Integrative and Comparative Biology Advance Access published June 23, 2010
Integrative and Comparative Biology, pp. 1–20
doi:10.1093/icb/icq074

SYMPOSIUM

Evolutionary Relationships Among Scyphozoan Jellyfish Families Based on Complete Taxon Sampling and Phylogenetic Analyses of 18S and 28S Ribosomal DNA

Keith M. Bayha,*[,1] Michael N Dawson,[2,*] Allen G. Collins,[†] Marcos S. Barbeitos[‡] and Steven H. D. Haddock[§]

*School of Natural Sciences, University of California at Merced, 5200 North Lake Road, Merced, CA 95343, USA; [†]National Systematics Laboratory of NOAA's Fisheries Service, National Museum of Natural History, MRC-153, Smithsonian Institution, PO Box 37012, Washington, DC 20013-7012, USA; [‡]University of Kansas, Department of Ecology and Evolutionary Biology, Haworth Hall, 1200 Sunnyside Avenue, Lawrence, KS 66045-7566, USA and [§]Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, USA

From the symposium "Assembling the Cnidarian Tree of Life" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2010, at Seattle, Washington.

[1]Present address: Dauphin Island Sea Lab, 101 Bienville Blvd, Dauphin Island, AL 36528 USA
[2]E-mail: mdawson@ucmerced.edu

Synopsis A stable phylogenetic hypothesis for families within jellyfish class Scyphozoa has been elusive. Reasons for the lack of resolution of scyphozoan familial relationships include a dearth of morphological characters that reliably distinguish taxa and incomplete taxonomic sampling in molecular studies. Here, we address the latter issue by using maximum likelihood and Bayesian methods to reconstruct the phylogenetic relationships among all 19 currently valid scyphozoan families, using sequence data from two nuclear genes: 18S and 28S rDNA. Consistent with prior morphological hypotheses, we find strong evidence for monophyly of subclass Discomedusae, order Coronatae, rhizostome suborder Kolpophorae and superfamilies Actinomyariae, Kampylomyariae, Krikomyariae, and Scapulatae. Eleven of the 19 currently recognized scyphozoan families are robustly monophyletic, and we suggest recognition of two new families pending further analyses. In contrast to long-standing morphological hypotheses, the phylogeny shows coronate family Nausithoidae, semaeostome family Cyaneidae, and rhizostome suborder Daktyliophorae to be nonmonophyletic. Our analyses neither strongly support nor strongly refute monophyly of order Rhizostomeae, superfamily Inscapulatae, and families Ulmaridae, Catostylidae, Lychnorhizidae, and Rhizostomatidae. These taxa, as well as familial relationships within Coronatae and within rhizostome superfamily Inscapulatae, remain unclear and may be resolved by additional genomic and taxonomic sampling. In addition to clarifying some historically difficult taxonomic questions and highlighting nodes in particular need of further attention, the molecular phylogeny presented here will facilitate more robust study of phenotypic evolution in the Scyphozoa, including the evolution characters associated with mass occurrences of jellyfish.

Introduction

Class Scyphozoa includes approximately 200 morphospecies of jellyfish that occur from brackish estuaries, across the epipelagic ocean, to the abyssal depths (Mayer 1910; Kramp 1961; Russell 1970; Arai 1997). These pelagic (*sensu lato*) cnidarians diversified in their roles as key predators in marine ecosystems throughout the Phanerozoic, unequivocally since the Middle Cambrian (Hagadorn et al. 2002; Cartwright et al. 2007; Hagadorn and Belt 2008) and most likely during the Neoproterozoic (Cartwright et al. 2007), although interpretations of Ediacaran body fossils as medusae (Wallcott 1911; Willoughby and Robison 1979; Rigby and Milsom 2000) remain in doubt (Young and Hagadorn in press). Marine fossil deposits from the Late Cambrian suggest an ancient origin of mass occurrences of scyphomedusae (Hagadorn et al. 2002), but

recently scyphozoans have been increasingly vilified as nuisance species that form immense accumulations with detrimental ecological and economic impacts around the world. Aggregations, blooms, and swarms can adversely affect important fisheries, sting, and injure swimmers, clog the water intakes of power plants, invade ecosystems, and elicit dysoxic conditions where large numbers of jellyfish carcasses are deposited (Arai 1997; Mills 2001; Hay 2006; Graham and Bayha 2007; Purcell et al. 2007; Pitt et al. 2009; Richardson et al. 2009; West et al. 2009). The causes of mass occurrences, however, remain poorly understood, in part because of the lack of a clear phylogenetic framework for interpreting the evolution of traits associated with these phenomena (Hamner and Dawson 2009).

Resolving a stable phylogeny of the Scyphozoa has likely been hindered by a relative dearth of morphological characters that reliably distinguish taxa of these simple soft-bodied invertebrates (Mayer 1910; Kramp 1961; e.g., Dawson 2003). Traditional taxonomic and morphological phylogenetic frameworks have only been successful in reliably delineating and reconstructing evolutionary relationships among a subset of taxa. Thus, a wealth of competing phylogenetic hypotheses exist, subject to frequent systematic revision at taxonomic levels from class to species (Kramp 1955, 1961; Werner 1975; Mills et al. 1987; Marques and Collins 2004; Figs. 1 and 2). For example, placement of Staurozoa as a class separate from Scyphozoa is based on only five nonhomoplasious morphological characters (Marques and Collins 2004), which, depending on how they are scored, can result in staurozoans being either derived or descended from the earliest divergence within Medusozoa (Marques and Collins 2004; cf. van Iten et al. 2006). Order Semaeostomeae is either reciprocally monophyletic to other scyphozoan orders or paraphyletic with respect to Rhizostomeae (Bigelow 1909; Kramp 1961; Arai 1997; Mianzan and Cornelius 1999; cf. Haeckel 1882; Mayer 1910; Uchida 1926; Thiel 1966; Marques and Collins 2004; Figs. 1 and 2). Within Semaeostomeae, morphology has inconsistently

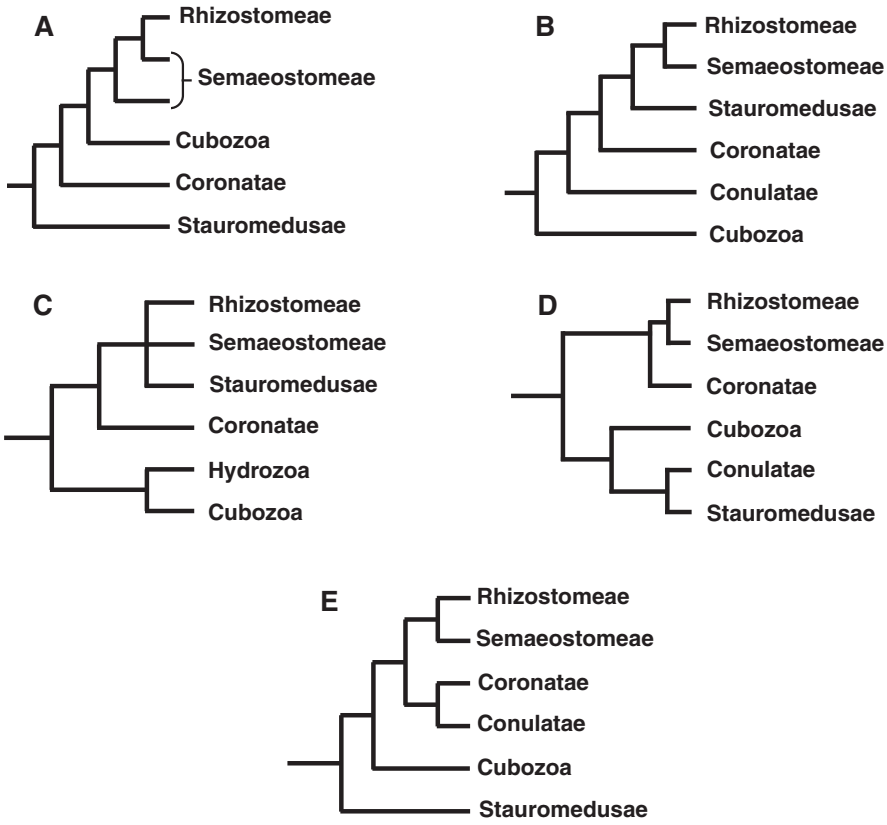

Fig. 1 Cladograms showing the diversity of relationships among classes and orders of medusae within Cnidaria that were hypothesized as a result of morphological analyses: (A) Hyman (1940), Thiel (1966); (B) Uchida (1963, 1972); (**C**) Werner (1973); (D) Marques and Collins (2004); (E) van Iten et al. (2006). Two major areas of debate have been whether Coronatae, Cubozoa, or Staurozoa is sister taxon to Discomedusae and, more relevant to this manuscript, whether Semaeostomeae is paraphyletic (Hyman 1940; Thiel 1966) or monophyletic (Uchida 1963, 1972; Werner 1973; Marques and Collins 2004) with respect to Rhizostomeae.

