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*)

Keith M. Bayha[1,2], Allen G. Collins[3] and Patrick M. Gaffney[4]

[1] Department of Invertebrate Zoology, Smithsonian Institution, National Museum of Natural History, Washington, DC, USA
[2] Department of Biological Sciences, University of Delaware, Newark, DE, USA
[3] National Systematics Laboratory of NOAA's Fisheries Service, Smithsonian Institution, Washington, DC, USA
[4] College of Earth, Ocean and Environment, University of Delaware, Lewes, DE, USA

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 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 and *16S* rDNA) sequence data from individuals of all four pelagiid genera, including 11 of 13 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 (*Chrysaora achlyos*, *Chrysaora colorata*, *Chrysaora fuscescens*, and *Chrysaora melanaster*). *Chrysaora 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 *Chrysaora 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 U.S. Coastal Atlantic *Chrysaora* species (U.S. Atlantic sea nettle), while the name *C. chesapeakei* Papenfuss 1936 applies to the U.S. Atlantic estuarine and Gulf of Mexico Chrysaora species (Atlantic bay nettle). We provide a detailed redescription, with designation of a neotype for *Chrysaora chesapeakei*, and clarify the description of *Chrysaora quinquecirrha*. Since Caribbean *Chrysaora* are genetically similar to *Chrysaora chesapeakei*, we provisionally term them *Chrysaora* c.f. *chesapeakei*. The presence of *Mawia benovici* off the coast of Western Africa provides a potential source region for jellyfish introduced into the Adriatic Sea in 2013.

INTRODUCTION

Scyphozoan jellyfishes (Cnidaria, class Scyphozoa), which include the conspicuous moon, lion's mane and sea nettle jellyfishes, exhibit significant and widespread economic and ecological impacts on a wide array of marine and estuarine communities. Jellyfish aggregations, blooms, and swarms damage economically important fisheries, close tourist beaches by stinging swimmers, clog intakes of coastal power and desalination plants, invade ecosystems, and can affect oxygen levels when mass numbers of carcasses are deposited (*Arai, 1997*; *Purcell, Uye & Lo, 2007*; *Richardson et al., 2009*; *Bayha & Graham, 2014*; *Qu et al., 2015*). On the other hand, jellyfish serve important roles as major prey items for some fish and sea turtles, in carbon capture and advection to the Deep Ocean, as important microhabitat for fish, invertebrates, and symbiotic algae, and as economic resources for humans (as food and therapeutic compounds) (*Doyle et al., 2014*, *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 *Chrysaora 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*), *Chrysaora 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* (eight 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) *Dactylometra 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 *Chrysaora 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 *Chrysaora quinquecirrha*.

In order to examine evolutionary relationships and taxonomic boundaries in the family Pelagiidae, with special focus on the genus *Chrysaora* and the species *Chrysaora 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 11 currently recognized species of *Chrysaora* and one species each of *Mawia* (*Mawia benovici*), *Pelagia* (*P. 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

Table 1 Geographic source regions of samples used for molecular analyses in this study, identified by taxon (original, morphologically based identification) and molecular ID (identification after molecular analyses).

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

Notes:
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).

(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; Fig. 1). An unknown and unidentified pelagiid specimen was collected from Dakar, Senegal and was accompanied by a photograph that did not allow
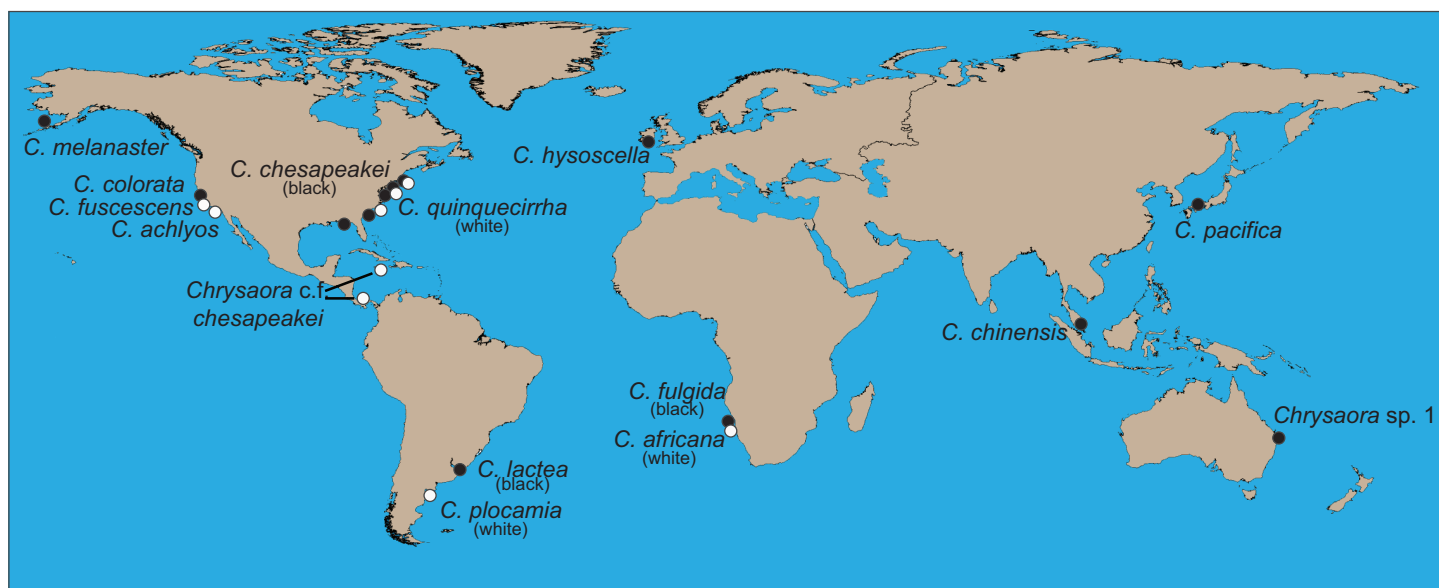
Bayha et al. (2017), *PeerJ*, DOI 10.7717/peerj.3863

5/43

Figure 1 **World map showing collecting sites of animals sequenced for this study.** Final species designations are employed. All aquarium samples (*Chrysaora achlyos*, *Chrysaora chinensis*, *Chrysaora colorata*, *Chrysaora fuscescens*, and *Chrysaora 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, 2015, personal communication).

for specific identification (Fig. S1). For *Chrysaora quinquecirrha*, samples were collected from 10 different sites along the Atlantic and Gulf of Mexico coasts (Table 1; Fig. 2), covering both coastal and estuarine environments, with the intention of capturing as many structural and color morphotypes as possible (Fig. 3). Both white (Table 1: NF1–NF3) and red-striped (Table 1: NF4–NF5) color morphs (Figs. 3C and 3D) 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*), 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 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
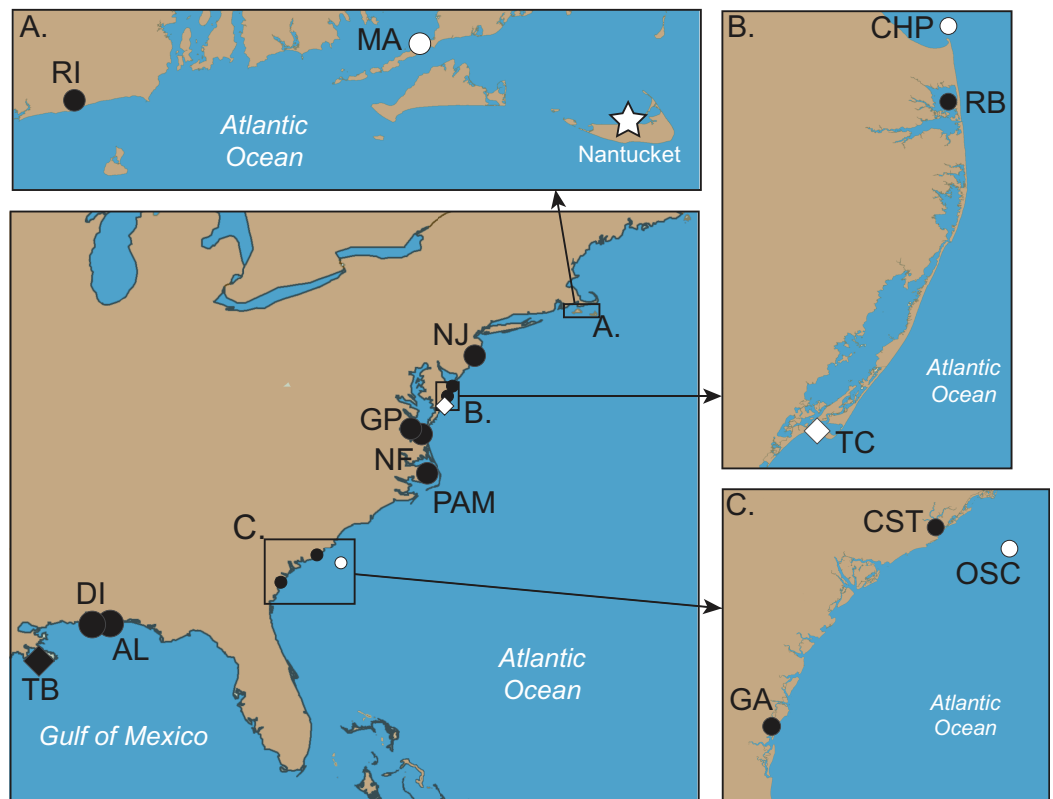
Figure 2 Collection locations of *Chrysaora quinquecirrha* s.l. medusae used in this study. Abbreviations all refer to Table 1 and Table S1. (A–C) are enlargements of rectangular inset regions. The star at Nantucket harbor indicates the type locality of *C. quinquecirrha* (*Desor, 1848*). Diamonds represent 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 Engelhard, 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 *Chrysaora quinquecirrha* are in white and those with individuals finally designated as *Chrysaora chesapeakei* in black.

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 600 s 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, USA) or Beckman-Coulter Genomics (Danvers, MA, USA). Sequences were assembled using Lasergene SeqMan Pro v. 8.1.5 (DNAStar, Inc., Madison, WI, USA) and then compared to the GenBank nucleotide database using BLASTn or BLASTx (*Altschul et al., 1997*) to
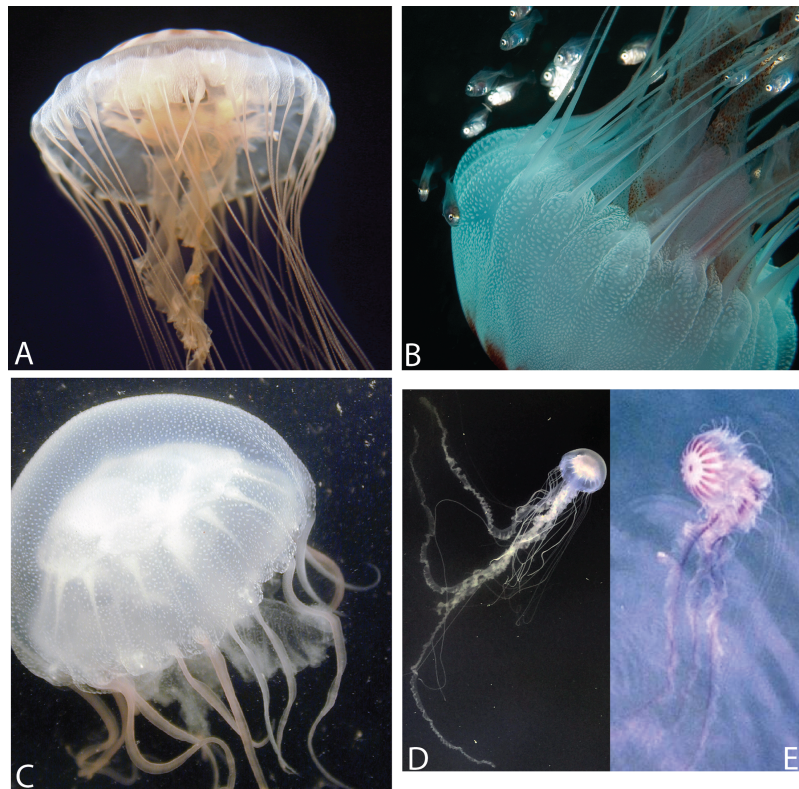
Figure 3 Various morphs of *Chrysaora quinquecirrha* s.l. (A) Offshore South Carolina (OSC); (B) Sample taken from offshore Georgia; (C) Engelhard, NC (PAM); (D) White Chesapeake Bay color morph (Broome's Island, MD—Patuxent River); (E) Red-striped Chesapeake Bay color morph (Solomons, MD—Patuxent River). Note that medusae from (A) to (B) have five tentacles per octant, while (C)–(E) have three tentacles per octant. Medusae in (A, C) were included in this study's phylogenetic analyses. (A: OSC1; C: PAM1). (A, B) represent individuals finally designated as *Chrysaora quinquecirrha*; (C–E) represent individuals finally designated as *Chrysaora chesapeakei*. Photo Credits: (A) Shannon Howard; (B) Greg McFall-NOAA; (E) Robert Condon.

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–MF141593; MF141595–MF141646; MF141648–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 MAAFT v7.245 employing the E-INS-I strategy (*Katoh & Standley, 2013*), since this strategy has been demonstrated to be effective for loci containing conserved motifs embedded within hypervariable regions (*Katoh & Toh, 2008*). Hypervariable regions of questionable alignment were removed from the

Original ID	Final ID	Location	Code	n		
				COI	16S	28S
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER1	KJ026191	–	–
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER2	KJ026212	–	–
Chrysaora melanaster	*C. melanaster*	Bering Sea	BER3	KJ026256	–	–
Chrysaora sp.	*Chrysaora* c.f. *chesapeakei*	Bocas del Toro, Panama	PAN	JN700941*	JN700941*	AY920779*
Chrysaora pacifica	*Chrysaora pacifica*	Kyoto, Japan	KYO	LC191577	–	–
Chrysaora quinquecirrha	*C. pacifica*	Geoje-do, Korea	KOR	HQ694730	HQ694730	–
Chrysaora sp.	*Chrysaora* sp. 1	Noosa Heads, Australia	AUS	DQ083524	–	–
Chrysaora sp.	*C. chinensis*	Malaysia	MAL1	–	JN184784	–
Chrysaora sp.	*C. chinensis*	Malaysia	MAL2	–	JN184785	–
Chrysaora sp.	*C. chinensis*	Malaysia	MAL3	–	JN184786	–
Pelagia benovici	*P. benovici*	Northern Adriatic Sea	ADR1	KJ573409	–	KJ573396
Pelagia benovici	*P. benovici*	Northern Adriatic Sea	ADR2	KJ573410	–	KJ573397
Pelagia benovici	*P. benovici*	Northern Adriatic Sea	ADR3	KJ573412	–	KJ573401
Pelagia noctiluca	*P. noctiluca*	Southern Tyrrhenian Sea, Italy	TYR	KJ573419	–	KJ573408
Pelagia noctiluca	*P. noctiluca*	Cape Town, South Africa	SA	JQ697961	–	–
Pelagia noctiluca	*P. noctiluca*	Dispensa Island, Costa Rica	CR1	JX235441	–	–
Pelagia noctiluca	*P. noctiluca*	Dispensa Island, Costa Rica	CR2	–	JX235404	–
Pelagia noctiluca	*P. noctiluca*	Dispensa Island, Costa Rica	CR3	–	JX235405	–
Pelagia c.f. *panopyra*	*Pelagia* c.f. *panopyra*	Papua, New Guinea	PNG	KJ573422	–	–

Note:
* Sequences came from the same individual.

