown pelagiid collected from the western
339 African coast (SEN) was nearly identical to the newly described *M. benovici* from the
340 Mediterranean for *COI* (0.0-0.3% difference) and *28S* (0.0-0.2% difference) (Figures 4, 6).
341
342 **Macromorphological and Nematocyst Analyses**
343 A total of 120 medusae (57 field collected and 63 museum specimens) (Table S1)
344 previously identified as *C. quinquecirrha* s.l. were observed for 20 quantitative and qualitative
345 macromorphological characters either taken from Gershwin and Collins (2002) or new to this
346 study (maximum oral arm length). Overall, three macromorphological characters differed
347 significantly: tentacle number, lappet number and maximum oral arm length vs. bell diameter
348 (Table 3). Animals collected from the estuarine Atlantic and all Gulf of Mexico sites (Table S1)
349 had an average of 3.07 +/- 0.07 (95% CI) tentacles per octant, excluding two aberrant individuals
350 (6 and 4.625-see Discussion) (Figure 8A; Table 3). In all instances when there were more than

three tentacles per octant (excluding aberrant individuals above), the additional tentacle(s) occurred between the secondary tentacles and the rhopalia (i.e. 3-2-1-2-3 octant tentacle orientation) and were typically undeveloped, being of similar size to nearby lappets. Animals collected from coastal regions along the U.S. Atlantic (Table S1) had an average of 5.28 +/- 0.48 (95% CI) tentacles per octant (Figure 8A; Table 3). Animals collected from the estuarine Atlantic and all Gulf of Mexico sites (Table S1) had oral arms that were on average 3.01 +/- 0.39 (95% CI) times as long as the bell diameter (Figure 8B; Table 3). Animals collected from coastal regions of the U.S. Atlantic (Table S1) had oral arms that were on average 1.24 +/- 0.27 (95% CI) times as long as bell diameter (Figure 8B; Table 3). Of the animals that were examined morphologically, a total of 38 individuals were also sequenced for *16S* to see which *Chrysaora* clade they fell into (K2P sequence divergence <~1.5%). Medusae examined morphologically that fell into the *C. chesapeakei* phylogenetic clade had an average of 2.99 +/- 0.03 tentacles per octant and oral arms that were 2.80 +/- 0.78 (95% CI) times as long as bell diameter on average, while all those that fell in the *C. quinquecirrha* clade had an average of 5.63 +/- 0.78 tentacles per octant and oral arms that were on average 0.93 +/- 0.18 (95% CI) times as long as bell diameter on average (Figure 8A, 8B).

We also studied the cnidome of medusae identified as *C. quinquecirrha*, examining the measurements of individual nematocyst types (Figure 8C, S1), as well as the representation of each type overall. Nematocyst measurements indicated significant grouping for holotrichous A-isorhizas, but not for other types. A-isorhiza measurements (Length vs. Width) showed two distinct groups, with one group containing only animals from U.S. Atlantic estuaries and the Gulf of Mexico and the other containing coastal Atlantic animals (Figure 8C). All sequenced animals in the smaller group (coastal Atlantic) were genetically similar to *C. quinquecirrha*,

while all those sequenced from the larger group (estuarine Atlantic and Gulf of Mexico) were genetically similar to *C. chesapeakei* (Figure 8C). For animals identified as *C. chesapeakei* (based on habitat, macromorphology and/or genetics), LDA analysis indicated that individual A-isorhiza measurements correctly identified species 97.8% of the time (2.2% of the time, they were incorrectly identified at *C. quinquecirrha*), while they were correctly identified 100% of the time using the mean of 10 nematocyst measurements. For animals previously identified as *C. quinquecirrha* (based on habitat, macromorphology and/or genetics), LDA correctly identified them 100% of the time, whether one or 10 nematocysts were used. Figure S1 (a-c) shows measurement graphs for a-isorhiza, O-isorhiza and heterotrichous microbasic rhopaloids, all of which indicate no significant groupings of measurements.

Nematocysts from proximal, medial and distal regions were typed and counted (200 total) for 10 individuals originally identified as *C. quinquecirrha*, chosen based on their previous molecular and macromorphological groupings (five from each group). All in all, heterotrichous microbasic rhopaloids were most frequent (62.1±9.8% [95% CI]), followed by O-isorhizas (13.4±5.0% [95% CI]), a-isorhizas (12.4±2.8% [95% CI]) and A-isorhizas (12.2±3.7% [95% CI]). As pilot studies indicated, nematocyst type proportions were different for different tentacles regions. While A-isorhizas and a-isorhizas were consistent over the entire tentacle, O-isorhizas were overrepresented in proximal regions and heterotrichous microbasic rhopaloids were overrepresented in the medial and distal regions (Figure S2A). Individuals varied considerably in proportions of nematocyst types (Figure S2B). Individuals collected from coastal Atlantic regions (circles) were generally clustered, including those genetically similar to *C. quinquecirrha*, while those from estuarine Atlantic and Gulf of Mexico regions (squares) were much more dispersed, as were those genetically similar to *C. chesapeakei* (Figure S2B). LDA

was moderately effective in distinguishing species using overall nematocyst proportions (4 of 5 *C. quinquecirrha* and 3 of 5 *C. chesapeakei* correctly classified) and this was almost entirely due to different proportions of A-isorhiza nematocysts. A-isorhiza proportions were significantly different (t=3.623, p-value=0.0068), with *C. chesapeakei* individuals averaging 16.5±3.4% for A-isorhiza and *C quinquecirrha* cnidomes averaging 7.8±3.4%.

DISCUSSION

Genus-level Systematic Inference

Our most robust phylogenetic hypothesis for Pelagiidae (Figure 7), based on the combined data set, directly contradicts current generic definitions, as well as earlier morphological-based phylogenies of the Pelagiidae. Both Gershwin & Collins (2002) and Morandini & Marques (2010) considered *Chrysaora* to be reciprocally monophyletic with respect to both *Sanderia* and *Pelagia*, with *Sanderia* in a basal position (Figure 9A, B). In contrast, our analyses indicate that *Chrysaora* is paraphyletic with respect to *Pelagia, Sanderia and* the newly erected *Mawia* (Figures 4-7, 9C). Mediterranean *M. benovici* is not in the combined analysis, but our Senegal pelagiid (SEN) can be treated as *M. benovici*, based on COI (Figure 4) and 28S (Figure 6) phylogenies (see below). Paraphyly of *Chrysaora* is not supported in morphological or genetic analyses in Avian et al. (2016) (Figure 9C, D), but this is almost certainly a result of incomplete taxon sampling. For example, their analysis based on combined morphological and genetic data only included *C. hysoscella* (Mediterranean), while the *28S* dataset included a subset of sequences published at the time, (*C. hysoscella*, *C. lactea*, and *C.* c.f. *chesapeakei* [see below]), all of which occur in a single clade in our analysis (Figure 7, 10E). Including fewer published sequences gave the appearance of *Chrysaora* monophyly, which may

have ~~facilitated~~ the ~~creation~~ of *Mawia*. For instance, throughout Avian et al. (2016), *Chrysaora* is often used as a singular entity (i.e. monophyletic), such as an entire section that examines characters at the "genus level". This more readily allows for the conclusion of a novel genus *Mawia*, as it sidesteps the difficult taxonomic questions raised by a paraphyletic *Chrysaora*. That notwithstanding, in agreement with both Piraino et al. (2014) and Avian et al. (2016), our analyses show *M. benovici* to be a close relative of *Sanderia malayensis* (Figures 4-7). Given the stark morphological differences between *Sanderia* and *Mawia* (Piraino et al. 2014; Avian et al. 2016), this relationship is more than a bit surprising.

Our working hypothesis for the relationships within Pelagiidae (Figure 7, 10), especially the paraphyletic *Chrysaora*, raises serious systematic questions for the genus level. To accept the validity of *Mawia*, as well as previously established *Pelagia* and *Sanderia*, each of which can be easily distinguished morphologically from those currently classified as *Chrysaora*, additional genera would have to be erected within Pelagiidae in order to maintain monophyly of these generic groupings. An initial question would be to which clade should the genus *Chrysaora* be limited. Because the type species of *Chrysaora* is *C. hysocella,* the genus would best be limited to the clade containing *C. hysocella*, *C. fulgida, C. lactea*, *C. plocamia*, *C. quinquecirrha,* and *C. chesapeakei* (see below). This then would leave three other lineages in need of new genera: 1) *C. africana* plus *C. melanaster*; 2) *C. chinensis*; and 3) *C. achlyos*, *C. colorata* and *C. fuscescens*. The latter grouping (*C. achlyos, C. colorata* and *C. fuscescens*) has a close relationship to *Pelagia noctiluca* (except for *COI*) and there is genetic support for generic designation. Unfortunately, we are currently aware of no clearly interpretable morphological characters that could be invoked to diagnose this clade, or other *Chrysaora* lineages, as has been the case in other studies seeking to reconcile jellyfish taxonomy based on morphology and molecular data

443 (Dawson & Martin 2001; Dawson 2003; Bayha & Dawson 2010). Future study will benefit from
444 more detailed morphological analyses to identify additional characters that could then be mapped
445 onto molecular phylogenies (e.g. Figure 7), as well as greater taxonomic sampling (e.g. two
446 additional *Chrysaora* species accepted and two declared *nomen dubium* in Morandini & Marques
447 (2010), more geographic samples of *Pelagia* and *Sanderia*). Both would allow for better
448 resolution to define genera and better explain their evolutionary relationships.
449
450 **Interspecific Evolutionary Relationships and Geographic Patterns**
451 While our molecular phylogenies bear almost no resemblance to the morphology-based
452 phylogenies within the currently defined genus *Chrysaora* (Gershwin & Collins 2002; Morandini
453 & Marques 2010) (Figure 9), there are some relationships that occur in all phylogenies. All
454 phylogenies agree on a close relationship between *C. achlyos* and *C. colorata* (Figure 9A, B, E).
455 Our phylogeny is in general agreement with Morandini and Marques (2010) in delineating their
456 basal 'Pacific' group (*C. achlyos, C. colorata, C. fuscescens, C. melanaster* and *C. plocamia*),
457 except that our *C. plocamia* samples came from the Atlantic and occur in an 'Atlantic' group
458 (Table 1; Figure 1). Morandini and Marques (2010) reasoned that this basal group may have
459 provided ancient species that then invaded the Atlantic, splitting into various Atlantic groups.
460 Our combined phylogeny (Figure 7) is in general agreement, with Pacific *Chrysaora* species
461 generally occupying a more basal position in the tree compared to the Atlantic species. Major
462 disagreements with Morandini & Marques (2010) include the placement of *C. chinensis* and *C.*
463 *pacifica* (both Pacific jellies) as closely related to *C. quinquecirrha* and *C. lactea*, with the *C.*
464 *pacifica* placement also a disagreement with Gershwin and Collins (2002). Likewise, the very
465 close relationship among *C. fulgida*, *C. hysoscella* and *C. plocamia* was not found in any of the

morphological phylogenies (Figure 9), though *C. hysoscella* and *C. plocamia* were closely related in Gershwin and Collins (2002).

One item of note here is our use of aquarium samples, which may be problematic where they are not confirmed with field-collected specimens. Aquarium collected specimens of *C. pacifica* (originally *C. melanaster*- see below) and *C. chinensis* are genetically confirmed, based on published sequences from field-collected specimens of known geographical origin (Figures 4-5). In addition, our aquarium-collected *C. fuscescens* is identical to published *16S* sequence of field-collected animals from Vancouver Island, Canada (NCBI JX393256). However, *C. colorata*, *C. achlyos* and *S. malayensis* are represented only by aquarium specimens and, therefore, conclusions based on these sequences should be made with care. Future studies incorporating field-collected specimens are necessary for confirming or refuting relationships shown here.

Species-level Systematic Inference

Chrysaora quinquecirrha and C. chesapeakei

The most striking conclusion revealed from this study is that *C. quinquecirrha*, one of the most studied and well-known U.S. Atlantic jellyfish, is made up of two distinct species, putting to rest taxonomic disagreements going back more than 100 years. This finding is supported by genetic (Figures 4-7), macromorphological (Figure 8A, 8B), and cnidome (Figure 8C) data. *C. quinquecirrha* occurred in two well-differentiated monophyletic groups, one containing all animals from estuarine Atlantic (RI, NJ, RB, NF, PAM, GA) and Gulf of Mexico (AL) regions and the other containing animals from coastal Atlantic regions (MA, CHP and OSC) (Figures 4-7). Average (COI: 13.1%; *16S*: 9.0%; *28S*: 2.5%) and minimum (COI: 12.1%; *16S*: 8.4%; *28S*:

2.4%; Table S3-S5) sequence divergences are well above what has been seen as delineating species in *Aurelia* (Dawson & Jacobs 2001; Dawson, Gupta & England 2005), *Cassiopea* (Holland et al. 2004), *Cyanea* (Dawson 2005a), and *Drymonema* (Bayha & Dawson 2010). More convincing is the fact that *C. fulgida* from Namibia (NAM), *C. plocamia* from Argentina (ARG) and *C. hysoscella* from Ireland (IRE) occur between these two species in all phylogenies (Figures 4-7). Additionally, animals representing these genetic clades (estuarine U.S. Atlantic/Gulf of Mexico and coastal Atlantic) were consistently differentiable based on tentacle number (Figure 8A), oral arm length (Figure 8B) and holotrichous A-isorhiza measurements (Figure 8C, 9). Two individuals (NMNH 33457a and NMNH 56703b) did not fit the typical pattern for tentacle number (Figure 8A). However, both exhibited anomalous tentacle morphologies (multiple tentacles emerging from within lappets instead of between lappets) and had typical patterns for holotrichous A-isorhiza measurements (NMNH 33457a: 27.59 x 20.98 um; NMNH 56703b: 27.04 x 21.75 um; Figure 8C) and/or oral arm length (NMNH 33457a: 4.54 times bell diameter; NMNH 56703b: sample too degraded; Figure 8B).

It appears that Bigelow (1880) was correct about Chesapeake Bay *Chrysaora* maturing at 24 tentacles and representing a distinct taxon from *Dactylometra quinquecirrha*. Our data refute the hypothesis that these individuals represent a growth stage toward the five-tentacled *C. quinquecirrha* described from the coast (Mayer, 1910; Calder, 1972). However, an important point is that it has been claimed that individuals only reach the “five-tentacled” stage after 130 mm (Agassiz & Mayer 1898; Mayer 1910), when small tentacles emerge between the secondary tentacles and the rhopalia (Mayer, 1910 Plate 64), termed Stage 5 in Calder (1972). In our data set, only a single individual larger than 130 mm was encountered and collected from the estuarine Atlantic or Gulf of Mexico (Dauphin Island, AL) and it had exactly three tentacles per

octant (Figure 8A). However, it is possible that within the estuarine Atlantic and Gulf of Mexico, these *Chrysaora* may develop small tertiary tentacles at very large sizes, though they likely never develop fully, as was observed in some animals examined here. Furthermore, in one case, Calder (1972) may have collected *Chrysaora* from an area (Broadkill River, DE) that experiences both species, albeit at different times of the day, seemingly supporting the hypothesis of development stages. The mouth of the Broadkill River experiences tidal inflows capable of pulling coastal *Chrysaora* into the inlet during high tide and outflows capable of pulling estuarine *Chrysaora* from the intercoastal waterway during low tide (K.M. Bayha, pers. obs.). In any case, the growth of small tertiary tentacles in large estuarine Atlantic and Gulf of Mexico *Chrysaora*, along with the dependence on a single morphological character (tentacle number), likely led to the historical taxonomic uncertainties we are clarifying here.