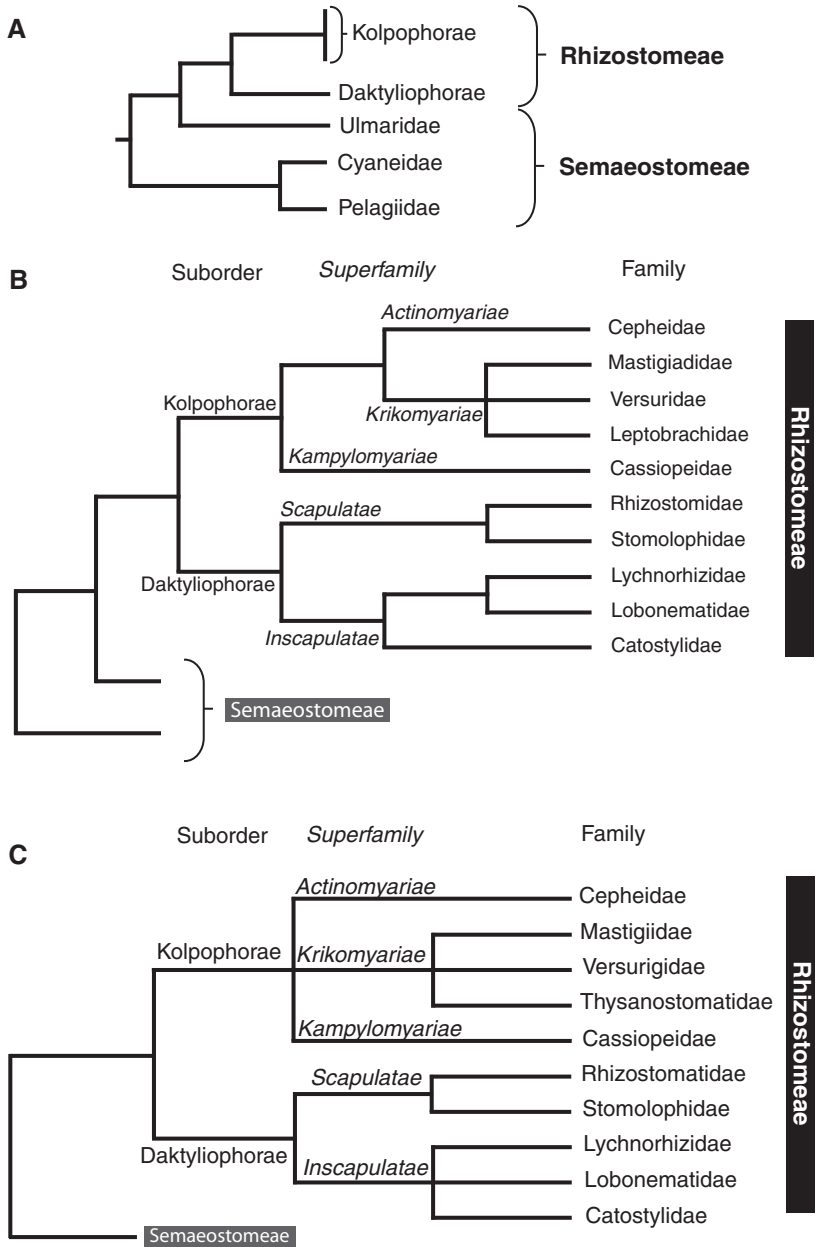

Fig. 2 Comparisons of some morphological hypotheses of relationships among orders and families of Scyphozoa: (A) Uchida (1926); (B) Stiasny (1921); (C) Kramp (1961). Differences among hypotheses regarding familial relationships within the Semaeostomeae and Rhizostomeae can be clearly seen. While Uchida (1926) and Stiasny (1921) both recognized the paraphyly of the Semaeostomeae, only Uchida (1926) showed this paraphyly to be due to the placement of Family Ulmaridae. Both Stiasny (1921) and Kramp (1961) indicate the Daktyliophorae and Kolpophorae to be reciprocally monophyletic groups.

resolved relationships among families Cyaneidae, Pelagiidae, and Ulmaridae (Haeckel 1882; Mayer 1910; Uchida 1926; Kramp 1961; Fig. 2), within family Pelagiidae (Agassiz 1862; Mayer 1910; Gershwin and Collins 2002; cf. Kramp 1955; Calder 1972), and within genera such as *Aurelia* (Mayer 1910; cf. Kramp 1968) and *Cyanea* (Agassiz 1862; Von Lendenfeld 1882; Haacke 1888; cf. Stiasny and van der Maaden 1943; Kramp 1961, 1965).

In recent years, molecular analyses have reduced systematic instability by providing strong evidence in favor of one or another morphological hypothesis at various nodes throughout the scyphozoan tree of life. For example, molecular analyses consistently identify Staurozoa as a basally branching class within the Medusozoa (Collins 2002; Dawson 2004; Collins et al. 2006), consistent with the morphological hypothesis of Van Iten et al. (2006).

Well-resolved molecular phylogenies indicate that semaeostomes are paraphyletic with respect to rhizostomes (Collins 2002; Collins et al. 2006; Hamner and Dawson 2009) because family Ulmaridae is sister taxon to order Rhizostomeae rather than to Cyaneidae and/or Pelagiidae (Collins 2002; Collins et al. 2006; Hamner and Dawson 2009). Likewise, molecular studies estimate species richness within *Aurelia* and *Cyanea* to be more similar to the earlier higher estimates of Mayer (1910) than the later lower estimates of Kramp (1961, 1968) who synonymized many taxa (Dawson and Jacobs 2001; Dawson 2003, 2005a, 2005b).

Despite recent progress, a number of morphological hypotheses remain unaddressed or poorly resolved by molecular studies. Particularly, familial relationships among scyphozoans have never been the focus of any molecular phylogenetic analysis even though they have been inconsistently resolved morphologically (Fig. 2) and a higher-level molecular analysis suggests paraphyletic and polyphyletic families may be commonplace (Dawson 2004). The lack of resolution in family-level molecular systematics of scyphozoans may result from inadequate taxonomic sampling, gene sampling, or choice of analytical methods (see discussions of these problems by Yoder and Irwin 1999; Zwickl and Hillis 2002; Telford et al. 2005; Heath et al. 2008; but see Regier et al. 2008; Seo and Kishino 2008). In previous molecular studies, taxonomic coverage has been variable and incomplete, ranging from as few as eight species in seven families (Collins 2002) to only 24 species in 12 families (Hamner and Dawson 2009), leaving one-third of family-level diversity unrepresented, principally absent from Coronatae and daktyliophoran rhizostomes. The molecular analyses with greatest taxon sampling had limited gene sampling and did not apply Bayesian statistical techniques (Hamner and Dawson 2009); those sampling more nucleotides and applying Bayesian inference were taxonomically less comprehensive (Collins et al. 2006). Although all used ribosomal DNA, none considered secondary structure in alignment or assessed character independence or homology (Telford et al. 2005; Regier et al. 2008; but see Seo and Kishino 2008).

Here, we estimate the relationships among 48 species of jellyfishes representing all 19 currently recognized scyphozoan families using maximum likelihood (ML) and Bayesian phylogenetic analyses of 18S and 28S rDNA aligned with and without reference to ribosomal secondary structure.

Materials and methods

Sample collection

Specimens were collected in the field or at public aquaria by the authors or professional biologists and tissue samples were either preserved immediately in liquid nitrogen and stored at −80°C, or preserved in 75–100% ethanol or DMSO–NaCl solution (Dawson et al. 1998) and stored at −15°C. Species were identified at the point of collection and, where possible, confirmed by the authors on receipt of voucher specimens or original photographs and videos (Table 1), except *Cassiopea andromeda* and *C. frondosa*, which were identified by comparing mitochondrial cytochrome *c* oxidase I (COI) sequences to those in Holland et al. (2004). In total, 55 specimens were collected from 48 species. All 19 families of Scyphozoa were represented by at least two specimens per family with two exceptions: only one specimen was available for Atorellidae and for Lobonematidae. In the case of monogeneric families (*Cassiopea*), we sampled one specimen from at least two species, and in the case of monospecific families or when only one species was available from a family we sampled at least two conspecific individuals usually from different locations or different times.

Amplification and sequencing

Genomic DNA was extracted using CTAB with phenol–chlorofom (Dawson et al. 1998), DNAzol (Chomczynski et al. 1997), or the Qiagen DNAeasy Kit (Invitrogen Inc). Polymerase chain reaction (PCR) amplifications were performed on an Applied Biosystems 2720 Thermal Cycler. Primers used to amplify nuclear 18S rDNA (SSU) and 28S rDNA (LSU) are provided in Table 2. Each 50 µL PCR consisted of 0.5 µM primers, 5.0 µL 10× buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs (Applied Biosystems, Foster City, CA, USA) and 1 µL of template DNA. We amplified SSU either as (1) a single amplicon using primers 18Sa and 18Sb with reaction conditions 94°C for 120 s, then 38 cycles of 94°C for 45 s, 48°C for 60 s and 72°C for 120 s, followed by a final step of 72°C for 600 s before storage at 4°C or (2) in two pieces using 18Sa with AaH18S_1318 and C with 18Sb and the same reaction conditions except the elongation step during cycling was reduced to 90 s. We amplified LSU with primers Aa_L28S_21 and Aa_H28S_1078 or LSUD1F and LSU4Ra; reaction conditions were 94°C for 120 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. PCR amplicons were directly sequenced using a combination of sequencing primers (Table 1). However, some

Table 1 List of specimens used in this study

Higher taxon	Family	Genus	Species	Geographic region	Site code	Approximate Lat. and Long.	Specimen code
Order Coronatae	Atollidae	*Atolla*	*tenella*	Beaufort Sea	USAKBFT	72.85°N 156.58°W	M0D06581C
		Atolla	*vanhoffeni*	San Pedro, CA, USA	USCASAP	—	M0D14620H
		Atolla	*wyvillei*	Monterey Bay, CA, USA	USCAMBA	36.70°N 122.05°W	M0D05955A
	Atorellidae	*Atorella*	*octogonos*	near Monterey Bay, CA, USA	USCAMBA	34.29°N 124.05°W	M0D09903W
	Linuchidae	*Linuche*	*aquila*	Julian Reef, Papua New Guinea	PGMBJUL	10.00°S 150.83°E	M0D00695S
		Linuche	*aquila*	Melekeok, Palau	PWMEMEK	7.30°N 134.63°E	M0D00890F
		Linuche	*unguiculata*	Bermuda	BMxxSAR	32.25°N 67.11°W	M0D13159C
		Stephanoscyphus	sp.	Duchess Island, Papua New Guinea	PGMBDUI	9.97°S 150.85°E	M0D00700X
		Stephanoscyphus	sp.	Raja Ampat, Indonesia	IDPADNG	0.45°S 130.49°E	M0D01336J
	Nausithoidae	*Nausithoe*	*atlantica*	near Monterey Bay, CA, USA	USCAMBA	36.58°N 122.49°W	M0D05968N
		Nausithoe	*rubra*	Channel Islands, CA, USA	USCACHN	—	M0D14621I
		Nausithoe	sp.	Risong, Palau	PWKORCA	7.30°N 134.48°E	M0D00682F
	Paraphyllinidae	*Paraphyllina*	*ransoni*	Gulf of California, Mexico	MXxxGOC	—	M0D09905Y
		Paraphyllina	*ransoni*	near Monterey Bay, CA, USA	USCAMBA	36.58°N 122.52°W	M0D05954Z
		Paraphyllina	*ransoni*	near Monterey Bay, CA, USA	USCAMBA	36.58°N 122.52°W	M0D05957C
	Periphyllidae	*Periphylla*	*periphylla*	Gulf of California, Mexico	MXxxGOC	25.45°N 109.83°W	M0D05956B
		Periphylla	*periphylla*	Sognefjorden, Norway	NOSFSOG	61.12°N 5.86°E	M0D00742N
Subclass Discomedusae							
Order Semaeostomeae	Cyaneidae	*Cyanea*	*annaskala*	Huon Estuary, Tasmania	AUTSHUE	43.26°S 147.09°E	M0D00908X
		Cyanea	*capillata*	Blomsterdalen, Norway	NOHOBLM	60.27°N 5.20°E	M0D14624L
		Desmonema	sp.	South Sandwich Islands	UKSGSSI	—	M0D13155Y
		Drymonema	*dalmatinum*	Foça, Turkey	TRIZFOC	38.67°N 26.75°E	M0D13158B
		Drymonema	sp.	Dauphin Island, AL, USA	USALDIS	30.20°N 88.19°W	M0D14609W
	Pelagiidae	*Chrysaora*	*fuscescens*	Aquarium[a]	USCAMBQ	N/A	M0D14619G
		Chrysaora	*lactea*	Rio de Janeiro, Brazil	BRRJRIO	22.94°S 43.16°W	M0D14610X
		Chrysaora	*melanaster*	Aquarium[b]	USCAMBQ	N/A	M0D14611Y
		Pelagia	*noctiluca*	Offshore of Delaware, USA	USDESAR	37.49°N 73.54°W	M0D14612Z
		Sanderia	*malayensis*	Aquarium[b]	USCAMBQ	N/A	M0D13625A
	Ulmaridae	*Aurelia*	*aurita*	Bjornefjorden, Norway	NOHOBLM	60.18°N 5.53°E	M0D14615C
		Aurelia	sp. 2	Cannaneia, Brazil	BRSPCAN	25.06°S 47.89°W	M0D14616D
		Deepstaria	*enigmatica*	Monterey Bay, CA, USA	USCAMBA	36.71°N 122.05°W	M0D05963I
		Phacellophora	*camtschatica*	Morro Bay, CA USA	USCAMOR	35.36°N 120.87°W	M0D02660H
		Poralia	*rufescens*	near Monterey Bay, CA, USA	USCAMBA	36.58°N 122.52°W	M0D05966L