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. ML phylogenetic trees were constructed using PhyML v3.0 (*Guindon et al., 2010*), employing the best-fit substitution models assessed using jModelTest v2.1.7 (*Darriba et al., 2012*) under Akaike (AIC) and Bayesian (BIC) information criteria, as well as decision theory performance-based selection (DT). For *COI* (TPMµf+I+G), *16S* (TIM2+I+G), and combined (GTR+I+G) datasets, selection criteria were unanimous, while BIC and DT chose TrNef+I+G for *28S*. A 1,000 bootstrap replicate analysis was performed in PhyML to obtain node support values. 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., San Jose, CA, USA). Mean interclade and intraclade, as well as minimum interclade sequence divergence values (Kimura 2-parameter) were determined using MEGAv7.0.14 (*Kumar, Stecher & Tamura, 2016*) and nucleotide statistics calculated in Seaview v4.6 (*Gouy, Guindon & Gascuel, 2010*).

Morphological analysis of *Chrysaora quinquecirrha*

While our study did not include a family-wide morphological analysis, we did perform morphological analyses on jellyfish identified as *Chrysaora quinquecirrha* from the U.S. Atlantic and Gulf of Mexico coasts. We examined a total of 57 formalin-preserved samples we collected from Charlestown Pond (RI), Cape Henlopen (DE), Rehoboth Bay (DE), York River (VA), Charleston (SC), and Dauphin Island (AL) (Table S1). In addition, we examined a total of 63 individuals housed at the Smithsonian Institution National Museum of Natural History (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, where preservation state allowed for its measurement (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 μL of distilled water in a 1.5 μL tube using a plastic microcentrifuge pestle until little visible intact tissue remained. A small drop was then

Table 3 Morphological characters examined for this study.

Character	*Chrysaora quinquecirrha*	*Chrysaora chesapeakei*
Macromorphology		
Bell diameter (average/median)	114 mm (59–176 mm)	62.2 mm (17–175 mm)
Tentacles/octant (average ± 95% CI)	**5.28 ± 0.45**	**3.07 ± 0.07**
Tentacles/octant (range)	**4.5–6.75**	**2.75–3.43**[*]
Lappets/octant (average ± 95% CI)	**6.26 ± 0.46**	**4.08 ± 0.06**
Lappets/octant (range)	**5.5–7.75**	**3.75–4.8**
Maximum oral arm length (average ± 95% CI)	**1.24 ± 0.27 times BD**	**3.00 ± 0.39 times BD**
Maximum oral arm length (range)	**0.68–1.81 times BD**	**1.21–5.58 times BD**
Lappets in size classes	Yes, rhopalar lappets larger	No, lappets of similar size
Rhopalia number	8	8
Rhopaliar pits	Deep	Deep
Septa shape	Bent	Bent
Septa termination	Near tentacle	Near tentacle
Spiral oral arms?	No	No
Manubrium length	Elongated	Elongated
Manubrium mass	Light	Light
Warts/papillae	Inconspicuous	Inconspicuous
Maximum bell diameter	<20 cm[^]	<20 cm[^]
Bell mass	Light	Light
Dominant color	White, colorless	Variable, white, colorless or red/brown bell
Exumbrellar marks	Minor bell marks in some cases	Variable, red or brown star shape conspicuous in some cases
Oral arm color	None	Variable, oral arms can be colored red/brown
Quadralinga	None	None
Gonads in pouch?	Yes	Yes
Gonad shape	Not finger-like	Not finger-like
Cnidome		
A isorhiza—length vs. width (avg)	**20.25 ± 0.38 × 11.27 ± 0.37 μm**	**26.21 ± 0.50 × 19.74 ± 0.55 μm**
A isorhiza—length vs. width (range)	**15.01–22.9 × 9.07–13.16 μm**	**20.54–33.79 × 15.03–29.77 μm**
a isorhiza—length vs. width (avg)	8.27 ± 0.19 × 4.22 ± 0.07 μm	7.88 ± 0.13 × 4.29 ± 0.07 μm
a isorhiza—length vs. width (range)	6.56–9.77 × 3.65–4.95 μm	6.32–9.9 × 3.59–5.46 μm
O isorhiza—length vs. width (avg)	21.64 ± 0.38 × 18.92 ± 0.77 μm	23.10 ± 0.43 × 20.75 ± 0.62 μm
O isorhiza—length vs. width (range)	17.64–23.97 × 16.08–21.74 μm	17.88–27.51 × 16.07–24.75 μm
Birhopaloids—length vs. width (avg)	13.58 ± 0.19 × 8.09 ± 0.09 μm	12.73 ± 0.22 × 8.29 ± 0.13 μm
Birhopaloids—length vs. width (range)	12.31–14.86 × 6.96–8.90 μm	10.96–15.27 × 7.1–10.23 μm

Notes:
Characters in bold are species diagnostic. All macromorphlogical characters and character states (except maximum oral arm length) are taken from *Gershwin & Collins (2002)*. Cnidome terminology is taken from *Morandini & Marques (2010)*, with average examples in Fig. 8C and Fig. S1.
[*] If two outlier specimens are included, the upper range is six tentacles/octant.
[^] Although maximum bell diameter for *Chrysaora quinquecirrha* has been recorded as great as 40 mm (*Gershwin & Collins, 2002*; *Morandini & Marques, 2010*), no animals >20 mm were observed in this study.

placed on a slide under cover slip and examined at 60× 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., Waltham, MA, USA).

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 *Chrysaora lactea* to 608 bp (*Chrysaora chinensis*). The MAAFT-aligned data set (included published sequences) was 628 bp, but the dataset was truncated to 582 bp (95.7%) after treatment with GBlocks. The *28S* dataset included 35 sequences, including 24 new sequences and 11 published sequences. New sequences ranged in size from 998 (*Chrysaora chinensis*) to 1,018 bp (*Chrysaora africana*). The MAFFT alignment (which included published sequences) was 1,027 bp, but the final data set was 1,015 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 (Figs. 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 *S. malayensis* (Bayesian support 100/ML support 100), with these two species forming a close relationship with *Chrysaora africana* and *Chrysaora pacifica* in the combined (88/67) and *28S* trees (100/61) (Figs. 6 and 7). Except for the *COI* tree, *P. noctiluca* formed a close relationship with a clade of Pacific jellies (*Chrysaora achlyos, Chrysaora colorata, Chrysaora fuscescens, and*
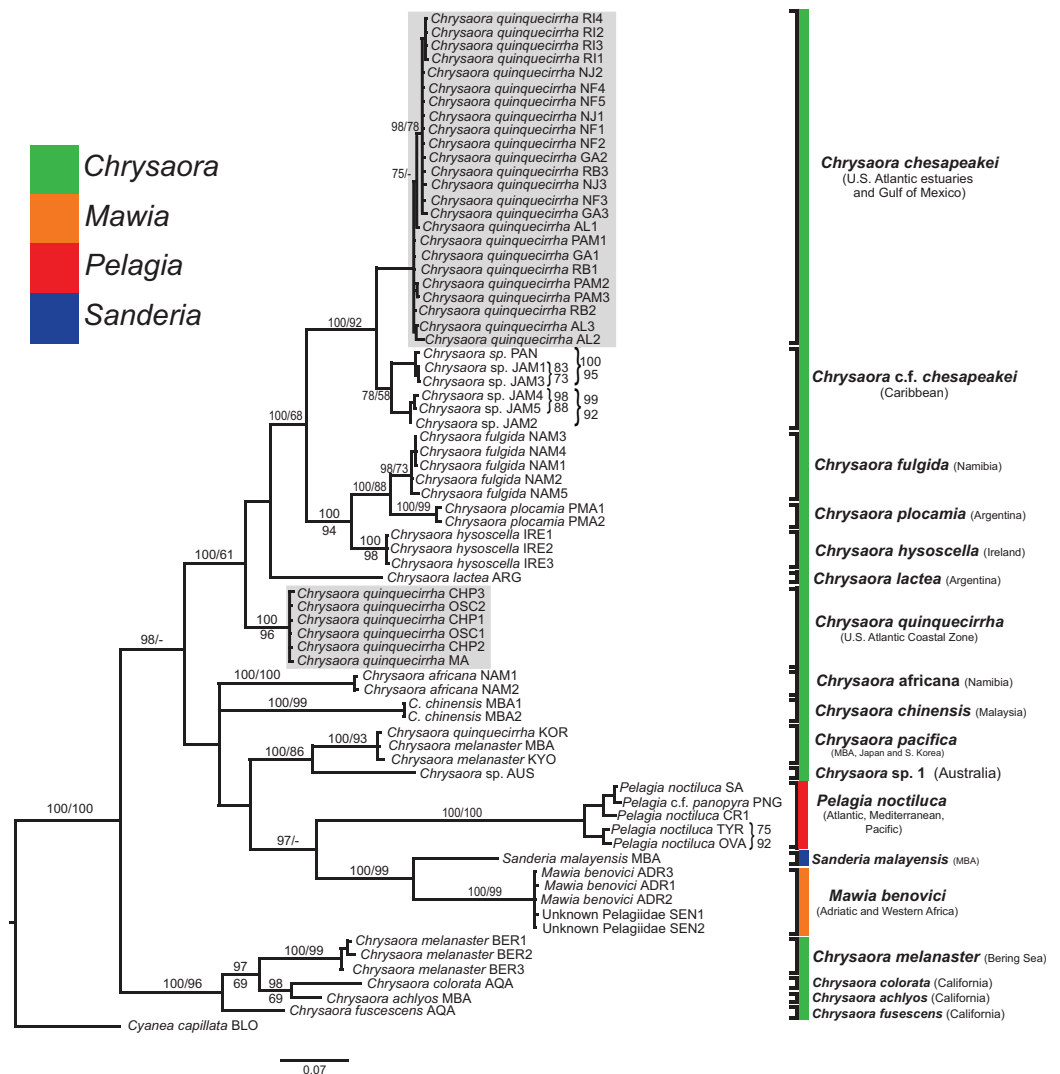
Figure 4 Pelagiidae *COI* Phylogeny. Bayesian inference (BI) *COI* tree reconstructed from CLUSTAL alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%. ML phylogeny was reconstructed using PhyML v3.0 (*Guindon et al., 2010*) applying the TPM2uf+I+G model of sequence evolution (-lnl 5451.81154) as determined by jMODELTEST v2.1.7 (*Darriba et al., 2012*). Abbreviations refer to Tables 1 and 2. Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *Chrysaora 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 (Figs. 3D and 3E).

Chrysaora melanaster) with high support values (combined: 100/99; *16S*: 100/92; *28S*: 82/58) (Figs. 5–7). For the combined analyses (100/100) and *28S* (100/100), a highly supported clade was composed of Atlantic species, including *Chrysaora quinquecirrha*, *Chrysaora lactea*, *Chrysaora plocamia*, *Chrysaora fulgida*, *Chrysaora hysoscella*, *Chrysaora chesapeakei* [see Discussion], and the Caribbean *Chrysaora*, while this clade was less supported for *COI* (100/61) and *16S* (75/60) (Figs. 4–7). *Chrysaora fulgida* (NAM), *Chrysaora plocamia* (PMA), and *Chrysaora hysoscella* (IRE) formed a closely related group
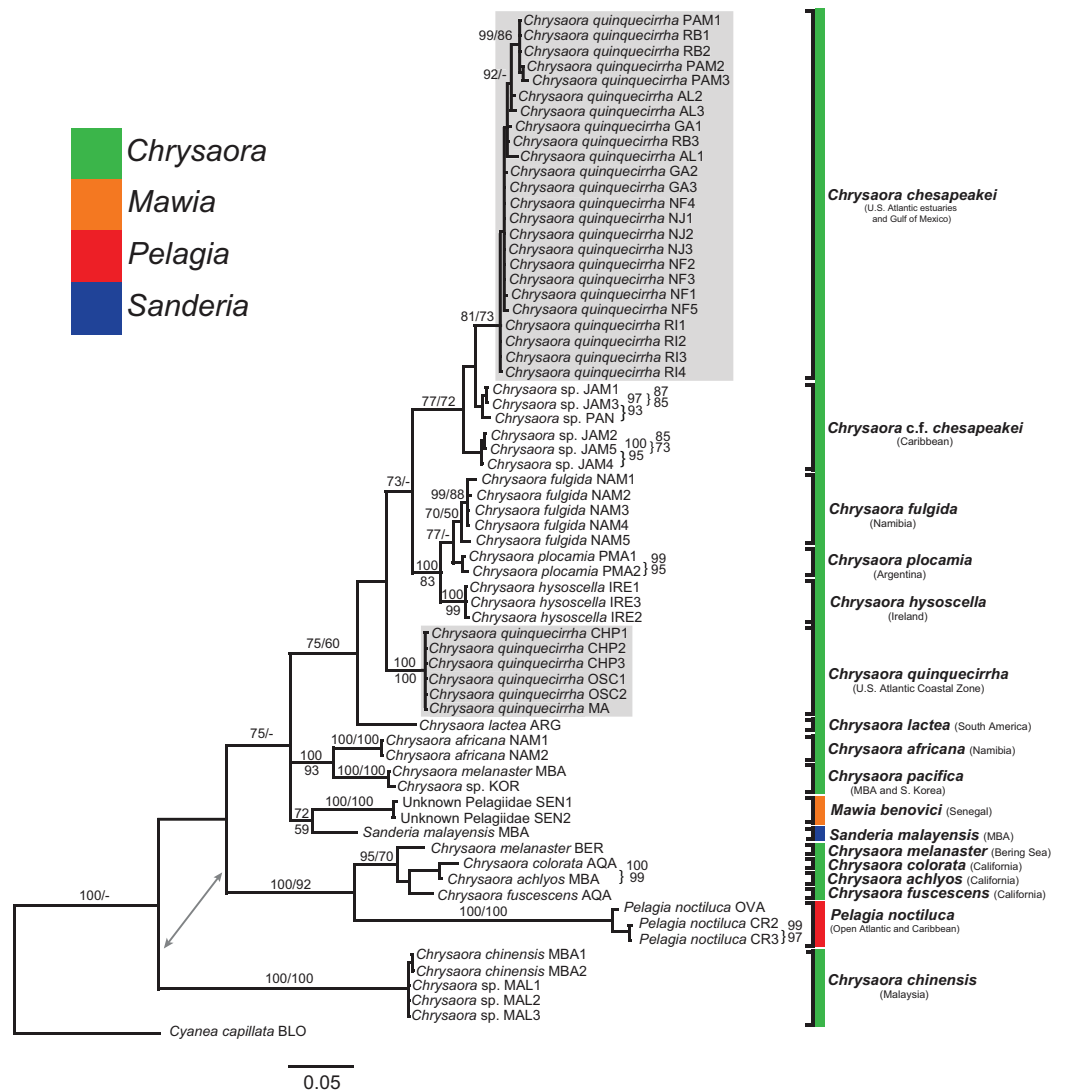
Figure 5 Pelagiidae *16S* Phylogeny. Bayesian inference (BI) *16S* tree reconstructed from MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%. ML phylogeny was reconstructed using PhyML v3.0 (*Guindon et al., 2010*) applying the TIM2+I+G model of sequence evolution (-lnl 3641.97519) as determined by jMODELTEST v2.1.7 (*Darriba et al., 2012*). Gray arrows indicate nodes that are alternated in the ML tree. Abbreviations refer to Tables 1 and 2. Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *Chrysaora quinquecirrha* s.l. Norfolk (VA) individuals NF1–NF3 were identified as white morph and individuals NF4–NF5 as red-striped bell morphs (Figs. 3D and 3E).

