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Comprehensive Analysis of the Jellyfish *Chrysaora pacifica* (Goette, 1886) (Semaeostomeae: Pelagiidae) with Description of the Complete rDNA Sequence

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Jinho Chae, Yoseph Seo, Won Bae Yu, Won Duk Yoon, Hye Eun Lee, Soo-Jung Chang, and Jang-Seu Ki (2018) The Scyphomedusae genus *Chrysaora* consists of highly diversified jellyfishes. Although morphological systematics of the genus has been documented over the past century, characterization of molecular taxonomy has been attempted only recently. In the present study, we sequenced an 8,167 bp region, encompassing a single ribosomal DNA (rDNA) repeat unit, from *Chrysaora pacifica*, and used it for phylogenetic analyses. The tandemly repeated rDNA units turned out to consist of both coding and noncoding regions, whose arrangement was found to be the same as that of a typical eukaryote. None of the 5S rRNA sequences were found among the repeat units. Comparative analyses of jellyfish rDNA sequences showed that the 28S locus is highly informative and divergent compared to the 18S locus. Phylogenetic analyses of the 18S and 28S loci revealed that the Semaeostomeae order of jellyfish is separated into taxonomic groups by families and genera, with a few exceptions. The family Pelagiidae was in a clade separate from other groups, thus forming a monophyletic lineage. All *Chrysaora* included here formed a strongly supported clade within the family Pelagiidae, and Pelagiidae manifested a sister relationship with *Cyanea*. Nonetheless, *Chrysaora* was found to be paraphyletic in both 18S and 28S phylogenies. *Chrysaora pacifica* was clearly distinct from close relatives *C. melanaster* and *C. quinquecirrha*. These results provide a special reference for the DNA taxonomy of Pelagiidae jellyfishes in terms of nuclear cistron rDNA sequences and improve our understanding of the molecular phylogenetic relationships among Semaeostomeae jellyfishes.

Key words: Jellyfish, Pelagiidae, *Chrysaora pacifica*, Ribosomal DNA, Transcription repeat unit.

BACKGROUND

The scyphozoan medusa genus *Chrysaora* Péron & Lesueur, 1810 (Semaeostomeae: Pelagiidae) is composed of many species, and frequently occurs in coastal waters around the

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world. Genera *Chrysaora* and *Pelagia* Péron & Lesueur, 1810 were both first described mainly on the basis of tentacle numbers. Studies on the developmental life cycle, including sexual maturity, showed that the genera are different from each other (Mayer 1910). Currently, species of *Chrysaora* are discriminated by morphological characteristics such as the tentacle number, shape of radial septa, order of tentacle development, coloration, and the form of nematocyst capsules (Morandini and Marques 2010). Nevertheless, these key features are obscure among preserved and live specimens owing to their fragile, transparent bodies and morphological differences depending on the environment. Thus, *Chrysaora* has been a subject of taxonomic confusion and species misidentification (Morandini and Marques 2010; Bayha at al. 2017). Recently, Morandini and Marques (2010) analyzed morphological features of various *Chrysaora* specimens and found 13 valid and two doubtful species. Although the taxonomic system was plausible, the species concepts and systematics of Morandini and Marques (2010) remain unstable due to inconsistency in morphological characteristics and the presence of undescribed and/or cryptic species.

Molecular approaches can help resolve the ambiguities of morphological taxonomy (*e.g.*, Collins et al. 2006; Ki et al. 2008; Lee at al. 2016). Molecular approaches include direct DNA sequencing, restriction fragment length polymorphism, and DNA chips (Ki et al. 2008 2010). Among these methods, DNA sequencing is quite effective in constructing phylogenies and elucidating evolutionary inferences in jellyfish. To date, DNA markers of many jellyfish species have been sequenced and are available in public databases. Species assignments and population-genetic and phylogenetic analyses of jellyfishes have been conducted based on DNA sequences of nuclear and mitochondrial genes (Schroth et al. 2002; Dawson et al. 2005; Collins et al. 2006; Ki et al. 2009; Ramšak et al. 2012; Lee et al. 2013; Glynn et al. 2015; Dong et al. 2016; van Walraven et al. 2016). Until now, these phylogenetic studies have been mostly carried out at higher taxonomic levels (family or higher) and have not been attempted at lower taxonomic levels such as genera and species. Indeed, existing reports contain little information on *Chrysaora* (Bayha et al. 2010; Rizman-Idid et al. 2016) because of a dearth of DNA sequence data on this genus. Nonetheless, DNA sequence data on the family Pelagiidae have been obtained for some members of genera *Chrysaora*, *Mawia* Avian, Ramšak, Tirelli, D'Ambra & Malej, 2016; *Sanderia* Goette, 1886; and *Pelagia* and used in taxonomic redescriptions (Avian et al. 2016). More recently, Bayha at al. (2017) extensively analyzed U.S. Atlantic specimens of *Chrysaora* and tried to decipher the molecular relationships among pelagiid species, particularly focusing on the features distinguishing *C. quinquecirrha* (Desor, 1848) from *C. chesapeakei* (Papenfuss, 1936).