Several lines of evidence support the U.S. Atlantic coastal *Chrysaora* group retaining the species name *C. quinquecirrha* and the estuarine Atlantic/Gulf of Mexico group requiring a different name. First, *Pelagia quinquecirrha (=C. quinquecirrha)* (Desor 1848) was described from a coastal zone region (Nantucket Harbor, MA) as having 40 tentacles and our coastal Atlantic animals were characterized by possessing 40 or more tentacles. Furthermore, one of our sampling sites and a museum specimen were from coastal waters (Buzzard's Bay, MA) near the *C. quinquecirrha* type locality. Assigning a species name to the U.S. Atlantic estuaries/Gulf of Mexico group is more problematic, owing to inconsistencies in Papenfuss (1936). Papenfuss (1936) compared two color morphs found within the Chesapeake Bay, a small, white morph (e.g. Figure 3D) and a larger red striped morph (e.g. Figure 3E), which she assumed to be *Dactylometra (=Chrysaora) quinquecirrha*. Papenfuss (1936) assigned the white morph to the new subspecies *Dactylometra quinquecirrha* var. *chesapeakei*, based on very small differences

535 in holotrichous a-isorhiza measurements, though without statistical support. However, for our
536 Norfolk (VA) samples, white (NF1-NF3) and red-striped (NF4-NF5) morphs occurred in the
537 same genetic clades for *16S* and *COI* (Figures 4-5) and we found no overall pattern of
538 differentiation in our holotrichous a-isorhiza measurements (Figure S1A). Furthermore, for
539 holotrichous A-isorhiza measurements, both morphs from Papenfuss (1936) are consistent with
540 our U.S. Atlantic estuary/Gulf of Mexico group (Figure 8C). In summary, evidence from
541 nematocyst measurements (Figure 8C), locality (Chesapeake Bay) and phylogenetic data (Figure
542 4-5) support the U.S. Atlantic estuarine/Gulf of Mexico group and both morphs from Papenfuss
543 (1936) as representing the same species. Even though Papenfuss (1936) may have been mistaken
544 in describing *D. quinquecirrha* var. *chesapeakei*, that name is taxonomically available based on
545 Article 45.6.4 of the International Code of Zoological Nomenclature (International Commission
546 on Zoological Nomenclature, 2015). As such, all animals from the U.S. Atlantic estuary/Gulf of
547 Mexico lineage should be assigned to the elevated species name *Chrysaora chesapeakei*
548 (Papenfuss, 1936). The placement of Gulf of Mexico medusae in *C. chesapeakei* differs from
549 Morandini & Marques (2010), who placed them in the species *C. lactea*, based on similarities in
550 octant tentacle orientation (2-3-1-3-2). However, our genetic data clearly separate these animals
551 from the distantly related *C. lactea* (Figure 4-7) and the number of tentacles (approximately 3)
552 found in the Gulf of Mexico animals observed here and in Morandini and Marques (2010)
553 (USNM 49733 and USNM 53826) make accurate determination of tertiary tentacle orientation
554 problematic.

555 In addition to their taxonomic value, it is possible that some of the morphological
556 characters that delineate *C. quinquecirrha* and *C. chesapeakei* may be related to adaptations to
557 different predominant prey items, especially for feeding on the ctenophore *Mnemiopsis leidyi*. In

558 general, *M. leidyi*, which is a major prey item for *Chrysaora* (Feigenbaum & Kelly 1984),
559 exhibits an inshore, estuarine preference and a seasonal spread from estuarine to coastal waters
560 (Costello et al., 2012; Beulieu et al., 2013). As such, *M. leidyi* may be a more frequent prey item
561 for estuarine Atlantic *Chrysaora* than for coastal animals. Larger oral arms, as exhibited in *C.*
562 *chesapeakei* (Figure 8B), have been argued to be an adaptation for scyphozoans that feed on
563 gelatinous prey (Bayha and Dawson, 2010). In addition, the larger and more numerous A-
564 isorhiza nematocysts found in estuarine *Chrysaora* might be better suited to efficiently attaching
565 to and feeding on very soft-bodied organisms such as *M. leidyi*. Since different nematocyst types
566 are assumed to have different functions based on morphological and discharge characteristics
567 (Rifkin and Endean, 1983; Purcell, 1984; Heeger and Moller, 1987; Purcell and Mills, 1988;
568 Colin and Costello, 2007), it has been proposed that nematocyst diversity within an organism can
569 be correlated to dietary preferences, at least in a coarse sense (Purcell, 1984; Purcell and Mills,
570 1988; Carrette et al., 2002). In particular, isorhiza nematocysts, which typically serve to entangle
571 hard prey or penetrate soft tissue (Purcell and Mills, 1988; Colin and Costello, 2007), are likely
572 important for feeding on gelatinous prey, since they are the only types found in some jelly-
573 feeding medusae, such as hydrozoan narcomedusae (Purcell and Mills, 1988) and the
574 scyphozoan *Drymonema larsoni* (K.M. Bayha, pers. obs.). A-isorhizas are about twice as
575 numerous in *C. chesapeakei* as in *C. quinquecirrha* (16.5±3.4% vs. 7.8±3.4%) and are
576 significantly larger (Figure 8C) in *C. chesapeakei.* It is possible that the more numerous A-
577 isorhizas, possessing longer tubules, could penetrate farther into the extremely soft-bodied *M.*
578 *leidyi*, resulting in greater capture efficiency.
579
580

581 *Chrysaora in the Caribbean*

582 *Chrysaora* medusae collected from the Caribbean Sea are genetically very similar to
583 *Chrysaora chesapeakei*. *Chrysaora* in the Caribbean have historically been included in the
584 species *C. lactea* (Mayer 1910; Morandini & Marques 2010), *C. quinquecirrha* (Perry & Larson
585 2004) or *Chrysaora* sp. (Persad et al. 2003). Our Caribbean samples, limited only to Jamaica and
586 the Bocas del Toro region of Panama, appear to be two lineages (both found in JAM) slightly
587 diverged from each other (4.4-5.1% for *COI*) and from *C. chesapeakei* (6.2-7.7% for *COI*) from
588 the US east coast estuaries and the Gulf of Mexico. These animals cannot be assigned to *C.*
589 *lactea* (type locality=Rio de Janiero, Brazil), as was previously done by Mayer (1910) and
590 Morandini and Marques (2010), since these animals are distantly related to *C. lactea* for most
591 genetic regions examined (Figure 4-7). At present, it is unclear if the Caribbean forms represent
592 distinct or incipient species and further study of them from across the region is necessary. For the
593 time being, we advocate referring to Caribbean animals as *Chrysaora* c.f. *chesapeakei* ahead of a
594 formal systematic redescription based on genetic and careful morphological examination.
595
596 *Chrysaora melanaster and C. pacifica*
597 Our phylogenetic data confirm the morphological conclusions in Morandini & Marques
598 (2010) that Japanese *Chrysaora* historically identified as *C. melanaster*, and labeled as such in
599 public aquaria worldwide for decades, are actually the distinct species *C. pacifica*. Kramp (1961)
600 synonymized the Pacific *Chrysaora* species *C. melanaster* (Brandt 1835) and the Japanese
601 jellyfish *C. pacifica* (Goette 1886) to *C. melanaster*. This identification convention made it into
602 jellyfish identification books (e.g. Wrobel and Mills, 1998) and subsequently Japanese
603 *Chrysaora* labeled as *C. melanaster* became a mainstay in early jellyfish exhibits, such as the

Monterey Bay Aquarium, and then in aquaria throughout the world (W. Patry, pers. comm.). Morandini & Marques (2010) separated *C. melanaster* and *C. pacifica* on morphological grounds (tentacle and lappet number) and deemed all aquarium specimens of Japanese origin to be *C. melanaster*. Our data (Figure 4-5) confirm this, as aquarium-collected jellyfish previously labeled *C. melanaster* (MBA) are distantly related to wild-caught *C. melanaster* (BER) from its type locality (Bering Sea), but are nearly genetically similar (sequence divergence: *COI*-0.5%; *16S*-0.6%) to wild-caught *Chrysaora* collected from the eastern Korean coast (KOR), where this jellyfish was recently redescribed as *C. pacifica* (Lee et al. 2016) and Kyoto, Japan (KYO), both near the type locality of Nagasaki, Japan (Goette 1886).

Chrysaora africana/fulgida

Our phylogenies support the resurrection of *Chrysaora* species along the southwestern coast of Africa. Three species of *Chrysaor*a were previously identified from the southwestern coast of Africa: *Chrysaora hysoscella* (Kramp 1955), *C. fulgida* (Reynaud 1830) and *C. africana* (Vanhöffen 1902). However, Kramp (1961) deemed *C. africana* a variant of *C. fulgida,* and Morandini & Marques (2010) placed all of these sightings within the species *C. fulgida*. All phylogenies indicate two distantly related species of *Chrysaora* from Namibian waters (Figures 4-7), with those appearing superficially similar to *C. fulgida* (brown striped) or to *C. africana* (red tentacles) placed provisionally into these species. These designations are consistent with upcoming re-descriptions of *C. fulgida* and *C. africana* of S. Neethling (unpublished data) based on morphological and genetic analyses. *Chrysaora* has increased over recent years in this area, with concomitant ecological perturbations (Lynam et al. 2006; Flynn et al. 2012; Roux et al. 2013), underscoring the importance of correct species identification.

627

628 *Mawia benovici*

629 In addition to revealing higher level phylogenetic relationships, our data add to our
630 knowledge regarding the distribution of *M. benovici*, indicating a possible source region for the
631 introduced species. Piraino et al. (2014) hypothesized that *Mawia benovici* (then *Pelagia*
632 *benovici*), likely arrived into the Adriatic Sea via ballast water. Our data indicate that two small
633 pelagiid jellyfishes collected from the beach near Dakar, Senegal are *M. benovici* based on *COI*
634 and *28S* phylogenies (Figure 4 and 6) (there are no published *16S* sequences for *M. benovici*).
635 While this is not definitive evidence that Mediterranean *M. benovici* populations originated from
636 the western coast of Africa, it raises the possibility. While many West African species have
637 arrived in the Mediterranean through the Strait of Gibraltar or occasionally inhabit the western
638 Mediterranean (Gofas & Zenetos 2003; Antit, Gofas & Azzouna 2010), there are examples of
639 animals introduced via shipping or fishing practices from West Africa to the Mediterranean (Ben
640 Souissi et al. 2004; Antit, Gofas & Azzouna 2010; Luque et al. 2012; Zenetos et al. 2012). If *M.*
641 *benovici* did originate from the western coast of Africa, it is more likely that it was a result of
642 shipping or fishing practices, since there are no records of *M. benovici* between Gibraltar and the
643 Adriatic Sea to our knowledge.

644

SYSTEMATICS

Chrysaora quinquecirrha Desor, 1848

Figure 3A, 3B, 4-9, S1-S2.

Pelagia quinquecirrha-Desor (1848): p. 76 (original description – Nantucket Sound, MA).

Dactylometra quinquecirrha: Agassiz (1862): 126, 166 [tentacle number]. Agassiz (1865): 48, 49 [tentacle number; Naushon, MA]. Fewkes (1881): 173, Pl. VIII Fig. 14 [tentacle number, drawing]. Brooks (1882): 137 [tentacles, drawing in Mayer, 1910; southern variety outside Beaufort Inlet]. Agassiz & Mayer (1898): 1-6, Plate I [tentacles, oral arms, drawing]. Fish (1925): 128, 130 [Vineyard Sound, MA; Nonamesset, MA; Lackeys Bay, MA]. Mayer (1910): 585-588, Pl. 64A [tentacles, drawing].

Chrysaora quinquecirrha: Kramp (1961): 327-328 [description fits both *C. quinquecirrha* and *C. chesapeakei*]. Calder (1972): 40-43, Figs. 5-6 [mouth of Broadkill River, DE]. Kraeuter & Setzler (1975): 69, Figs. 1-2 [offshore samples, Sea Buoy]. Calder (2009): 24-28 [offshore animals collected on continental shelf possibly *C. quinquecirrha*].

Diagnosis: Living medusae up to 40 cm (observed 59.0 - 176.0 mm) (Figures 3A, 3B); tentacles typically 40 or more; 5.28 ± 0.45 (95% CI) tentacles/octant on average (Table 3; Figure 8A); primary tentacle central, secondary and tertiary tentacles laterally (3-2-1-2-3); lappets rounded typically 48 or more; 6.26 ± 0.46 lappets/octant on average; rhopalar lappets slightly larger than tentacular lappets; can be differentiated from *C. chesapeakei* based on 1) smaller size of holotrichous A-isorhiza nematocysts: average: 20.25 [± 0.38] µm x 11.27 [± 0.37] µm (Table 3;

667 Figure 8C); 2) larger tentacle number (more than 5 per octant) and 3) typically shorter maximum
668 oral arm length (average: 1.24 ± 0.27 time bell diameter).
669
670 **Material Examined**: NMNH 24496 (*n*=1; Buzzard's Bay, MA), NMNH 53860 (*n*=1;
671 Assateague Island, VA), NMNH 53861 (*n*=1; Assateague Island, VA), NMNH 54511 (*n*=2;
672 Cape Henlopen, DE), NMNH 56702 (*n*=1; Cape Henlopen, DE), KMBCDE1-KMBCDE5 (*n*=5;
673 Cape Henlopen, DE).
674
675 **Description of holotype**: No holotype located, no neotype designated.
676
677 **Description of specimens**: Bell diameter up to approximately 40 cm (observed 59.0-176.0 mm),
678 almost hemispherical. Exumbrellar finely granulated with small, inconspicuous marks (papillae);
679 exumbrellar color varies from entirely transparent white to white with inconspicuous radial
680 markings. Tentacle number approximately 5 tentacles per octant, but can be more (average 5.28
681 ± 0.48) (Table 3; Figure 8A); primary tentacle central, secondary and tertiary tentacles laterally
682 (3-2-1-2-3) with additional tentacles originating toward the rhopalia; lappets rounded typically
683 48 or more (average 6.26 ± 0.46 per octant); Tentacle clefts of varied depth with primary clefts
684 deeper than secondary clefts. Radial and ring musculature not obvious. Brachial disc circular.
685 Pillars evident. No quadralinga. Subgenital ostia rounded, approximately 1/8 of bell diameter.
686 Oral arms v-shaped with frills emanating from tube-like structure; straight without spiral; curved,
687 frilled edges taper toward distal end of oral arms. Oral arms short, approximately the same length
688 as bell diameter (average 1.24 ± 0.27 times bell diameter). Oral arms typically transparent white.
689 4 semi-circular gonads, white, pinkish or slightly orange, well developed within pouch outlining

gastric filaments. 16 stomach pouches bounded by 16 septae. Septae bent at 45-degree angle distally towards the rhopalia terminating near tentacle in rhopalar lappet, resulting in tentacular pouches being somewhat larger than rhopalar pouches distally.

Cnidome (tentacle). Average Dimensions (Length ± 95% CI x Width ± 95% CI)

Holotrichous A-isorhizas: 20.15 ± 0.33 x 11.13 ± 0.24 μm;

Holotrichous a-isorhizas: 8.27 ± 0.49 x 4.22 ± 0.07 μm;

Holotrichous O-isorhizas: 21.63 ± 0.39 x 18.91 ± 0.78 μm;

Heterotrichous microbasic rhopaloids: 13.58 ± 0.19 x 8.09 ± 0.09 μm;

Type Locality: Nantucket Bay, Nantucket Island, Massachusetts, East Coast of USA.

Habitat: Medusae are found in open coastal waters on the US Atlantic coast.

Distribution: Western North Atlantic, east coast of the USA south of Cape Cod in coastal Atlantic waters at least as far south as Georgia/northern Florida.

DNA sequence: Mitochondrial *COI* and *16S* and Nuclear *28S* sequence data are available in NCBI GenBank under accession numbers MF141552-MF141556, MF141608, MF141613-MF141614, MF141628, MF141635, MF141642-MF141646, MF141688-MF141689, MF141697.

Phylogeny: *C. quinquecirrha* and *C. chesapeakei* sequences form reciprocally monophyletic groups for *16S*, *COI*, *28S* and combined analyses (Figures 4-7). Minimum sequence divergences between *C. quinquecirrha* and *C. chesapeakei* clades (COI: 12.1%, *16S*: 8.4%, *28S*: 2.4%) were

much larger than the maximum within clades for *C. quinquecirrha* (COI: 0.2%, *16S*: 0.1%, *28S*: 0.0%) or *C. chesapeakei* (COI: 0.7%, *16S*: 0.6%, *28S*: 0.4%). *C. quinquecirrha* sequences did not form monophyletic groups with any other species (Figures 4-7).