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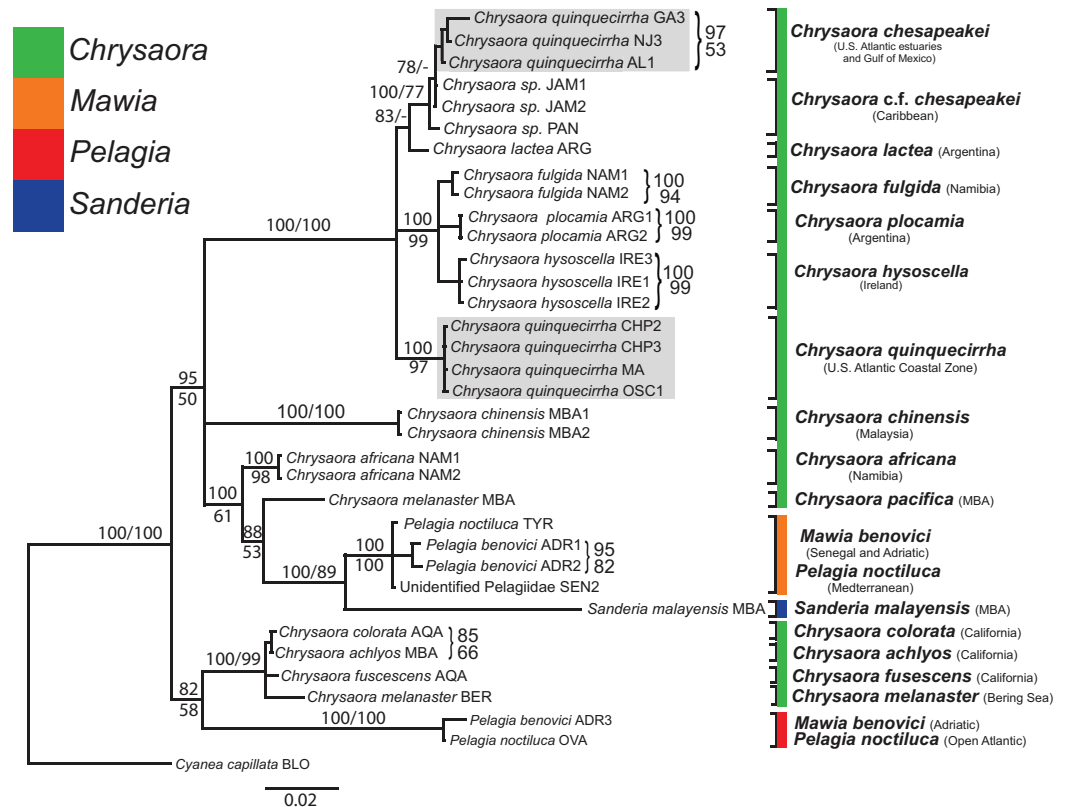
Figure 6 Pelagiidae *28S* Phylogeny. Bayesian inference (BI) *28S* tree reconstructed from MAFFT alignment using Mr. Bayes v3.2.4 and applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a percentage), followed by bootstrap support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

At the species level, our analyses highlighted multiple species boundaries, and showed the samples identified as *Chrysaora quinquecirrha* to be polyphyletic. In all analyses, *Chrysaora quinquecirrha* sequences fell into two distinct, highly diverged clades (Figs. 4–7; Tables S3–S5), with one clade (*Chrysaora chesapeakei*—see "Discussion" and "Systematics") made up of animals from U.S. Atlantic estuaries and the Gulf of Mexico animals and another (*Chrysaora quinquecirrha*—see "Discussion" and "Systematics") made up of U.S. coastal Atlantic animals. Caribbean *Chrysaora* (Jamaica and Panama) formed a clade closely related to *Chrysaora chesapeakei* in all analyses (Figs. 4–7). Aquarium animals previously identified as *Chrysaora melanaster* (AQA) were genetically distinct from *Chrysaora melanaster* collected from the Bering Sea (BER) in all analyses where both were included (Figs. 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 *Chrysaora chinensis* formed a well-supported clade with field collected *Chrysaora chinensis* (MAL), analyses differed in where this species fell out in
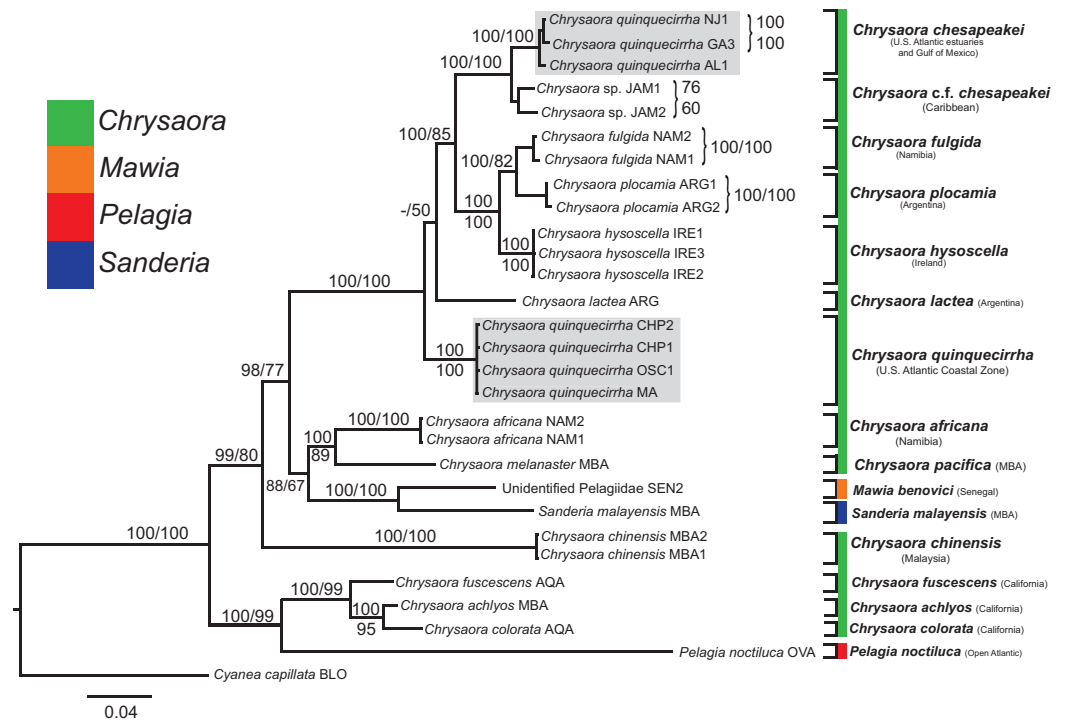
Figure 7 Pelagiidae combined phylogeny. Bayesian inference (BI) tree of the combined dataset applying the GTR+I+G model of sequence evolution. Numbers adjacent to branches show bootstrap support if ≥0.70 (presented as a percentage), followed by bootstrap support from maximum likelihood (ML) analysis if ≥50%. ML phylogeny was reconstructed using PhyML v3.0 (*Guindon et al., 2010*) applying the GTR+I+G model of sequence evolution (−lnl 11924.23655) as determined by jMODELTEST v2.1.7 (*Darriba et al., 2012*). Specific identification to the right of the tree indicates final species designations. Clades colored in gray were originally identified as *Chrysaora quinquecirrha.*

the trees (Figs. 4–7). The unknown pelagiid collected from the Western African coast (SEN) was nearly identical to the newly described *Mawia benovici* from the Mediterranean for *COI* (0.0–0.3% difference) and *28S* (0.0–0.2% difference) (Figs. 4 and 6).

Macromorphological and nematocyst analyses

A total of 120 medusae (57 field collected and 63 museum specimens) (Table S1) previously identified as *Chrysaora quinquecirrha* s.l. were examined for 19 quantitative and qualitative macromorphological characters taken from *Gershwin & 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") (Fig. 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
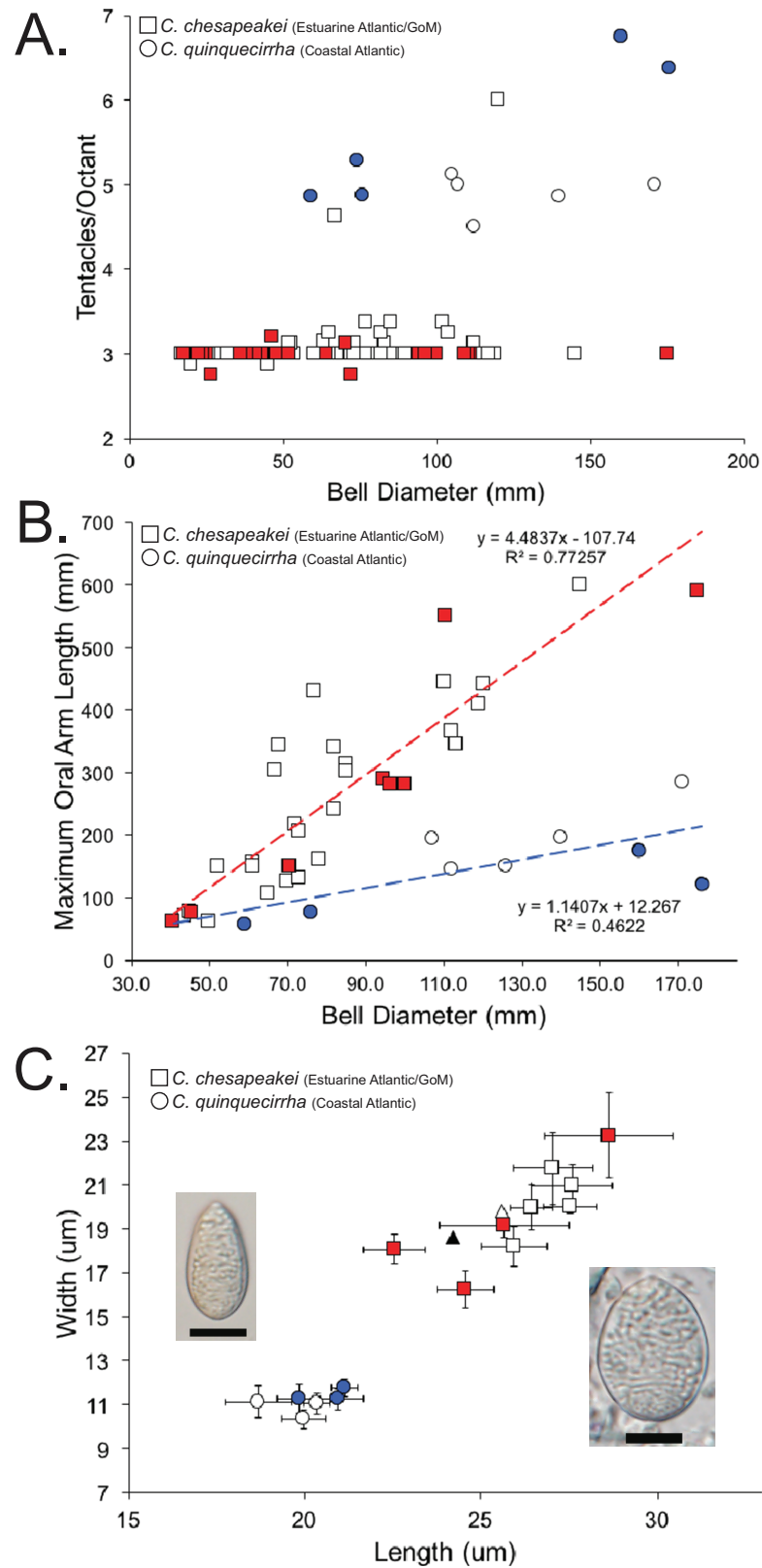
Figure 8 Morphological evidence separating *Chrysaora quinquecirrha* and *Chrysaora chesapeakei*. (A) Tentacle counts. Graph represents tentacles per octant against bell diameter (mm) for field collected and museum specimens. Squares represent animals taken from estuarine Atlantic and Gulf of Mexico regions (*Chrysaora chesapeakei*), while circles represent animals taken from coastal Atlantic regions (*Chrysaora quinquecirrha*). All animals with *16S* sequences matching the *Chrysaora chesapeakei* clade appear in red, while those whose sequences matched the *Chrysaora 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 (*Chrysaora chesapeakei*), while circles represent animals taken from coastal Atlantic regions (*Chrysaora quinquecirrha*). Only animals with fully intact and extended oral arms were included. All animals with *16S* sequences matching the *Chrysaora chesapeakei* clade appear in red, while those whose sequences matched the *Chrysaora quinquecirrha* clade appear in blue. (C) Average size measurements for holotrichous A-isrohiza nematocysts (length vs. width), based on 10 nematocysts per. Error bars represent 95% CI (2*standard error). Squares represent nematocysts from estuarine Atlantic and Gulf of Mexico medusae (*Chrysaora chesapeakei*), while circles represent nematocysts from coastal Atlantic medusae (*Chrysaora quinquecirrha*). Photograph of an average sized A-isorhiza from *Chrysaora quinquecirrha* appears on the left and a photograph of an average size A-isorhiza from *Chrysaora chesapeakei* appears on the right. Scale bars = 10 μm. 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 *Chrysaora chesapeakei* clade appear in red, while those whose sequences matched the *Chrysaora 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).

