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 hinders progress in studying their biology and evolutionary adaptations that make them nuisance species, as well as 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 of all four pelagiid genera, including eleven of thirteen currently recognized species of *Chrysaora*. To 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 from 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 indicates that *Chrysaora quinquecirrha* Desor 1848 applies to the US Coastal Atlantic *Chrysaora* species (US Atlantic sea nettle), while the name *C. chesapeakei* Papenfuss 1936 applies to the US Atlantic estuarine and Gulf of Mexico *Chrysaora* species (US 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 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 hinders progress in understanding their biology and the evolutionary adaptations that make them nuisance species, as well as 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 of all four pelagiid genera, including eleven of thirteen currently recognized species of *Chrysaora*. To 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 from 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

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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; Diaz Briz et al. 2017). 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; but see Kayal et al. 2017) 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 2005; 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 (Gegenbaur 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, a 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; Hedgepeth 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 (Gegenbaur 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 Péron & Lesueur (1810), though both included species not recognized today as pelagiids. Gegenbaur (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 Janeiro. Based on established generic definitions, Piraino et al. (2014) placed an undescribed, presumably non-indigenous 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*, *Mawia* 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 (Figure S1). 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 2005; 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* (Blomsterdalen, Norway) 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 MAFFT v7.245 employing the E-INS-I strategy (Kato & 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* (TPM_{uf}+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 MEGA v7.0.14 (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 (USNM) 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; Östman & 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, formalin-preserved 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 MAFFT-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*

sequences fell into two distinct, highly diverged clades (Figures 4-7; Tables S3-S5), with one clade (*C. chesapeakei* – see Discussion and Systematics) made up of animals from U.S. Atlantic estuaries and the Gulf of Mexico animals and another (*C. quinquecirrha* – see Discussion and Systematics) made up of U.S. coastal Atlantic animals. Caribbean *Chrysaora* (Jamaica and Panama) formed a clade closely related to *C. chesapeakei* in all analyses (Figures 4-7). Aquarium animals previously identified as *C. melanaster* (AQA) were genetically distinct from *C. melanaster* collected from the Bering Sea (BER) in all analyses where both were included (Figures 4-6) and formed a clade with *Chrysaora pacifica* collected from South Korea (KOR) and Japan (KYO) for *COI* and/or *16S*. While aquarium collected *C. chinensis* formed a well-supported clade with field collected *C. chinensis* (MAL), analyses differed in where this species fell out in the trees (Figures 4-7). The unknown pelagiid collected from the western African coast (SEN) was nearly identical to the newly described *M. benovici* from the Mediterranean for *COI* (0.0-0.3% difference) and *28S* (0.0-0.2% difference) (Figures 4, 6).

Macromorphological and Nematocyst Analyses

A total of 120 medusae (57 field collected and 63 museum specimens) (Table S1) previously identified as *C. quinquecirrha* s.l. were examined for 19 quantitative and qualitative macromorphological characters taken from Gershwin and Collins (2002) and one new to this study (maximum oral arm length) (Table 3). Overall, three macromorphological characters differed significantly: tentacle number, lappet number and maximum oral arm length vs. bell diameter (Table 3). Animals collected from the estuarine Atlantic and all Gulf of Mexico sites (Table S1) had an average of 3.07 +/- 0.07 (95% CI) tentacles per octant, excluding two aberrant individuals (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 $\pm$ 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 3.00 $\pm$ 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 $\pm$ 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 $\pm$ 0.03 tentacles per octant and oral arms that were 2.80 $\pm$ 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 $\pm$ 0.78 tentacles per octant and oral arms that were on average 0.93 $\pm$ 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* for

16S, while all jellyfish from the larger group (estuarine Atlantic and Gulf of Mexico) that were sequenced for 16S 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 as *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 S2 (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 ($62.1 \pm 9.8\%$ [95% CI]), followed by O-isorhizas ($13.4 \pm 5.0\%$ [95% CI]), a-isorhizas ($12.4 \pm 2.8\%$ [95% CI]) and A-isorhizas ($12.2 \pm 3.7\%$ [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 S3A). Individuals varied considerably in proportions of nematocyst types (Figure S3B). 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 S3B). 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 ($t=3.623$, $p\text{-value}=0.0068$), with *C. chesapeakei* individuals averaging $16.5\pm3.4\%$ for A-isorhiza and *C. quinquecirrha* cnidomes averaging $7.8\pm3.4\%$.