Biological Data: Although the name *Chrysaora quinquecirrha* applies to the US coastal Atlantic species, almost no ecological studies have been done on the coastal species, apart from (Kraeuter & Setzler 1975), which found the largest C. *quinquecirrha* individual was found in a coastal area about 90 km offshore in full seawater (Salinity >30).

Notes: Since this species retains the scientific name *C. quinquecirrha*, we advocate it retaining the common name "U.S. Atlantic sea nettle", since it is also a coastal and open ocean species.

Chrysaora chesapeakei Papenfuss, 1936

Figures 3C, 3D, 3E, 4-9, S1-S2

Dactylometra quinquecirrha: (Bigelow 1880): 66 [white colored morph, Chesapeake Bay]. Brooks (1882): 137 [Chesapeake Bay –USA]. (Agassiz & Mayer 1898): 48-49 [upper Narragansett Bay (RI)]. Mayer (1910): 585-588, Pl.63-64 [24 tentacle morph, white, red/brown striped morph, Tampa Bay (FL), Hampton Roads (VA), St. Mary's (MD)]. Papenfuss (1936): 14-17, Figs. 7, 11, 16, 20 [lower Chesapeake Bay; red striped morph based on A-isorhiza measurements]. Littleford & Truitt (1937): 91 [Chesapeake Bay]. Littleford (1939): 368-381, Pls. I-III [Chesapeake Bay]. Hedgepeth (1954): 277-278 [Tampa Bay (FL), Gulf of Mexico]. *Dactylometra quinquecirrha* var. *chesapeakei*: Papenfuss (1936): 14-17, Figs. 12, 21

736 [Chesapeake Bay; white colored morph based on A-isorhiza measurements].

737 *Chrysaora quinquecirrha*: Kramp (1961): 327-328 [parts of description covers both *C.*

738 *quinquecirrha* and *C. chesapeakei*]. Rice & Powell (1970): 180-186 [Chesapeake Bay]. Burke

739 (1976): 20, 22-28 [Mississippi Sound, Gulf of Mexico]. Calder (1971): 270-274 [Gloucester

740 Point (VA) – Chesapeake Bay]. Calder (1972): 40-43, Figs. 1-4 [Chesapeake Bay, Pamlico

741 Sound, Gulf of Mexico]. Loeb (1972): 279-291 [Chesapeake Bay]. Loeb (1973): 144-147

742 [Chesapeake Bay]. Loeb & Blanquet (1973): 150-157 [Chesapeake Bay]. Calder (1974b): 326-

743 333 [Chesapeake Bay]. Loeb (1974): 423-432 [Chesapeake Bay]. Blanquet & Wetzel (1975):

744 181-192 [Chesapeake Bay]. Cargo (1975): 145-154 [Chesapeake Bay]. Kraeuter & Setzler

745 (1975): 69, Figs. 1-2 [Doboy Sound (GA)]. Loeb & Gordon (1975): 37-41 [Chesapeake Bay].

746 Lin & Zubkoff (1976): 37-41 [Chesapeake Bay]. Calder (1977): 13-19 [Gloucester Point, MD –

747 Chesapeake Bay]. Clifford & Cargo (1978): 58-60 [Patuxent River, MD – Chesapeake Bay].

748 Cargo (1979): 279-286 [Chesapeake Bay]. Cargo & Rabenold (1980): 20-26 [Patuxent River

749 (MD)]. Hutton et al. (1986): 154-155 [Chesapeake Bay]. Cargo & King (1990): 486-491

750 [Chesapeake Bay]. Purcell et al. (1991): 103-111 [Choptank River, MD – Chesapeake Bay].

751 Nemazie, Purcell & Glibert (1993): 451-458 [Chesapeake Bay]. Purcell, White & Roman (1994):

752 263-278 [Chesapeake Bay]. Burnett et al. (1996): 1377-1383 [Chesapeake Bay]; Houck et al.

753 (1996): 771-778 [St. Margaret's, MD – Chesapeake Bay]. Olesen, Purcell & Stoecker (1996):

754 149-158 [Broad Creek (MD) – Chesapeake Bay]. Ford et al. (1997): 355-361 (Choptank River

755 (MD) – Chesapeake Bay]. Kreps, Purcell & Heidelberg (1997): 441-446 [Choptank River (MD)

756 – Chesapeake Bay]. Wright & Purcell (1997): 332-338 [Patuxent River (MD) – Chesapeake

757 Bay]. Suchman & Sullivan (1998): 237-244 [Green Hill Pond (RI)]. Purcell, Malej & Benović

758 (1999): 241-263 [Chesapeake Bay]. Purcell et al. (1999): 187-196 [Choptank River (MD) –

759 Chesapeake Bay]. Bloom, Radwan & Burnett (2001): 75-90 [St. Mary's (MD) – Chesapeake
760 Bay]. Condon, Decker & Purcell (2001): 89-95 [Choptank River (MD) – Chesapeake Bay].
761 Graham (2001): 97-111 [Gulf of Mexico]. Johnson, Perry & Burke (2001): 213-221 [Gulf of
762 Mexico]. Matanoski, Hood & Purcell (2001): 191-200 [Choptank River (MD) – Chesapeake
763 Bay]. Segura-Puertas, Suárez-Morales & Celis (2003): 9 [Gulf of Mexico]. Ishikawa et al.
764 (2004): 895-899 [Gibson Island (MD) – Chesapeake Bay]. Grove & Breitburg (2005): 185-198
765 [Patuxent River (MD) – Chesapeake Bay]. Purcell & Decker (2005): 376-385 [Chesapeake
766 Bay]. Thuesen et al. (2005): 2475-2482 [Chesapeake Bay]. Breitburg & Fulford (2006): 776-784
767 [Solomon's Island [MD] – Chesapeake Bay]. Kimmel, Roman & Zhang (2006): 131-141 [mid to
768 upper Chesapeake Bay]. Decker et al. (2007): 99-113 [Chesapeake Bay]. Condon & Steinberg
769 (2008): 153-168 [York River (VA) – Chesapeake Bay]. Calder (2009): 24-28 [estuarine
770 animals]. Matanoski & Hood (2006): 595-608 [Choptank River (MD) – Chesapeake Bay].
771 Purcell (2007): 184, 190-192 [Chesapeake Bay]. Purcell (2009): 23-50 [Chesapeake Bay]. Duffy,
772 Epifanio & Fuiman (1997): 123-131 [Port Aransas (TX) – Gulf of Mexico]. Bayha & Graham
773 (2009): 217-228 [Rhode Island, New Jersey, Chesapeake Bay, Georgia, Alabama]. Sexton et al.
774 (2010): 125-133 [Choptank River (MD) – Chesapeake Bay]. Birsa, Verity & Lee (2010): 426-
775 430 [Skidaway River (GA), Wassow Sound (GA)]. Condon, Steinberg & Bronk (2010): 153-170
776 [York River (VA) – Chesapeake Bay]. Condon et al. (2011): 10225-10230 [Chesapeake Bay].
777 Frost et al. (2012): 247-256 [Steinhatchee River (FL) – Gulf of Mexico]. Duarte et al. (2012):
778 91-97 [St. Leonard's (MD) – Chesapeake Bay]. Kimmel, Boynton & Roman (2012): 76-85
779 [Solomon's Island (MD) – Chesapeake Bay]. Sexton (2012): 1-153 [Chesapeake Bay]. Brown et
780 al. (2013): 113-125 [Chesapeake Bay]. Robinson & Graham (2013): 235-253 [Gulf of Mexico].
781 Breitburg & Burrell (2014): 183-200 [Patuxent River (MD) – Chesapeake Bay]. Kaneshiro-

Pineiro & Kimmel (2015): 1965-1975 [Pamlico Sound (NC). Meredith, Gaynor & Bologna (2016): 6248-6266 [Barnegat Bay (NJ)]. Tay & Hood (2017): 227-242 [Choptank River (MD), Chesapeake Bay].

Diagnosis: Living medusae up to 20 cm (observed 17.0-175.0 mm; average: 63.0 mm); Tentacles typically number 24 or 3 per octant (average 3.07±0.07); primary tentacle central and secondary tentacles lateral (2-1-2); rarely, additional tentacles arise lateral to secondary tentacles (3-2-1-2-3) and are typically undeveloped; marginal lappets rounded and typically 32 or 4 per octant (average 4.08 ± 0.06); rhopalar lappets are typically about the same size as tentacular lappets; can be differentiated from *C. quinquecirrha* based on 1) larger size of holotrichous A-isorhiza nematocysts: 26.21 [± 0.50] µm x 19.74 [± 0.55] µm; 2) smaller tentacle number (~3 tentacles per octant); and 3) larger maximum oral arm length (average: 3.00 ± 0.39 times bell diameter).

Material Examined. Neotype: - KMBGVA8 – (Gloucester Point, MD – Chesapeake Bay). Other comparative specimens: NMNH 57925 (*n*=9; Orange Inlet, NC), NMNH 56758 (*n*=5; Charlestown Pond, RI), NMNH 33456 (*n*=4; Plum Point, MD), NMNH 49733 (*n*=1; Alligator Harbor, FL), NMNH 53826 (*n*=2; Timbalier Bay, LA), NMNH 56703 (*n*=2; Chesapeake Bay 37.23 N 76.04 W), NMNH 56704 (*n*=4; Chesapeake Bay 37.23 N 76.04 W), NMNH 53870 (*n*=3; Beaufort, NC), NMNH 53828 (*n*=2; Drum Point, MD), NMNH 33458 (*n*=3; Plum Point, MD), NMNH 33457 (*n*=4; Plum Point, MD), NMNH 55621 (*n*=6; near Chesapeake Beach, MD), NMNH 53867 (*n*=1; Arundel on the Bay, MD), NMNH 54404 (*n*=1; Chesapeake Bay 37.23 N 76.04 W), NMNH 33121 (*n*=6; Arundel on the Bay, MD), NMNH

42155 (*n*=2; Louisiana, Gulf of Mexico), NMNH 54372 (*n*=1; Lake Pontchartrain, LA); (KMBCSC1-KMBCSC7 (*n*=7; Charleston Harbor, SC), KMBGVA1-KMBGVA12 (*n*=12; Gloucester Point, VA), KMBCRI1-KMBCRI14 (*n*=14; Charlestown Pond, RI), KMBRDE1-KMBRDE16 (*n*=16; Rehoboth Bay, DE), KMBDAL2-3 (*n*=3; Dauphin Island, AL).

Description of neotype specimen: KMBGVA8. Bell diameter 110.4 mm, almost hemispherical. Exumbrella white/clear with granulated surface of small white marks. 8 rhopalia. No ocelli. Deep rhopalar clefts; deep sensory pits. Marginal lappets rounded, 32 total or 4 per octant made up of two rhopalar lappets and two tentacular lappets. Lappet size barely heterogeneous, with rhopalar lappets about the same width as tentacular lappets but longer. Tentacle number 24 or 3 per octant, with primary tentacle surrounded by two secondary tentacles (2-1-2), primary tentacle longer than secondary tentacles, up to 3-4 times bell diameter. Tentacles are white, slightly pinkish. Tentacle clefts of varied depth with primary clefts deeper than secondary clefts. Radial and ring musculature not obvious. Brachial disc circular. Pillars evident. No quadralinga. Subgenital ostia rounded, approximately 1/10 of bell diameter. Oral arms white, v-shaped with frills emanating from tube-like structure. Oral arms straight without spiral curved, frilled edges taper toward distal end of oral arms. Orals arms long, approximately 5 (4.98) times bell diameter. 4 semi-circular gonads, white (a bit orange), well developed within pouch outlining gastric filaments. 16 stomach pouches bounded by 16 septae. Septae bent at 45-degree angle distally towards the rhopalia terminating near tentacle in rhopalar lappet, resulting in tentacular pouches being somewhat larger than rhopalar pouches distally.

Cnidome (tentacle): Average dimensions (Length ± 95% CI x Width ± 95% CI)

Holotrichous A-isorhizas: 25.66 ± 0.83 x 19.16 ± 0.54 μm;

828 Holotrichous a-isorhizas 7.77 ± 0.20 x 4.17 ± 0.10 μm;

829 Holotrichous O-isorhizas 22.02 ± 0.30 x 19.95 ± 0.24 μm;

830 Heterotrichous microbasic rhopaloids 12.35 ± 0.47 μm x 8.55 ± 0.55 μm.

831

832 **Description of other specimens**: Bell diameter up to approximately 20 cm (observed 17.0-
833 175.0 mm), almost hemispherical but flattened in small individuals. Exumbrellar finely
834 granulated with small, inconspicuous marks (papillae); exumbrellar color varies considerably,
835 varying from all white to a completely brown or red colored bell, to a bell with radial lines of
836 red/brown with a spot in the center of the bell. Radial lines may be relatively inconspicuous
837 without a noticeable spot in the center. Tentacles typically number 24 or 3 per octant (average
838 3.07 ± 0.07), with primary tentacle surrounded by two secondary tentacles (2-1-2), primary
839 tentacle longer than secondary tentacles, up to 3-4 times bell diameter. In some rare cases, small
840 tentacles may occur laterally to secondary tentacle, occurring between the secondary tentacle and
841 rhopalium. In almost all cases, this tentacle is similar in size to or smaller than the lappets
842 surrounding it. In very rare cases (twice observed), about 5 or more tentacles per octant have
843 been seen, though these medusae had aberrant tentacle patterns overall (e.g. more than one
844 tentacle emerging from same spot, tentacles emerging below lappet). Tentacles are white,
845 slightly pinkish. Marginal lappets rounded and typically 32 or 4 per octant (average 4.08 ± 0.06).
846 Tentacle clefts of varied depth with primary clefts deeper than secondary clefts, which are deeper
847 than rare tertiary clefts. Radial and ring musculature not obvious. Brachial disc circular. Pillars
848 evident. No quadralinga. Subgenital ostia rounded, approximately 1/10 of bell diameter. Oral
849 arms v-shaped with frills emanating from tube-like structure; straight without spiral; curved,
850 frilled edges taper toward proximal end of oral arms. Oral arms long, approximately 3 times bell

851 diameter on average (as much as 5.6 times bell diameter). Oral arms vary in color, from
852 transparent white, to red or brown colored tubule surrounded by pinkish frilled edges. 4 semi-
853 circular gonads, white, pinkish or slightly orange, well developed within pouch outlining gastric
854 filaments. 16 stomach pouches bounded by 16 septae. Septae bent at 45-degree angle distally
855 towards the rhopalia terminating near tentacle in rhopalar lappet, resulting in tentacular pouches
856 being somewhat larger than rhopalar pouches distally.
857 Cnidome (tentacle). Average Dimensions (Length ± 95% CI x Width ± 95% CI)
858 Holotrichous A-isorhizas: 26.21 ± 0.50 x 19.74 ± 0.55 μm;
859 Holotrichous a-isorhizas: 7.88 ± 0.13 x 4.29 ± 0.07 μm;
860 Holotrichous O-isorhizas: 23.10 ± 0.43 x 20.75 ± 0.62 μm;
861 Heterotrichous microbasic rhopaloids: 12.73 ± 0.22 x 8.29 ± 0.13 μm;
862
863 **Type Locality**: Gloucester Point (VA), Chesapeake Bay, east coast of USA.
864
865 **Habitat**: Medusae are found in estuarine waters on the US Atlantic coast and estuarine and
866 nearshore waters of the Gulf of Mexico.
867
868 **Distribution**: Western North Atlantic, east coast of the USA south of New England to
869 the Gulf of Mexico, restricted to estuarine waters on the Atlantic coast, known to exist outside of
870 estuaries in the Gulf of Mexico.
871
872 **Remarks**: Since *C. chesapeakei* is commonly found in estuarine waters, we advocate the
873 common name "U.S. Atlantic bay nettle" to distinguish it from the "U.S. Atlantic sea nettle" (*C.*

874 *quinquecirrha*). The specific name *chesapeakei* originates from *Dactylometra quinquecirrha* var.
875 *chesapeakei* of Papenfuss (1936). For Papenfuss (1936), it is clear that: 1) the manuscript likely
876 compared nematocyst measurements between two color morphs of *C. chesapeakei* and did not
877 include *C. quinquecirrha* s. str. (see Discussion; Figure 8C); and 2) differences invoked for
878 holotrichous a-isorhizas are in question, since the nematocysts are small (~1.5 um), making
879 identifying differences difficult even with more precise, modern instruments, and the data are not
880 accompanied by any statistics or measurement error. Regardless, based on Article 35.6.4 of the
881 International Code of Zoological Nomenclature 4th Edition (ICZN 1999), the specific name
882 *chesapeakei* has taxonomic priority and *C. chesapeakei* applies to the Chesapeake Bay animals,
883 as well as estuarine Atlantic and Gulf of Mexico animals that are genetically similar, and have
884 similar macromorphological and cnidome characteristics (Figures 4-9). Papenfuss (1936) did not
885 designate a type specimen for *Dactylometra* (=*Chrysaora*) *quinquecirrha* var. *chesapeakei*. We
886 designate the specimen KMBGVA8 as a neotype specimen so that a physical specimen, along
887 with preserved tissue for genetic analysis, will be available to objectively define *C. chesapeakei*
888 [see Article 75 of the International Code for Zoological Nomenclature (ICZN 1999)], which will
889 be necessary given the close genetic relationship between this species and specimens from the
890 Caribbean (see below). Our neotype specimen originates from Gloucester Bay (VA), within the
891 Chesapeake Bay, where Papenfuss (1936) hypothesized *Dactylometra* (=*Chrysaora*)
892 *quinquecirrha* var. *chesapeakei* to be confined.
893
894 **DNA sequence**: Mitochondrial *COI* and *16S* and Nuclear *28S* sequence data are available in
895 GenBank under accession numbers MF141564-MF141587, MF141615-MF141617, MF141637-
896 MF141639, MF141649-MF141669, MF141699-MF141718, MF167556-MF167568.