[5.28 ± 0.48] 95% CI tentacles per octant (Fig. 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 ± 0.39 (95% CI) times as long as the bell diameter (Fig. 8B; Table 3). Animals collected from coastal regions of the U.S. Atlantic (Table S1) had oral arms that were on average [1.24 ± 0.27] 95% CI times as long as bell diameter (Fig. 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 *Chrysaora chesapeakei* phylogenetic clade had an average of 2.99 ± 0.03 tentacles per octant and oral arms that were [2.80 ± 0.78] 95% CI times as long as bell diameter on average, while all those that fell in the *Chrysaora quinquecirrha* clade had an average of 5.63 ± 0.78 tentacles per octant and oral arms that were on average 0.93 ± 0.18 (95% CI) times as long as bell diameter on average (Figs. 8A and 8B).

We also studied the cnidome of medusae identified as *Chrysaora quinquecirrha*, examining the measurements of individual nematocyst types (Fig. 8C; Fig. 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 (Fig. 8C). All sequenced animals in the smaller group (coastal Atlantic) were genetically similar to *Chrysaora 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 *Chrysaora chesapeakei* (Fig. 8C). For animals identified as *Chrysaora chesapeakei* (based on habitat, macromorphology, and/or genetics), LDA analysis

indicated that individual A-isorhiza measurements correctly identified species 97.8% of the time (2.2% of the time, they were incorrectly identified at *Chrysaora quinquecirrha*), while they were correctly identified 100% of the time using the mean of 10 nematocyst measurements. For animals previously identified as *Chrysaora 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 *Chrysaora quinquecirrha*, chosen based on their previous molecular and macromorphological groupings (five from each group). All in all, heterotrichous microbasic rhopaloids were most frequent ([62.1 ± 9.8%] 95% CI), followed by O-isorhizas ([13.4 ± 5.0%] 95% CI), a-isorhizas ([12.4 ± 2.8%] 95% CI) and A-isorhizas ([12.2 ± 3.7%] 95% CI). As pilot studies indicated, nematocyst type proportions were different for different tentacles regions. While A-isorhizas and a-isorhizas were consistent over the entire tentacle, O-isorhizas were overrepresented in proximal regions and heterotrichous microbasic rhopaloids were overrepresented in the medial and distal regions (Fig. S3A). Individuals varied considerably in proportions of nematocyst types (Fig. S3B). Individuals collected from coastal Atlantic regions (circles) were generally clustered, including those genetically similar to *Chrysaora quinquecirrha*, while those from estuarine Atlantic and Gulf of Mexico regions (squares) were much more dispersed, as were those genetically similar to *Chrysaora chesapeakei* (Fig. S3B). LDA was moderately effective in distinguishing species using overall nematocyst proportions (four of five *Chrysaora quinquecirrha* and three of five *Chrysaora chesapeakei* correctly classified) and this was almost entirely due to different proportions of A-isorhiza nematocysts. A-isorhiza proportions were significantly different ($t = 3.623$, $p = 0.0068$), with *Chrysaora chesapeakei* individuals averaging 16.5 ± 3.4% for A-isorhiza and *Chrysaora quinquecirrha* cnidomes averaging 7.8 ± 3.4%.

DISCUSSION

Genus-level systematic inference

Our most robust phylogenetic hypothesis for Pelagiidae (Fig. 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 (Figs. 9A and 9B). In contrast, our analyses indicate that *Chrysaora* is paraphyletic with respect to *Pelagia*, *Sanderia*, and the newly erected *Mawia* (Figs. 4–7 and 9E). Mediterranean *Mawia benovici* is not in the combined analysis, but our Senegal pelagiid (SEN) can be treated as *Mawia benovici*, based on *COI* (Fig. 4) and *28S* (Fig. 6) phylogenies (see below). Paraphyly of *Chrysaora* is not supported in morphological or genetic analyses in *Avian et al. (2016)* (Figs. 9C and 9D), but this is almost certainly a result of incomplete taxon sampling. For example, their analysis based on combined morphological and genetic data only included *Chrysaora*
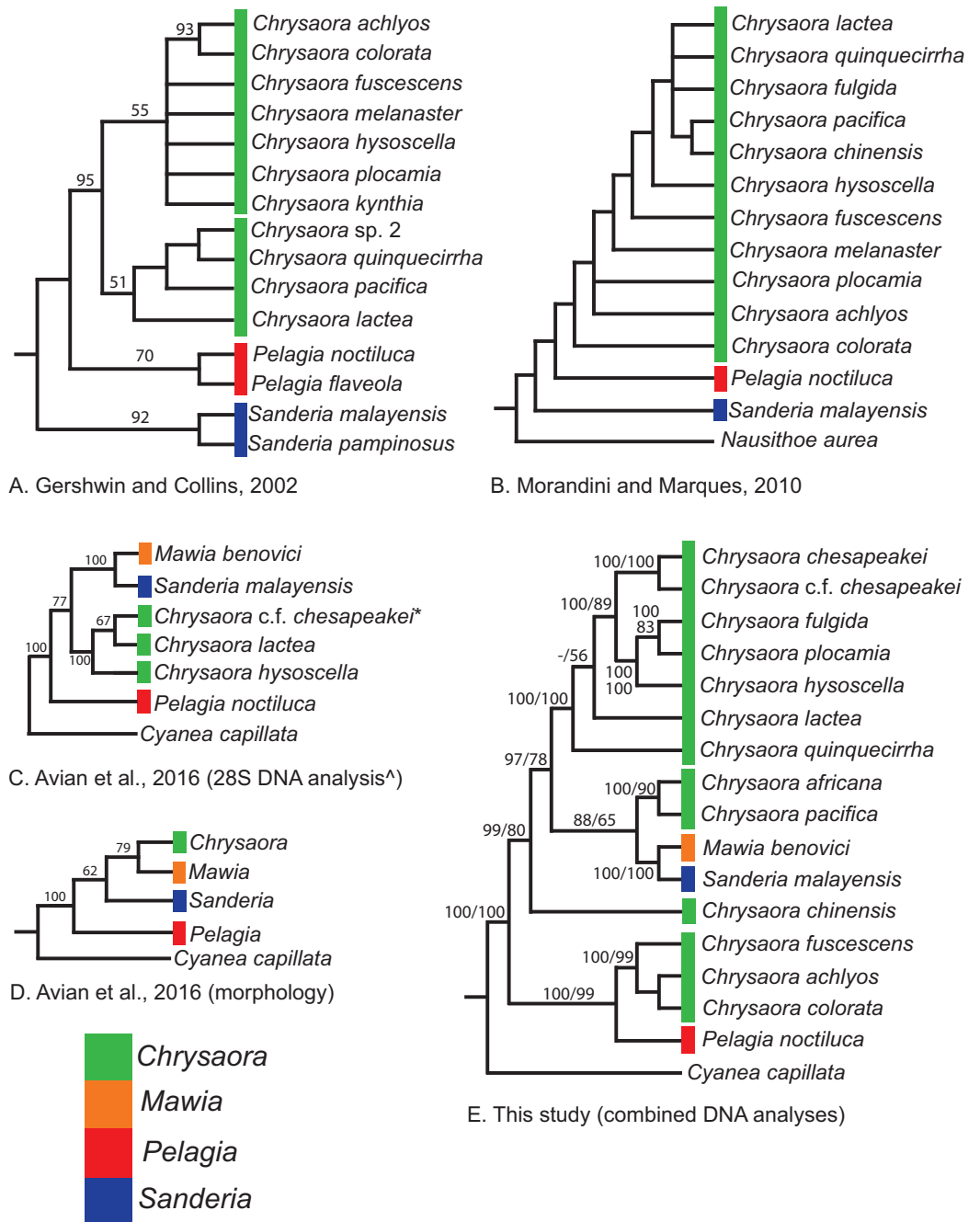
Figure 9 Pelagiidae evolution. Cladograms showing genus-level relationships within the Pelagiidae family. Colors represent individual genera as shown. (A) *Gershwin & Collins (2002)*; (B) *Morandini & Marques (2010)*; (C) *Avian et al. (2016)*: DNA analysis based on nuclear *28S*; (D) *Avian et al. (2016)*: 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*.

hysoscella (Mediterranean), while the *28S* dataset included a subset of sequences published at the time, (*Chrysaora hysoscella*, *Chrysaora lactea*, and *Chrysaora* c.f. *chesapeakei* [see below]), all of which occur in a single clade in our analysis (Figs. 7 and 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 *Mawia benovici* to be a close relative of *S. malayensis* (Figs. 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 (Figs. 7 and 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 *Chrysaora hysoscella*, the genus would best be limited to the clade containing *Chrysaora hysoscella*, *Chrysaora fulgida*, *Chrysaora lactea*, *Chrysaora plocamia*, *Chrysaora quinquecirrha*, and *Chrysaora chesapeakei* (see below). This then would leave three other lineages in need of new genera: (1) *Chrysaora africana* plus *Chrysaora melanaster*; (2) *Chrysaora chinensis*; and (3) *Chrysaora achlyos*, *Chrysaora colorata*, and *Chrysaora fuscescens*. The latter grouping (*Chrysaora achlyos*, *Chrysaora colorata*, and *Chrysaora fuscescens*) has a close relationship to *P. noctiluca* (except for *COI*) and there is genetic support for generic designation. Unfortunately, none of the morphological characters employed in this study clearly 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., Fig. 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*) (Fig. 9), there are some relationships that occur in all phylogenies. All phylogenies agree on a close relationship between *Chrysaora achlyos* and

Chrysaora colorata (Figs. 9A, 9B and 9E). Our phylogeny is in general agreement with *Morandini & Marques (2010)* in delineating their basal "Pacific" group (*Chrysaora achlyos*, *Chrysaora colorata*, *Chrysaora fuscescens*, *Chrysaora melanaster*, and *Chrysaora plocamia*), except that our *Chrysaora plocamia* samples came from the Atlantic and occur in an "Atlantic" group (Table 1; Fig. 1). *Morandini & 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 (Fig. 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 *Chrysaora chinensis* and *Chrysaora pacifica* (both Pacific jellies) as closely related to *Chrysaora quinquecirrha* and *Chrysaora lactea*, with the *Chrysaora pacifica* placement also a disagreement with *Gershwin & Collins (2002)*. Likewise, the very close relationship among *Chrysaora fulgida*, *Chrysaora hysoscella*, and *Chrysaora plocamia* was not found in any of the morphological phylogenies (Fig. 9), though *Chrysaora hysoscella* and *Chrysaora plocamia* were closely related in *Gershwin & 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 *Chrysaora pacifica* (originally *Chrysaora melanaster*—see below) and *Chrysaora chinensis* are genetically confirmed, based on published sequences from field-collected specimens of known geographical origin (Figs. 4 and 5). In addition, our aquarium-collected *Chrysaora fuscescens* is identical to published *16S* sequence of field-collected animals from Vancouver Island, Canada (NCBI JX393256). However, *Chrysaora colorata*, *Chrysaora 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 an aquarium system. Future studies incorporating field-collected specimens are necessary for confirming or refuting relationships shown here.

Species-level systematic inference

Chrysaora quinquecirrha and Chrysaora chesapeakei

The most striking conclusion revealed from this study is that *Chrysaora 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 (Figs. 4–7), macromorphological (Figs. 8A and 8B), and cnidome (Fig. 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) (Figs. 4–7). Average (*COI*: 13.1%; *16S*: 9.0%; *28S*: 2.5%) and minimum (*COI*: 12.1%; *16S*: 8.4%; *28S*: 2.4%; Tables 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 *Chrysaora fulgida* from Namibia (NAM), *Chrysaora plocamia* from Argentina (ARG),

and *Chrysaora hysoscella* from Ireland (IRE) occur between these two species in all phylogenies (Figs. 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 (Fig. 8A), oral arm length (Fig. 8B), and holotrichous A-isorhiza measurements (Figs. 8C and 9). Two individuals (USNM 33457a and USNM 56703b) did not fit the typical pattern for tentacle number (Fig. 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 × 20.98 μm; USNM 56703b: 27.04 × 21.75 μm; Fig. 8C) and/or oral arm length (USNM 33457a: 4.54 times bell diameter; USNM 56703b: sample too degraded; Fig. 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 *Chrysaora 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 (Fig. 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, 1994–2004, personal observation). 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 *Chrysaora 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 *Chrysaora 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., Fig. 3D) and a

larger red-striped morph (e.g., Fig. 3E), which the author 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* (Figs. 4 and 5) and we found no overall pattern of differentiation in our holotrichous a-isorhiza measurements (Fig. S2A). Furthermore, for holotrichous A-isorhiza measurements, both morphs from *Papenfuss (1936)* are consistent with our U.S. Atlantic estuary/Gulf of Mexico group (Fig. 8C). In summary, evidence from nematocyst measurements (Fig. 8C), locality (Chesapeake Bay), and phylogenetic data (Figs. 4 and 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 *Dactylometra quinquecirrha* var. *chesapeakei*, that name is taxonomically available based on Article 45.6.4 of the International Code of Zoological Nomenclature (*ICZN, 1999*). 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 *Chrysaora chesapeakei* differs from *Morandini & Marques (2010)*, who placed them in the species *Chrysaora 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 *Chrysaora lactea* (Figs. 4–7) and the number of tentacles (approximately three) and lack of tertiary tentacles in the Gulf of Mexico animals observed here and in *Morandini & 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 *Chrysaora quinquecirrha* and *Chrysaora chesapeakei* may be related to adaptations to different predominant prey items, especially for feeding on the ctenophore *Mnemiopsis leidyi*. In general, *Mnemiopsis 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, *Mnemiopsis leidyi* may be a more frequent prey item for estuarine Atlantic *Chrysaora* than for coastal animals. Larger oral arms, as exhibited in *Chrysaora chesapeakei* (Fig. 8B), have been argued to be an adaptation for scyphozoans that feed on gelatinous prey (*Bayha & 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 *Mnemiopsis leidyi*. Since different nematocyst types are assumed to have different functions based on morphological and discharge characteristics (*Rifkin & Endean, 1983*; *Purcell, 1984*; *Heeger & Möller, 1987*; *Purcell & Mills, 1988*; *Colin & 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 & Mills, 1988*; *Carrette, Alderslade & Seymour, 2002*). In particular, isorhiza nematocysts, which typically serve to entangle hard prey or penetrate soft tissue (*Purcell & Mills, 1988*; *Colin & 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 & Mills, 1988*) and the scyphozoan *Drymonema larsoni* (KM Bayha, personal observation). A-isorhizas are about twice as numerous in *Chrysaora chesapeakei* as in *Chrysaora quinquecirrha* (16.5 ± 3.4% vs. 7.8 ± 3.4%) and are significantly larger (Fig. 8C) in *Chrysaora chesapeakei*. It is possible that the more numerous A-isorhizas, possessing longer tubules, could penetrate farther into the extremely soft-bodied *Mnemiopsis leidyi*, resulting in greater capture efficiency.