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, 9E). 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, 9E). Including fewer published sequences gave the appearance of *Chrysaora* monophyly, which may

have biased the establishment 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 the paraphyly of *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, 9), 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 matter would be to which clade should the genus *Chrysaora* should 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 (Dawson & Martin 2001; Dawson 2003; Bayha & Dawson 2010). Future study will benefit from more detailed morphological analyses to identify additional characters that could then be mapped onto molecular phylogenies (e.g. Figure 7), as well as greater taxonomic sampling (e.g. two additional *Chrysaora* species accepted and two declared *nomen dubium* in Morandini & Marques (2010), more geographic samples of *Pelagia* and *Sanderia*). Both would allow for better resolution to define genera and better explain their evolutionary relationships.

Interspecific Evolutionary Relationships and Geographic Patterns

While our molecular phylogenies bear almost no resemblance to the morphology-based phylogenies within the currently defined genus *Chrysaora* (Gershwin & Collins 2002; Morandini & Marques 2010) (Figure 9), there are some relationships that occur in all phylogenies. All phylogenies agree on a close relationship between *C. achlyos* and *C. colorata* (Figure 9A, B, E). Our phylogeny is in general agreement with Morandini and Marques (2010) in delineating their basal ‘Pacific’ group (*C. achlyos*, *C. colorata*, *C. fuscescens*, *C. melanaster* and *C. plocamia*), except that our *C. plocamia* samples came from the Atlantic and occur in an ‘Atlantic’ group (Table 1; Figure 1). Morandini and Marques (2010) reasoned that this basal group may have provided ancient species that then invaded the Atlantic, splitting into various Atlantic groups. Our combined phylogeny (Figure 7) is in general agreement, with Pacific *Chrysaora* species generally occupying a more basal position in the tree compared to the Atlantic species. Major disagreements with Morandini & Marques (2010) include the placement of *C. chinensis* and *C. pacifica* (both Pacific jellies) as closely related to *C. quinquecirrha* and *C. lactea*, with the *C. pacifica* placement also a disagreement with Gershwin and Collins (2002). Likewise, the very 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, given questions surrounding geographic provenance and any unnatural interbreeding that might occur in a the aquarium system. 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. *Chrysaora 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 2005), 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 (USNM 33457a and USNM 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 (USNM 33457a: 27.59 x 20.98 um; USNM 56703b: 27.04 x 21.75 um; Figure 8C) and/or oral arm length (USNM 33457a: 4.54 times bell diameter; USNM 56703b: sample too degraded; Figure 8B).

It appears that Bigelow (1880) was correct that Chesapeake Bay *Chrysaora* that matured at 24 tentacles represented 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 in holotrichous a-isorhiza measurements, though without statistical support. However, for our Norfolk (VA) samples, white (NF1-NF3) and red-striped (NF4-NF5) morphs occurred in the same genetic clades for *16S* and *COI* (Figures 4-5) and we found no overall pattern of differentiation in our holotrichous a-isorhiza measurements (Figure S2A). Furthermore, for holotrichous A-isorhiza measurements, both morphs from Papenfuss (1936) are consistent with our U.S. Atlantic estuary/Gulf of Mexico group (Figure 8C). In summary, evidence from nematocyst measurements (Figure 8C), locality (Chesapeake Bay) and phylogenetic data (Figure 4-5) support the U.S. Atlantic estuarine/Gulf of Mexico group and both morphs from Papenfuss (1936) as representing the same species. Even though Papenfuss (1936) may have been mistaken in describing *D. quinquecirrha* var. *chesapeakei*, that name is taxonomically available based on Article 45.6.4 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 2015). As such, all animals from the U.S. Atlantic estuary/Gulf of Mexico lineage should be assigned to the elevated species name *Chrysaora chesapeakei* (Papenfuss, 1936). The placement of Gulf of Mexico medusae in *C. chesapeakei* differs from Morandini & Marques (2010), who placed them in the species *C. lactea*, based on similarities in octant tentacle orientation (2-3-1-3-2). However, our genetic data clearly separate these animals from the distantly related *C. lactea* (Figure 4-7) and the number of tentacles (approximately 3) and lack of tertiary tentacles in the Gulf of Mexico animals observed here and in Morandini and Marques (2010) (USNM 49733 and USNM 53826) make accurate determination of tertiary tentacle orientation problematic.

In addition to their taxonomic value, it is possible that some of the morphological characters that delineate *C. quinquecirrha* and *C. chesapeakei* may be related to adaptations to