(continued)

Table 1 Continued

Higher taxon	Family	Genus	Species	Geographic region	Site code	Approximate Lat. and Long.	Specimen code
Order Rhizostomeae							
Suborder Daktyliophorae							
Superfamily Inscapulata	Catostylidae	*Catostylus*	*mosaicus*	Botany Bay, Australia	AUNSSYD	33.99°S 151.18°E	M0D00935Y
		Crambionella	*orsini*	Gulf of Oman, Iran	IRxxGOM	—	M0D00881W
		Crambione	*mastigophora*	Kwajalein Atoll, Marshall Islands	MHKWKWA	9.04°N 167.51°E	M0D02295G
		Acromitus	sp.	Goa, India	INGAGOA	15.43°N 73.80°E	M0D00978P
	Lobonematidae	Lobonematidae	sp.	La Paz, Mexico	MXBSLAP	24.19°N 110.38°W	M0D06067I
	Lychnorhizidae	*Lychnorhiza*	*lucerna*	San Clemente del Tuyu, Argentina	ARBASCT	36.35°S 56.71°W	M0D13183A
		Pseudorhiza	*haeckeli*	Port Phillip Bay, Australia	AUVIPPB	38.08°S 145.05°E	M0D12394R
Superfamily Scapulata							
	Rhizostomatidae	*Rhizostoma*	*pulmo*	Gulf of Trieste, Slovenia	SIISPIR	45.52°N 13.56°E	M0D05985E
		Rhopilema	*verrilli*	Dauphin Island, AL, USA	USALDIS	30.20°N 88.19°W	M0D06018L
		Rhopilema	*esculentum*	Bohai Bay, China (Aquacultured)	CNAQED	N/A	M0D05980Z
	Stomolophidae	*Stomolophus*	*meleagris*	Jacksonville Beach, FL, USA	USFLJAC	30.37°N 81.40°W	M0D09902V
		Stomolophus	*meleagris*	Dauphin Island, AL, USA	USALDIS	30.20°N 88.19°W	M0D14618F
Suborder Kolpophorae							
Superfamily Actinomyariae	Cepheidae	*Cephea*	*cephea*	Koror, Palau	PWKOSIA	7.28°N 134.25°E	M0D00129Y
		Cotylorhiza	*tuberculata*	Aquarium[b]	USCAMBA	N/A	M0D05945Q
Superfamily Kampylomyariae	Cassiopeidae	*Cassiopea*	*andromeda*	Key West, FL, USA	USFLKWS	24.60°N 81.65°E	M0D14622J
		Cassiopea	*frondosa*	Key West, FL, USA	USFLKWS	24.60°N 81.65°E	M0D14623K
		Cassiopea	*ornata*	Koror, Palau	PWKOMDD	7.34°N 134.47°E	M0D02666N
Superfamily Krikomyariae	Mastigiidae	*Mastigias*	*papua*	Ongael Lake, Palau	PWKOOLO	7.26°N 134.24°E	M0D06000T
		Phyllorhiza	*punctata*	Sydney, Australia	AUNSSYD	33.86°S 151.24°E	M0D00662L
	Thysanostomatidae	*Thysanostoma*	*thysanura*	Melekeok, Palau	PWKOCEM	7.48°N 134.63°E	M0D00736H
		Thysanostoma	*thysanura*	Raja Ampat, Indonesia	IDPADNL	0.45°S 130.49°E	M0D01235M
	Versurigidae	*Versuriga*	*anadyomene*	Cemetary Reef, Palau	PWKOCEM	7.24°N 134.37°E	M0D00095Q
		Versuriga	*anadyomene*	Koror, Palau	PWKONEC	7.34°N 134.46°E	M0D00059G

[a]Samples from the Aquarium of the Americas.
[b]Samples from the Monterey Bay Aquarium.
Where GPS coordinates were not available, latitude and longitude were estimated from satellite imagery using Google Earth Version 5.1.3533.1731. Latitude and Longitude values labeled N/A indicate samples from aquaria of unknown source location, while those labeled—represent those without site data.

Table 2 PCR primers employed in this study

Region	Primer name	Sequence (5′–3′)	Primer purpose	Reference
SSU	18Sa	AACCTGGTTGATCCTGCCAGT	A, S	Medlin et al. 1988
	18Sb	GATCCTTCTGCAGGTTCACCTAC	A, S	Medlin et al. 1988
	Aa_L18S_12	TCCTGCCAGTAGTCATATGCTTG	A, S	This study
	Aa_L18S_88	GCGAATGGCTCATTAAATCAGTT	S	This study
	Aa_H18S_1798	CCTACGGAAACCTTGTTACGA	A, S	This study
	L	CCAACTACGAGCTTTTTAACTG	S	Apakupakul et al. 1999
	C	CGGTAATTCCAGCTCCAATAG	S	Apakupakul et al. 1999
	Aa_L18S_1159	CGGAAGGGCACCACCAGGAG	S	This study
	Aa_H18S_1318	CAGACAAATCACTCCACCAAC	S	This study
LSU	Aa_L28S_21	GAACRGCTCAAGCTTRAAATCT	A, S	This study
	Aa_H28S_1078	GAAACTTCGGAGGGAACCAGCTAC	A, S	This study
	LSUD1F	ACCCGCTGAATTTAAGCATA	A, S	Matsumoto et al. 2003
	LSU4Ra	AACCAGCTACTAGRYGGTTCGAT	A, S	Matsumoto et al. 2003
	Aa_L28S_48	GCTTGCAACAGCGAATTGTA	S	This study
	Aa_H28S_1039	GTCTTTCGCCCCTATACCCA	S	This study
	Aa_L28S_260	ATAGCGAACAAGTACCGTGA	S	This study
	Aa_H28S_775	ACTTGCGCACATGTTAGACT	S	This study

A: PCR amplification primer; S: sequencing primer.

amplifications proved difficult to sequence directly and were first cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen Inc.) and then sequenced using primers T3 (ATTAACCCTCACTAA AGGGA) and T7 (TAATACGACTCACTATAGGG) and inner sequencing primers (Table 2). DNA sequencing was performed by Cogenics Inc. (Houston, TX, USA) or University of Washington High-Throughput Genomics Unit (Seattle, WA, USA). Sequences were compared to the GENBANK nucleotide database via BLASTn (Altschul et al. 1997) to confirm the correct region had been sequenced, then assembled in Sequencher v4.8 (Gene Codes Corp., Ann Arbor) and checked by eye to remove primers and to correct base calls. All sequences were deposited in Genbank (HM194768-HM194875 and HM215008-HM215009).

Phylogenetic reconstruction

Due to concerns regarding the general lack of objectivity and repeatability of time-intensive manual DNA sequence alignments, yet also the poor performance of some computational methods in successfully aligning sequences containing nonuniformly distributed nonindependent gaps (Kjer et al. 2006), we used three alignment strategies with four phylogenetic reconstruction methods to estimate evolutionary relationships within Scyphozoa. Our primary goal was to reconstruct a robust phylogeny while, secondarily, gaining some insight into the relative merits of using secondary structure in phylogenetic analyses of Scyphozoa and assessing which parts of the tree are sensitive to the choice of reconstruction method.

Alignment

Alignment strategy #1 (AS1)

Sequences were aligned using ClustalX v.2.0 (Larkin et al. 2007), because it is the most widely used alignment tool (Higgins and Lemey 2009), and MAFFT using the E-INS-I strategy (Katoh and Kuma 2002) which uses iterative refinement methods applicable to loci containing conserved motifs embedded within hypervariable regions (Katoh and Toh 2008). ClustalX alignments used four different gap-opening:gap-extension penalty combinations (6.7 : 15, 10 : 5, 10 : 2, 2 : 1) and MAFFT used four different gap-opening:offset-value penalty combinations (1.53 : 0, 2.0 : 0.25, 2.5 : 0.5, 3.0 : 0.75, 3.0 : 1.0). Alignment quality was evaluated by obtaining column scores in ClustalX v.2.0 and the alignment with the highest normalized product of total column scores (TCS) and average column scores (ACS), calculated as $TCS/TCS_{max} \times ACS/ACS_{max}$, was chosen for subsequent analyses.

Alignment strategy #2 (AS2)

SSU and LSU sequences were aligned manually to secondary-structure models for Cnidaria designed using a DCSE annotated SSU template from the European ribosomal RNA Database (http://bioinformatics.psb.ugent.be/webtools/rRNA/index.html) and a LSU template from Schnare et al. (1996), hand-coded using DCSE (De Rijk and Wachter 1993) notation onto a sequence of the scleractinian coral *Montastraea franksi* (GenBank accession AY026375) and both further refined comparatively using alignments with representatives of all orders within cnidarians (995 species for SSU and

517 species for LSU—M. Barbeitos, University of Kansas). Experimental sequences were manually aligned to the structural models using Genedoc v.2.6.002 (Nicholas et al. 1997). The putative secondary structures of regions that could not be confidently aligned to the template were estimated using RNAalifold (Hofacker 2003) and further refined using MFold (Zuker 2003). Base pairing in stem regions was evaluated using the PERL script *ReNATon* v0.88 (M. Barbeitos, University of Kansas) and alignment issues were corrected in Genedoc and recursively reevaluated with *ReNATon*. From this final, overall alignment, two datamatrices were generated: (1) Alignment strategy #2i (AS2i) included structurally conserved regions concatenated with machine-aligned hypervariable loop regions, excluding poorly aligned loop regions (defined as those in which neither CLUSTALX nor MAFFT always scored highest for ACS, TCS and normalized product). (2) Alignment strategy 2ii (AS2ii) included only structurally conserved regions. For both AS2i and AS2ii, the final DCSE files were parsed by another PERL script (*dsce2jRNA*) into a NEXUS file compatible with the PERL package *jRNA* (http://hymenoptera.tamu.edu/rna/). *jRNA* was used to compile alignments for regions that were structurally conserved as PHYLIP and NEXUS files, the latter with assignment of paired positions in stems.