Chrysaora in the Caribbean

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

Chrysaora melanaster and Chrysaora pacifica

Our phylogenetic data confirm the morphological conclusions in *Morandini & Marques (2010)* that Japanese *Chrysaora* historically identified as *Chrysaora melanaster*, and labeled as such in public aquaria worldwide for decades, are actually the distinct species *Chrysaora pacifica*. *Kramp (1961)* synonymized the Pacific *Chrysaora* species *C. melanaster* (Brandt, 1835) and the Japanese jellyfish *C. pacifica* (*Goette, 1886*) to *Chrysaora melanaster*. This identification convention made it into jellyfish identification books (*Wrobel & Mills, 1998*) and subsequently Japanese *Chrysaora* labeled as *Chrysaora melanaster* became a mainstay in early jellyfish exhibits, such as the Monterey Bay Aquarium (MBA), and then in aquaria throughout the world (W Patry, personal communication). *Morandini & Marques (2010)* separated *Chrysaora melanaster* and *Chrysaora pacifica* on morphological grounds (tentacle and lappet number) and deemed all aquarium specimens of Japanese origin to be *Chrysaora pacifica*. Our data (Figs. 4 and 5) confirm this, as aquarium-collected jellyfish previously labeled *Chrysaora melanaster* (MBA) are distantly related to wild-caught *Chrysaora melanaster* (BER) from its type locality (Bering Sea), but are nearly genetically similar (sequence divergence: *COI*: 0.5%; *16S*: 0.6%) to wild-caught *Chrysaora* collected from the Eastern Korean coast (KOR), where this jellyfish was recently redescribed as *C. pacifica*

(*Lee et al., 2016*) and Kyoto, Japan (KYO), both near the type locality of Nagasaki, Japan (*Goette, 1886*).

Chrysaora africana/fulgida

Our phylogenies support the resurrection of *Chrysaora* species along the southwestern coast of Africa. Three species of *Chrysaor*a were previously identified from the southwestern coast of Africa: *Chrysaora hysoscella* (Kramp, 1955), *C. fulgida* (*Reynaud, 1830*) and *C. africana* (*Vanhöffen, 1902*). However, *Kramp (1961)* deemed *Chrysaora africana* a variant of *Chrysaora fulgida*, and *Morandini & Marques (2010)* placed all of these sightings within the species *Chrysaora fulgida*. All phylogenies indicate two distantly related species of *Chrysaora* from Namibian waters (Figs. 4–7), with those appearing superficially similar to *Chrysaora fulgida* (brown striped) or to *Chrysaora africana* (red tentacles) placed provisionally into these species. These designations are consistent with upcoming redescriptions of *Chrysaora fulgida* and *Chrysaora africana* of S. Neethling, 2014, unpublished data based on morphological and genetic analyses. *Chrysaora* has increased over recent years in this area, with concomitant ecological perturbations (*Lynam et al., 2006*; *Flynn et al., 2012*; *Roux et al., 2013*), underscoring the importance of correct species identification.

Mawia benovici

In addition to revealing higher level phylogenetic relationships, our data add to our knowledge regarding the distribution of *Mawia 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 *Mawia benovici* based on *COI* and *28S* phylogenies (Figs. 4 and 6) (there are no published *16S* sequences for *Mawia benovici*). While this is not definitive evidence that Mediterranean *Mawia 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 *Mawia 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 *Mawia benovici* between Gibraltar and the Adriatic Sea to our knowledge.

SYSTEMATICS

Chrysaora quinquecirrha Desor, 1848
Figs. 3A, 3B, 4–9; Figs. S1 and 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 *Chrysaora quinquecirrha* and *Chrysaora 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 *Chrysaora quinquecirrha*].

Diagnosis: Living medusae up to 40 cm (observed 59.0–176.0 mm) (Figs. 3A and 3B); tentacles typically 40 or more; 5.28 ± 0.45 (95% CI) tentacles/octant on average (Table 3; Fig. 8A); 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 *Chrysaora chesapeakei* based on (1) smaller size of holotrichous A-isorhiza nematocysts: average: 20.25 [±0.38] × 11.27 [±0.37] µm (Table 3; Fig. 8C); (2) larger tentacle number (more than five 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 five tentacles per octant, but can be more (average 5.28 ± 0.48) (Table 3; Fig. 8A); 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. Four semi-circular gonads, white, pinkish, or slightly orange, well developed within pouch outlining gastric filaments. About 16 stomach pouches bounded by 16 septae. Septae bent at 45° angle distally toward the rhopalia terminating near tentacle in rhopalar lappet, resulting in tentacular pouches being somewhat larger than rhopalar pouches distally.

Cnidome (tentacle): Average dimensions (length ± 95% CI × width ± 95% CI)

Holotrichous A-isorhizas: $20.15 \pm 0.33 \times 11.13 \pm 0.24$ μm;

Holotrichous a-isorhizas: $8.27 \pm 0.49 \times 4.22 \pm 0.07$ μm;

Holotrichous O-isorhizas: $21.63 \pm 0.39 \times 18.91 \pm 0.78$ μm;

Heterotrichous microbasic rhopaloids: $13.58 \pm 0.19 \times 8.09 \pm 0.09$ μm;

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

Habitat: Medusae are found in open coastal waters on the U.S. 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: *Chrysaora quinquecirrha* and *Chrysaora chesapeakei* sequences form reciprocally monophyletic groups for *16S, COI, 28S*, and combined analyses (Figs. 4–7). Minimum sequence divergences between *Chrysaora quinquecirrha* and *Chrysaora chesapeakei* clades (*COI*: 12.1%, *16S*: 8.4%, *28S*: 2.4%) were much larger than the maximum within clades for *Chrysaora quinquecirrha* (*COI*: 0.2%, *16S*: 0.1%, *28S*: 0.0%) or *Chrysaora chesapeakei* (*COI*: 0.7%, *16S*: 0.6%, *28S*: 0.4%). *Chrysaora quinquecirrha* sequences did not form monophyletic groups with any other species (Figs. 4–7).

Biological data: Although the name *Chrysaora quinquecirrha* applies to the U.S. coastal Atlantic species, almost no ecological studies have been done on the coastal species, apart from (*Kraeuter & Setzler, 1975*), which found the largest *Chrysaora 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 *Chrysaora 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
Figs. 3C–3E and 4–9; Figs. S1 and 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 [Chesapeake Bay; white colored morph based on A-isorhiza measurements].

Chrysaora quinquecirrha: *Kramp (1961)*: 327–328 [parts of description covers both *Chrysaora quinquecirrha* and *Chrysaora chesapeakei*]. *Rice & Powell (1970)*: 180–186 [Chesapeake Bay]. *Burke (1976)*: 20, 22–28 [Mississippi Sound, Gulf of Mexico]. *Calder (1971)*: 270–274 [Gloucester Point (VA)—Chesapeake Bay]. *Calder (1972)*: 40–43, Figures 1–4 [Chesapeake Bay, Pamlico Sound, Gulf of Mexico]. *Loeb (1972)*: 279–291 [Chesapeake Bay]. *Loeb (1973)*: 144–147 [Chesapeake Bay]. *Loeb & Blanquet (1973)*: 150–157 [Chesapeake Bay]. *Calder (1974b)*: 326–333 [Chesapeake Bay]. *Loeb (1974)*: 423–432 [Chesapeake Bay]. *Blanquet & Wetzel (1975)*: 181–192 [Chesapeake Bay]. *Cargo (1975)*: 145–154 [Chesapeake Bay]. *Kraeuter & Setzler (1975)*: 69, Figures 1–2 [Doboy Sound (GA)]. *Loeb & Gordon (1975)*: 37–41 [Chesapeake Bay]. *Lin & Zubkoff (1976)*: 37–41 [Chesapeake Bay]. *Calder (1977)*: 13–19 [Gloucester Point, MD—Chesapeake Bay]. *Clifford & Cargo (1978)*: 58–60 [Patuxent River, MD—Chesapeake Bay]. *Cargo (1979)*: 279–286 [Chesapeake Bay]. *Cargo & Rabenold (1980)*: 20–26 [Patuxent River (MD)]. *Hutton et al. (1986)*: 154–155 [Chesapeake Bay]. *Cargo & King (1990)*: 486–491 [Chesapeake Bay]. *Purcell et al. (1991)*: 103–111 [Choptank River, MD—Chesapeake Bay]. *Nemazie, Purcell & Glibert (1993)*: 451–458 [Chesapeake Bay]. *Purcell, White & Roman (1994)*: 263–278 [Chesapeake Bay]. *Burnett et al. (1996)*: 1377–1383 [Chesapeake Bay]; *Houck et al. (1996)*: 771–778 [St. Margaret's, MD—Chesapeake Bay]. *Olesen, Purcell & Stoecker (1996)*: 149–158 [Broad Creek (MD)—Chesapeake Bay]. *Ford et al. (1997)*: 355–361 (Choptank River (MD)—Chesapeake Bay]. *Kreps, Purcell & Heidelberg (1997)*: 441–446 [Choptank River (MD)—Chesapeake Bay]. *Wright & Purcell (1997)*: 332–338 [Patuxent River (MD)—Chesapeake Bay]. *Suchman & Sullivan (1998)*: 237–244 [Green Hill Pond (RI)]. *Purcell, Malej & Benović (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-Pineiro & Kimmel (2015)*: 1965–1975 [Pamlico Sound (NC). *Meredith, Gaynor & Bologna (2016)*: 6248–6266 [Barnegat Bay (NJ)]. *Tay & Hood (2017)*: 227–242 [Choptank River (MD), Chesapeake Bay].

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

Material examined: Neotype: USNM 1454948—(Gloucester Point, MD—Chesapeake Bay). Other comparative specimens: USNM 57925 (n = 9; Orange Inlet, NC), USNM 56758 (n = 5; Charlestown Pond, RI), USNM 33456 (n = 4; Plum Point, MD), USNM 49733 (n = 1; Alligator Harbor, FL), USNM 53826 (n = 2; Timbalier Bay, LA), USNM 56703 (n = 2; Chesapeake Bay 37.23 N 76.04 W), USNM 56704 (n = 4; Chesapeake Bay 37.23 N 76.04 W), USNM 53870 (n = 3; Beaufort, NC), USNM 53828 (n = 2; Drum Point, MD), USNM 33458 (n = 3; Plum Point, MD), USNM 33457 (n = 4; Plum Point, MD), USNM 55621 (n = 6; near Chesapeake Beach, MD), USNM 53867 (n = 1; Arundel on the Bay, MD), USNM 54404 (n = 1; Chesapeake Bay 37.23 N 76.04 W), USNM 33121 (n = 6; Arundel on the Bay, MD), USNM 42155 (n = 2; Louisiana, Gulf of Mexico), USNM 54372 (n = 1; Lake Pontchartrain, LA); USNM 1454941–USNM 1454943, KMBCSC1, KMBCSC4–KMBCSC5, KMBCSC7 (n = 7; Charleston Harbor, SC), USNM 1454944–USNM 1454951, KMBGVA1, KMBGVA5, KMBGVA7, KMBGVA10 (n = 12; Gloucester Point, VA), KMBCRI1–KMBCRI14 (n = 14; Charlestown Pond, RI), KMBRDE1–KMBRDE16 (n = 16; Rehoboth Bay, DE), USNM 1454956, KMBDIA2–KMBDIA3 (n = 3; Dauphin Island, AL).

Description of neotype specimen: USNM 1454948. Bell diameter 110.4 mm, almost hemispherical. Exumbrella white/clear with granulated surface of small white marks. Eight rhopalia. No ocelli. Deep rhopalar clefts; deep sensory pits. Marginal lappets rounded, 32 total or 4 per octant made up of two rhopalar lappets and two tentacular lappets. Lappet size barely heterogeneous, with rhopalar lappets about the same width as tentacular lappets but longer. Tentacle number 24 or 3 per octant, with primary tentacle surrounded by two secondary tentacles (2-1-2), primary tentacle longer than secondary tentacles, up to 3–4 times bell diameter. Tentacles are white, slightly pinkish. Tentacle clefts of varied depth with primary clefts deeper than secondary clefts. Radial and ring musculature not obvious. Brachial disc circular. Pillars evident. No quadralinga.

Subgenital ostia rounded, approximately 1/10 of bell diameter. Oral arms white, v-shaped with frills emanating from tube-like structure. Oral arms straight without spiral curved, frilled edges taper toward distal end of oral arms. Orals arms long, approximately five (4.98) times bell diameter. Four semi-circular gonads, white (a bit orange), well developed within pouch outlining gastric filaments. About 16 stomach pouches bounded by 16 septae. Septae bent at 45° angle distally toward the rhopalia terminating near tentacle in rhopalar lappet, resulting in tentacular pouches being somewhat larger than rhopalar pouches distally.