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

581

582 *Chrysaora in the Caribbean*

583 *Chrysaora medusae* collected from the Caribbean Sea are genetically very similar to
 584 *Chrysaora chesapeakei*. *Chrysaora* in the Caribbean have historically been included in the
 585 species *C. lactea* (Mayer 1910; Morandini & Marques 2010), *C. quinquecirrha* (Perry & Larson
 586 2004) or *Chrysaora* sp. (Persad et al. 2003). Our Caribbean samples, limited only to Jamaica and
 587 the Bocas del Toro region of Panama, appear to be two lineages (both found in JAM) slightly
 588 diverged from each other (4.4-5.1% for *COI*) and from *C. chesapeakei* (6.2-7.7% for *COI*) from
 589 the US east coast estuaries and the Gulf of Mexico. These animals cannot be assigned to *C.*
 590 *lactea* (type locality=Rio de Janiero, Brazil), as was previously done by Mayer (1910) and
 591 Morandini and Marques (2010), since these animals are distantly related to *C. lactea* for most
 592 genetic regions examined (Figure 4-7). At present, it is unclear if the Caribbean forms represent
 593 distinct or incipient species and further study of them from across the region is necessary. For the
 594 time being, we advocate referring to Caribbean animals as *Chrysaora* c.f. *chesapeakei* ahead of a
 595 formal systematic redescription based on genetic and careful morphological examination.

596

597 *Chrysaora melanaster* and *C. pacifica*

598 Our phylogenetic data confirm the morphological conclusions in Morandini & Marques
 599 (2010) that Japanese *Chrysaora* historically identified as *C. melanaster*, and labeled as such in
 600 public aquaria worldwide for decades, are actually the distinct species *C. pacifica*. Kramp (1961)
 601 synonymized the Pacific *Chrysaora* species *C. melanaster* (Brandt 1835) and the Japanese
 602 jellyfish *C. pacifica* (Goette 1886) to *C. melanaster*. This identification convention made it into
 603 jellyfish identification books (e.g. Wrobel and Mills, 1998) and subsequently Japanese

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

614

615 *Chrysaora africana/fulgida*

616 Our phylogenies support the resurrection of *Chrysaora* species along the southwestern
 617 coast of Africa. Three species of *Chrysaora* were previously identified from the southwestern
 618 coast of Africa: *Chrysaora hysoscella* (Kramp 1955), *C. fulgida* (Reynaud 1830) and *C. africana*
 619 (Vanhöffen 1902). However, Kramp (1961) deemed *C. africana* a variant of *C. fulgida*, and
 620 Morandini & Marques (2010) placed all of these sightings within the species *C. fulgida*. All
 621 phylogenies indicate two distantly related species of *Chrysaora* from Namibian waters (Figures
 622 4-7), with those appearing superficially similar to *C. fulgida* (brown striped) or to *C. africana*
 623 (red tentacles) placed provisionally into these species. These designations are consistent with
 624 upcoming re-descriptions of *C. fulgida* and *C. africana* of S. Neethling (unpublished data) based
 625 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.

Mawia benovici

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

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, Figures 5-6 [mouth of Broadkill River, DE]. Kraeuter & Setzler (1975): 69, Figures 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: $20.25 [\pm 0.38] \mu\text{m} \times 11.27 [\pm 0.37] \mu\text{m}$ (Table 3;

Figure 8C); 2) larger tentacle number (more than 5 per octant) and 3) typically shorter maximum oral arm length (average: 1.24 ± 0.27 time bell diameter).

Material Examined: USNM 24496 ($n=1$; Buzzard's Bay, MA), USNM 53860 ($n=1$; Assateague Island, VA), USNM 53861 ($n=1$; Assateague Island, VA), USNM 54511 ($n=2$; Cape Henlopen, DE), USNM 56702 ($n=1$; Cape Henlopen, DE), USNM 1454776 - USNM 1454778, KMBCDE2, KMBCDE4 ($n=5$; Cape Henlopen, DE).

Description of holotype: No holotype located, no neotype designated.

Description of specimens: Bell diameter up to approximately 40 cm (observed 59.0-176.0 mm), almost hemispherical. Exumbrella finely granulated with small, inconspicuous marks (papillae); exumbrellar color varies from entirely transparent white to white with inconspicuous radial markings. Tentacle number approximately 5 tentacles per octant, but can be more (average 5.28 ± 0.48) (Table 3; Figure 8A); primary tentacle central, secondary and tertiary tentacles laterally (3-2-1-2-3) with additional tentacles originating toward the rhopalia; lappets rounded typically 48 or more (average 6.26 ± 0.46 per octant); 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 $1/8$ of bell diameter. Oral arms v-shaped with frills emanating from tube-like structure; straight without spiral; curved, frilled edges taper toward distal end of oral arms. Oral arms short, approximately the same length as bell diameter (average 1.24 ± 0.27 times bell diameter). Oral arms typically transparent white. 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 $\pm$ 95% CI x Width $\pm$ 95% CI)

Holotrichous A-isorhizas: $20.15 \pm 0.33 \times 11.13 \pm 0.24 \mu\text{m}$;

Holotrichous a-isorhizas: $8.27 \pm 0.49 \times 4.22 \pm 0.07 \mu\text{m}$;

Holotrichous O-isorhizas: $21.63 \pm 0.39 \times 18.91 \pm 0.78 \mu\text{m}$;