Phylogeny: *C. chesapeakei* and *C. quinquecirrha* sequences form reciprocally monophyletic groups for *16S*, *COI*, *28S* and combined analyses (Figures 4-7). Minimum sequence divergences between *C. chesapeakei* and *C. quinquecirrha* clades (COI: 12.1%, *16S*: 8.4%, *28S*: 2.5%) were much larger than the maximum within clades for *C. quinquecirrha* (COI: 0.3%, *16S*: 0.1%, *28S*: 0.0%) or *C. chesapeakei* (COI: 2.2%, *16S*: 1.9%, *28S*: 0.7%). *C. chesapeakei* sequences do not form monophyletic groups with any other species (Figures 4-7).

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920 **FIGURES**
921
922 **Figure 1**: World map showing collecting sites of animals sequenced for this study. Final
923 species designations are employed. All aquarium samples (*C. achlyos, C. chinensis, C. colorata,*
924 *C. fuscescens* and *C. pacifica*) originated from cultures at the Monterey Bay Aquarium, although
925 some were obtained from the Aquarium of the Americas. Their locations on the map are based
926 on original collection locations for the aquarium cultures (W. Patry, pers. comm.).
927
928 **Figure 2**: Collection locations of *Chrysaora quinquecirrha* s.l. medusae used in this study.
929 Abbreviations all refer to Tables 1 and S1. Figures 2 (A-C) are enlargements of rectangular inset
930 regions. The star at Nantucket harbor indicates the type locality of *C. quinquecirrha* (Desor,
931 1848). Diamonds represent important museum collection sites (Table S1). Site RI is within the
932 enclosed Charlestown Pond, RI (41.364.765 N, 71.628865 W). Site NJ is at Ocean Gate Yacht
933 Club (39.930490 N, 74.140448 W) on Toms River, inside Barnegat Bay. Site RB was collected
934 from inside Rehoboth Bay, DE (38.688091 N, 75.077114 W). All Chesapeake Bay samples (NF
935 and Gloucester Point, VA) were taken from well within the Chesapeake Bay. Site PAM was
936 collected from Englehard, NC (35.509102 N, 75.989712 W), well within Pamlico Sound. CST
937 was taken from within Charleston Harbor (32.786995 N, 79.909297 W). Site GA was taken from
938 Fancy Bluff Creek, upstream from Saint Simons Sound, GA (31.166291 N, 81.416032 W).
939 Sample sites with individuals finally designated as *C. quinquecirrha* are in white and those with
940 individuals finally designated as *C. chesapeakei* in black.
941
942 **Figure 3**: Various morphs of *C. quinquecirrha s.l.* A) Offshore South Carolina (OSC); B)
943 Sample taken from offshore Georgia; C) Englehard, NC (PAM); D) White Chesapeake Bay color

944 morph (Choptank River, MD); E) Red-striped Chesapeake Bay color morph (York River, VA).
945 Note that medusae from A-B have 5 tentacles per octant, while C-E appear have three tentacles
946 per octant. Medusae in 3A and 3C were included in this study's phylogenetic analyses. (3A:
947 OSC1; 3C: PAM1). A-B represent individuals finally designated as *C. quinquecirrha*; C-E
948 represent individuals finally designated as *C. chesapeakei*.
949
950 **Figure 4**: Pelagiidae *COI* Phylogeny. Bayesian Inference (BI) *COI* tree reconstructed from
951 CLUSTAL alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence
952 evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a
953 percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%.
954 ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the
955 TPM2uf+I+G model of sequence evolution (-lnl 5451.81154) as determined by jMODELTEST
956 v2.1.7 (Darriba et al. 2012). Abbreviations refer to Tables 1-2. Specific identification to the right
957 of the tree indicates final species designations. Clades colored in gray were originally identified
958 as *C. quinquecirrha*. Norfolk (VA) individuals NF1-NF3 were identified as white Chesapeake
959 Bay color morph and individuals NF4-NF5 as red-striped Chesapeake Bay color morph (Figure
960 3D-E).
961
962 **Figure 5**: Pelagiidae *16S* Phylogeny. Bayesian Inference (BI) *16S* tree reconstructed from
963 MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence
964 evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a
965 percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%.
966 ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the

967 TIM2+I+G model of sequence evolution (-lnl 3641.97519) as determined by jMODELTEST
968 v2.1.7 (Darriba et al. 2012). Gray arrows indicate nodes that are alternated in the ML tree.
969 Abbreviations refer to Tables 1-2. Specific identification to the right of the tree indicates final
970 species designations. Clades colored in gray were originally identified as *C. quinquecirrha* s.l.
971 Norfolk (VA) individuals NF1-NF3 were identified as white morph and individuals NF4-NF5 as
972 red-striped bell morphs (Figure 3D-E).
973
974 **Figure 6**: Pelagiidae *28S* Phylogeny. Bayesian Inference (BI) *28S* tree reconstructed from
975 MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence
976 evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a
977 percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%.
978 ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the
979 TrNef+I+G model of sequence evolution (-lnl 3817.02691) as determined by jMODELTEST
980 v2.1.7 (Darriba et al. 2012). Specific identification to the right of the tree indicates final species
981 designations. Clades colored in gray were originally identified as *C. quinquecirrha*.
982
983 **Figure 7**: Pelagiidae Combined Phylogeny. Bayesian Inference (BI) tree of the combined dataset
984 applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show
985 bootstrap support if ≥0.70 (presented as a percentage), followed by bootstrap support from
986 maximum likelihood (ML) analysis if ≥50%. ML phylogeny was reconstructed using PhyML
987 v3.0 (Guindon et al. 2010) applying the GTR+I+G model of sequence evolution (-lnl
988 11924.23655) as determined by jMODELTEST v2.1.7 (Darriba et al. 2012). Specific

identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *C. quinquecirrha*.

Figure 8: Morphological evidence separating *C. quinquecirrha* and *C. chesapeakei*. A) Tentacle counts. Graph represents tentacles per octant against bell diameter (mm) for field collected and museum specimens. Squares represent animals taken from estuarine Atlantic and Gulf of Mexico regions (*C. chesapeakei*), while circles represent animals taken from coastal Atlantic regions (*C. quinquecirrha*). All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. B) Maximum oral arm measurements. Graph represents maximum oral arm length against bell diameter (mm) for field-collected and museum specimens. Squares represent animals taken from U.S. Atlantic estuaries and the Gulf of Mexico (*C. chesapeakei*), while circles represent animals taken from coastal Atlantic regions (*C. quinquecirrha*). Only animals with fully intact and extended oral arms were included. All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. C) Average size measurements for holotrichous A-isrohiza nematocysts (length vs. width), based on 10 nematocysts per. Error bars represent 95% confidence intervals (2*standard error). Squares represent nematocysts from estuarine Atlantic and Gulf of Mexico medusae (*C. chesapeakei*), while circles represent nematocysts from coastal Atlantic medusae (*C. quinquecirrha*). Photograph of an average sized A-isorhiza from *C. quinquecirrha* appears on the left and a photograph of an average size A-isorhiza from *C. chesapeakei* appears on the right. Scale bars=10 um. Photographs have been resized so that all error bars are the same size on the page to allow size comparisons. All animals with *16S* sequences matching the *C. chesapeakei*

clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. Triangles represent average values from Papenfuss (1936) for morphs identified as *Dactylometra quinquecirrha* (gray) or *Dactylometra quinquecirrha* var. *chesapeakei* (white).

Figure 9: Pelagiidae Evolution. Cladograms showing genus-level relationships within the Pelagiidae family. Colors represent individual genera as shown. A) Gershwin and Collins (2002); B) Morandini and Marques (2010); C) Avian et al. (2016): DNA analysis based on nuclear *28S*; D) Avian et al. (2016): morphological analyses only; E) This study: Combined DNA analysis using sequence data from *COI, 16S* and *28S*. *In Avian et al. (2016), this sequence is marked as *Chrysaora* sp. AY920779. This sequence is included in our analysis and is part of the clade that we call *Chrysaora* c.f. *chesapeakei*. ^We include the *28S* phylogeny from Avian et al. (2016) because it has more species than their combined analysis but their generic conclusions are identical. Note that all previous hypotheses include a monophyletic *Chrysaora*.

Figure S1: Tentacle Nematocyst Sizes. Average size measurements based on 10 nematocysts per individual (length vs. width) for nematocysts: A) a-isorhizas; B) O-isorhizas; C) heterotrichous microbasic rhopaloids. Error bars represent standard deviation values. Squares represent nematocysts from estuarine Atlantic and Gulf of Mexico medusae (*C. chesapeakei*), while circles represent nematocysts from coastal Atlantic medusae (*C. quinquecirrha*). All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. Triangles represent average values from Papenfuss (1936) for morphs identified as *Dactylometra quinquecirrha* (gray) or *Dactylometra quinquecirrha var. chesapeakei* (white). Nematocyst examples are to the right of each graph. All

nematocysts are of average size for the nematocyst type and species. Photographs have been resized so that all error bars are the same size on the page to allow size comparisons.

Figure S2: Tentacle Nematocyst Diversity. A) Mosaic plot showing the relative proportions of nematocyst types in distal, medial and proximal tentacle regions. O-isorhiza and rhopaloid nematocysts vary markedly in abundance across regions. Plot drawn using R package vcd (Meyer, Zeileis & Hornik 2016). Proportions of nematocysts types vary significantly across tentacle regions; shading indicates significant departures from expected values (red = negative residuals, blue = positive residuals).
B) Non-metric multidimensional scaling of similarities in overall (proximal, medial and distal regions) proportions of all four nematocyst types. Squares represent nematocysts from estuarine Atlantic and Gulf of Mexico medusae, while circles represent nematocysts from coastal Atlantic medusae. All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue.

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Table 1(on next page)

Geographic source regions of samples used for molecular analyses in this study, identified by taxon (original, morphologically based identification) and molecular ID (identification after molecular analyses).

Table 1: Geographic source regions of samples used for molecular analyses in this study, identified by taxon (original, morphologically based identification) and molecular ID (identification after molecular analyses). For six individuals, 28S sequences from those individuals were published previously. For *S. malayensis*, 16S/COI and 28S sequences came from the same culture, but two different individuals. For some aquarium specimens, the geographic source region for the culture is known: *near Los Angeles, CA (USA); ^Northern Malaysia; $^{+}$near Monterey Bay, CA (USA).

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				n		
Original ID	**Final ID**	**Location**	**Code**	**COI**	**16S**	**28S**
Chrysaora achlyos	*C. achlyos*	Monterey Bay Aquarium*	MBA	1	1	1
Chrysaora africana	*C. africana*	Coastal Namibia	NAM	2	2	2
Chrysaora chinensis	*C. chinensis*	Monterey Bay Aquarium^	MBA	2	2	2
Chrysaora colorata	*C. colorata*	Aquarium of the Americas+	AQA	1	1	1
Chrysaora fulgida	*C. fulgida*	Coastal Namibia	NAM	5	5	2
Chrysaora fuscescens	*C. fuscescens*	Aquarium of the Americas+	AQA	1	1	HM194868
Chrysaora hysoscella	*C. hysoscella*	Cork, Ireland	IRE	3	3	3
Chrysaora lactea	*Chrysaora* c.f. *chesapeakei*	Kingston, Jamaica	JAM	5	5	2
Chrysaora lactea	*C. lactea*	Rio de la Plata, Argentina	ARG	1	1	1
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER	-	1	AY920780
Chrysaora melanaster	*C. pacifica*	Monterey Bay Aquarium	MBA	1	1	HM194864
Chrysaora plocamia	*C. plocamia*	Puerto Madryn, Argentina	PMA	2	2	2
Chrysaora quinquecirrha	*C. quinquecirrha*	Buzzard's Bay, MA (USA)	MA	1	1	1
Chrysaora quinquecirrha	*C. quinquecirrha*	Cape Henlopen, DE (USA)	CHP	3	3	2
Chrysaora quinquecirrha	*C. quinquecirrha*	Offshore South Carolina (USA) (32.60 N, 79.21 W)	OSC	2	2	1
Chrysaora quinquecirrha	*C. chesapeakei*	Charlestown Pond, RI (USA)	RI	4	4	-
Chrysaora quinquecirrha	*C. chesapeakei*	Tom's River Harbor, NJ (USA)	NJ	3	3	1
Chrysaora quinquecirrha	*C. chesapeakei*	Rehoboth Bay, DE (USA)	RB	3	3	-
Chrysaora quinquecirrha	*C. chesapeakei*	Norfolk, VA (USA)	NF	5	5	-
Chrysaora quinquecirrha	*C. chesapeakei*	Pamlico Sound, NS (USA)	PAM	3	3	-
Chrysaora quinquecirrha	*C. chesapeakei*	St. Simon's Island, GA (USA)	GA	3	3	1
Chrysaora quinquecirrha	*C. chesapeakei*	Perdido Pass, AL (USA)	AL	3	3	1
Pelagia noctiluca	*P. noctiluca*	Offshore Virginia (USA) (37.81 N, 73.91 W)	OVA	1	1	HM194865
Sanderia malayensis	*S. malayensis*	Monterey Bay Aquarium	MBA	1	1	HM194861
Unknown Pelagiidae	*M. benovici*	Dakar, Senegal	SEN	2	2	1

Table 2(on next page)

Geographic source regions of previously published sequences used in in this study identified by taxon

Table 2: Geographic source regions of previously published sequences used in in this study identified by taxon (previous identification) and Molecular ID (identification after molecular analyses). *Sequences came from the same individual.