Alignment strategy #3 (AS3)

The unaligned concatenated SSU and LSU sequences were used as input into the program *MUSCLE* (Edgar 2004). To mask poorly aligned regions, the output of this process was run through *GBLOCKS*, which removes gapped positions and regions of questionable alignment (Castresana 2000), with minimum block size 10 and gaps present in up to half of the positions (i.e., parameters $-b4 = 10$ and $-b5 = h$).

Tree reconstruction

The SSU and LSU data sets were analyzed, independently and conjoined, in ML and Bayesian phylogenetic frameworks using the substitution models identified as best fitting the data by hLRT or AIC in Modeltest v.3.7 (Posada and Crandall 1998). Conflict between the SSU and LSU data partitions was tested *a priori* using the partition homogeneity test (Farris et al. 1994, 1995) in PAUP* 4.0b10 (Swofford 2003) applying unweighted maximum parsimony, heuristic TBR search, and 100 homogeneity replicates. The partitions were not re-sampled to adjust for unequal sizes so reported *P*-values are likely smaller than the true *P*-value (Lee 2001). We also assessed conflict between the SSU and LSU data partitions *a posteriori* (see Clade stability below). All trees were unrooted and drawn with the longest internal branch placed centrally.

Tree reconstruction method A (TRA)

ML analyses of Alignment strategy #1 (AS1) and AS2i alignments were undertaken in GARLI v0.96 (Zwickl 2006) run on the CIPRES Portal 2.0 (Miller et al. 2009). For SSU alone, LSU alone, and SSU and LSU conjoined in a single "total evidence" datamatrix, we used the GTR+I+Γ substitution model (Lanave et al. 1984), with substitution rates and invariant sites estimated from the data and four rate categories, beginning each of 20 replicate searches from a random starting tree topology. Default values were used for initialization, population, branch-length optimization, mutation prior weighting, mutation details, logs, and run termination parameters. We subsequently ran bootstrap analyses using the same parameters, except doing only two replicate searches for each of 200 bootstrap repetitions; bootstrap searches were repeated twice to give a total of 400 bootstrap trees. Bootstrap trees were renumbered, concatenated in a single file for each data set, and imported into PAUP* 4.0b10 (Swofford 2003) to calculate bootstrap values on a majority rule consensus tree. The ML tree was drawn in Figtree v.1.1.2 (Rambaut 2009a) then annotated with bootstrap values in Adobe Illustrator CS3 (Adobe Systems Incorporated, San Jose, CA, USA).

Tree reconstruction method B (TRB)

Bayesian Markov Chain Monte Carlo analyses of AS1 alignments were undertaken using the BEAST v.1.5.3 software pipeline (Drummond and Rambaut 2007). Preliminary analyses were set up in BEAUTi v1.5.3 (Drummond and Rambaut 2007), run in BEAST, and visualized using TRACER v.1.5 (Rambaut and Drummond 2009) to estimate burn-in times and run times that would generate stable, Gaussian, posterior density distributions in phylogenetic reconstruction. Subsequently, for SSU, each phylogenetic reconstruction in BEAST employed the GTR+I+Γ substitution model with empirical base frequencies, gamma site heterogeneity with four rate categories, with all parameters unlinked, and an uncorrelated exponential relaxed clock with substitution rate = 1.0 and all tip dates set to zero. Starting trees were randomly generated, and the tree prior assumed the Yule speciation process. The Markov chain Monte Carlo (MCMC) was 10^7 steps long and parameters were sampled every 10^3 steps after

a burn-in of 10^6 steps. The same analysis was run two times using different random seeds and convergence within and between runs examined using the trace plot in TRACER. Convergence of independent MCMC runs in SSU, LSU, and combined data sets performed under the GTR+I+Γ model was assessed by visually ascertaining stationarity of log-likelihood values using TRACER v1.5. We also used the "cumulative", "compare" and "var" diagnostic tools implemented in AWTY (Nylander et al. 2008) (with default settings) to verify topological convergence among chains. These analyses were then repeated using exactly the same conditions except employing the TN93 substitution model instead of GTR; we report the combined results of these runs. The same suite of four searches and checks of convergence were run for LSU. Finally, for the combined SSU and LSU data set we employed the same search and convergence criteria, except running three searches per substitution model, first on the concatenated "total evidence" data set and then using two data partitions, one comprised of SSU and the other LSU. For each data set, a summary tree was generated by combining the 9001 trees per run with LogCombiner v1.5.3 (Drummond and Rambaut 2007) then calculating the maximum clade credibility tree with median node heights and >50% posterior probabilities in TreeAnnotator v1.5.3 (Drummond and Rambaut 2007). The summary trees were drawn in FigTree v.1.1.2 (Rambaut 2009a).

Tree reconstruction method C (TRC)

The posterior distribution of trees was obtained using the MPI version of MrBayes 3.1 (Altekar et al. 2004) by combining trees yielded by four independent runs. All analyses were conducted at the Ohio State University's Supercomputer Center (OSC). We employed the GTR+I+Γ model with the gamma distribution being approximated by four discrete categories. Base co-variation in stems was taken into account by enforcing the doublet model (Schöniger and von Haeseler 1994). Parameters were unlinked across different genes (in the total evidence analyses) and across loop and stem partitions within each gene. Chain convergence was also assessed using Tracer v1.4 (Rambaut and Drummond 2007) and AWTY (Nylander et al. 2008). Runs were interrupted after convergence was verified using those criteria or after the maximum wall time allowed by OSC (168 h) was exceeded.

Tree reconstruction method D (TRD)

If convergence was not achieved, alignment *Alignment strategy #3* (AS3) was converted to PHYLIP format using the authors' *encodename.pl* program, and evaluated using *modelgenerator* (Keane et al. 2006) to determine the best likelihood model for subsequent analyses. Based on hLRT, AIC1, and AIC2 criteria, the best model was GTR+I+Γ, whereas the Bayesian BIC selected TrN+I+Γ. Given the consensus of most criteria, we used GTR+I+Γ within the program *PhyML* (Guindon and Gascuel 2003) to perform 400 bootstraps with the following command line options: "0 i 1 400 GTR e e 8 e BIONJ y y", indicating eight rate categories, and that all parameters (ts/tv, Pinv, alpha shape parameter) were to be estimated. Upon completion of the run, the tree names were converted back to their full names using the *decodename.pl* script, and trees were visualized and annotated using FigTree 1.2.2 (Rambaut 2009b) and Adobe Illustrator CS3.

Clade stability

Support for taxa and congruence or conflict between clades in different reconstructions, were assessed at the 95% bootstrap support and 95% posterior probability levels. These values were chosen as benchmarks that correspond, approximately, to an excess of at least three characters for versus against a clade (Felsenstein, 1985; Avise, 2000) and 95% confidence intervals under a set of simplifying assumptions (Felsenstein 1985; Hillis and Bull 1993; Huelsenbeck et al. 2002; Alfaro et al. 2003; Huelsenbeck and Rannala 2004).

Results

Alignment AS1 with tree reconstructions TRA and TRB

The length of SSU sequences ranged from 1674 to 1775 nucleotides. MAFFT, employing a gap-opening:offset-value penalty of 2.5:0.5, produced the best alignment (normalized product score = 0.998). This alignment was 1785 characters long, of which 197 (i.e., 11.0%) were variable, nongapped, and nondegenerate and 166 (9.3%) were parsimony informative. The LSU sequences ranged in length from 927 to 1154 nucleotides. ClustalX, employing a gap-opening:gap-extension penalty of 6.7 : 15, produced the best alignment (normalized product = 0.983). This LSU alignment was 1244 characters long, with 336 sites (i.e., 27.0%) being variable, nongapped, and nondegenerate and

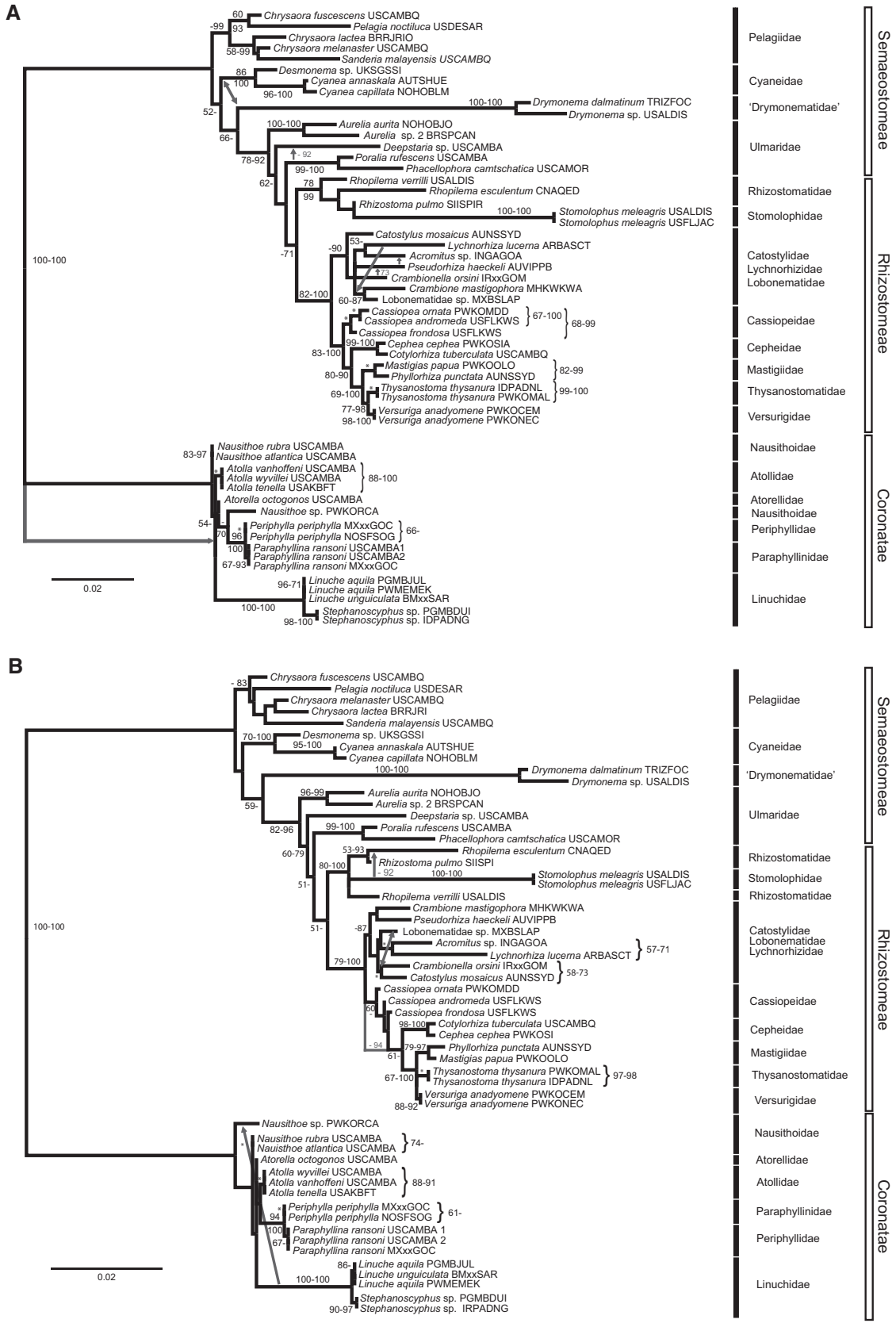

Fig. 3 (A) The maximum likelihood SSU gene tree for Scyphozoa reconstructed from a MAFFT alignment (gap-opening:offset-value penalties 2.5:0.5) using GARLI (20 heuristic replicates) and applying the GTR+I+Γ model of sequence evolution [alignment strategy AS1 and tree reconstruction method A (TRA)]. Gray arrows show alternative topologies present in Bayesian analyses applying the

(continued)

291 (23.4%) parsimony informative. The partition-homogeneity test did not reject congruence between the SSU and LSU partitions ($P = 0.12$).