Heterotrichous microbasic rhopaloids: $13.58 \pm 0.19 \times 8.09 \pm 0.09 \mu\text{m}$;

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, Figures 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, Figures 12, 21

737 [Chesapeake Bay; white colored morph based on A-isorhiza measurements].
 738 *Chrysaora quinquecirrha*: Kramp (1961): 327-328 [parts of description covers both *C.*
 739 *quinquecirrha* and *C. chesapeakei*]. Rice & Powell (1970): 180-186 [Chesapeake Bay]. Burke
 740 (1976): 20, 22-28 [Mississippi Sound, Gulf of Mexico]. Calder (1971): 270-274 [Gloucester
 741 Point (VA) – Chesapeake Bay]. Calder (1972): 40-43, Figures 1-4 [Chesapeake Bay, Pamlico
 742 Sound, Gulf of Mexico]. Loeb (1972): 279-291 [Chesapeake Bay]. Loeb (1973): 144-147
 743 [Chesapeake Bay]. Loeb & Blanquet (1973): 150-157 [Chesapeake Bay]. Calder (1974b): 326-
 744 333 [Chesapeake Bay]. Loeb (1974): 423-432 [Chesapeake Bay]. Blanquet & Wetzel (1975):
 745 181-192 [Chesapeake Bay]. Cargo (1975): 145-154 [Chesapeake Bay]. Kraeuter & Setzler
 746 (1975): 69, Figures 1-2 [Doboy Sound (GA)]. Loeb & Gordon (1975): 37-41 [Chesapeake Bay].
 747 Lin & Zubkoff (1976): 37-41 [Chesapeake Bay]. Calder (1977): 13-19 [Gloucester Point, MD –
 748 Chesapeake Bay]. Clifford & Cargo (1978): 58-60 [Patuxent River, MD – Chesapeake Bay].
 749 Cargo (1979): 279-286 [Chesapeake Bay]. Cargo & Rabenold (1980): 20-26 [Patuxent River
 750 (MD)]. Hutton et al. (1986): 154-155 [Chesapeake Bay]. Cargo & King (1990): 486-491
 751 [Chesapeake Bay]. Purcell et al. (1991): 103-111 [Choptank River, MD – Chesapeake Bay].
 752 Nemazie, Purcell & Glibert (1993): 451-458 [Chesapeake Bay]. Purcell, White & Roman (1994):
 753 263-278 [Chesapeake Bay]. Burnett et al. (1996): 1377-1383 [Chesapeake Bay]; Houck et al.
 754 (1996): 771-778 [St. Margaret's, MD – Chesapeake Bay]. Olesen, Purcell & Stoecker (1996):
 755 149-158 [Broad Creek (MD) – Chesapeake Bay]. Ford et al. (1997): 355-361 (Choptank River
 756 (MD) – Chesapeake Bay]. Kreps, Purcell & Heidelberg (1997): 441-446 [Choptank River (MD)
 757 – Chesapeake Bay]. Wright & Purcell (1997): 332-338 [Patuxent River (MD) – Chesapeake
 758 Bay]. Suchman & Sullivan (1998): 237-244 [Green Hill Pond (RI)]. Purcell, Malej & Benović
 759 (1999): 241-263 [Chesapeake Bay]. Purcell et al. (1999): 187-196 [Choptank River (MD) –

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

783 Pineiro & Kimmel (2015): 1965-1975 [Pamlico Sound (NC). Meredith, Gaynor & Bologna
 784 (2016): 6248-6266 [Barnegat Bay (NJ)]. Tay & Hood (2017): 227-242 [Choptank River (MD),
 785 Chesapeake Bay].
 786
 787 **Diagnosis:** Living medusae up to 20 cm (observed 17.0-175.0 mm; average: 63.0
 788 mm); Tentacles typically number 24 or 3 per octant (average 3.07 ± 0.07); primary tentacle central
 789 and secondary tentacles lateral (2-1-2); rarely, additional tentacles arise lateral to secondary
 790 tentacles (3-2-1-2-3) and are typically undeveloped; marginal lappets rounded and typically 32 or
 791 4 per octant (average 4.08 ± 0.06); rhopalar lappets are typically about the same size as
 792 tentacular lappets; can be differentiated from *C. quinquecirrha* based on 1) larger size of
 793 holotrichous A-isorhiza nematocysts: $26.21 [\pm 0.50] \mu\text{m} \times 19.74 [\pm 0.55] \mu\text{m}$; 2) smaller tentacle
 794 number (~3 tentacles per octant); and 3) larger maximum oral arm length (average: 3.00 ± 0.39
 795 times bell diameter).