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				n		
Original ID	**Final ID**	**Location**	**Code**	**COI**	**16S**	**28S**
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER1	KJ026191	-	-
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER2	KJ026212	-	-
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER3	KJ026256	-	-
Chrysaora sp.	*Chrysaora* c.f. *chesapeakei*	Bocas del Toro, Panama	PAN	JN700941*	JN700941*	AY920779*
Chrysaora pacifica	*Chrysaora pacifica*	Kyoto, Japan	KYO	LC191577	-	-
Chrysaora quinquecirrha	*C. pacifica*	Geoje-do, Korea	KOR	HQ0694730	HQ0694730	-
Chrysaora sp.	*Chrysaora* sp. 1	Noosa Heads, Australia	AUS	DQ083524	-	-
Chrysaora sp.	*C. chinensis*	Malaysia	MAL1	-	JN184784	-
Chrysaora sp.	*C. chinensis*	Malaysia	MAL2	-	JN184785	-
Chrysaora sp.	*C. chinensis*	Malaysia	MAL3	-	JN184786	-
Pelagia benovici	*P. benovici*	Northern Adriatic Sea	ADR1	KJ573409	-	KJ573396
Pelagia benovici	*P. benovici*	Northern Adriatic Sea	ADR2	KJ573410	-	KJ573397
Pelagia benovici	*P. benovici*	Northern Adriatic Sea	ADR3	KJ573412	-	KJ573401
Pelagia noctiluca	*P. noctiluca*	Southern Tyrrhenian Sea, Italy	TYR	KJ573419	-	KJ573408
Pelagia noctiluca	*P. noctiluca*	Cape Town, South Africa	SA	JQ697961	-	-
Pelagia noctiluca	*P. noctiluca*	Dispensa Island, Costa Rica	CR1	JX235441	-	-
Pelagia noctiluca	*P. noctiluca*	Dispensa Island, Costa Rica	CR2	-	JX235404	-
Pelagia noctiluca	*P. noctiluca*	Dispensa Island, Costa Rica	CR3	-	JX235405	-
Pelagia c.f. panopyra	*Pelagia c.f. panopyra*	Papua, New Guinea	PNG	KJ573422	-	-

Table 3(on next page)

Morphological characters examined for this study

Table 3: Morphological characters examined for this study. Characters in bold are species diagnostic. All macromorpholgical characters and character states (except maximum oral arm length) are taken from Gershwin and Collins (2004). Cnidome terminology is taken from Morandini and Marques (2010), with average examples in Figure 8C, S1. *If two outlier specimens are included, the upper range is 6 tentacles/octant. ^Although maximum bell diameter for *C. quinquecirha* has been recorded as great as 40 mm (Gershwin and Collins, 2004; Morandini and Marques, 2010), no animals >20 mm were observed in this study.

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Character	*Chrysaora quinquecirrha*	*C. chesapeakei*
Macromorphology		
Bell Diameter (average/median)	114 mm (59-176 mm)	62.2 mm (17-175 mm)
Tentacles / octant (average ± 95% CI)	**5.28 ± 0.45**	**3.07 ± 0.07**
Tentacles / octant (range)	**4.5 - 6.75**	**2.75 - 3.43***
Lappets / octant (average ± 95% CI)	**6.26 ± 0.46**	**4.08 ± 0.06**
Lappets / octant (range)	**5.5 – 7.75**	**3.75 - 4.8**
Maximum Oral Arm Length (average ± 95% CI)	**1.24 ± 0.27 times BD**	**3.00 ± 0.39 times BD**
Maximum Oral Arm Length (range)	**0.68 to 1.81 times BD**	**1.21 to 5.58 timed BD**
Lappets in Size Classes	Yes, rhopalar lappets larger	No, lappets of similar size
Rhopalia Number	8	8
Rhopaliar Pits	deep	deep
Septa Shape	bent	bent
Septa Termination	near tentacle	near tentacle
Spiral Oral Arms?	No	No
Manubrium Length	elongated	elongated
Manubrium Mass	light	light
Warts/Papillae	inconspicuous	inconspicuous
Maximum Bell Diameter	< 20 cm^	< 20 cm^
Bell Mass	light	light
Dominant color	White, colorless	Variable, white, colorless or red/brown bell
Exumbrellar marks	Minor bell marks in some cases	Variable, red or brown star shape conspicuous in some cases,
Oral arm color	None	Variable, Oral arms can be colored red/brown
Quadralinga	None	None
Gonads in Pouch?	Yes	Yes
Gonad Shape	Not finger-like	Not finger-like
Cnidome		
A isorhiza - Length vs. Width (avg)	**20.25 ± 0.38 x 11.27 ± 0.37 µm**	**26.21 ± 0.50 x 19.74 ± 0.55 µm**
A isorhiza - Length vs. Width (range)	**15.01–22.9 x 9.07–13.16 µm**	**20.54–33.79 x 15.03–29.77 µm**
a isorhiza – Length vs. Width (avg)	8.27 ± 0.19 x 4.22 ± 0.07 µm	7.88 ± 0.13 x 4.29 ± 0.07 µm
a isorhiza – Length vs. Width (range)	6.56-9.77 x 3.65-4.95 µm	6.32-9.9 x 3.59-5.46 µm
O isorhiza – Length vs. Width (avg)	21.64 ± 0.38 x 18.92 ± 0.77 µm	23.10 ± 0.43 x 20.75 ± 0.62 µm
O isorhiza – Length vs. Width (range)	17.64-23.97 x 16.08-21.74 µm	17.88-27.51 x 16.07-24.75 µm
Birhopaloids – Length vs. Width (avg)	13.58 ± 0.19 x 8.09 ± 0.09 µm	12.73 ± 0.22 x 8.29 ± 0.13 µm
Birhopaloids – Length vs. Width (range)	12.31-14.86 x 6.96-8.90 µm	10.96-15.27 x 7.1-10.23 µm

9

Figure 1(on next page)

World map showing collecting sites of animals sequenced for this study

Figure 1: World map showing collecting sites of animals sequenced for this study . Final species designations are employed. All aquarium samples (*C. achlyos, C. chinensis, C. colorata, C. fuscescens* and *C. pacifica*) originated from cultures at the Monterey Bay Aquarium, although some were obtained from the Aquarium of the Americas. Their locations on the map are based on original collection locations for the aquarium cultures (W. Patry, pers. comm.).

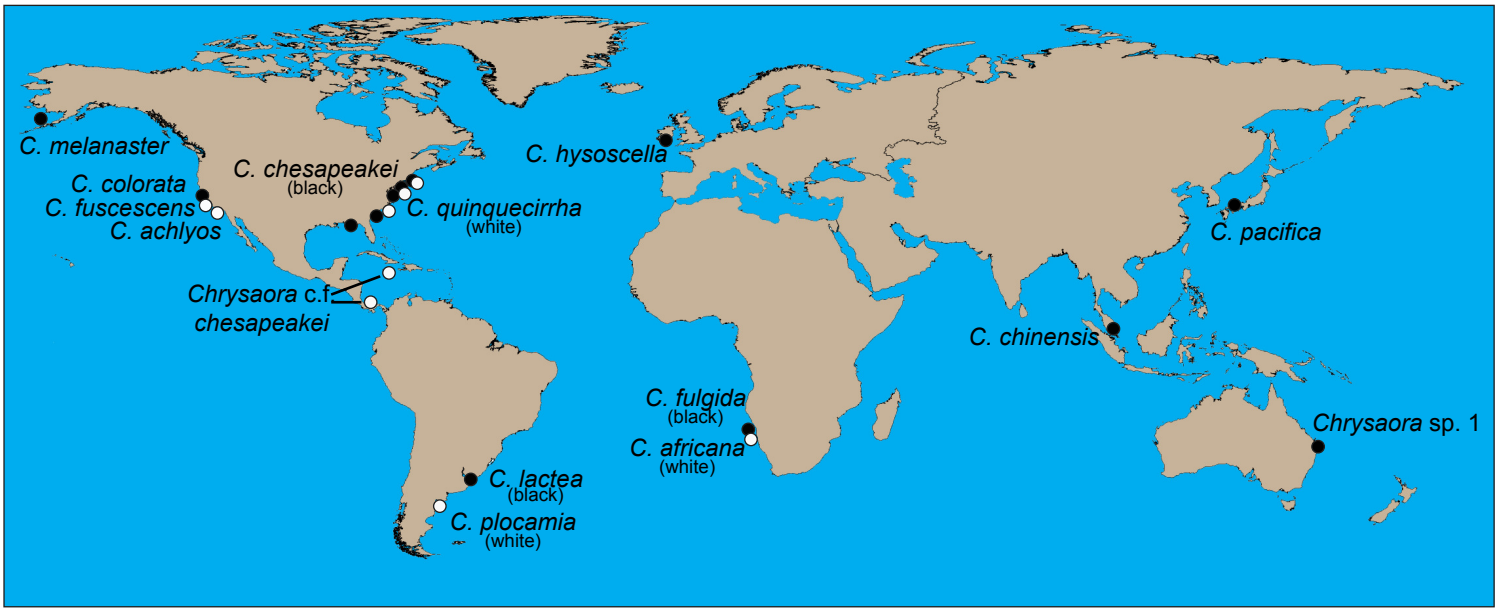

C. melanaster
C. colorata
C. fuscescens
C. achlyos
C. chesapeakei
(black)
C. quinquecirrha
(white)
Chrysaora c.f.
chesapeakei
C. hysoscella
C. fulgida
(black)
C. africana
(white)
C. lactea
(black)
C. plocamia
(white)
C. pacifica
C. chinensis
Chrysaora sp. 1

Figure 2(on next page)

Collection locations of *Chrysaora quinquecirrha* s.l. medusae

Figure 2: Collection locations of *Chrysaora quinquecirrha* s.l. medusae used in this study. Abbreviations all refer to Tables 1 and S1. Figures 2 (A-C) are enlargements of rectangular inset regions. The star at Nantucket harbor indicates the type locality of *C. quinquecirrha* (Desor, 1848). Diamonds represent important museum collection sites (Table S1). Site RI is within the enclosed Charlestown Pond, RI (41.364.765 N, 71.628865 W). Site NJ is at Ocean Gate Yacht Club (39.930490 N, 74.140448 W) on Toms River, inside Barnegat Bay. Site RB was collected from inside Rehoboth Bay, DE (38.688091 N, 75.077114 W). All Chesapeake Bay samples (NF and Gloucester Point, VA) were taken from well within the Chesapeake Bay. Site PAM was collected from Englehard, NC (35.509102 N, 75.989712 W), well within Pamlico Sound. CST was taken from within Charleston Harbor (32.786995 N, 79.909297 W). Site GA was taken from Fancy Bluff Creek, upstream from Saint Simons Sound, GA (31.166291 N, 81.416032 W). Sample sites with individuals finally designated as *C. quinquecirrha* are in white and those with individuals finally designated as *C. chesapeakei* in black.

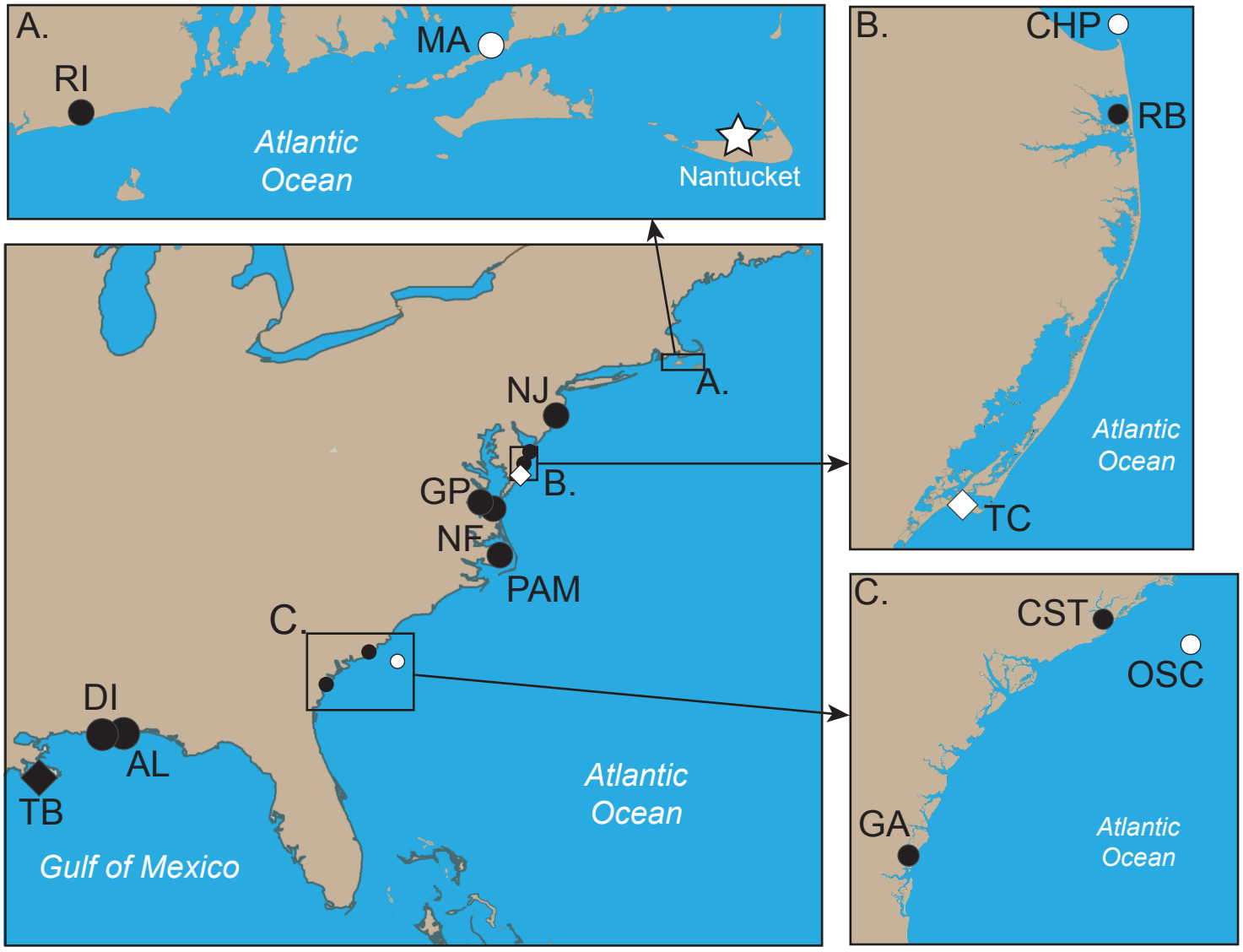

A.
RI
MA
Atlantic Ocean
Nantucket
B.
CHP
RB
Atlantic Ocean
TC
NJ
A.
GP
B.
NF
PAM
C.
DI
AL
TB
Gulf of Mexico
Atlantic Ocean
C.
CST
OSC
GA
Atlantic Ocean

Figure 3(on next page)

Various morphs of *C. quinquecirrha s.l*.

Figure 3: Various morphs of *C. quinquecirrha s.l*. A) Offshore South Carolina (OSC); B) Sample taken from offshore Georgia; C) Englehard, NC (PAM); D) White Chesapeake Bay color morph (Choptank River, MD); E) Red-striped Chesapeake Bay color morph (York River, VA). Note that medusae from A-B have 5 tentacles per octant, while C-E appear have three tentacles per octant. Medusae in 3A and 3C were included in this study's phylogenetic analyses. (3A: OSC1; 3C: PAM1). A-B represent individuals finally designated as *C. quinquecirrha*; C-E represent individuals finally designated as *C. chesapeakei*.