ML analyses of SSU alone, LSU alone, and concatenated SSU–LSU each produced a primarily dichotomously branching tree with moderate to high bootstrap support at a majority of nodes (Figs. 3A, 4A, and 5A). Repeat BMCMC runs converged on similar posterior distributions in analyses of SSU, LSU, or conjoined SSU–LSU. Trace plots showed tree posterior probabilities reached stationarity during burn-in and adequate mixing during sampling; effective sample sizes for the tree posterior probability ranged from 133 (for LSU) to 806 (for SSU) in individual replicates and were >300 in all summary analyses that combined replicates. Bivariate plots of split frequencies showed high correlation with points deviating less than a few percentage points from the predicted 1 : 1 line evincing convergence between BMCMC runs, although variance was greater in conjoined analyses than in analyses of individual loci. Topologies within and among runs had absolute difference scores that were all <1 and differences among runs were often the same as, and always <5×, the differences within runs; difference scores changed ≤0.2 during the final 5×10^6 steps. BMCMC analyses of all data sets thus reconstructed primarily dichotomously branching trees with moderate to high posterior probability for a majority of nodes. These topologies were mostly congruent with ML trees differing only in regions where nodes received generally low support (Figs. 3–5).

Alignment AS2 with tree reconstructions TRA and TRC

Manual alignment of 1674–1775 nucleotides long SSU sequences yielded a datamatrix 1790 characters long. Thirty-seven positions were scored as inconsistently aligned and excluded from the data set; 184 (10.5%) positions were variable, nongapped, and nondegenerate and 154 (8.8%) were parsimony informative. Manual alignment of the 927–1154 nucleotides long partial LSU sequences yielded a datamatrix 1407 characters long. One hundred and ten nonstem positions were scored as inconsistently alignable, another 91 were primarily missing, and both were excluded from the data set. Of the remaining 1209 positions, 347 (28.7%) were variable, nongapped, and nondegenerate and 301 (24.9%) were parsimony informative.

Alignment AS2i with tree reconstruction TRA

The partition-homogeneity test did not refute congruence between the SSU and LSU partitions in the AS2i alignment ($P = 0.25$). ML analyses of SSU, LSU, and combined SSU–LSU alignments (data set AS2i) each produced a primarily dichotomously branching tree with bootstrap support >50% for a majority of nodes (Figs. 3B, 4B, and 5B).

Alignment AS2ii with tree reconstruction TRC

The two runs executed for each gene separately showed good convergence according to every criterion used (average standard deviation of split frequencies, likelihood trace files and the AWTY diagnostics) and produced phylogenetic trees with similar topologies and nodal support (posterior probabilities >0.70) to those created with ML (Figs. 3B, 4B, and 5B). However, in the case of concatenated analysis under the doublet model, convergence was not achieved according to any of the above evaluation criteria before the wall time of 168 h was exceeded and analysis of the concatenated data set under the doublet model is not included.

Alignment AS3 with tree reconstruction TRD

The MUSCLE alignment of the 1674–1775 nucleotides of SSU and 927–1154 nucleotides of LSU resulted in a concatenated data alignment 3165 base pairs long. Employing GBLOCKS trimmed this data set to 2609 nucleotides (82% of original data set), of which 536 (20.5%) were variable, nongapped, and nondegenerate and 449 (17.2%) were parsimony informative.

Fig. 3 Continued

GTR+I+Γ or TN93+I+Γ substitution models [alignment strategy AS1 and tree reconstruction method B (TRB)]. Numbers adjacent to branches show bootstrap support if >50% (from 400 bootstraps) followed, after a hyphen, by posterior probability if >0.70 presented as a percentage. Where space for annotating a branch is lacking, statistical support for a clade is indicated by an asterisk and the support values are presented to the right of the tree bracketing the relevant leaves. Familial and ordinal affiliations of species are indicated by vertical bars to the right of the tree. (B) The maximum likelihood SSU gene tree for Scyphozoa reconstructed from a manual secondary structure alignment using GARLI (20 heuristic replicates) and applying the GTR+I+Γ model of sequence evolution (alignment strategy AS2ii and tree reconstruction method TRA). Gray arrows show alternative topologies present in Bayesian analyses employing the doublet model [alignment strategy AS2ii and tree reconstruction method C (TRC)]. All annotation is the same as described for part A.

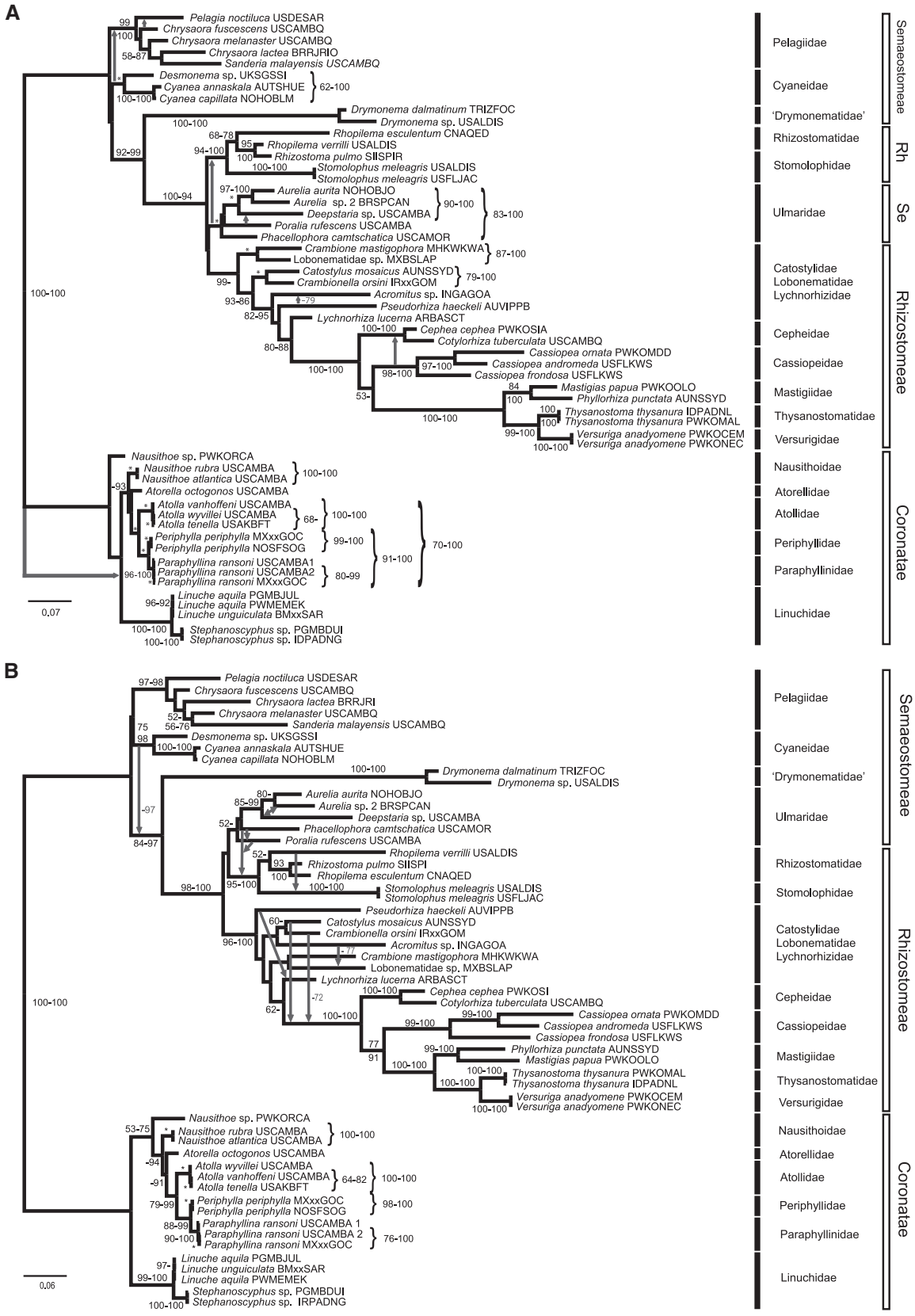

Fig. 4 (A) The maximum likelihood LSU gene tree for Scyphozoa reconstructed from a CLUSTALX alignment (gap-opening:-gap-extension penalty of 6.7:15) using GARLI (20 heuristic replicates) and applying the GTR+I+Γ model of sequence evolution (alignment strategy AS1 and tree reconstruction method TRA). Gray arrows show alternative topologies present in Bayesian analyses applying the GTR+I+Γ or TN93+I+Γ substitution models (alignment strategy AS1 and tree reconstruction method TRB). (B) The (continued)

The ostensibly improved alignment provided by MUSCLE as compared with *ClustalX* led to the rearrangement of several nodes and affected the sister taxon relationships of a few taxa, particularly within the Rhizostomeae (Fig. 5B). It did not, however, change the major relationships within the tree, attesting to the robustness of this parallel approach regarding genes and methodologies.

Clade stability

Analyses of the conjoined data set produced phylogenetic trees with overall greater resolution (49.1–70.9% [average: 57.8 ± 11.2%; median: 60%] of the maximum possible nodes [for a fully dichotomous tree with 55 OTU's] received bootstrap support and/or posterior probability ≥95%) than either LSU (range: 40–60%; average: 52.1 ± 8.7%; median: 54.5%) or the less variable SSU (range: 16.4–50.9%; average: 34.5 ± 13.1%; median: 34.5%). A majority of trees in analyses of SSU, LSU, and SSU+LSU supported (≥95% support value) monophyly of (1) Subclass Discomedusa; (2) Order Coronatae; (3) Suborder Kolpophorae; (4) superfamilies Actinomyariae, Kampylomyariae, Krikomyariae, and Scapulatae; and (5) families Atollidae, Cassiopeidae, Cepheidae, Linuchidae, Mastigiidae, Paraphyllinidae, Pelagiidae, Periphyllidae, Thysanostomatidae, and Versurigidae. A majority of trees in analyses of SSU, LSU, and SSU + LSU refuted monophyly of Semaeostomeae, Daktyliophorae, Cyaneidae, and Nausithoidae, instead revealing consistently ≥95% support for at least one branch that established paraphyly or polyphyly of these groups. A majority of trees neither strongly supported nor strongly refuted monophyly of Rhizostomeae, Inscapulatae, Catostylidae, Lychnorhizidae, Rhizostomatidae, or Ulmaridae. Only analyses of Inscapulatae and Ulmaridae yielded results that conflicted, with three trees strongly refuting and two to five trees strongly supporting monophyly of each taxon.