796
 797 **Material Examined.** Neotype: - USNM 1454948 – (Gloucester Point, MD –
 798 Chesapeake Bay). Other comparative specimens: USNM 57925 ($n=9$; Orange Inlet, NC), USNM
 799 56758 ($n=5$; Charlestown Pond, RI), USNM 33456 ($n=4$; Plum Point, MD), USNM 49733 ($n=1$;
 800 Alligator Harbor, FL), USNM 53826 ($n=2$; Timbalier Bay, LA), USNM 56703 ($n=2$;
 801 Chesapeake Bay 37.23 N 76.04 W), USNM 56704 ($n=4$; Chesapeake Bay 37.23 N 76.04 W),
 802 USNM 53870 ($n=3$; Beaufort, NC), USNM 53828 ($n=2$; Drum Point, MD), USNM 33458 ($n=3$;
 803 Plum Point, MD), USNM 33457 ($n=4$; Plum Point, MD), USNM 55621 ($n=6$; near Chesapeake
 804 Beach, MD), USNM 53867 ($n=1$; Arundel on the Bay, MD), USNM 54404 ($n=1$; Chesapeake
 805 Bay 37.23 N 76.04 W), USNM 33121 ($n=6$; Arundel on the Bay, MD), USNM 42155 ($n=2$;

806 Louisiana, Gulf of Mexico), USNM 54372 ($n=1$; Lake Pontchartrain, LA); USNM 1454941 -
 807 USNM 1454943, KMBCSC1, KMBCSC4-KMBCSC5, KMBCSC7 ($n=7$; Charleston Harbor,
 808 SC), USNM 1454944 – USNM 1454951, KMBGVA1, KMBGVA5, KMBGVA7, KMBGVA10
 809 ($n=12$; Gloucester Point, VA), KMBCRI1-KMBCRI14 ($n=14$; Charlestown Pond, RI),
 810 KMBRDE1-KMBRDE16 ($n=16$; Rehoboth Bay, DE), USNM 1454956, KMBDIA2-KMBDIA3
 811 ($n=3$; Dauphin Island, AL).

812

813 **Description of neotype specimen:** USNM 1454948. Bell diameter 110.4 mm, almost
 814 hemispherical. Exumbrella white/clear with granulated surface of small white marks. 8 rhopalia.
 815 No ocelli. Deep rhopalar clefts; deep sensory pits. Marginal lappets rounded, 32 total or 4 per
 816 octant made up of two rhopalar lappets and two tentacular lappets. Lappet size barely
 817 heterogeneous, with rhopalar lappets about the same width as tentacular lappets but longer.
 818 Tentacle number 24 or 3 per octant, with primary tentacle surrounded by two secondary tentacles
 819 (2-1-2), primary tentacle longer than secondary tentacles, up to 3-4 times bell diameter.
 820 Tentacles are white, slightly pinkish. Tentacle clefts of varied depth with primary clefts deeper
 821 than secondary clefts. Radial and ring musculature not obvious. Brachial disc circular. Pillars
 822 evident. No quadralinga. Subgenital ostia rounded, approximately 1/10 of bell diameter. Oral
 823 arms white, v-shaped with frills emanating from tube-like structure. Oral arms straight without
 824 spiral curved, frilled edges taper toward distal end of oral arms. Orals arms long, approximately
 825 5 (4.98) times bell diameter. 4 semi-circular gonads, white (a bit orange), well developed within
 826 pouch outlining gastric filaments. 16 stomach pouches bounded by 16 septae. Septae bent at 45-
 827 degree angle distally towards the rhopalia terminating near tentacle in rhopalar lappet, resulting
 828 in tentacular pouches being somewhat larger than rhopalar pouches distally.

829 Cnidome (tentacle): Average dimensions (Length $\pm$ 95% CI x Width $\pm$ 95% CI)

830 Holotrichous A-isorhizas: $25.66 \pm 0.83 \times 19.16 \pm 0.54 \mu\text{m}$;

831 Holotrichous a-isorhizas $7.77 \pm 0.20 \times 4.17 \pm 0.10 \mu\text{m}$;

832 Holotrichous O-isorhizas $22.02 \pm 0.30 \times 19.95 \pm 0.24 \mu\text{m}$;

833 Heterotrichous microbasic rhopaloids $12.35 \pm 0.47 \mu\text{m} \times 8.55 \pm 0.55 \mu\text{m}$.

834

835 **Description of other specimens:** Bell diameter up to approximately 20 cm (observed 17.0-

836 175.0 mm), almost hemispherical but flattened in small individuals. Exumbrellar finely

837 granulated with small, inconspicuous marks (papillae); exumbrellar color varies considerably,

838 varying from all white to a completely brown or red colored bell, to a bell with radial lines of

839 red/brown with a spot in the center of the bell. Radial lines may be relatively inconspicuous

840 without a noticeable spot in the center. Tentacles typically number 24 or 3 per octant (average