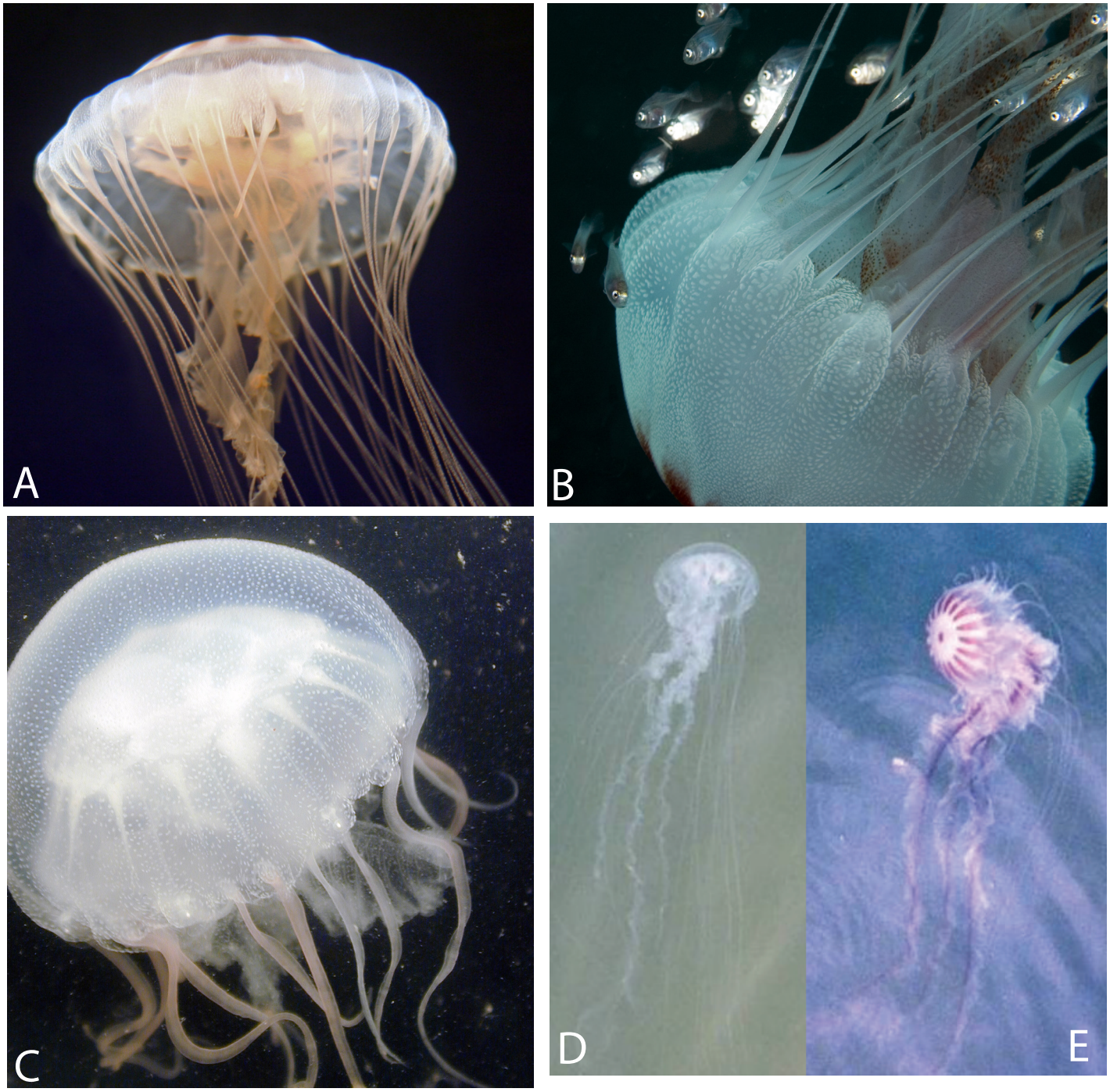

A
B
C
D
E

Figure 4(on next page)

Pelagiidae *COI* Phylogeny

Figure 4: Pelagiidae *COI* Phylogeny . Bayesian Inference (BI) *COI* tree reconstructed from CLUSTAL alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥ 0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥ 50%. ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the TPM2uf+I+G model of sequence evolution (-lnl 5451.81154) as determined by jMODELTEST v2.1.7 (Darriba et al. 2012) . Abbreviations refer to Tables 1-2. Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *C. quinquecirrha*. Norfolk (VA) individuals NF1-NF3 were identified as white Chesapeake Bay color morph and individuals NF4-NF5 as red-striped Chesapeake Bay color morph (Figure 3D-E).

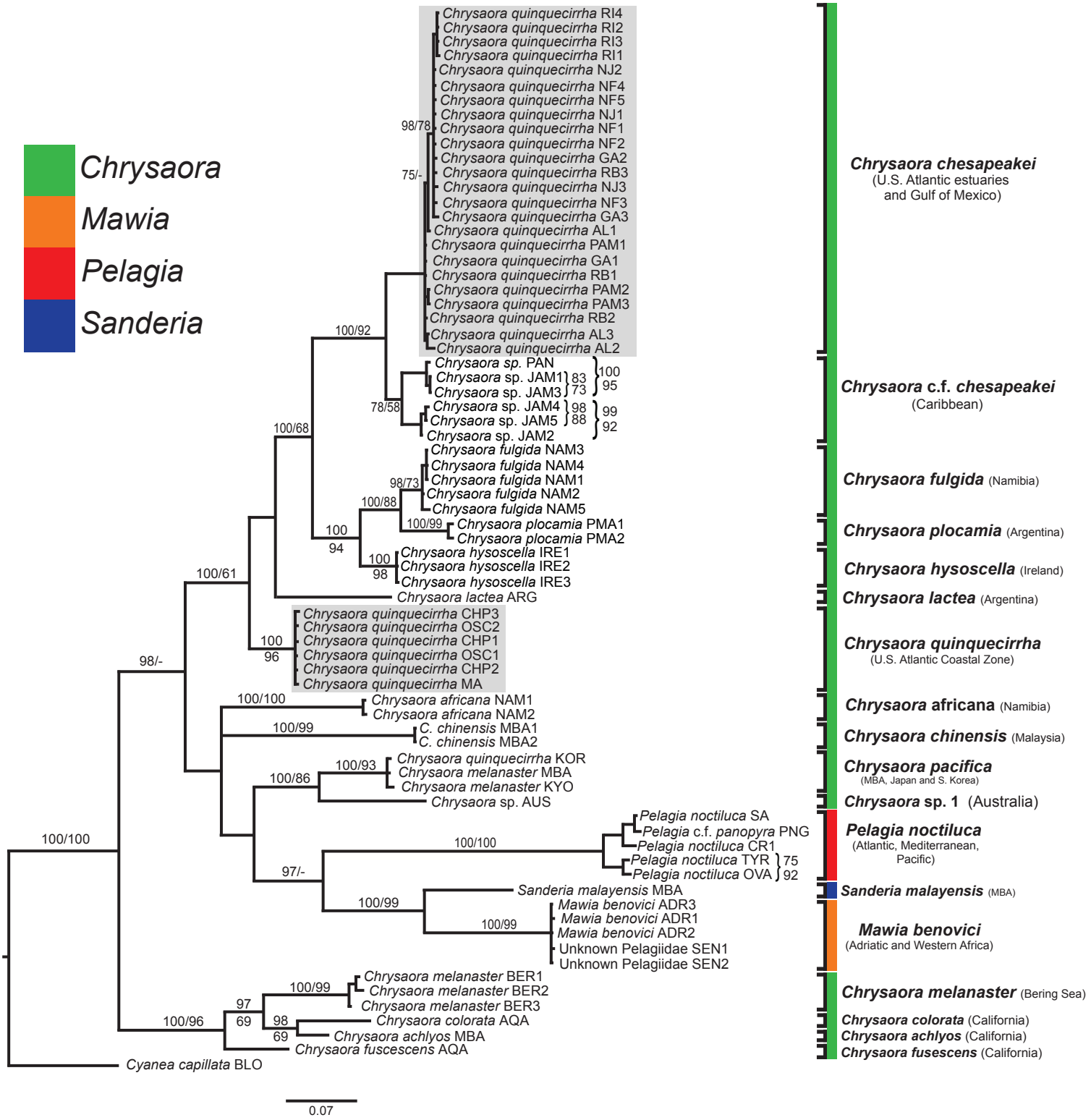

Chrysaora
Mawia
Pelagia
Sanderia
Chrysaora quinquecirrha RI4
Chrysaora quinquecirrha RI2
Chrysaora quinquecirrha RI3
Chrysaora quinquecirrha RI1
Chrysaora quinquecirrha NJ2
Chrysaora quinquecirrha NF4
Chrysaora quinquecirrha NF5
Chrysaora quinquecirrha NJ1
Chrysaora quinquecirrha NF1
Chrysaora quinquecirrha NF2
Chrysaora quinquecirrha GA2
Chrysaora quinquecirrha RB3
Chrysaora quinquecirrha NJ3
Chrysaora quinquecirrha NF3
Chrysaora quinquecirrha GA3
Chrysaora quinquecirrha AL1
Chrysaora quinquecirrha PAM1
Chrysaora quinquecirrha GA1
Chrysaora quinquecirrha RB1
Chrysaora quinquecirrha PAM2
Chrysaora quinquecirrha PAM3
Chrysaora quinquecirrha RB2
Chrysaora quinquecirrha AL3
Chrysaora quinquecirrha AL2
Chrysaora sp. PAN
Chrysaora sp. JAM1
Chrysaora sp. JAM3
Chrysaora sp. JAM4
Chrysaora sp. JAM5
Chrysaora sp. JAM2
Chrysaora fulgida NAM3
Chrysaora fulgida NAM4
Chrysaora fulgida NAM1
Chrysaora fulgida NAM2
Chrysaora fulgida NAM5
Chrysaora plocamia PMA1
Chrysaora plocamia PMA2
Chrysaora hysoscella IRE1
Chrysaora hysoscella IRE2
Chrysaora hysoscella IRE3
Chrysaora lactea ARG
Chrysaora quinquecirrha CHP3
Chrysaora quinquecirrha OSC2
Chrysaora quinquecirrha CHP1
Chrysaora quinquecirrha OSC1
Chrysaora quinquecirrha CHP2
Chrysaora quinquecirrha MA
Chrysaora africana NAM1
Chrysaora africana NAM2
C. chinensis MBA1
C. chinensis MBA2
Chrysaora quinquecirrha KOR
Chrysaora melanaster MBA
Chrysaora melanaster KYO
Chrysaora sp. AUS
Pelagia noctiluca SA
Pelagia c.f. panopyra PNG
Pelagia noctiluca CR1
Pelagia noctiluca TYR
Pelagia noctiluca OVA
Sanderia malayensis MBA
Mawia benovici ADR3
Mawia benovici ADR1
Mawia benovici ADR2
Unknown Pelagiidae SEN1
Unknown Pelagiidae SEN2
Chrysaora melanaster BER1
Chrysaora melanaster BER2
Chrysaora melanaster BER3
Chrysaora colorata AQA
Chrysaora achlyos MBA
Chrysaora fuscescens AQA
Cyanea capillata BLO
0.07
Chrysaora chesapeakei (U.S. Atlantic estuaries and Gulf of Mexico)
Chrysaora c.f. chesapeakei (Caribbean)
Chrysaora fulgida (Namibia)
Chrysaora plocamia (Argentina)
Chrysaora hysoscella (Ireland)
Chrysaora lactea (Argentina)
Chrysaora quinquecirrha (U.S. Atlantic Coastal Zone)
Chrysaora africana (Namibia)
Chrysaora chinensis (Malaysia)
Chrysaora pacifica (MBA, Japan and S. Korea)
Chrysaora sp. 1 (Australia)
Pelagia noctiluca (Atlantic, Mediterranean, Pacific)
Sanderia malayensis (MBA)
Mawia benovici (Adriatic and Western Africa)
Chrysaora melanaster (Bering Sea)
Chrysaora colorata (California)
Chrysaora achlyos (California)
Chrysaora fusescens (California)

Figure 5(on next page)

Pelagiidae *16S* Phylogeny

Figure 5: Pelagiidae *16S* Phylogeny . Bayesian Inference (BI) *16S* tree reconstructed from MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥ 0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥ 50%. ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the TIM2+I+G model of sequence evolution (-lnl 3641.97519) as determined by jMODELTEST v2.1.7 (Darriba et al. 2012) . Gray arrows indicate nodes that are alternated in the ML tree. Abbreviations refer to Tables 1-2. Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *C. quinquecirrha* s.l. Norfolk (VA) individuals NF1-NF3 were identified as white morph and individuals NF4-NF5 as red-striped bell morphs (Figure 3D-E).