Cnidome (tentacle): Average dimensions (length ± 95% CI × width ± 95% CI)

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

Holotrichous a-isorhizas: 7.77 ± 0.20 × 4.17 ± 0.10 μm;

Holotrichous O-isorhizas: 22.02 ± 0.30 × 19.95 ± 0.24 μm;

Heterotrichous microbasic rhopaloids: 12.35 ± 0.47 × 8.55 ± 0.55 μm.

Description of other specimens: Bell diameter up to approximately 20 cm (observed 17.0–175.0 mm), almost hemispherical but flattened in small individuals. Exumbrella finely granulated with small, inconspicuous marks (papillae); exumbrellar color varies considerably, varying from all white to a completely brown or red colored bell, to a bell with radial lines of red/brown with a spot in the center of the bell. Radial lines may be relatively inconspicuous without a noticeable spot in the center. Tentacles typically number 24 or 3 per octant (average 3.07 ± 0.07), with primary tentacle surrounded by two secondary tentacles (2-1-2), primary tentacle longer than secondary tentacles, up to 3–4 times bell diameter. In some rare cases, small tentacles may occur laterally to secondary tentacle, occurring between the secondary tentacle and rhopalium. In almost all cases, this tentacle is similar in size to or smaller than the lappets surrounding it. In very rare cases (twice observed), about five or more tentacles per octant have been seen, though these medusae had aberrant tentacle patterns overall (e.g., more than one tentacle emerging from same spot, tentacles emerging below lappet). Tentacles are white, slightly pinkish. Marginal lappets rounded and typically 32 or 4 per octant (average 4.08 ± 0.06). Tentacle clefts of varied depth with primary clefts deeper than secondary clefts, which are deeper than rare tertiary clefts. Radial and ring musculature not obvious. Brachial disc circular. Pillars evident. No quadralinga. Subgenital ostia rounded, approximately 1/10 of bell diameter. Oral arms 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 three 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. Four semi-circular gonads, white, pinkish or slightly orange, well developed within pouch outlining gastric filaments. About 16 stomach pouches bounded by 16 septae. Septae bent at 45° angle distally toward the rhopalia terminating near tentacle in rhopalar lappet, resulting in tentacular pouches being somewhat larger than rhopalar pouches distally.

Cnidome (tentacle): Average dimensions (length ± 95% CI × width ± 95% CI)

Holotrichous A-isorhizas: 26.21 ± 0.50 × 19.74 ± 0.55 μm;

Holotrichous a-isorhizas: 7.88 ± 0.13 × 4.29 ± 0.07 μm;

Holotrichous O-isorhizas: 23.10 ± 0.43 × 20.75 ± 0.62 μm;

Heterotrichous microbasic rhopaloids: 12.73 ± 0.22 × 8.29 ± 0.13 μm.

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

Habitat: Medusae are found in estuarine waters on the U.S. 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.

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

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: *Chrysaora chesapeakei* and *Chrysaora quinquecirrha* sequences form reciprocally monophyletic groups for *16S*, *COI*, *28S*, and combined analyses (Figs. 4–7). Minimum sequence divergences between *Chrysaora chesapeakei* and *Chrysaora quinquecirrha* clades (*COI*: 12.1%, *16S*: 8.4%, *28S*: 2.5%) were much larger than the

maximum within clades for *Chrysaora quinquecirrha* (*COI*: 0.3%, *16S*: 0.1%, *28S*: 0.0%) or *Chrysaora chesapeakei* (*COI*: 2.2%, *16S*: 1.9%, *28S*: 0.7%). *Chrysaora chesapeakei* sequences do not form monophyletic groups with any other species (Figs. 4–7).

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Grant Disclosures

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Keith M. Bayha conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Allen G. Collins conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
- Patrick M. Gaffney conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

MF141552–MF141718; MF167556–MF167568

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.3863#supplemental-information.

REFERENCES

Agassiz A. 1865. North American Acelephae. *Illustrated Catalogue of the Museum of Comparative Zoology at Harvard College* **2**:1–234.

Agassiz A, Mayer A. 1898. On *Dactylometra. Bulletin of the Museum of Comparative Zoology at Harvard College* **32**:1–11.

Agassiz L. 1862. *Contributions to the Natural History of the United States of America. IV.* Boston: Little, Brown and Company.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25(17)**:3389 DOI 10.1093/nar/25.17.3389.

Antit M, Gofas S, Azzouna A. 2010. A gastropod from the tropical Atlantic becomes an established alien in the Mediterranean. *Biological Invasions* **12(5)**:991–994 DOI 10.1007/s10530-009-9532-2.

Arai M. 1997. *A Functional Biology of Scyphozoa.* London: Chapman and Hall.

Arai MN. 2005. Predation on pelagic coelenterates: a review. *Journal of the Marine Biological Association of the United Kingdom* **85(3)**:523–536 DOI 10.1017/s0025315405011458.

Ausubel FM, Brent R, Kingston RF, Moore DD, Seidman JG, Smith JA, Struhl K. 1989. *Current Protocols in Molecular Biology.* New York: Wiley and Sons.

Avian M, Ramšak A, Tirelli V, D'Ambra I, Malej A. 2016. Redescription of *Pelagia benovici* into a new jellyfish genus, *Mawia*, gen. nov., and its phylogenetic position within Pelagiidae (Cnidaria: Scyphozoa: Semaeostomeae). *Invertebrate Systematics* **30**:523–546 DOI 10.1071/IS16010.

Bayha KM, Dawson MN. 2010. New family of allomorphic jellyfishes, Drymonematidae (Scyphozoa, Discomedusae), emphasizes evolution in the functional morphology and trophic ecology of gelatinous zooplankton. *Biological Bulletin* **219(3)**:249–267 DOI 10.1086/bblv219n3p249.

Bayha KM, Dawson MN, Collins AG, Barbeitos MS, Haddock SHD. 2010. Evolutionary relationships among scyphozoan jellyfish families based on complete taxon sampling and phylogenetic analyses of *18S* and *28S* ribosomal DNA. *Integrative and Comparative Biology* **50(3)**:436–455 DOI 10.1093/icb/icq074.

Bayha KM, Graham WM. 2009. A new Taqman© PCR-based method for the detection and identification of scyphozoan jellyfish polyps. *Hydrobiologia* **616**:217–228 DOI 10.1007/s10750-008-9590-y.

Bayha KM, Graham WM. 2014. Nonindigenous marine jellyfish: invasiveness, invasibility, and impacts. In: Pitt KA, Lucas CH, eds. *Jellyfish Blooms.* Dordrecht: Springer, 45–77.

Ben Souissi J, Zaouali J, Rezig M, Bradai M, Quignard J, Rudman B. 2004. Contribution à l'étude de quelques récentes migrations d'espèces exotiques dans les eaux tunisiennes. *Rapports de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* **37**:312.

Beulieu WT, Costello JH, Klein-Macphee G, Sullivan BK. 2013. Seasonality of the ctenophore *Mnemiopsis leidyi* in Narragansett Bay, Rhode Island. *Journal of Plankton Research* **35(4)**:785–791 DOI 10.1093/plankt/fbt041.

Bigelow RP. 1880. A new Chrysaoran medusa. *Johns Hopkins Circular* **9**:66.

Birsa LM, Verity PG, Lee RF. 2010. Evaluation of the effects of various chemicals on discharge of and pain caused by jellyfish nematocysts. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **151(4)**:426–430 DOI 10.1016/j.cbpc.2010.01.007.

Blanquet RS, Wetzel B. 1975. Surface ultrastructure of the scyphopolyp, *Chrysaora quinquecirrha*. *Biological Bulletin* **148(2)**:181–192 DOI 10.2307/1540541.

Bloom D, Radwan F, Burnett J. 2001. Toxinological and immunological studies of capillary electrophoresis fractionated *Chrysaora quinquecirrha* (Desor) fishing tentacle and *Chironex fleckeri* Southcott nematocyst venoms. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **128(1)**:75–90 DOI 10.1016/s1532-0456(00)00180-0.

Breitburg D, Burrell R. 2014. Predator-mediated landscape structure: seasonal patterns of spatial expansion and prey control by *Chrysaora quinquecirrha* and *Mnemiopsis leidyi*. *Marine Ecology Progress Series* **510**:183–200 DOI 10.3354/meps10850.

Breitburg DL, Fulford RS. 2006. Oyster-sea nettle interdependence and altered control within the Chesapeake Bay ecosystem. *Estuaries and Coasts* **29(5)**:776–784 DOI 10.1007/bf02786528.

Brooks WK. 1882. List of medusae found at Beaufort, NC, during the summers of 1880 and 1881. *Studies from the Biological Laboratory of the Johns Hopkins University* **2**:135–146.

Brotz L, Pauly D. 2012. Jellyfish populations in the Mediterranean Sea. *Acta Adriatica* **53**:213–230.

Brown CW, Hood RR, Long W, Jacobs J, Ramers DL, Wazniak C, Wiggert JD, Wood R, Xu J. 2013. Ecological forecasting in Chesapeake Bay: using a mechanistic–empirical modeling approach. *Journal of Marine Systems* **125**:113–125 DOI 10.1016/j.jmarsys.2012.12.007S.

Burke WD. 1976. Biology and distribution of the macrocoelenterates of Mississippi Sound and adjacent waters. *Gulf and Caribbean Research* **5**:17–28 DOI 10.18785/grr.0502.03.

Burnett JW, Bloom DA, Imafuku S, Houck H, Vanucci S, Aurelian L, Morris SC. 1996. Coelenterate venom research 1991–1995: clinical, chemical and immunological aspects. *Toxicon* **34(11–12)**:1377–1383 DOI 10.1016/s0041-0101(96)00096-7.

Calder DR. 1971. Nematocysts of polyps of *Aurelia*, *Chrysaora*, and *Cyanea*, and their utility in identification. *Transactions of the American Microscopical Society* **90(3)**:269–274 DOI 10.2307/3225186.

Calder DR. 1972. Development of the sea nettle *Chrysaora quinquecirrha* (Scyphozoa, Semaeostomeae). *Chesapeake Science* **13(1)**:40–44 DOI 10.2307/1350549.

Calder DR. 1974a. Nematocysts of the coronate scyphomedusa, *Linuche unguiculata*, with a brief reexamination of scyphozoan nematocyst classification. *Chesapeake Science* **15(3)**:170–173 DOI 10.2307/1351039.

Calder DR. 1974b. Strobilation of the sea nettle, *Chrysaora quinquecirrha*, under field conditions. *Biological Bulletin* **146(3)**:326–334 DOI 10.2307/1540408.

Calder DR. 1977. Nematocysts of the ephyra stages of *Aurelia*, *Chrysaora*, *Cyanea*, and *Rhopilema* (Cnidaria, Scyphozoa). *Transactions of the American Microscopical Society* **96(1)**:13–19 DOI 10.2307/3225958.

Calder DR. 2009. Cubozoan and scyphozoan jellyfishes of the Carolinian biogeographic province, southeastern USA. *Royal Ontario Museum Contributions in Science* **3**:1–58.

Canepa A, Fuentes V, Sabatés A, Piraino S, Boero F, Gili J-M. 2014. *Pelagia noctiluca* in the Mediterranean Sea. In: Pitt KA, Lucas CH, eds. *Jellyfish Blooms*. Dordrecht: Springer, 237–266.

Cargo DG. 1979. Observations on the settling behavior of planular larvae of *Chrysaora quinquecirrha*. *International Journal of Invertebrate Reproduction* **1(5)**:279–287 DOI 10.1080/01651269.1979.10553325.

Cargo DG. 1975. Comments on the laboratory culture of Scyphozoa. In: Smith WL, Chanley MH, eds. *Culture of Marine Invertebrate Animals*. New York: Plenum Press, 145–154.

Cargo DG, King DR. 1990. Forecasting the abundance of the sea nettle, *Chrysaora quinquecirrha*, in the Chesapeake Bay. *Estuaries* **13(4)**:486–491 DOI 10.2307/1351793.

Cargo DG, Rabenold GE. 1980. Observations on the asexual reproductive activities of the sessile stages of the sea nettle *Chrysaora quinquecirrha* (Scyphozoa). *Estuaries* **3(1)**:20–27 DOI 10.2307/1351931.

Cargo DG, Schultz LP. 1966. Notes on the biology of the sea nettle, *Chrysaora quinquecirrha*, in Chesapeake Bay. *Chesapeake Science* **7(2)**:95–100 DOI 10.2307/1351129.

Carrette T, Alderslade P, Seymour J. 2002. Nematocyst ratio and prey in two Australian cubomedusans, *Chironex fleckeri* and *Chiropsalmus* sp. *Toxicon* **40**:1547–1551 DOI 10.1016/S0041-0101(02)00168-X.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17(4)**:540–552 DOI 10.1093/oxfordjournals.molbev.a026334.

Castro JJ, Santiago JA, Santana-Ortega AT. 2002. A general theory on fish aggregation to floating objects: an alternative to the meeting point hypothesis. *Reviews in Fish Biology and Fisheries* **11**:255–277 DOI 10.1023/A:1020302414472.

Clifford HC, Cargo DG. 1978. Feeding rates of the sea nettle, *Chrysaora quinquecirrha*, under laboratory conditions. *Estuaries* **1(1)**:58–61 DOI 10.2307/1351651.

Colin S, Costello JH. 2007. Functional characteristic of nematocysts found on the scyphomedusa *Cyanea capillata*. *Journal of Experimental Marine Biology and Ecology* **351**:114–120 DOI 10.1016/j.jembe.2007.06.033.

Condon RH, Decker MB, Purcell JE. 2001. Effects of low dissolved oxygen on survival and asexual reproduction of scyphozoan polyps (*Chrysaora quinquecirrha*). *Hydrobiologia* **451**:89–95 DOI 10.1023/A:1011892107211.

Condon RH, Duarte CM, Pitt KA, Robinson KL, Lucas CH, Sutherland KR, Mianzan HW, Bogeberg M, Purcell JE, Decker MB. 2013. Recurrent jellyfish blooms are a consequence of global oscillations. *Proceedings of the National Academy of Sciences of the United States of America* **110**:1000–1005 DOI 10.1073/pnas.1210920110.

Condon RH, Steinberg DK. 2008. Development, biological regulation, and fate of ctenophore blooms in the York River estuary, Chesapeake Bay. *Marine Ecology Progress Series* **369**:153–168 DOI 10.3354/meps07595.

Condon RH, Steinberg DK, Bronk DA. 2010. Production of dissolved organic matter and inorganic nutrients by gelatinous zooplankton in the York River estuary, Chesapeake Bay. *Journal of Plankton Research* **32(2)**:153–170 DOI 10.1093/plankt/fbp109.