841 3.07 ± 0.07), with primary tentacle surrounded by two secondary tentacles (2-1-2), primary

842 tentacle longer than secondary tentacles, up to 3-4 times bell diameter. In some rare cases, small

843 tentacles may occur laterally to secondary tentacle, occurring between the secondary tentacle and

844 rhopalium. In almost all cases, this tentacle is similar in size to or smaller than the lappets

845 surrounding it. In very rare cases (twice observed), about 5 or more tentacles per octant have

846 been seen, though these medusae had aberrant tentacle patterns overall (e.g. more than one

847 tentacle emerging from same spot, tentacles emerging below lappet). Tentacles are white,

848 slightly pinkish. Marginal lappets rounded and typically 32 or 4 per octant (average 4.08 ± 0.06).

849 Tentacle clefts of varied depth with primary clefts deeper than secondary clefts, which are deeper

850 than rare tertiary clefts. Radial and ring musculature not obvious. Brachial disc circular. Pillars

851 evident. No quadralinga. Subgenital ostia rounded, approximately 1/10 of bell diameter. Oral

arms v-shaped with frills emanating from tube-like structure; straight without spiral; curved, frilled edges taper toward proximal end of oral arms. Oral arms long, approximately 3 times bell diameter on average (as much as 5.6 times bell diameter). Oral arms vary in color, from transparent white, to red or brown colored tubule surrounded by pinkish frilled edges. 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 $\pm$ 95% CI x Width $\pm$ 95% CI)

Holotrichous A-isorhizas: $26.21 \pm 0.50 \times 19.74 \pm 0.55 \mu\text{m}$;

Holotrichous a-isorhizas: $7.88 \pm 0.13 \times 4.29 \pm 0.07 \mu\text{m}$;

Holotrichous O-isorhizas: $23.10 \pm 0.43 \times 20.75 \pm 0.62 \mu\text{m}$;

Heterotrichous microbasic rhopaloids: $12.73 \pm 0.22 \times 8.29 \pm 0.13 \mu\text{m}$;

Type Locality: Gloucester Point (VA), Chesapeake Bay, east coast of USA.

Habitat: Medusae are found in estuarine waters on the US Atlantic coast and estuarine and nearshore waters of the Gulf of Mexico.

Distribution: Western North Atlantic, east coast of the USA south of New England to the Gulf of Mexico, restricted to estuarine waters on the Atlantic coast, known to exist outside of estuaries in the Gulf of Mexico.

875 **Notes:** Since *C. chesapeakei* is commonly found in estuarine waters, we advocate the common
 876 name “U.S. Atlantic bay nettle” to distinguish it from the “U.S. Atlantic sea nettle” (*C.*
 877 *quinquecirrha*). The specific name *chesapeakei* originates from *Dactylometra quinquecirrha* var.
 878 *chesapeakei* of Papenfuss (1936). For Papenfuss (1936), it is clear that: 1) the manuscript likely
 879 compared nematocyst measurements between two color morphs of *C. chesapeakei* and did not
 880 include *C. quinquecirrha* s. str. (see Discussion; Figure 8C); and 2) differences invoked for
 881 holotrichous a-isorhizas are in question, since the nematocysts are small (~1.5 um), making
 882 identifying differences difficult even with more precise, modern instruments, and the data are not
 883 accompanied by any statistics or measurement error. Regardless, based on Article 35.6.4 of the
 884 International Code of Zoological Nomenclature 4th Edition (ICZN 1999), the specific name
 885 *chesapeakei* has taxonomic priority and *C. chesapeakei* applies to the Chesapeake Bay animals,
 886 as well as estuarine Atlantic and Gulf of Mexico animals that are genetically similar, and have
 887 similar macromorphological and cnidome characteristics (Figures 4-9). Papenfuss (1936) did not
 888 designate a type specimen for *Dactylometra* (= *Chrysaora*) *quinquecirrha* var. *chesapeakei*. We
 889 designate the specimen USNM 1454948 as a neotype specimen so that a physical specimen,
 890 along with preserved tissue for genetic analysis, will be available to objectively define *C.*
 891 *chesapeakei* [see Article 75 of the International Code for Zoological Nomenclature (ICZN
 892 1999)], which will be necessary given the close genetic relationship between this species and
 893 specimens from the Caribbean (see below). Our neotype specimen originates from Gloucester
 894 Bay (VA), within the Chesapeake Bay, where Papenfuss (1936) hypothesized *Dactylometra*
 895 (= *Chrysaora*) *quinquecirrha* var. *chesapeakei* to be confined.

896

DNA sequence: Mitochondrial *COI* and *16S* and Nuclear 28S sequence data are available in GenBank under accession numbers MF141564-MF141587, MF141615-MF141617, MF141637-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 micropscopic assistance. We are thankful to Phillipe Bouchet, Dale Calder and Steve Cairns for
 921 their critical nomenclatural advice.