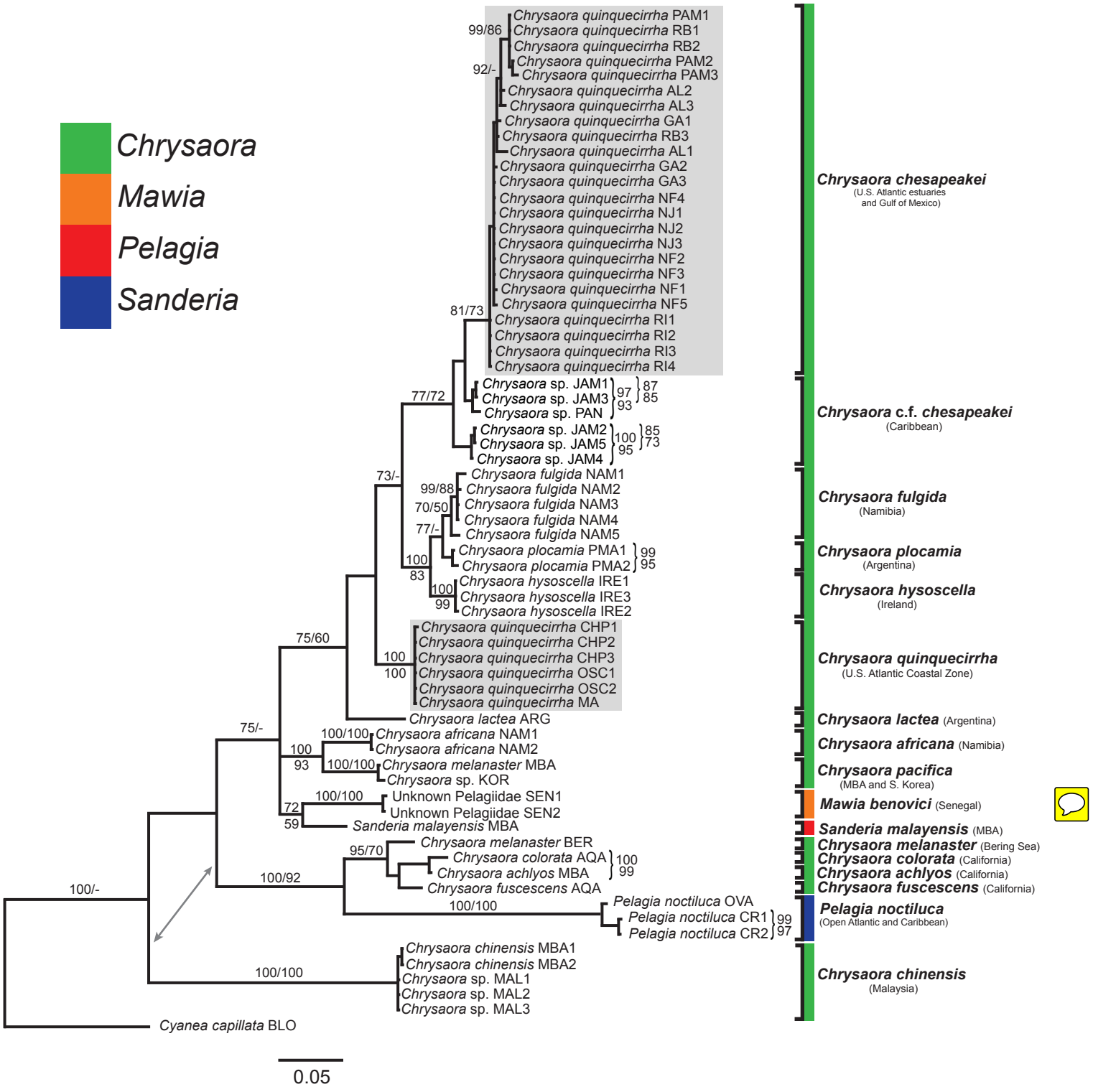

Chrysaora
Mawia
Pelagia
Sanderia
Chrysaora quinquecirrha PAM1
Chrysaora quinquecirrha RB1
Chrysaora quinquecirrha RB2
Chrysaora quinquecirrha PAM2
Chrysaora quinquecirrha PAM3
Chrysaora quinquecirrha AL2
Chrysaora quinquecirrha AL3
Chrysaora quinquecirrha GA1
Chrysaora quinquecirrha RB3
Chrysaora quinquecirrha AL1
Chrysaora quinquecirrha GA2
Chrysaora quinquecirrha GA3
Chrysaora quinquecirrha NF4
Chrysaora quinquecirrha NJ1
Chrysaora quinquecirrha NJ2
Chrysaora quinquecirrha NJ3
Chrysaora quinquecirrha NF2
Chrysaora quinquecirrha NF3
Chrysaora quinquecirrha NF1
Chrysaora quinquecirrha NF5
Chrysaora quinquecirrha RI1
Chrysaora quinquecirrha RI2
Chrysaora quinquecirrha RI3
Chrysaora quinquecirrha RI4
Chrysaora sp. JAM1
Chrysaora sp. JAM3
Chrysaora sp. PAN
Chrysaora sp. JAM2
Chrysaora sp. JAM5
Chrysaora sp. JAM4
Chrysaora fulgida NAM1
Chrysaora fulgida NAM2
Chrysaora fulgida NAM3
Chrysaora fulgida NAM4
Chrysaora fulgida NAM5
Chrysaora plocamia PMA1
Chrysaora plocamia PMA2
Chrysaora hysoscella IRE1
Chrysaora hysoscella IRE3
Chrysaora hysoscella IRE2
Chrysaora quinquecirrha CHP1
Chrysaora quinquecirrha CHP2
Chrysaora quinquecirrha CHP3
Chrysaora quinquecirrha OSC1
Chrysaora quinquecirrha OSC2
Chrysaora quinquecirrha MA
Chrysaora lactea ARG
Chrysaora africana NAM1
Chrysaora africana NAM2
Chrysaora melanaster MBA
Chrysaora sp. KOR
Unknown Pelagiidae SEN1
Unknown Pelagiidae SEN2
Sanderia malayensis MBA
Chrysaora melanaster BER
Chrysaora colorata AQA
Chrysaora achlyos MBA
Chrysaora fuscescens AQA
Pelagia noctiluca OVA
Pelagia noctiluca CR1
Pelagia noctiluca CR2
Chrysaora chinensis MBA1
Chrysaora chinensis MBA2
Chrysaora sp. MAL1
Chrysaora sp. MAL2
Chrysaora sp. MAL3
Cyanea capillata BLO
0.05
Chrysaora chesapeakei
(U.S. Atlantic estuaries and Gulf of Mexico)
Chrysaora c.f. chesapeakei
(Caribbean)
Chrysaora fulgida
(Namibia)
Chrysaora plocamia
(Argentina)
Chrysaora hysoscella
(Ireland)
Chrysaora quinquecirrha
(U.S. Atlantic Coastal Zone)
Chrysaora lactea (Argentina)
Chrysaora africana (Namibia)
Chrysaora pacifica
(MBA and S. Korea)
Mawia benovici (Senegal)
Sanderia malayensis (MBA)
Chrysaora melanaster (Bering Sea)
Chrysaora colorata (California)
Chrysaora achlyos (California)
Chrysaora fuscescens (California)
Pelagia noctiluca
(Open Atlantic and Caribbean)
Chrysaora chinensis
(Malaysia)

Figure 6(on next page)

Pelagiidae *28S* Phylogeny

Figure 6: Pelagiidae *28S* Phylogeny . Bayesian Inference (BI) *28S* tree reconstructed from MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥ 0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥ 50%. ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the TrNef+I+G model of sequence evolution (-lnl 3817.02691) as determined by jMODELTEST v2.1.7 (Darriba et al. 2012) . Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *C. quinquecirrha*.

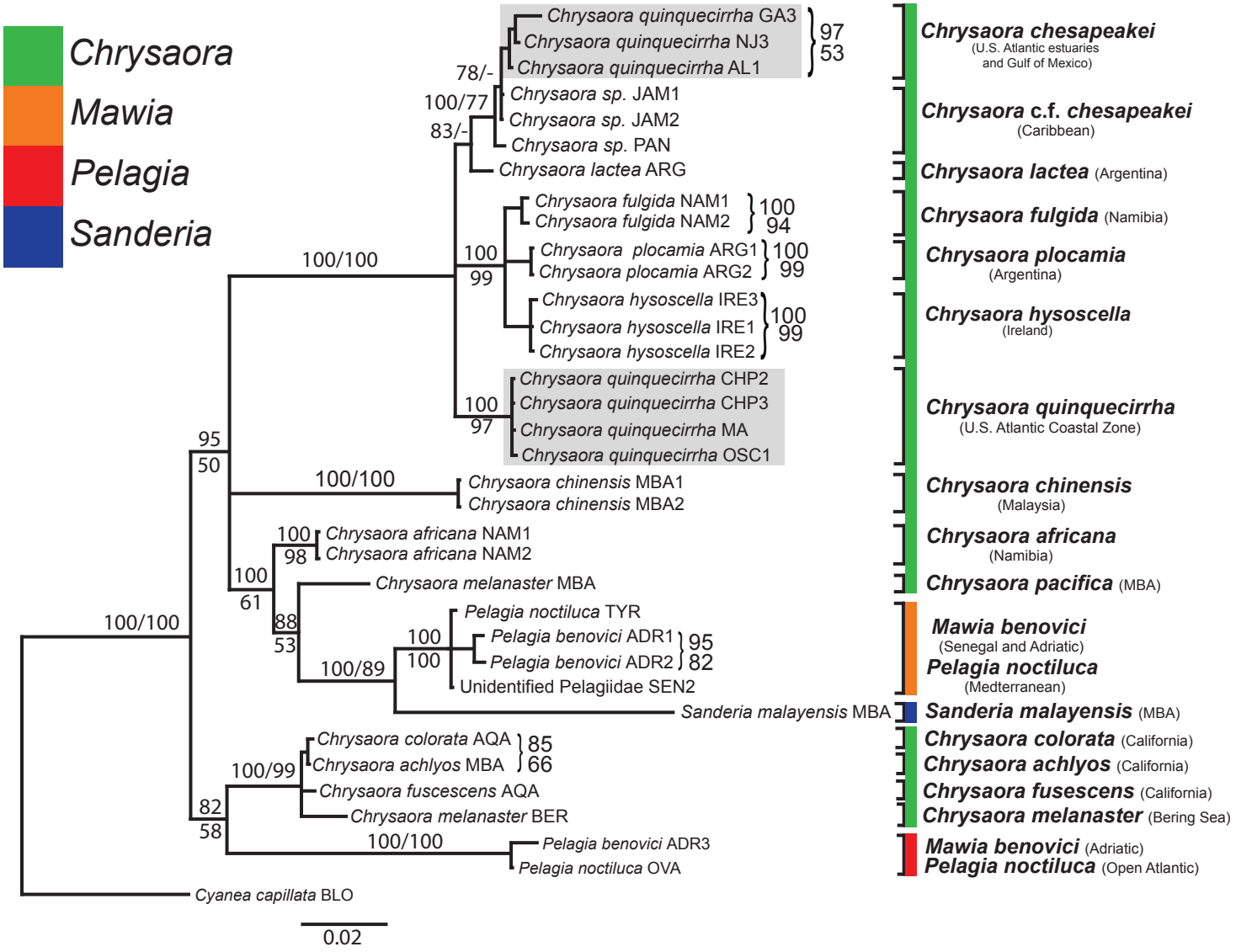

Chrysaora
Mawia
Pelagia
Sanderia
Chrysaora quinquecirrha GA3
Chrysaora quinquecirrha NJ3
Chrysaora quinquecirrha AL1
Chrysaora sp. JAM1
Chrysaora sp. JAM2
Chrysaora sp. PAN
Chrysaora lactea ARG
Chrysaora fulgida NAM1
Chrysaora fulgida NAM2
Chrysaora plocamia ARG1
Chrysaora plocamia ARG2
Chrysaora hysoscella IRE3
Chrysaora hysoscella IRE1
Chrysaora hysoscella IRE2
Chrysaora quinquecirrha CHP2
Chrysaora quinquecirrha CHP3
Chrysaora quinquecirrha MA
Chrysaora quinquecirrha OSC1
Chrysaora chinensis MBA1
Chrysaora chinensis MBA2
Chrysaora africana NAM1
Chrysaora africana NAM2
Chrysaora melanaster MBA
Pelagia noctiluca TYR
Pelagia benovici ADR1
Pelagia benovici ADR2
Unidentified Pelagiidae SEN2
Sanderia malayensis MBA
Chrysaora colorata AQA
Chrysaora achlyos MBA
Chrysaora fuscescens AQA
Chrysaora melanaster BER
Pelagia benovici ADR3
Pelagia noctiluca OVA
Cyanea capillata BLO
0.02
Chrysaora chesapeakei (U.S. Atlantic estuaries and Gulf of Mexico)
Chrysaora c.f. chesapeakei (Caribbean)
Chrysaora lactea (Argentina)
Chrysaora fulgida (Namibia)
Chrysaora plocamia (Argentina)
Chrysaora hysoscella (Ireland)
Chrysaora quinquecirrha (U.S. Atlantic Coastal Zone)
Chrysaora chinensis (Malaysia)
Chrysaora africana (Namibia)
Chrysaora pacifica (MBA)
Mawia benovici (Senegal and Adriatic)
Pelagia noctiluca (Medterranean)
Sanderia malayensis (MBA)
Chrysaora colorata (California)
Chrysaora achlyos (California)
Chrysaora fusescens (California)
Chrysaora melanaster (Bering Sea)
Mawia benovici (Adriatic)
Pelagia noctiluca (Open Atlantic)

Figure 7 (on next page)

Pelagiidae Combined Phylogeny

Figure 7: Pelagiidae Combined Phylogeny . Bayesian Inference (BI) tree of the combined dataset applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%. ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the GTR+I+G model of sequence evolution (-lnl 11924.23655) as determined by jMODELTEST v2.1.7 (Darriba et al. 2012) . Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *C. quinquecirrha*.

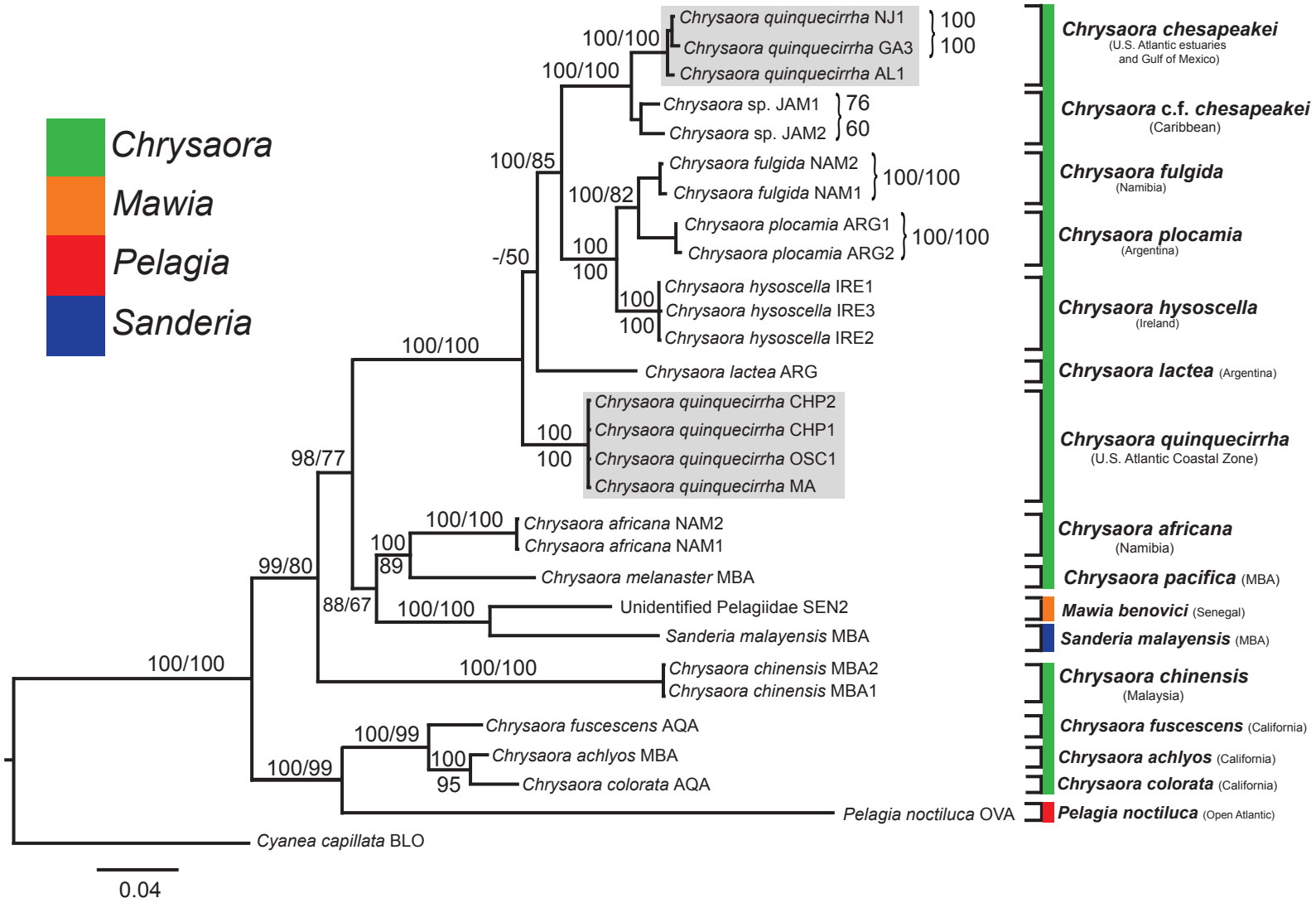
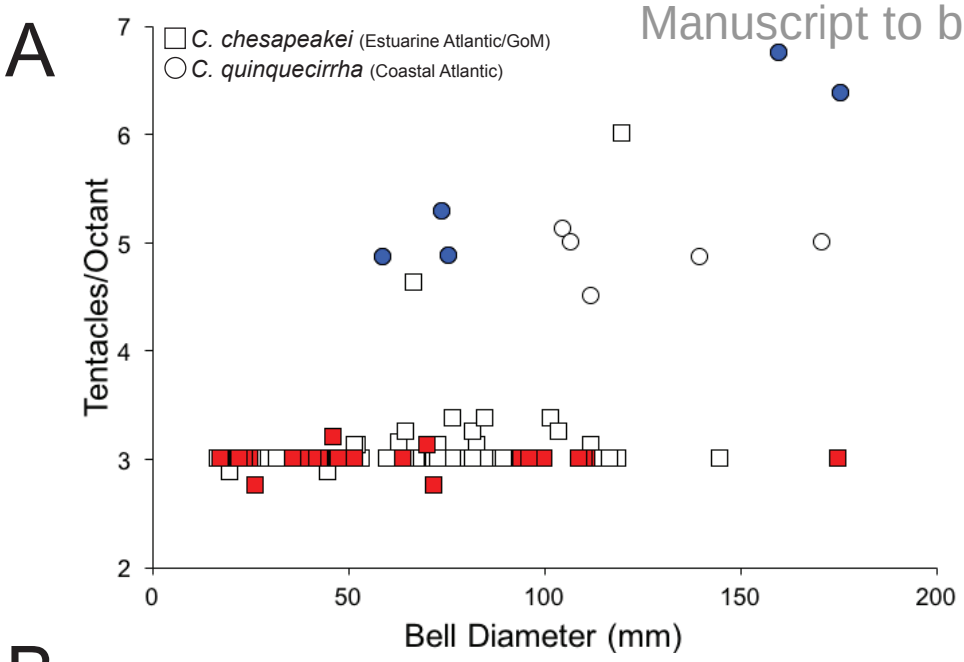
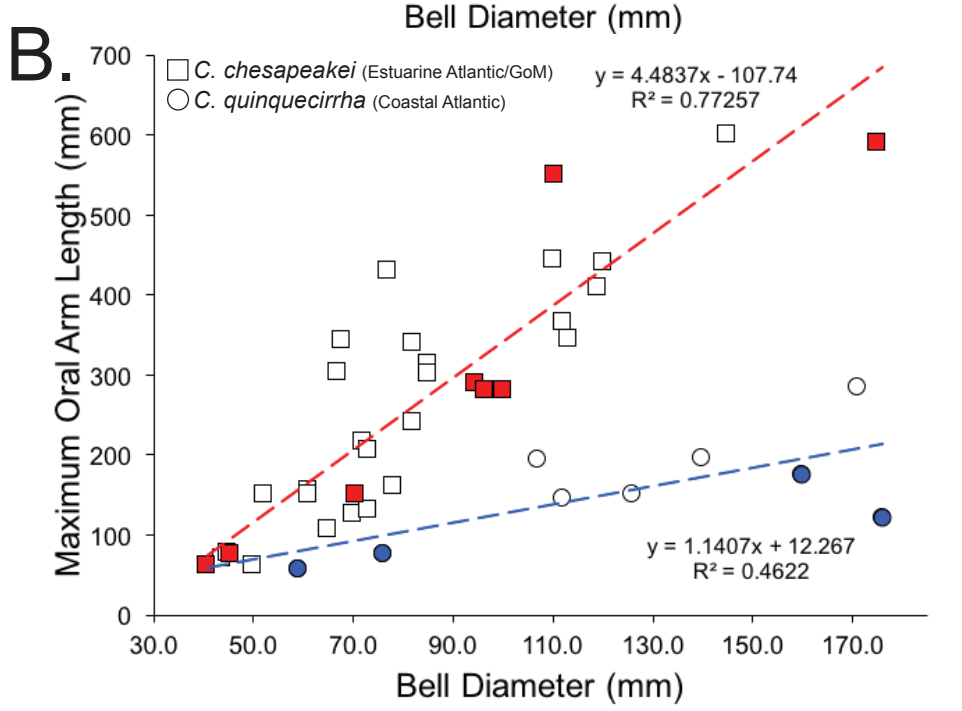