Discussion

Methodological inferences

Our analyses, using three different alignments of nuclear ribosomal DNA loci and four different approaches to tree estimation, reveal that, given our taxonomic sampling, molecular phylogenetic inference of evolutionary relationships among scyphozoans was generally robust to methodological issues that may confound other studies (Telford et al. 2005; Erpenbeck et al. 2007; Fleck et al. 2008; Ripplinger and Sullivan 2008). The cumulative effects of differences in loci, alignments, models of substitution, reconstruction methods, and optimality criteria produce primarily concordant topologies, yielding contradictory inferences about only two groups—Inscapulatae and Ulmaridae—among a minority of trees (Figs. 3–5). Nodes that are inconsistently recovered in analyses of different loci are the same nodes that receive inconsistent support across different phylogenetic methods and weak support within a single gene tree. On the other hand, nodes that are strongly supported in any single analysis are typically supported in the large majority of analyses (≥11 of 17 trees) irrespective of the method applied to estimate the phylogeny from either locus despite 2- to 3-fold difference in the percentage of variable positions. The trees reconstructed using combined analyses of SSU and LSU (Fig. 5) were the most highly resolved, albeit in places still ambiguous, and provide our preferred hypothesis to compare with prior morphological phylogenetic hypotheses.

Systematic inferences

Our molecular phylogenetic analysis of the class Scyphozoa provides greater resolution than previous molecular analyses performed with fewer taxa (Collins 2002; Dawson 2004; Collins et al. 2006; Hamner and Dawson 2009) and therefore stronger affirmation of some morphological phylogenetic hypotheses (e.g., aspects of Stiasny 1921; Uchida 1926; Hyman 1940; Thiel 1966) and stronger refutation of others (e.g., aspects of Kramp 1961).

Affirmation

Our molecular phylogenetic analyses are consistent with prior analyses that found class Scyphozoa comprises two reciprocally monophyletic groups: Coronatae and Semaeostomeae-plus-Rhizostomeae. This supports the morphological phylogenetic hypotheses of Mayer (1910), Stiasny (1921), Hyman (1940), Thiel (1966), Marques and Collins (2004), and van Iten et al. (2005) and favors application of the term Discomedusae to the clade containing

Fig. 4 Continued
maximum likelihood LSU gene tree for Scyphozoa reconstructed from a manual secondary structure alignment using GARLI (20 heuristic replicates) and applying the GTR+I+Γ model of sequence evolution (alignment strategy AS2ii and tree reconstruction method TRA). Gray arrows show alternative topologies present in Bayesian analyses employing the doublet model (Alignment strategy AS2ii and tree reconstruction method TRC). All annotation is the same as described for Fig. 3.

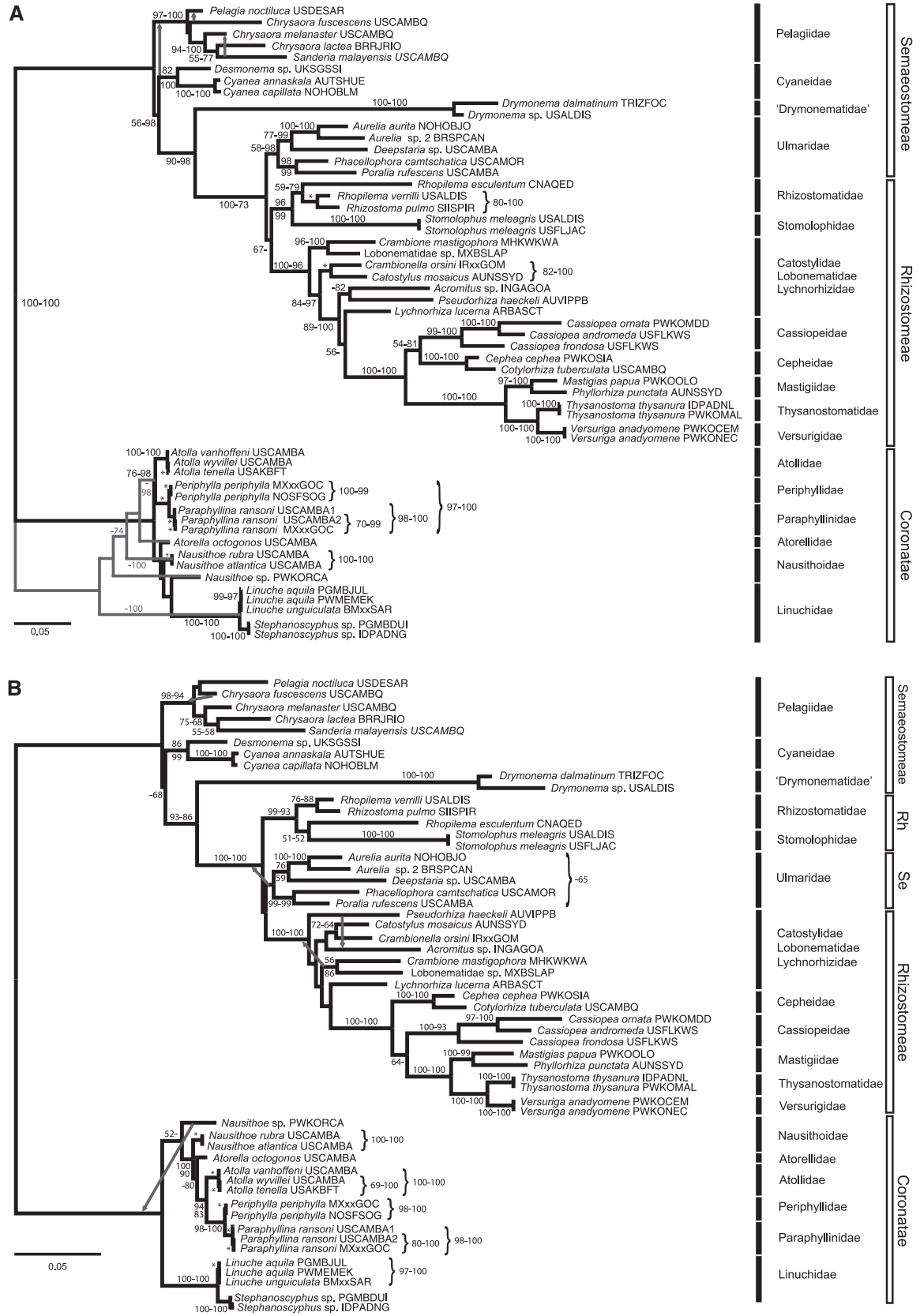

Fig. 5 (A) The maximum likelihood phylogeny for Scyphozoa reconstructed from concatenated SSU MAFFT and LSU CLUSTALX alignments using GARLI (20 heuristic replicates) and applying the GTR+I+Γ model of sequence evolution (alignment strategy AS1 and tree reconstruction method TRA). Gray arrows show alternative topologies present in Bayesian analyses of the concatenated data set,

(continued)

semaeostomes and rhizostomes but excluding the coronates (Maas 1907; Naumov 1961; Dawson 2004; cf. Haeckel 1879, 1882; Bigelow 1904).

Four of the six currently recognized families in order Coronatae are supported as reciprocally monophyletic groups (Atollidae, Linuchidae, Paraphyllinidae, and Periphyllidae). However, interfamilial relationships are poorly resolved due to low sequence divergence within Coronatae. The molecular phylogenetic results do complement morphological analyses that reassigned Stephanoscyphistoma to *Atorella* and *Nausithoe* (Morandini and Jarms 2005), thus bringing recent evidence into full agreement with prior observations that *Atorella*, *Nausithoe*, and *Linuche* scyphomedusae may emanate from *Stephanoscyphus* scyphopolyps (Werner 1967, 1971, 1974, 1979).

Discomedusae contains 15 robustly reconstructed reciprocally monophyletic clades of which five correspond with higher taxonomic groups proposed on morphological criteria: Superfamily Scapulatae, Suborder Kolpophorae, and Superfamily Krikomyariae plus the superfamilies Actinomyariae and Kampylomyariae (which are represented by single families). Thus, of the 13 discomedusan families proposed on morphological grounds (Kramp 1961), seven form strongly supported reciprocally monophyletic clades in our molecular analyses (Cassiopeidae, Cepheidae, Mastigiidae, Pelagiidae, Stomolophidae, Thysanostomatidae, and Versurigidae).

Irresolution

Relationships among coronate families (except Paraphyllinidae and Periphyllidae), among semaeostome families near the base of Discomedusae (particularly Cyaneidae and Pelagiidae), and among families at the base of Kolpophorae (particularly Cassiopeidae and Cepheidae) are not well-resolved. Our trees also provide no strong evidence, in terms of reciprocal monophyly, for or against five families—Atorellidae (Order Coronatae), Lychnorhizidae, Catostylidae, Lobonematidae (Order Rhizostomeae, Superfamily Inscapulatae)—due to poor resolution of relevant nodes or incomplete taxonomic sampling below the family level.

Reclassification

Our molecular phylogeny conflicts with the current morphological classification of Scyphozoa by Kramp (1961) in five ways.

(1) Order Semaeostomeae is paraphyletic with respect to the Rhizostomeae (Figs. 3–5), supporting previous morphological studies (Haeckel 1882; Mayer 1910; Uchida 1926; Hyman 1940; Thiel 1966) and consistent with molecular analyses of fewer taxa (Collins 2002; Dawson 2004; Collins et al. 2006; Hamner and Dawson 2009). Further, our trees endorse the hypothesis that paraphyly is due to a sister taxa relationship between Ulmaridae and Rhizostomeae (Agassiz 1862; Haeckel 1882; Uchida 1926; Collins 2002; Collins et al. 2006; Hamner and Dawson 2009).

(2) Suborder Daktyliophorae is paraphyletic with respect to the Kolpophorae (Figs. 3–5). Although the preponderance of morphological hypotheses have posited reciprocal monophyly of the rhizostome suborders Daktyliophorae and Kolpophorae (Mayer 1910; Stiasny 1921; Kramp 1961; but see Uchida 1926) the inscapulate daktyliophorans are paraphyletic with respect to Kolpophorae.

(3) Family Cyaneidae is polyphyletic (Figs. 3–5). Genus *Drymonema*, a member of Cyaneidae since first being described as a new genus by Ernst Haeckel (1880), is unequivocally reciprocally monophyletic with respect to all other clades of semaeostomes—Pelagiidae, *Cyanea*+*Desmonema*, and Ulmaridae. Although its relationship to other clades within Semaeostomeae is poorly resolved, morphological and genetic data warrant recognition of *Drymonema* as the member of a new family, Drymonematidae (Bayha and Dawson submitted for publication).