922

FIGURES

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.).

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.

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 (Broome's Island, MD - Patuxent River); E) Red-striped Chesapeake Bay color morph (Solomons, MD - Patuxent River). Note that medusae from A-B have 5 tentacles per octant, while C-E 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*. Photo Credits: 3A) Shannon Howard; 3B) Greg McFall-NOAA; 3E) Robert Condon.

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 $\geq 50\%$. 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).

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 $\geq 50\%$.

970 ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the
 971 TIM2+I+G model of sequence evolution (-lnl 3641.97519) as determined by jMODELTEST
 972 v2.1.7 (Darriba et al. 2012). Gray arrows indicate nodes that are alternated in the ML tree.
 973 Abbreviations refer to Tables 1-2. Specific identification to the right of the tree indicates final
 974 species designations. Clades colored in gray were originally identified as *C. quinquecirrha* s.l.
 975 Norfolk (VA) individuals NF1-NF3 were identified as white morph and individuals NF4-NF5 as
 976 red-striped bell morphs (Figure 3D-E).

977

978 **Figure 6: Pelagiidae 28S Phylogeny.** Bayesian Inference (BI) 28S tree reconstructed from
 979 MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence
 980 evolution. Numbers adjacent to branches show bootstrap support if ≥ 0.70 (presented as a
 981 percentage), followed by bootstrap support from maximum likelihood (ML) analysis if $\geq 50\%$.
 982 ML phylogeny was reconstructed using PhyML v3.0 (Guindon et al. 2010) applying the
 983 TrNef+I+G model of sequence evolution (-lnl 3817.02691) as determined by jMODELTEST
 984 v2.1.7 (Darriba et al. 2012). Specific identification to the right of the tree indicates final species
 985 designations. Clades colored in gray were originally identified as *C. quinquecirrha*.
 986

987 **Figure 7: Pelagiidae Combined Phylogeny.** Bayesian Inference (BI) tree of the combined dataset
 988 applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show
 989 bootstrap support if ≥ 0.70 (presented as a percentage), followed by bootstrap support from
 990 maximum likelihood (ML) analysis if $\geq 50\%$. ML phylogeny was reconstructed using PhyML
 991 v3.0 (Guindon et al. 2010) applying the GTR+I+G model of sequence evolution (-lnl
 992 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-isorhiza 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*.

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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).

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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Original ID	Final ID	Location	Code	n		
				COI	16S	28S
<i>Chrysaora achlyos</i>	<i>C. achlyos</i>	Monterey Bay Aquarium*	MBA	1	1	1
<i>Chrysaora africana</i>	<i>C. africana</i>	Coastal Namibia	NAM	2	2	2
<i>Chrysaora chinensis</i>	<i>C. chinensis</i>	Monterey Bay Aquarium^	MBA	2	2	2
<i>Chrysaora colorata</i>	<i>C. colorata</i>	Aquarium of the Americas+	AQA	1	1	1
<i>Chrysaora fulgida</i>	<i>C. fulgida</i>	Coastal Namibia	NAM	5	5	2
<i>Chrysaora fuscescens</i>	<i>C. fuscescens</i>	Aquarium of the Americas+	AQA	1	1	HM194868
<i>Chrysaora hysoscella</i>	<i>C. hysoscella</i>	Cork, Ireland	IRE	3	3	3
<i>Chrysaora lactea</i>	<i>Chrysaora</i> c.f. <i>chesapeakei</i>	Kingston, Jamaica	JAM	5	5	2
<i>Chrysaora lactea</i>	<i>C. lactea</i>	Rio de la Plata, Argentina	ARG	1	1	1
<i>Chrysaora melanaster</i>	<i>C. melanaster</i>	Bering Sea	BER	-	1	AY920780
<i>Chrysaora melanaster</i>	<i>C. pacifica</i>	Monterey Bay Aquarium	MBA	1	1	HM194864
<i>Chrysaora plocamia</i>	<i>C. plocamia</i>	Puerto Madryn, Argentina	PMA	2	2	2
<i>Chrysaora quinquecirrha</i>	<i>C. quinquecirrha</i>	Buzzard's Bay, MA (USA)	MA	1	1	1
<i>Chrysaora quinquecirrha</i>	<i>C. quinquecirrha</i>	Cape Henlopen, DE (USA)	CHP	3	3	2
<i>Chrysaora quinquecirrha</i>	<i>C. quinquecirrha</i>	Offshore South Carolina (USA) (32.60 N, 79.21 W)	OSC	2	2	1
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	Charlestown Pond, RI (USA)	RI	4	4	-
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	Tom's River Harbor, NJ (USA)	NJ	3	3	1
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	Rehoboth Bay, DE (USA)	RB	3	3	-
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	Norfolk, VA (USA)	NF	5	5	-
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	Pamlico Sound, NS (USA)	PAM	3	3	-
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	St. Simon's Island, GA (USA)	GA	3	3	1
<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>	Perdido Pass, AL (USA)	AL	3	3	1
<i>Pelagia noctiluca</i>	<i>P. noctiluca</i>	Offshore Virginia (USA) (37.81 N, 73.91 W)	OVA	1	1	HM194865
<i>Sanderia malayensis</i>	<i>S. malayensis</i>	Monterey Bay Aquarium	MBA	1	1	HM194861
Unknown Pelagiidae	<i>M. benovici</i>	Dakar, Senegal	SEN	2	2	1
<i>Cyanea capillata</i>	<i>C. capillata</i>	Blomsterdalen, Norway	BLO	1	1	HM194873