Nuclear ribosomal DNA (rDNA) in eukaryotes is typically structured as tandem arrays of a basic unit that contains a transcription unit (18S, 5.8S, and 28S) and an intervening intergenic spacer (IGS) region (Hill et al. 1990). The different subunits and loci of rDNA, therefore, have different degrees of sequence variability and varying suitability for comparison at the inter-genus level or inter-species level (Ki et al. 2009). In addition, these properties offer various options for data construction in phylogenetic analyses. Because the coding regions evolve at a slower rate, they allow phylogenies to be constructed for more distant divergences. Conversely, the high variability of the noncoding regions is useful for reconstructing relatively recent evolutionary events (Hillis and Dixon 1991). Previously, we (Ki et al. 2009) reported for the first time the complete rDNA sequence of the moon jelly *Aurelia coerulea* von Lendenfeld, 1884 (formerly *Aurelia* sp. 1). Except for this example, the complete rDNA region has not been sequenced in other jellyfish species, and thus, the rDNA sequences of jellyfishes are characterized insufficiently.

In the present study, we determined the complete nucleotide sequence of a single unit of tandemly repeated rDNA of *Chrysaora pacifica* (Goette, 1886) and characterized the molecular features of various rDNA components according to individual molecules. Comparative analyses of parsimony and dot plot analyses were performed on already known complete and/or partial rDNA sequences to gain a better understanding of jellyfish rDNA characteristics. Finally, we studied the phylogenetic relationships among members of the order Semaeostomeae, in particular focusing on the genus *Chrysaora*.

MATERIALS AND METHODS

Sample collection, morphology, and DNA extraction

Chrysaora pacifica specimens were examined and collected from the waters of Namildae, Tongyeong, Jindong, Jangmok, Busan, and Yangyang (southern and south-eastern coasts of Korea) and showed no conspicuous morphological differences (Lee et al. 2016). Jellyfish specimens for this study were collected in Tongyeong Bay (34°55.59N, 128°5.79E), Korea, on 14 August 2013. Before sample collection, we photographed the jellyfish specimens (Olympus OM-D E-M5 with an underwater housing) in their natural habitat to record live morphology. For genetic analyses, oral-arm and gonad tissues were preserved in 100% ethanol (Merck, Darmstadt, Germany).

Prior to genomic-DNA extraction, the alcohol-preserved specimens were washed in distilled water to remove all ethanol, and this procedure was repeated several times during the night. Total genomic DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method described in Ausubel et al. (1989).

Polymerase chain reaction (PCR)

Nuclear rDNA sequences were amplified by the long PCR technique with two sets of eukaryotic universal primers (forward 18F01, 5'-TAT CTG GTT GAT CCT GCC AGT AG-3' and reverse 28R691, 5'-CTT GGT CCG TGT TTC AAG AC-3'; forward 28F01, 5'-CCG CTG AAT TTA AGC ATA TAA GTA AGC-3', reverse 18R, 5'-GCT ATT GGA GCT GGA ATT ACC-3'), according to our previous study (Ki et al. 2009). PCR was carried out in 20 μL reaction mixtures containing 12.9 μL of sterile distilled water, 2 μL of 10× Ex PCR buffer (TaKaRa, Shiga, Japan), 2 μL of a dNTP mix (4 mM each), 1 μL of each primer (10 pmoles), 0.1 μL Ex *Taq* polymerase (2.5 U), and 1 μL of a template. PCR cycling was performed on a Bio-Rad iCycler via the following program: 94°C for 5 min; followed by 35 cycles of 94°C for 20 sec, 55°C for 30 sec, and 68°C for 5 min; with a final extension at 72°C for 10 min. Resulting PCR products were subjected to electrophoresis in a 1.0% agarose gel (Promega, USA), stained with ethidium bromide, and visualized under ultraviolet light on a transilluminator.