Condon RH, Steinberg DK, Del Giorgio PA, Bouvier TC, Bronk DA, Graham WM, Ducklow HW. 2011. Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems. *Proceedings of the National Academy of Sciences of the United States of America* **108**:10225–10230 DOI 10.1073/pnas.1015782108.

Costello JH, Bayha KM, Mianzan HW, Shiganova TA, Purcell JE. 2012. Transitions of *Mnemiopsis leidyi* (Ctenophora: Lobata) from a native to an exotic species: a review. *Hydrobiologia* **690(1)**:21–46 DOI 10.1007/s10750-012-1037-9.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9(8)**:772–772 DOI 10.1038/nmeth.2109.

Dawson MN. 2003. Macro-morphological variation among cryptic species of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). *Marine Biology* **143**:369–379 DOI 10.1007/s00227-003-1070-3.

Dawson MN. 2005. *Cyanea capillata* is not a cosmopolitan jellyfish: morphological and molecular evidence for *C. annaskala* and *C. rosea* (Scyphozoa: Semaeostomeae: Cyaneidae) in south-eastern Australia. *Invertebrate Systematics* **19(4)**:361–370 DOI 10.1071/is03035.

Dawson MN, Gupta AS, England MH. 2005. Coupled biophysical global ocean model and molecular genetic analyses identify multiple introductions of cryptogenic species. *Proceedings of the National Academy of Sciences of the United States of America* **102(34)**:11968–11973 DOI 10.1073/pnas.0503811102.

Dawson MN, Jacobs DK. 2001. Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). *Biological Bulletin* **200(1)**:92–96 DOI 10.2307/1543089.

Dawson MN, Martin LE. 2001. Geographic variation and ecological adaptation in *Aurelia* (Scyphozoa, Semaeostomeae): some implications from molecular phylogenetics. *Hydrobiologia* **451**:259–273 DOI 10.1007/978-94-010-0722-1_21.

Dawson MN, Raskoff KA, Jacobs DK. 1998. Field preservation of marine invertebrate tissue for DNA analyses. *Molecular Marine Biology and Biotechnology* **7**:145–152.

Decker MB, Brown CW, Hood RR, Purcell JE, Gross TF, Matanoski JC, Bannon RO, Setzler-Hamilton EM. 2007. Predicting the distribution of the scyphomedusa *Chrysaora quinquecirrha* in Chesapeake Bay. *Marine Ecology Progress Series* **329**:99–113 DOI 10.3354/meps329099.

Desor E. 1848. Hydroids from Nantucket. *Proceedings of the Boston Society of Natural History* **3**:65–66.

Diaz Briz L, Sánchez F, Marí N, Mianzan H, Genzano G. 2017. Gelatinous zooplankton (ctenophores, salps and medusae): an important food resource of fishes in the temperate SW Atlantic Ocean. *Marine Biology Research* **13(6)**:630–644 DOI 10.1080/17451000.2016.1274403.

Doyle TK, Hays GC, Harrod C, Houghton JD. 2014. Ecological and societal benefits of jellyfish. In: Pitt KA, Lucas CH, eds. *Jellyfish Blooms*. Dordrecht: Springer, 105–127.

Duarte CM, Pitt KA, Lucas CH, Purcell JE, Uye S-I, Robinson K, Brotz L, Decker MB, Sutherland KR, Malej A. 2012. Is global ocean sprawl a cause of jellyfish blooms? *Frontiers in Ecology and the Environment* **11(2)**:91–97 DOI 10.1890/110246.

Duffy JT, Epifanio CE, Fuiman LA. 1997. Mortality rates imposed by three scyphozoans on red drum (*Sciaenops ocellatus* Linnaeus) larvae in field enclosures. *Journal of Experimental Marine Biology and Ecology* **212(1)**:123–131 DOI 10.1016/s0022-0981(96)02741-4.

Eschscholtz F. 1829. *System der Acalephen. Eine ausführliche Beschreibung aller medusen artigen Strahltiere.* Berlin: Ferdinand Dümmler.

Feigenbaum D, Kelly M. 1984. Changes in the lower Chesapeake Bay food chain in presence of the sea nettle *Chrysaora quinquecirrha* (Scyphomedusa). *Marine Ecology Progress Series* **19**:39–47 DOI 10.3354/meps019039.

Fewkes JW. 1881. Studies of the jelly-fishes of Narragansett Bay. *Bulletin of the Museum of Comparative Zoology at Harvard College* **8**:141–182.

Fish CJ. 1925. Seasonal distribution of the plankton of the Woods Hole region. *Bulletin of the Bureau of Fisheries* **41**:91–179.

Flynn B, Richardson A, Brierley A, Boyer D, Axelsen B, Scott L, Moroff N, Kainge P, Tjizoo B, Gibbons M. 2012. Temporal and spatial patterns in the abundance of jellyfish in the northern Benguela upwelling ecosystem and their link to thwarted pelagic fishery recovery. *African Journal of Marine Science* **34(1)**:131–146 DOI 10.2989/1814232x.2012.675122.

Ford M, Costello J, Heidelberg K, Purcell J. 1997. Swimming and feeding by the scyphomedusa *Chrysaora quinquecirrha*. *Marine Biology* **129(2)**:355–362 DOI 10.1007/s002270050175.

Frost JR, Jacoby CA, Frazer TK, Zimmerman AR. 2012. Pulse perturbations from bacterial decomposition of *Chrysaora quinquecirrha* (Scyphozoa: Pelagiidae). *Hydrobiologia* **690(1)**:247–256 DOI 10.1007/s10750-012-1042-z.

Gegenbaur C. 1856. Versuch eines Systemes der Medusen, mit Bescheibung neuer oder wenig gekannter Formen. *Zeitschrift für wissenschaftliche Zoologie* **8**:202–273.

Gershwin L, Collins A. 2002. A preliminary phylogeny of Pelagiidae (Cnidaria, Scyphozoa), with new observations of *Chrysaora colorata* comb. nov. *Journal of Natural History* **36(2)**:127–148 DOI 10.1080/00222930010003819.

Goette A. 1886. Verzeichniss der Medusen welche von Dr Sander, Stabsarzt auf S.M.S. "Prinz Adalbert" gesammelt wurden. *Sitzungsberichte der preussischen Akademie der Wissenschaften* **7**:831–837.

Gofas S, Zenetos A. 2003. Exotic molluscs in the Mediterranean basin: current status and perspectives. In: Gibson R, Atkinson R, eds. *Oceanography and Marine Biology, An Annual Review.* London: Taylor and Francis, 237–277.

Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27(2)**:221–224 DOI 10.1093/molbev/msp259.

Graham W. 2001. Numerical increases and distributional shifts of *Chrysaora quinquecirrha* (Desor) and *Aurelia aurita* (Linné) (Cnidaria: Scyphozoa) in the northern Gulf of Mexico. *Hydrobiolgia* **451**:97–111 DOI 10.1023/A:1011844208119.

Grove M, Breitburg DL. 2005. Growth and reproduction of gelatinous zooplankton exposed to low dissolved oxygen. *Marine Ecology Progress Series* **301**:185–198 DOI 10.3354/meps301185.

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59(3)**:307–321 DOI 10.1093/sysbio/syq010.

Hedgepeth J. 1954. Scyphozoa. *Fishery Bulletin of the Fish and Wildlife Service, US* **55**:277–278.

Heeger T, Möller H. 1987. Ultrastructural observations on prey capture and digestion in the scyphomedusa *Aurelia aurita*. *Marine Biology* **96**:391–400 DOI 10.1007/BF00412523.

Holland BS, Dawson MN, Crow GL, Hofmann DK. 2004. Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. *Marine Biology* **145(6)**:1119–1128 DOI 10.1007/s00227-004-1409-4.

Houck HE, Lipsky MM, Marzella L, Burnett JV. 1996. Toxicity of sea nettle (*Chrysaora quinquecirrha*) fishing tentacle nematocyst venom in cultured rat hepatocytes. *Toxicon* **34(7)**:771–778 DOI 10.1016/0041-0101(96)00004-9.

Houghton JD, Doyle TK, Wilson MW, Davenport J, Hays GC. 2006. Jellyfish aggregations and leatherback turtle foraging patterns in a temperate coastal environment. *Ecology* **87(8)**:1967–1972 DOI 10.1890/0012-9658(2006)87[1967:jaaltf]2.0.co;2.

Hutton CH, Delisle PF, Roberts MH, Hepworth DA. 1986. *Chrysaora quinquecirrha*: a predator on mysids (*Mysidopsis bahia*) in culture. *Progressive Fish Culturist* **48(2)**:154–155 DOI 10.1577/1548-8640(1986)48<154:cq>2.0.co;2.

International Commission on Zoological Nomenclature (ICZN). 1999. *International Code of Zoological Nomenclature*. Fourth Edition. Padvoa: Tipografia La Garangola.

Ishikawa T, Vucenik I, Shamsuddin A, Niculescu F, Burnett JW. 2004. Two new actions of sea nettle (*Chrysaora quinquecirrha*) nematocyst venom: studies on the mechanism of actions on complement activation and on the central nervous system. *Toxicon* **44(8)**:895–899 DOI 10.1016/j.toxicon.2004.08.017.

Johnson DR, Perry HM, Burke WD. 2001. Developing jellyfish strategy hypotheses using circulation models. *Hydrobiologia* **451**:213–221 DOI 10.1023/A:1011880121265.

Kaneshiro-Pineiro MY, Kimmel DG. 2015. Local wind dynamics influence the distribution and abundance of *Chrysaora quinquecirrha* in North Carolina, USA. *Estuaries and Coasts* **38**:1965–1975 DOI 10.1007/s12237-014-9935-x.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30(4)**:772–780 DOI 10.1093/molbev/mst010.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9(4)**:286–298 DOI 10.1093/bib/bbn013.

Kayal E, Bentlage B, Pankey MS, Ohdera A, Medina M, Pachetzki DC, Collins AG, Ryan JF. 2017. Comprehensive phylogenomic analyses resolve cnidarian relationships and the origins of key organismal traits. *PeerJ Preprints* **5**:e3172v3171 DOI 10.7287/peerj.preprints.3172v1.

Kayal E, Roure B, Philippe H, Collins AG, Lavrov DV. 2013. Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology* **13(1)**:5 DOI 10.1186/1471-2148-13-5.

Kimmel DG, Boynton WR, Roman MR. 2012. Long-term decline in the calanoid copepod *Acartia tonsa* in central Chesapeake Bay, USA: an indirect effect of eutrophication? *Estuarine, Coastal and Shelf Science* **101**:76–85 DOI 10.1016/j.ecss.2012.02.019.

Kimmel DG, Roman MR, Zhang X. 2006. Spatial and temporal variability in factors affecting mesozooplankton dynamics in Chesapeake Bay: evidence from biomass size spectra. *Limnology and Oceanography* **51(1)**:131–141 DOI 10.4319/lo.2006.51.1.0131.

Kishinouye K. 1902. Some new Scyphomedusae of Japan. *Journal of the College of Science, Imperial University, Tokyo* **17**:1–17.

Kolbasova GD, Zalevsky AO, Gafurov AR, Gusev PO, Ezhova MA, Zheludkevich AA, Konovalova OP, Kosobokova KN, Kotlov NU, Lanina NO. 2015. A new species of *Cyanea* jellyfish sympatric to *C. capillata* in the White Sea. *Polar Biology* **38**:1439–1451 DOI 10.1007/s00300-015-1707-y.

Kraeuter JN, Setzler EM. 1975. The seasonal cycle of Scyphozoa and Cubozoa in Georgia estuaries. *Bulletin of Marine Science* **25**:66–74.

Kramp PL. The medusae of the tropical west coast of Africa. *Atlantide Report* **3**:239–324.

Kramp PL. 1961. Synopsis of the medusae of the world. *Journal of the Marine Biological Association of the United Kingdom* **40**:7–382 DOI 10.1017/s0025315400007347.

Kreps TA, Purcell J, Heidelberg K. 1997. Escape of the ctenophore *Mnemiopsis leidyi* from the scyphomedusa predator *Chrysaora quinquecirrha*. *Marine Biology* **128(3)**:441–446 DOI 10.1007/s002270050110.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetic analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33(7)**:1870–1874 DOI 10.1093/molbev/msw054.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**:2947 DOI 10.1093/bioinformatics/btm404.

Larson RJ. 1976. *Marine Flora and Fauna of the Northeastern United States, Cnidaria: Scyphozoa. NOAA Technical Report NMFS Circular 397*. Washington, D.C.: US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Lebrato M, Pitt KA, Sweetman AK, Jones DO, Cartes JE, Oschlies A, Condon RH, Molinero JC, Adler L, Gaillard C. 2012. Jelly-falls historic and recent observations: a review to drive future research directions. *Hydrobiologia* **690(1)**:227–245 DOI 10.1007/s10750-012-1046-8.

Lee HE, Yoon WD, Chae J, Ki J-S. 2016. Re-description of *Chrysaora pacifica* (Goette, 1886) (Cnidaria, Scyphozoa) from Korean Coastal waters: morphology and molecular comparisons. *Ocean and Polar Research* **38(4)**:295–301 DOI 10.4217/opr.2016.38.4.295.

Lin AL, Zubkoff PL. 1976. Malate dehydrogenase isozymes of different stages of Chesapeake Bay jellyfish. *Biological Bulletin* **150(2)**:268–278 DOI 10.2307/1540473.

Littleford RA. 1939. The life cycle of *Dactylometra quinquecirrha*, L. Agassiz in the Chesapeake Bay. *Biological Bulletin* **77(3)**:368–381 DOI 10.2307/1537646.

Littleford RA, Truitt R. 1937. Variation of *Dactylometra quinquecirrha*. *Science* **86(2236)**:426–427 DOI 10.1126/science.86.2236.426.

Loeb MJ. 1972. Strobilation in the Chesapeake Bay sea nettle *Chrysaora quinquecirrha*. I. The effects of environmental temperature changes on strobilation and growth. *Journal of Experimental Zoology* **180(2)**:279–291 DOI 10.1002/jez.1401800214.

Loeb MJ. 1973. The effect of light on strobilation in the Chesapeake Bay sea nettle *Chrysaora quinquecirrha*. *Marine Biology* **20(2)**:144–147 DOI 10.1007/bf00351452.