Chrysaora
Mawia
Pelagia
Sanderia
Chrysaora quinquecirrha NJ1
Chrysaora quinquecirrha GA3
Chrysaora quinquecirrha AL1
Chrysaora sp. JAM1
Chrysaora sp. JAM2
Chrysaora fulgida NAM2
Chrysaora fulgida NAM1
Chrysaora plocamia ARG1
Chrysaora plocamia ARG2
Chrysaora hysoscella IRE1
Chrysaora hysoscella IRE3
Chrysaora hysoscella IRE2
Chrysaora lactea ARG
Chrysaora quinquecirrha CHP2
Chrysaora quinquecirrha CHP1
Chrysaora quinquecirrha OSC1
Chrysaora quinquecirrha MA
Chrysaora africana NAM2
Chrysaora africana NAM1
Chrysaora melanaster MBA
Unidentified Pelagiidae SEN2
Sanderia malayensis MBA
Chrysaora chinensis MBA2
Chrysaora chinensis MBA1
Chrysaora fuscescens AQA
Chrysaora achlyos MBA
Chrysaora colorata AQA
Pelagia noctiluca OVA
Cyanea capillata BLO
0.04
Chrysaora chesapeakei (U.S. Atlantic estuaries and Gulf of Mexico)
Chrysaora c.f. chesapeakei (Caribbean)
Chrysaora fulgida (Namibia)
Chrysaora plocamia (Argentina)
Chrysaora hysoscella (Ireland)
Chrysaora lactea (Argentina)
Chrysaora quinquecirrha (U.S. Atlantic Coastal Zone)
Chrysaora africana (Namibia)
Chrysaora pacifica (MBA)
Mawia benovici (Senegal)
Sanderia malayensis (MBA)
Chrysaora chinensis (Malaysia)
Chrysaora fuscescens (California)
Chrysaora achlyos (California)
Chrysaora colorata (California)
Pelagia noctiluca (Open Atlantic)

Figure 8(on next page)

Morphological evidence separating *C. quinquecirrha* and *C. chesapeakei*

Figure 8: Morphological evidence separating *C. quinquecirrha* and *C. chesapeakei* . A) Tentacle counts. Graph represents tentacles per octant against bell diameter (mm) for field collected and museum specimens. Squares represent animals taken from estuarine Atlantic and Gulf of Mexico regions (*C. chesapeakei*), while circles represent animals taken from coastal Atlantic regions (*C. quinquecirrha*). All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. B) Maximum oral arm measurements. Graph represents maximum oral arm length against bell diameter (mm) for field-collected and museum specimens. Squares represent animals taken from U.S. Atlantic estuaries and the Gulf of Mexico (*C. chesapeakei*), while circles represent animals taken from coastal Atlantic regions (*C. quinquecirrha*). Only animals with fully intact and extended oral arms were included. All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. C) Average size measurements for holotrichous A-isrohiza nematocysts (length vs. width), based on 10 nematocysts per. Error bars represent 95% confidence intervals (2*standard error). Squares represent nematocysts from estuarine Atlantic and Gulf of Mexico medusae (*C. chesapeakei*), while circles represent nematocysts from coastal Atlantic medusae (*C. quinquecirrha*). Photograph of an average sized A-isorhiza from *C. quinquecirrha* appears on the left and a photograph of an average size A-isorhiza from *C. chesapeakei* appears on the right. Scale bars=10 um. Photographs have been resized so that all error bars are the same size on the page to allow size comparisons. All animals with *16S* sequences matching the *C. chesapeakei* clade appear in red, while those whose sequences matched the *C. quinquecirrha* clade appear in blue. Triangles represent average values from Papenfuss (1936) for morphs identified as *Dactylometra quinquecirrha* (gray) or *Dactylometra quinquecirrha* var. *chesapeakei* (white).


A
C. chesapeakei (Estuarine Atlantic/GoM)
C. quinquecirrha (Coastal Atlantic)
Tentacles/Octant
Bell Diameter (mm)


B.
C. chesapeakei (Estuarine Atlantic/GoM)
C. quinquecirrha (Coastal Atlantic)
y = 4.4837x - 107.74
R² = 0.77257
y = 1.1407x + 12.267
R² = 0.4622
Maximum Oral Arm Length (mm)
Bell Diameter (mm)

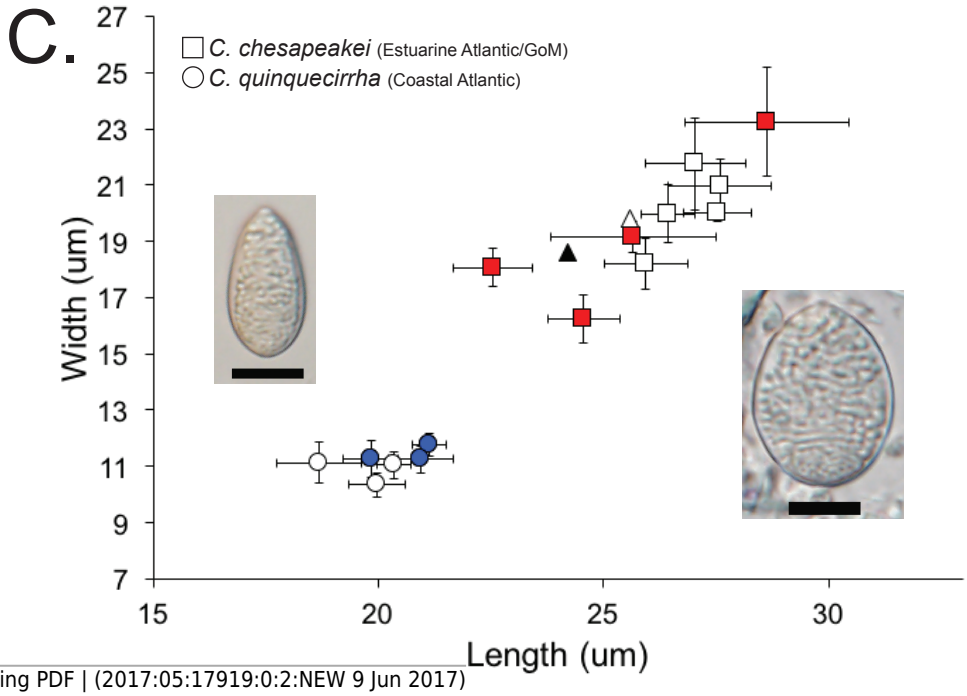
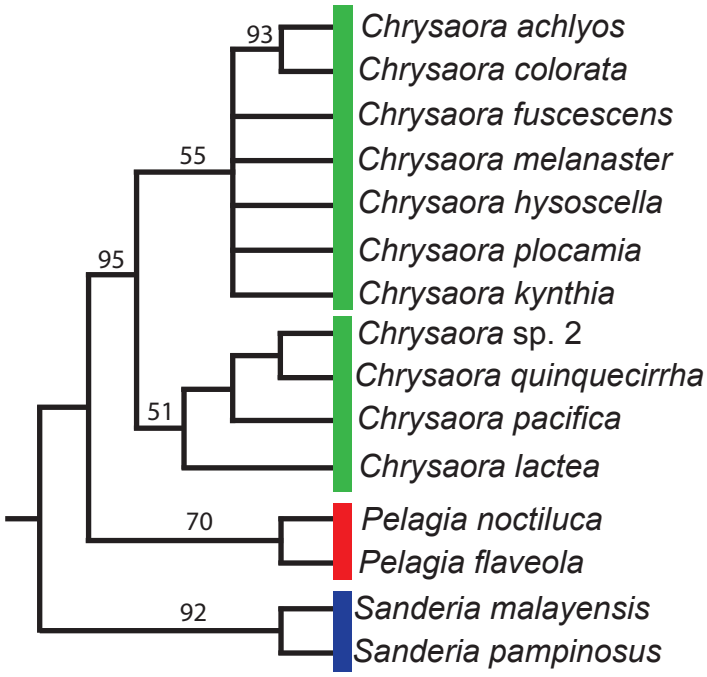

C.
C. chesapeakei (Estuarine Atlantic/GoM)
C. quinquecirrha (Coastal Atlantic)
Width (um)
Length (um)

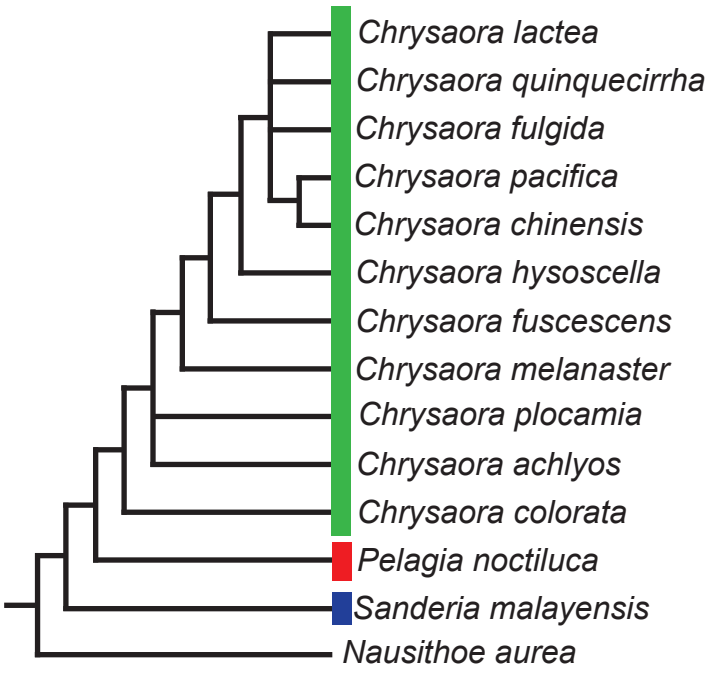
Figure 9(on next page)

Pelagiidae Evolution

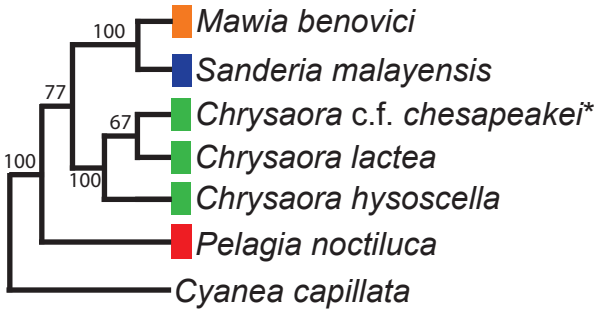
Figure 9: Pelagiidae Evolution . Cladograms showing genus-level relationships within the Pelagiidae family. Colors represent individual genera as shown. A) Gershwin and Collins (2002); B) Morandini and Marques (2010); C) Avian et al. (2016): DNA analysis based on nuclear *28S*; D) Avian et al. (2016): morphological analyses only; E) This study: Combined DNA analysis using sequence data from *COI, 16S* and *28S*. *In Avian et al. (2016), this sequence is marked as *Chrysaora* sp. AY920779. This sequence is included in our analysis and is part of the clade that we call *Chrysaora* c.f. *chesapeakei*. ^We include the *28S* phylogeny from Avian et al. (2016) because it has more species than their combined analysis but their generic conclusions are identical. Note that all previous hypotheses include a monophyletic *Chrysaora*.



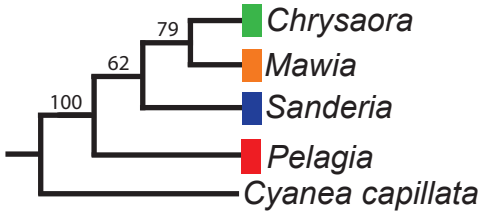
A. Gershwin and Collins, 2002



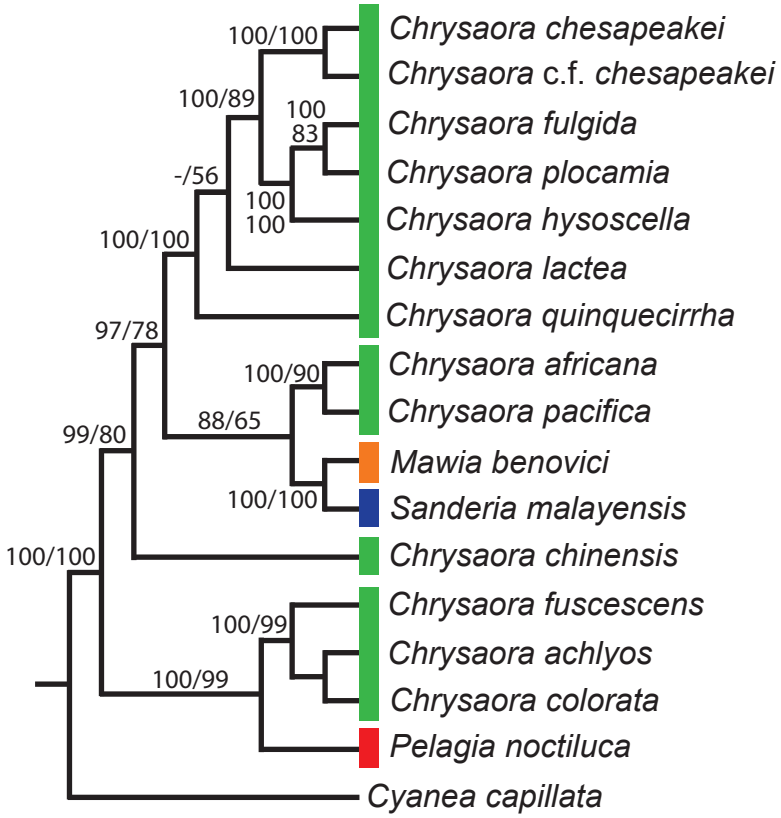
B. Morandini and Marques, 2010



C. Avian et al., 2016 (28S DNA analysis^)



D. Avian et al., 2016 (morphology)



E. This study (combined DNA analyses)