The amplicons were then purified with the QIAquick PCR Purification Kit (Qiagen GmbH, Germany), and DNA sequencing reactions were run with the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, CA) using the PCR products and PCR primers. After that, the remaining DNA sequences were determined by primer walking. Labeled DNA fragments were analyzed on an automated DNA sequencer (Model 3700, Applied Biosystems, CA).

Editing and contig assembly of the rDNA sequence fragments were carried out in Sequencher 4.7 software (Gene Codes, MI). The coding rDNA genes were identified with the help of the NCBI database and *Aurelia coerulea* sequence (GenBank No. EU276014). All the sequences determined here were deposited in the GenBank database.

Data analyses

General molecular features of the *Chrysaora pacifica* rDNA were calculated in Genetyx ver. 7.0 (Hitachi Engineering Co., Tokyo, Japan) and MEGA ver. 5.0 (Tamura et al. 2011). In addition, nucleic acid distribution, sequence complexity, and entropy across the entire rDNA nucleotides of *C. pacifica* were calculated by means of BioAnnotator in Vector NTI Advance ver. 10.3.0 (Invitrogen, San Diego, CA). The repeat sequence pattern in the rDNA IGS sequences was analyzed in Genetyx ver. 7.0 and Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.basic.submit.html).

A comparison of molecular features was performed by comparing the rDNA sequence of *Chrysaora pacifica* with that of the moon jelly *Aurelia coerulea* (Scyphozoa, EU276014). Dot plot analysis of both rDNA regions was carried out in the MegAlign software, ver. 5.01 (DNAstar Inc Madison, WI). In addition, parsimony analyses were performed on 11 DNA sequences of jellyfish 18S and the corresponding 28S rDNA loci (Table S1). DNA sequences were aligned on the online server of MAFFT (https://mafft.cbrc.jp/alignment/server/; Katoh and Standley 2013). Genetic distances were calculated using the aligned DNA sequences via the Kimura 2-parameter model in DNASIS ver. 3.5 (Hitachi Software Engineering America, San Bruno, CA). Molecular similarity was measured by means of BioEdit ver. 5.09. Further comparative analyses, such as determination of parsimony-informative sites and of the transition/transversion ratio, were performed on the above data matrix in MEGA ver. 5.0.

Putative signals of assumed termination (*e.g.*, poly(T) tract) and promoter signals (bi-repeats)

within the IGS rDNA were analyzed in Genetyx ver. 7.0. Additionally, putative secondary structures of the assumed signals were estimated in DNA sequences of their flanking regions using web-based software Mfold ver. 3.2 (http://unafold.rna.albany.edu/?q=mfold) according to Zuker (2003).

Phylogenetic analyses

For phylogenetic reconstruction procedures, the 18S rDNA sequence was determined and the remaining sequences were obtained from the DDBJ/EMBL/GenBank database (Table S1). These sequences were aligned in MAFFT (Katoh and Standley 2013), and ambiguous regions were removed on the Gblocks server with the least stringent settings (http://molevol.cmima.csic.es/castresana/Gblocks_server.html; Castresana 2000). Maximum likelihood (ML) analysis was conducted on an 18S data matrix (1,483 alignment sites) in RAxML ver. 8.0 using the GTR+G nucleotide substitution model (Stamatakis 2014). Branch support was assessed with 5,000 bootstrap replicates. Additional Bayesian analysis of the same dataset was implemented in MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001) via the GTR+G nucleotide substitution model. The Markov chain Monte Carlo (MCMC) process was set to four chains, and 1,000,000 generations were carried out with the sampling frequency of one per 100 generations. After this analysis, the first 2,000 trees were deleted as burn-in, and a consensus tree was constructed. Bayesian posterior probabilities (> 0.50) are indicated at each branch node in the figures. Phylogenetic trees were visualized in TreeView ver. 1.6.6 (Page 1996).