Loeb MJ. 1974. Strobilation in the Chesapeake Bay sea nettle *Chrysaora quinquecirrha*—III. Dissociation of the neck-inducing factor from strobilating polyps. *Comparative Biochemistry and Physiology Part A: Physiology* **49(3)**:423–432 DOI 10.1016/0300-9629(74)90558-1.

Loeb MJ, Blanquet RS. 1973. Feeding behavior in polyps of the Chesapeake Bay sea nettle, *Chrysaora quinquecirrha* (Desor, 1848). *Biological Bulletin* **145(1)**:150–158 DOI 10.2307/1540355.

Loeb MJ, Gordon CM. 1975. Strobilation in the Chesapeake Bay sea nettle, *Chrysaora quinquecirrha*—IV. Tissue levels of iodinated high molecular weight component and nif in relation to temperature change-induced behavior. *Comparative Biochemistry and Physiology Part A: Physiology* **51(1)**:37–42 DOI 10.1016/0300-9629(75)90410-7.

Luque ÁA, Barrajón A, Remón JM, Moreno D, Moro L. 2012. *Marginella glabella* (Mollusca: Gastropoda: Marginellidae): a new alien species from tropical West Africa established in southern Mediterranean Spain through a new introduction pathway. *Marine Biodiversity Records* **5**:e17 DOI 10.1017/s1755267212000012.

Lynam CP, Brierley AS. 2007. Enhanced survival of 0-group gadoid fish under jellyfish umbrellas. *Marine Biology* **150(6)**:1397–1401 DOI 10.1007/s00227-006-0429-7.

Lynam CP, Gibbons MJ, Axelsen BE, Sparks CA, Coetzee J, Heywood BG, Brierley AS. 2006. Jellyfish overtake fish in a heavily fished ecosystem. *Current Biology* **16(13)**:R492–R493 DOI 10.1016/j.cub.2006.06.018.

Matanoski J, Hood R, Purcell J. 2001. Characterizing the effect of prey on swimming and feeding efficiency of the scyphomedusa *Chrysaora quinquecirrha*. *Marine Biology* **139(1)**:191–200 DOI 10.1007/s002270100558.

Matanoski JC, Hood RR. 2006. An individual-based numerical model of medusa swimming behavior. *Marine Biology* **149(3)**:595–608 DOI 10.1007/s00227-006-0244-1.

Mayer AG. 1910. *Medusae of the World, III: The Scyphomedusae*. Washington: Carnegie Institute.

Meredith RW, Gaynor JJ, Bologna PA. 2016. Diet assessment of the Atlantic Sea Nettle *Chrysaora quinquecirrha* in Barnegat Bay, New Jersey, using next-generation sequencing. *Molecular Ecology* **25(24)**:6248–6266 DOI 10.1111/mec.13918.

Meyer D, Zeileis A, Hornik K. 2016. *vcd: Visulaizing Categorical Data. R Package Version 14-3.*

Mianzan H, Quiñones J, Palma S, Schiariti A, Acha EM, Robinson KL, Graham WM. 2014. *Chrysaora plocamia*: a poorly understood jellyfish from South American waters. In: Pitt KA, Lucas CH, eds. *Jellyfish Blooms*. Dordrecht: Springer, 219–236.

Morandini AC, Marques AC. 2010. Revision of the genus *Chrysaora* Péron & Lesueur, 1810 (Cnidaria: Scyphozoa). *Zootaxa* **2464**:1–97.

Nemazie D, Purcell J, Glibert P. 1993. Ammonium excretion by gelationous zooplankton and their contribution to the ammonium requirements of microplankton in Chesapeake Bay. *Marine Biology* **116(3)**:451–458 DOI 10.1007/bf00350062.

Ohta N, Sato M, Ushida K, Kokubo M, Baba T, Taniguchi K, Urai M, Kihira K, Mochida J. 2009. Jellyfish mucin may have potential disease-modifying effects on osteoarthritis. *BMC Biotechnology* **9(1)**:98 DOI 10.1186/1472-6750-9-98.

Olesen NJ, Purcell JE, Stoecker DK. 1996. Feeding and growth by ephyrae of scyphomedusae *Chrysaora quinquecirrha*. *Marine Ecology Progress Series* **137**:149–159 DOI 10.3354/meps137149.

Omori M, Nakano E. 2001. Jellyfish fisheries in southeast Asia. *Hydrobiologia* **451**:19–26 DOI 10.1023/A:1011879821323.

Östman C, Hydman J. 1997. Nematocyst analysis of *Cyanea capillata* and *Cyanea lamarckii* (Scyphozoa, Cnidaria). *Scientia Marina* **61**:313–344.

Papenfuss EJ. 1936. The utility of the nematocysts in the classification of certain scyphomedusae. I. *Cyanea capillata*, *Cyanea palmstruchii*, *Dactylometra quinquecirrha*, *Dactylometra quinquecirrha* var. *chesapeakei*, and *Chrysaora hysoscella*. *Acta Universitatis Lundensis, Nova Series* **31**:19–26.

Péron F, Lesueur C. 1810. Tableau des caractères génériques et spécifiques de toutes les espèces de Méduses connues jusqu'à ce jour. *Annales du Muséum National d'Histoire Naturelle, Paris* **14**:325–366.

Perry HM, Larson K. 2004. A picture guide to shelf invertebrates from the Northern Gulf of Mexico. *Available at http://www.gsmfc.org/seamap-pg.php* (accessed 13 March 2017).

Persad G, Hopcroft RR, Webber MK, Roff JC. 2003. Abundance, biomass and production of ctenophores and medusae off Kingston, Jamaica. *Bulletin of Marine Science* **73**:379–396.

Piraino S, Aglieri G, Martell L, Mazzoldi C, Melli V, Milisenda G, Scorrano S, Boero F. 2014. *Pelagia benovici* sp. nov.(Cnidaria, Scyphozoa): a new jellyfish in the Mediterranean Sea. *Zootaxa* **3794(3)**:455–468 DOI 10.11646/zootaxa.3794.3.7.

Purcell JE. 1992. Effects of predation by the scyphomedusan *Chrysaora quinquecirrha* on zooplankton populations in Chesapeake Bay, USA. *Marine Ecology Progress Series* **87**:65–76 DOI 10.3354/meps087065.

Purcell JE. 1997. Pelagic cnidarians and ctenophores as predators: selective predation, feeding rates, and effects on prey populations. *Annales de l'Institut océanographique* **73**:125–137.

Purcell JE. 1984. The functions of nematocysts in prey capture by epipelagic siphonophores (Coelenterata, Hydrozoa). *Biological Bulletin* **166**:310–327 DOI 10.2307/1541219.

Purcell JE. 2007. Environmental effects on asexual reproduction rates of the scyphozoan *Aurelia labiata*. *Marine Ecology Progress Series* **348**:183–196 DOI 10.3354/meps07056.

Purcell JE. 2009. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. *Hydrobiologia* **616(1)**:23–50 DOI 10.1007/s10750-008-9585-8.

Purcell JE, Cresswell FP, Cargo DG, Kennedy VS. 1991. Differential ingestion and digestion of bivalve larvae by the scyphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi*. *Biological Bulletin* **180(1)**:103–111 DOI 10.2307/1542433.

Purcell JE, Decker MB. 2005. Effects of climate on relative predation by scyphomedusae and ctenophores on copepods in Chesapeake Bay during 1987–2000. *Limnology and Oceanography* **50(1)**:376–387 DOI 10.4319/lo.2005.50.1.0376.

Purcell JE, Malej A, Benović A. 1999. Potential links of jellyfish to eutrophication and fisheries. In: Malone TC, Malej A, Harding LW Jr, Smodlaka N, Eugene Turner R, eds. *Ecosystems at the Land-Sea Margin: Drainage Basin to Coastal Sea: Drainage Basis to Coastal Sea Coastal and Estuarine Studies*. Vol. 55. Washington: American Geophysical Union, 241–263.

Purcell JE, Mills CE. 1988. The correlation of nematocyst types to diets in pelagic Hydrozoa. In: Hessinger DA, Lenhoff HM, eds. *The Biology of Nematocysts*. San Diego: Academic Press, 463–485.

Purcell JE, Uye S, Lo WT. 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Marine Ecology Progress Series* **350**:153–174 DOI 10.3354/meps07093.

Purcell JE, White JR, Nemazie DA, Wright DA. 1999. Temperature, salinity and food effects on asexual reproduction and abundance of the scyphozoan *Chrysaora quinquecirrha*. *Marine Ecology Progress Series* **180**:187–196 DOI 10.3354/meps180187.

Purcell JE, White JR, Roman MR. 1994. Predation by gelatinous zooplankton and resource limitation as potential controls of *Acartia tonsa* copepod populations in Chesapeake Bay. *Limnology and Oceanography* **39(2)**:263–278 DOI 10.4319/lo.1994.39.2.0263.

Qu C-F, Song J-M, Li N, Li X-G, Yuan H-M, Duan L-Q, Ma Q-X. 2015. Jellyfish (*Cyanea nozakii*) decomposition and its potential influence on marine environments studied via simulation experiments. *Marine Pollution Bulletin* **97(1–2)**:199–208 DOI 10.1016/j.marpolbul.2015.06.016.

Rambaut A. 2014. Figtree v1.4.2. (accessed March 2017). *Available at http://tree.bio.ed.ac.uk/ software/figtree/*.

Reynaud AAM. 1830. Medusa (*Rhyzostoma*) *fulgida*. In: Lesson RP, ed. *Centurie Zoologique, ou choix d'animaux rares, nouveaux ou imparfaitement connus*. Paris: F.G. Levrault, 79–80.

Rice NE, Powell WA. 1970. Observations on three species of jellyfishes from Chesapeake Bay with special reference to their toxins. I. *Chrysaora* (*Dactylometra*) *quinquecirrha*. *Biological Bulletin* **139(1)**:180–187 DOI 10.2307/1540135.

Richardson AJ, Bakun A, Hays GC, Gibbons MJ. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology & Evolution* **24(6)**:312–322 DOI 10.1016/j.tree.2009.01.010.

Rifkin J, Endean R. 1983. The structure and function of the nematocysts of *Chironex fleckeri* Southcott, 1956. *Cell Tissue Research* **233**:563–577.

Robinson KL, Graham WM. 2013. Long-term change in the abundances of northern Gulf of Mexico scyphomedusae *Chrysaora* sp. and *Aurelia* spp. with links to climate variability. *Limnology and Oceanography* **58(1)**:235–253 DOI 10.4319/lo.2013.58.1.0235.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61(3)**:539–542 DOI 10.1093/sysbio/sys029.

Roux J-P, van der Lingen CD, Gibbons MJ, Moroff NE, Shannon LJ, Smith AD, Cury PM. 2013. Jellyfication of marine ecosystems as a likely consequence of overfishing small pelagic fishes: lessons from the Benguela. *Bulletin of Marine Science* **89(1)**:249–284 DOI 10.5343/bms.2011.1145.

Schroth W, Jarms G, Streit B, Schierwater B. 2002. Speciation and phylogeography in the cosmopolitan marine moon jelly, *Aurelia* sp. *BMC Evolutionary Biology* **2**:1 DOI 10.1186/1471-2148-2-1.

Schultz LP, Cargo DG. 1969. *Sea Nettle Barriers for Bathing Beaches in Upper Chesapeake Bay.* Solomons: Natural Resources Institute, University of Maryland.

Segura-Puertas L, Suárez-Morales E, Celis L. 2003. A checklist of the Medusae (Hydrozoa, Scyphozoa and Cubozoa) of Mexico. *Zootaxa* **194(1)**:1–15 DOI 10.11646/zootaxa.194.1.1.

Sexton MA. 2012. Factors influencing appearance, disappearance, and variability of abundance of the sea nettle *Chrysaora quinquecirrha* in Chesapeake Bay. PhD dissertation. University of Delaware.

Sexton MA, Hood RR, Sarkodee-adoo J, Liss AM. 2010. Response of *Chrysaora quinquecirrha* medusae to low temperature. *Hydrobiologia* **645(1)**:125–133 DOI 10.1007/s10750-010-0222-y.

Stiasny G. 1930. Über *Dactylometra fulgida* (Renaud) von der Walfischbai. *Zoologische Anzeiger* **126**:172–185.

Suchman CL, Sullivan BK. 1998. Vulnerability of the copepod *Acartia tonsa* to predation by the scyphomedusa *Chrysaora quinquecirrha*: effect of prey size and behavior. *Marine Biology* **132(2)**:237–245 DOI 10.1007/s002270050389.

Sutton JS, Burnett JW. 1969. A light and electron microscopic study of nematocytes of *Chrysaora quinquecirrha*. *Journal of Ultrastructure Research* **28(4)**:214–234 DOI 10.1016/s0022-5320(69)90081-1.

Tay J, Hood RR. 2017. Abundance and patchiness of *Chrysaora quinquecirrha* medusae from a high-frequency time series in the Choptank River, Chesapeake Bay, USA. *Hydrobiologia* **792(1)**:227–242 DOI 10.1007/s10750-016-3060-8.

Thuesen EV, Rutherford LD, Brommer PL, Garrison K, Gutowska MA, Towanda T. 2005. Intragel oxygen promotes hypoxia tolerance of scyphomedusae. *Journal of Experimental Biology* **208(13)**:2475–2482 DOI 10.1242/jeb.01655.

Vanhöffen E. 1902. Die Acraspeden Medusen de deutschen Tiefsee-expedition 1898–1899. In: *Wissenschaftliche Ergebnisse der deutschen Tiefsee-expedition auf dem dampfer Valdivia 1898–1899.* Vol. 3. Jen: Gustav Fischer, 3–52.

Venables WN, Ripley BD. 2002. *Modern Applied Statistics with S.* Fourth Edition. New York: Springer.

Weill R. 1934. Contribution à l' étude des cnidaires et de leurs nématocystes. I, II. *Travaux de la Station Zoologique de Wimereux* **10/11**:1–701.

Wright DA, Purcell DA. 1997. Effect of salinity on ionic shifts in mesohaline scyphomedusae, *Chrysaora quinquecirrha*. *Biological Bulletin* **192(2)**:332–339 DOI 10.2307/1542726.

Wrobel D, Mills C. 1998. *Pacific coast pelagic invertebrates: a guide to the common gelatinous animals.* Monterey: Sea Challengers and Monterey Bay Aquarium.

Zenetos A, Gofas S, Morri C, Rosso A, Violanti D, Raso J, Çinar M, Almogi-Labin A, Ates A, Azzurro E. 2012. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterranean Marine Science* **13(2)**:328–352 DOI 10.12681/mms.327.