(4) Families Catostylidae, Lobonematidae, and Lychnorhizidae are polyphyletic and/or paraphyletic (Figs. 3–5). Our analyses did not separate catostylid, lobonematid, or lychnorhizid scyphozoans into reciprocally monophyletic groups,

Fig. 5 Continued
or SSU and LSU data partitions, applying the GTR+I+Γ or TN93+I+Γ substitution models (alignment strategy AS1 and tree reconstruction method TRB). (B) The maximum likelihood phylogeny for Scyphozoa reconstructed from concatenated manual secondary-structure alignment using GARLI (20 heuristic replicates) and applying the GTR+I+Γ model of sequence evolution (alignment strategy AS2ii and tree reconstruction method TRA). All annotation is the same as described for Fig. 3, with the exception that Bayesian analysis are not included (due to nonconvergence of the data sets). In its place, bootstrap values and alternative topologies (gray arrows) are indicated for maximum likelihood analysis of the *muscle*-aligned data set trimmed using *Gblocks* and analyzed using *PhyML* (alignment strategy AS3 and tree reconstruction method TRD).

thus resembling Uchida's (1926) more inclusive family Lychnorhizidae rather than recent taxonomic treatments including three distinct families (Kramp 1961).

(5) Family Nausithoidae is polyphyletic and/or paraphyletic (Figs. 3–5). Despite unclear polarity and relationships within the coronates, the phylogenetic positions of tropical shallow-water *Nausithoe* sp. and genetically distant deepwater *Nausithoe atlantica and Nausithoe rubra* establish Nausithoidae as nonmonophyletic and raise the possibility of a cryptic coronate family. Similarly, classification of *Atorella* as a nausithoid (Mills et al. 1987) would render Nausithoidae nonmonophyletic, and we interpret our trees as supporting recognition of Family Atorellidae (Eggers and Jarms 2007).

Evolutionary implications

The relationships described in Fig. 5 represent the most complete and statistically well-resolved molecular phylogenetic hypothesis for class Scyphozoa to date. This phylogeny provides the analytically most robust framework for family-level evolutionary analyses of Scyphozoa, enabling studies of, for example, evolutionary transitions in behavioral, biogeographic, ecological, life history, morphologic, and physiological characters that allow some scyphozoans to aggregate, bloom, or swarm (Dawson and Hamner 2009; Hamner and Dawson 2009). The phylogeny also will further our understanding of adaptation, facilitating studies of rates of evolution and the rates and modes of speciation in determining radiations (Sanderson and Donoghue 1996; Hamner and Dawson 2009; Rabosky and McCune 2010). Understanding apparent substitution rate differences in, for example, Linuchidae versus other coronates and in Kolpophorae versus most other discomedusae, may elucidate general patterns, such as whether these rate differences may be a consequence of the evolution of photosymbiosis (Lutzoni and Pagel 1997; Allen et al. 2006), temperature (Lumbsch et al. 2008), or solar irradiation (Willis et al. 2009). Taxa such as "Drymonematidae" and Stomolophidae, which are morphologically and genetically very distinct from their closest relatives, raise the question of correlated rates of genotypic and phenotypic evolution. In places, however—for example within Coronatae, at the base of Discomedusae, and base of the rhizostomes—phylogenetic relationships encompassing key evolutionary transitions, including the origin of mass-occurring taxa, remain unresolved and in need of additional taxonomic and genomic sampling.

Acknowledgments

We thank O. Angulo, U. Båmstedt, L. Bell, J. Browne, L. Chiaverano, L. Christianson, P. Colin, R. Daryanabard, E. Demir, T. Demir, M. Davis, J. Dong, W. Graham, L. Gomez-Daglio, G. Jarms, R. Johnsson, T. Knowles, K. Kuhn, A. Malej, L. Martin, M. Martinussen, G. Matsumoto, M. Miller, A. Morandini, W. Patry, K. Raskoff, K. Santanillano, T. Skau, R. Sreepada, J. Taylor, E. Thuesen, and C. Widmer for providing and/or identifying scyphozoan specimens. We also thank the Coral Reef Research Foundation (CRRF), the Monterey Bay Aquarium Research Institute (MBARI), the Monterey Bay Aquarium, the Aquarium of the Americas, and the crews of the R/V Western Flyer and R/V Cape Henlopen for assisting collections. We are grateful to an anonymous reviewer and Claudia Mills for critical comments that improved the manuscript. We acknowledge the Society for Integrative and Comparative Biology (SICB), as well as the Divisions of Invertebrate Zoology (DIZ) and Systematic and Evolutionary Biology (DSEB), for supporting the "Assembling the Cnidarian Tree of Life" symposium.

Funding

Cnidarian Tree of Life (CnidTOL) project, National Science Foundation (grant EF-0531779 to P. Cartwright, A. G. C., and D. Fautin); the David and Lucile Packard Foundation; and a Revisionary Syntheses in Systematics project, National Science Foundation (grant DEB-0717078, to M. N. D. and A. G. C.).

References

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

Alfaro ME, Zoller S, Lutzoni F. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol Biol Evol 20:255–66.

Allen AP, Gillooly JF, Savage VM, Brown JH. 2006. Kinetic effects of temperature on rates of genetic divergence and speciation. P Natl Acad Sci USA 103:9130–5.

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F. 2004. Parallel Metropolis-coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20:407–415.

Altschul SF, Madden TL, Schäffer 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 Res 25:3389–402.

Apakupakul K, Siddall ME, Burrelson E. 1999. Higher-level relationships of leeches based on morphology and gene sequences. Mol Phylogenet Evol 12:350–9.

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

Avise JC. 2000. Phylgeography: the history and formation of species. Cambridge (MD): Harvard University Press.

Bigelow HB. 1904. Medusae from the Maldive Islands. Bull Mus Comp Zool Harv 39:245–69.

Bigelow HB. 1909. Reports on the scientific results of the expedition to the eastern tropical Pacific, U.S. Fisheries Commission Steam "Albatross" 1904–1905. Mem Mus Comp Zool Harv 39:1–243.

Calder DR. 1972. Development of the sea nettle *Chrysaora quinquecirrha* (Scyphozoa, Semaeostomeae). Chesapeake Science 13:40–4.

Cartwright P, Halgedahl SL, Hendricks JR, Jarrard RD, Marques AC, Collins AG, Lieberman BS. 2007. Exceptionally preserved jellyfishes from the Middle Cambrian. PLoS ONE 2:e1121.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molec Biol Evol 17:540–52.

Chomczynski P, Mackey K, Drews R, Wilfinger W. 1997. DNAzol: a reagent for the rapid isolation of genomic DNA. Biotechniques 22:550–3.

Collins AG. 2002. Phylogeny of Medusozoa and the evolution of cnidarian life cycles. J Evolution Biol 15:418–32.

Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. Syst Biol 55:97–115.

Dawson MN. 2003. Macro-morphological variation among cryptic species of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). Mar Biol 143:369–79.

Dawson MN. 2004. Some implications of molecular phylogenetics for understanding biodiversity in jellyfishes, with an emphasis on Scyphozoa. Hydrobiologia 530/531:249–60.

Dawson MN. 2005a. Morphologic and molecular redescription of *Catostylus mosaicus conservativus* (Scyphozoa, Rhizostomeae, Catostylidae) from southeast Australia. J Mar Biol Assoc UK 85:723–32.

Dawson MN. 2005b. *Cyanea capillata* is not a cosmopolitan jellyfish: morphological and molecular evidence for *C. annaskala* and *C. rosea* (Scyphozoa, Semaeostomeae, Cyaneidae) in southeast Australia. Invertebr Syst 19:361–70.

Dawson MN, Hamner WM. 2009. A character-based analysis of the evolution of jellyfish blooms: adaptation and exaptation. Hydrobiologia 616:193–215.

Dawson MN, Jacobs DK. 2001. Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). Biol Bull 200:92–6.

Dawson MN, Raskoff KA, Jacobs DK. 1998. Preservation of marine invertebrate tissues for DNA analyses. Mol Mar Biol Biotech 7:145–52.

De Rijk P, Wachter RD. 1993. DCSE, an interactive tool for sequence alignment and secondary structure research. Comput Appl Biosci 9:735–40.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–7.

Eggers N, Jarms G. 2007. The morphogenesis of ephyra in Coronatae (Cnidaria, Scyphozoa). Mar Biol 152:495–502.

Erpenbeck D, Nichols SA, Voigt O, Dohrmann M, Degnan BM, Hooper JNA, Wörheide G. 2007. Phylogenetic analyses under secondary structure-specific substitution models outperform traditional approaches: case studies with diploblast LSU. J Mol Evol 64:543–57.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Constructing a significance test for incongruence. Syst Biol 44:570–2.

Farris JS, Källersjö M, Kluge AG, Bult C. 1995. Testing significance of incongruence. Cladistics 10:315–9.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–91.

Fleck G, Ullrich B, Brenk M, Wallinsch C, Orland M, Bleidissel S, Misof B. 2008. A phylogeny of anisopterous dragonflies (Insecta, Odonata) using mtRNA genes and mixed nucleotide/doublet models. J Zool Syst Evol Res 46:310–22.

Gershwin L, Collins AG. 2002. A preliminary phylogeny of Pelagiidae (Cnidaria, Scyphozoa), with new observations of *Chrysaora colorata* comb. nov. J Nat Hist 36:127–48.

Graham WM, Bayha KM. 2007. Biological invasions by marine jellyfish. In: Nentwig W, editor. Ecological studies, Biological invasions, Vol. 193. Berlin: Springer. p. 240–55.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704.

Haacke W. 1888. Die Scyphomedusen des St. Vincent Golfes. Jena Z Naturw 20:588–638.

Haeckel E. 1879. Das System der Medusen: Erster Theil einer Monographie der Medusen. Jena: Gustav Fischer.

Haeckel E. 1880. System der Acraspeden. Zweite Halfte des System der Medusen. Jena: Verlag von Gustav Fischer. p. 361–672.

Haeckel E. 1882. Report on the deep-sea medusae dredged by H.M.S. Challenger during the years 1873–1876. Rep Sci Res Challenger (Zoology) 4:1–154.

Hagadorn JW, Belt ES. 2008. Stranded in upstate New York: Cambrian scyphomedusae from the Potsdam Sandstone. Palaios 23:424–41.

Hagadorn JW, Dott RH, Damrow D. 2002. Stranded on a Late Cambrian shoreline: medusae from central Wisconsin. Geology 30:147–150.

Hamner WM, Dawson MN. 2009. A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. Hydrobiologia 616:161–91.

Hay S. 2006. Gelatinous bells may ring change in marine ecosystems. Curr Biol 16:679–82.

Heath TA, Hedtke SM, Hillis DM. 2008. Taxon sampling and the accuracy of phylogenetic analyses. J Syst Evol 46:239–57.