Table 2(on next page)

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

Table 3 (on next page)

Morphological characters examined for this study

Characters in bold are species diagnostic. All macromorphological characters and character states (except maximum oral arm length) are taken from Gershwin and Collins (2002). 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. quinquecirrha* has been recorded as great as 40 mm (Gershwin and Collins, 2002; Morandini and Marques, 2010), no animals >20 mm were observed in this study.

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5 tentacles/octant. ^Although maximum bell diameter for *C. quinquecirrha* has been recorded as great as 40
6 mm (Gershwin and Collins, 2002; Morandini and Marques, 2010), no animals >20 mm were observed in
7 this study.
8

Character	<i>Chrysaora quinquecirrha</i>	<i>C. chesapeakei</i>
<u>Macromorphology</u>		
Bell Diameter (average/median)	114 mm (59-176 mm)	62.2 mm (17-175 mm)
Tentacles / octant (average $\pm$ 95% CI)	5.28 $\pm$ 0.45	3.07 $\pm$ 0.07
Tentacles / octant (range)	4.5 - 6.75	2.75 - 3.43*
Lappets / octant (average $\pm$ 95% CI)	6.26 $\pm$ 0.46	4.08 $\pm$ 0.06
Lappets / octant (range)	5.5 – 7.75	3.75 - 4.8
Maximum Oral Arm Length (average $\pm$ 95% CI)	1.24 $\pm$ 0.27 times BD	3.00 $\pm$ 0.39 times BD
Maximum Oral Arm Length (range)	0.68 to 1.81 times BD	1.21 to 5.58 times BD
Lappets in Size Classes	Yes, rhopalar lappets larger	No, lappets of similar size
Rhopalia Number	8	8
Rhopalial 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
<u>Cnidome</u>		
A isorhiza - Length vs. Width (avg)	20.25 $\pm$ 0.38 x 11.27 $\pm$ 0.37 μm	26.21 $\pm$ 0.50 x 19.74 $\pm$ 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 $\pm$ 0.19 x 4.22 $\pm$ 0.07 μ m	7.88 $\pm$ 0.13 x 4.29 $\pm$ 0.07 μ m
a isorhiza – Length vs. Width (range)	6.56-9.77 x 3.65-4.95 μ m	6.32-9.9 x 3.59-5.46 μ m
O isorhiza – Length vs. Width (avg)	21.64 $\pm$ 0.38 x 18.92 $\pm$ 0.77 μ m	23.10 $\pm$ 0.43 x 20.75 $\pm$ 0.62 μ m
O isorhiza – Length vs. Width (range)	17.64-23.97 x 16.08-21.74 μ m	17.88-27.51 x 16.07-24.75 μ m
Birhopaloids – Length vs. Width (avg)	13.58 $\pm$ 0.19 x 8.09 $\pm$ 0.09 μ m	12.73 $\pm$ 0.22 x 8.29 $\pm$ 0.13 μ m
Birhopaloids – Length vs. Width (range)	12.31-14.86 x 6.96-8.90 μ m	10.96-15.27 x 7.1-10.23 μ m

Figure 1(on next page)

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.).

Figure 2 (on next page)

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.

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 (Broome's Island, MD - Patuxent River); E) Red-striped Chesapeake Bay color morph (Solomons, MD-Patuxent River). Note that medusae from A-B have 5 tentacles per octant, while C-E 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*. Photo Credits: 3A) Shannon Howard; 3B) Greg McFall-NOAA; 3E) Robert Condon.

Figure 4(on next page)

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 $\geq 50\%$. 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).

Figure 5(on next page)

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 $\geq 50\%$. 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).

Figure 6(on next page)

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 $\geq 50\%$. 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*.

Figure 7 (on next page)

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 $\geq 50\%$. 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*.

Figure 8(on next page)

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-isorhiza 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(on next page)

Pelagiidae Evolution

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)