In addition, phylogenetic analyses of scyphozoan 28S rDNA sequences were performed on a 28S data matrix (843 alignment sites) via the GTR+G model, and an identical methodology was used for analysis of the 18S rDNA region.

RESULTS

The complete rDNA sequence of *Chrysaora pacifica*

In this study, we performed morphological examination of the collected specimens before proceeding with alcohol preservation. Jellyfish specimens from the study site (off Tongyeong) had typical morphological characteristics of Korean *Chrysaora pacifica*. The bell diameter was approximately 15 cm, marginal lappets were rounded, and there were 32 brownish radiating strips and 40 tentacles. Live specimens (Fig. 1) possessed conspicuously extended oral arms and tentacles as compared to the formalin-fixed specimens.

In addition, we determined the sequence of the full-length 8,167 bp single rDNA repeat unit of Korean *Chrysaora pacifica* (GenBank No. KY212123). It was found to be organized in the typical rDNA fashion of eukaryotes, *i.e.*, 18S-ITS1-5.8S-ITS2-28S-IGS (Fig. 2A). The rDNA region of the species was found to have the following structure: 1,810 bp 18S, 246 bp ITS1, 158 bp 5.8S, 182 bp ITS2, 3,609 bp 28S, and 2,162 bp IGS. Intron-like sequences were not detected in 18S and 28S rDNA. Upon comparison, we found that each coding region was nearly identical in length between our study specimen and *Aurelia coerulea* (GenBank No. EU276014); however, noncoding regions (*i.e.*, ITSs and IGS) considerably differed in length. Particularly, the IGS of *C. pacifica* (2,162 bp) is much longer than that of *A. coerulea* (1,603 bp). G+C content of the full-length region turned out to be 46.5% (A, 25.2%; T, 32.5%; G, 23.6%; and C, 18.7%). Cytosine (C) content of each rDNA sequence was the lowest. Nucleotide composition was found to differ between coding and noncoding regions of rDNA.

Sequence complexity and nucleic-acid distribution were determined using sliding windows of 100 nucleotides along the entire rDNA sequence (Fig. 2B). The distribution of G+C content was ~50% across the complete rDNA sequence. Nevertheless, some sites in the IGS showed considerably lower G+C content and high fluctuation due to the presence of GC- and AT-rich loci and poly(T) tracts (Fig. 3). In addition, sequence variability was analyzed via sequence complexity and entropy plotting. Overall, the two variables fluctuated against one another along the rDNA. These observations revealed a clear difference in profiles between the coding and noncoding regions (ITS and IGS). Sequence complexity was considerably higher in the noncoding regions, such as ITS and IGS, compared to the coding regions. The locus that manifested the lowest complexity corresponded to GC- and AT-rich sequences within the IGS.

Highly informative characteristics of IGS rDNA

The IGS rDNA of *Chrysaora pacifica* contains 2,162 nucleotides (Fig. 3), and the nucleotide

frequencies of A, T, G, and C were measured and turned out to be 26.5%, 31.5%, 21.3%, and 20.6%, respectively. Overall, A+T content was higher than G+C content owing to the presence of poly(T) tracts and an AT-rich sequence. In addition, we detected a GC-rich sequence in the IGS. After a comparison with the 5S rDNA database (http://combio.pl/rrna/, accessed 20 Mar. 2018), none of the 5S rDNA sequences were found to be present within the IGS locus. Rather, the IGS consisted of transcription termination and bi-repeated sequences. We detected two poly(T) tracts (5'-TAT TTT TTT T-3', 5'-TTA TTT TTT TCT TT-3') in the 5' external transcribed site (ETS) area adjacent to the end of 28S rDNA (Fig. 3), possibly serving as a termination signal. As a putative RNA polymerase I transcription initiation site, many promoters of RNA polymerase I are bipartite, consisting of a proximal promoter domain and an upstream control element (UCE) (Chen et al. 2000). In the present study, we identified a promoter candidate pattern of bi-repeated sequences (5'-*CTG ATA TAG AG*-TAG AGC ATG GCT TAG-*CTG ATA TAG AG*-3' (underlined nucleotides likely form a hairpin structure) within the IGS.