Higgins D, Lemey P. 2009. Multiple sequence alignment. In: Lemey P, Salemi M, Vandamme AM, editors. The Phylogenetic Handbook. Cambridge, UK: University Press. p. 68–108.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42:182–92.

Hofacker IL. 2003. Vienna RNA secondary structure server. Nucleic Acids Res 31:3429–31.

Holland BS, Dawson MN, Crow GL, Hofmann DK. 2004. Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple Hawaiian invasions. Mar Biol 145:1119–28.

Huelsenbeck JP, Larget B, Miller RE, Ronquist F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst Biol 51:673–88.

Huelsenbeck JP, Rannala B. 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst Biol 53:904–13.

Hyman LH. 1940. Metazoa of the tissue grade of construction – the radiate phyla – Phylum Cnidaria. In: Hyman LH, editor. The Invertebrates: Protozoa through Ctenophora. New York: McGraw-Hill. p. 365–661.

Katoh M, Kuma M. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–66.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9:286–98.

Keane TM, Creevey CJ, Pentony MM, Naughton TJ, Mclnerney JO. 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evol Biol 6:29.

Kjer KM, Gillespie JJ, Ober KA. 2006. Structural homology in ribosomal RNA, and a deliberation on POY. Arthrop Syst Phylog 64:71–6.

Kramp PL. 1955. The medusae of the tropical west coast of Africa. Atlantide Report 3:239–324.

Kramp PL. 1961. Synopsis of the medusae of the world. J Mar Biol Assoc UK 40:1–469.

Kramp PL. 1965. Some medusae (mainly scyphomedusae) from Australian coastal waters. T Roy Soc South Aust 89:257–78.

Kramp PL. 1968. The scyphomedusae collected by the Galathea expedition 1950-52. Vidensk Medd Dan Naturhist Foren 131:67–98.

Lanave CG, Preparata C, Saccone C, Serio G. 1984. A new method for calculating evolutionary substitution rates. J Mol Evol 20:86–93.

Larkin MA, et al. 2007. Clustal W and clustal X version 2.0. Bioinformatics 23:2947–8.

Lee MSY. 2001. Uninformative characters and apparent conflict between molecules and morphology. Mol Biol Evol 18:676–80.

Lumbsch HT, Hipp AL, Divakar PK, Blanco O, Crespo A. 2008. Accelerated evolutionary rates in tropical and oceanic parmelioid lichens. BMC Evol Biol 8:257.

Lutzoni F, Pagel M. 1997. Accelerated evolution as a consequence of transitions to mutualism. Proc Natl Acad Sci USA 94:11422–7.

Maas O. 1907. Die Scyphomedusen. Ergeb Fortschritte der Zool 1:189–238.

Marques AC, Collins AG. 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr Biol 123:23–42.

Matsumoto GI, Raskoff KA, Lindsay DJ. 2003. *Tiburonia granrojo* n. sp., a mesopelagic scyphomedusa from the Pacific Ocean representing the type of a new subfamily (class Scyphozoa: order Semaeostomeae: family Ulmaridae: subfamily Tiburoniinae subfam. nov.). Mar Biol 143:73–7.

Mayer AG. 1910. Medusae of the World, III – the Scyphomedusae. Washington, DC: Carnegie Institute.

Medlin LK, Elwood HJ, Stickel S, Sogin ML. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71:491–9.

Mianzan HW, Cornelius PFS. 1999. Cubomedusae and Scyphomedusae. In: Boltovskoy D, editor. South Atlantic zooplankton. 1. Leiden: Backhuys Press. p. 513–59.

Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T. 2009. The CIPRES Portals. CIPRESURL: Accessed: 2010-01-05. http://www.phylo.org/sub_sections/portal.

Mills CE. 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? Hydrobiologia 451 (Developments in Hydrobiology 155):55–68.

Mills CE, Larson RJ, Youngbluth MJ. 1987. A new species of coronate scyphomedusae from the Bahamas, *Atorella octogonos*. B Mar Sci 40:423–7.

Morandini AC, Jarms G. 2005. New combinations for two coronate polyp species (Atorellidae and Nausithoidae, Coronatae, Scyphozoa, Cnidaria). Contrib Zool 74:117–23.

Naumov DV. 1961. Stsifoidnye meduzy morei SSSR (Scyphomedusae of the seas of the USSR). Opredelitel po Faune SSSR 75:1–98.

Nicholas KB, Nicholas HB Jr, Deerfield II DW. 1997. GeneDoc: analysis and visualization of genetic variation. EMBnet.news 4:14.

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581–3.

Pitt KA, Welsh DT, Condon RH. 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. Hydrobiologia 616:133–49.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–8.

Purcell JE, Uye S, Lo W. 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Mar Ecol Prog Ser 350:153–74.

Rabosky DL, McCune AR. 2010. Reinventing species selection with molecular phylogenies. Trends Ecol Evol 25:68–74.

Rambaut A. 2009a. Figtree v1.1.2. Available from http://tree.bio.ed.ac.uk/software/figtree (Accessed February 2010).

Rambaut A. 2009b. Figtree v1.2.2. Available from http://tree.bio.ed.ac.uk/software/figtree (Accessed February 2010).

Rambaut A, Drummond AJ. 2007. Tracer v1.4. Available from http://beast.bio.ed.ac.uk/Tracer (Accessed February 2010).

Rambaut A, Drummond AJ. 2009. Tracer v1.5. Available from http://beast.bio.ed.ac.uk/Tracer (Accessed February 2010).

Regier JC, et al. 2008. Resolving arthropod phylogeny: exploring phylogenetic signal with 41 kb of protein-coding nuclear gene sequence. Syst Biol 57:920–38.

Richardson AJ, Bakun A, Hays GC, Gibbons MJ. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends Ecol Evol 24:312–22.

Rigby S, Milsom CV. 2000. Origins, evolution, and diversification of zooplankton. Annu Rev Ecol Syst 31:293–313.

Ripplinger J, Sullivan J. 2008. Does choice in model selection affect maximum likelihood analysis? Syst Biol 57:76–85.

Russell FS. 1970. The Medusae of the British Isles. II. Pelagic Scyphozoa with a Supplement to the First Volume on Hydromedusae. Cambridge: Cambridge University Press.

Sanderson MJ, Donoghue MJ. 1996. Reconstructing shifts in diversification rates on phylogenetic trees. Trends Ecol Evol 11:15–20.

Schnare MN, Damberger SH, Gray MW, Gutell R. 1996. Comprehensive comparison of structural characteristics in eukaryotic cytoplasmic large subunit (23S-like) ribosomal RNA. J Mol Biol 256:701–19.

Schöniger M, von Haeseler A. 1994. A stochastic model for the evolution of autocorrelated DNA sequences. Mol Phylogenet Evol 3:240–7.

Seo TK, Kishino H. 2008. Synonymous substitutions substantially improve evolutionary inference from highly diverged proteins. Syst Biol 57:367–77.

Stiasny G. 1921. Studien über Rhizostomeen. In: van Oort ED, editor. Capita Zoologica. Gravenhage: Martinus Njhoff.

Stiasny G, van der Maaden H. 1943. Über Scyphomedusen aus dem Ochotskischen und Kamtschatka Meer nebst einer Kritik der Genera *Cyanea* und *Desmonema*. Zool Jb 76:227–66.

Swofford DL. 2003. PAUP phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sunderland: Sinauer Associates.

Telford MJ, Wise MJ, Gowri-Shankar V. 2005. Consideration of RNA secondary structure significantly improves likelihood-based estimates of phylogeny: examples from the Bilateria. Mol Biol and Evol 22:1129–36.

Thiel H. 1966. The evolution of the Scyphozoa, a review. In: Rees WJ, editor. Cnidaria and their evolution. London: Academic Press. p. 77–177.

Uchida T. 1926. The anatomy and development of a Rhizostome medusa *Mastigias papua* L. Agassiz, with observations on the phylogeny of Rhizostomeae. J Fac Sci, Tokyo Univ, Sect IV. Zool 1:45–95.

Uchida T. 1963. The systematic position of the Hydrozoa. Japan J Zool 14:1–14.

Uchida T. 1972. The systematic position of the Stauromedusae. Publ Seto Mar Biol Lab 20:133–9.

van Iten H, Moraes Leme J, Simões MG, Marques AC, Collins AG. 2006. Reassessment of the phylogenetic position of conulariids (?Ediacaran–Triassic) within the subphylum Medusozoa (phylum Cnidaria). J Syst Palaeontol 4:109–18.

von Lendenfeld R. 1882. Über Coelenteraten der Südsee. I. *Cyanea annaskala*, sp. n. Z wiss Zool 37:465–52.

Walcott CD. 1911. Cambrian geology and paleontology II, No. 3 - Middle Cambrian holothurians and medusae. Smith Misc Coll 57:41–69.

Werner B. 1967. Morphologie, systematik und Lebensgeschichte von *Stephanoscyphus* (Scyphozoa, Coronatae), sowie seine Bedeutung für die Evolution der Scyphozoa. Zool Anz Suppl 30:297–319.

Werner B. 1971. *Stephanoscyphus planulophorus* n. spec., ein neuer Scyphopolyp mit einem neuen Entwicklungsmodus. Helgoländer wiss Meeresunters 22:120–40.

Werner B. 1973. New investigations on systematics and evolution of the class Scyphozoa and the phylum Cnidaria. Publ Seto Mar Biol Lab 20:35–61.

Werner B. 1974. *Stephanoscyphus eumedusoides* n. spec. (Scyphozoa, Coronatae), ein Höhlenpolyp mit einem neuen Entwicklungsmodus. Helgoländer wiss Meeresunters 26:434–63.

Werner B. 1975. Bau und Lebensgeschichte des Polypen von *Tripedalia cystophora* (Cubozoa, class. nov., Carybdeidae) und seine Bedeutung für die Evolution der Cnidaria. Helgoländ Wiss Meersunters 27:461–504.

Werner B. 1979. Coloniality in the Scyphozoa: Cnidaria. In: Larwood G, Rosen BR, editors. Biology and systematics of colonial organisms. London: Academic Press. p. 81–103.

West EJ, Welsh DT, Pitt KA. 2009. Influence of decomposing jellyfish on the sediment oxygen demand and nutrient dynamics. Hydrobiologia 616:151–60.

Willis KJ, Bennett KD, Birks HJB. 2009. Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation. J Biogeogr 36:1630–44.

Willoughby RH, Robison RA. 1979. Medusoids from the Middle Cambrian of Utah. J Paleontol 53:494–500.

Yoder AD, Irwin JA. 1999. Phylogeny of the Lemuridae: effects of character and taxon sampling on resolution of species relationships within *Eulemur*. Cladistics 15:351–61.

Young GA, Hagadorn JW. 2010. The fossil record of cnidarian medusae. Proceedings of the 10th International Conference on Fossil Cnidaria and Porifera.

Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31:3406–15.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Dissertation. Auston, TX: The University of Texas at Austin.

Zwickl DJ, Hillis DM. 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst Biol 51:588–98.