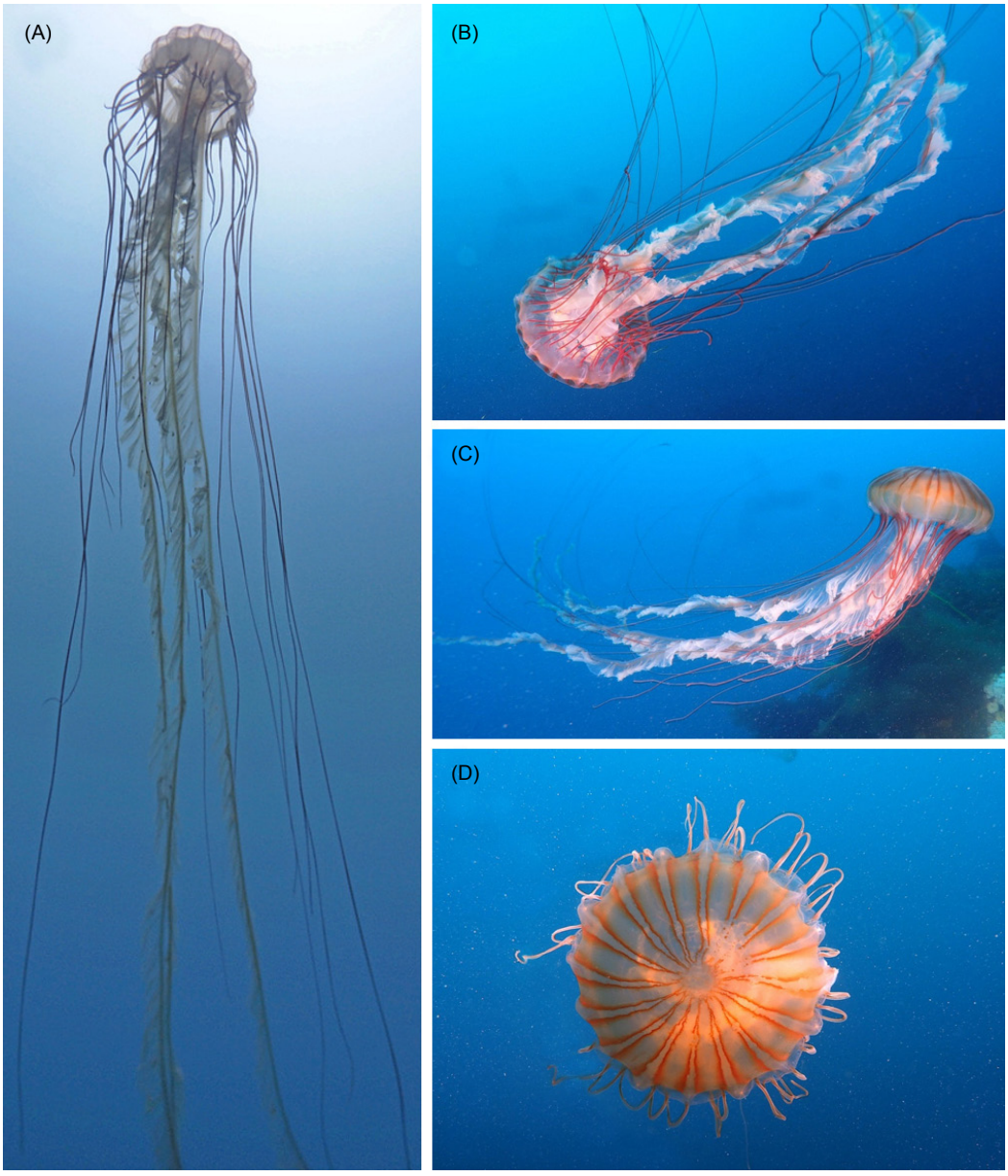

Fig. 1. Live *Chrysaora pacifica* in natural habitat: basolateral (A and B), lateral (C) and apical view (D).

Intra- and interspecific comparisons of complete rDNA

We compared the rDNA of our study specimen, *Chrysaora pacifica* (collected in 2013 in Tongyeong, Korea), to other Korean specimens collected on the Namildae Beach (in Sacheon), Tongyeong, Jindong, and Jangmok in Korea during August 2014 (Lee et al. 2016) and found that all of them had the same DNA sequence, implying that

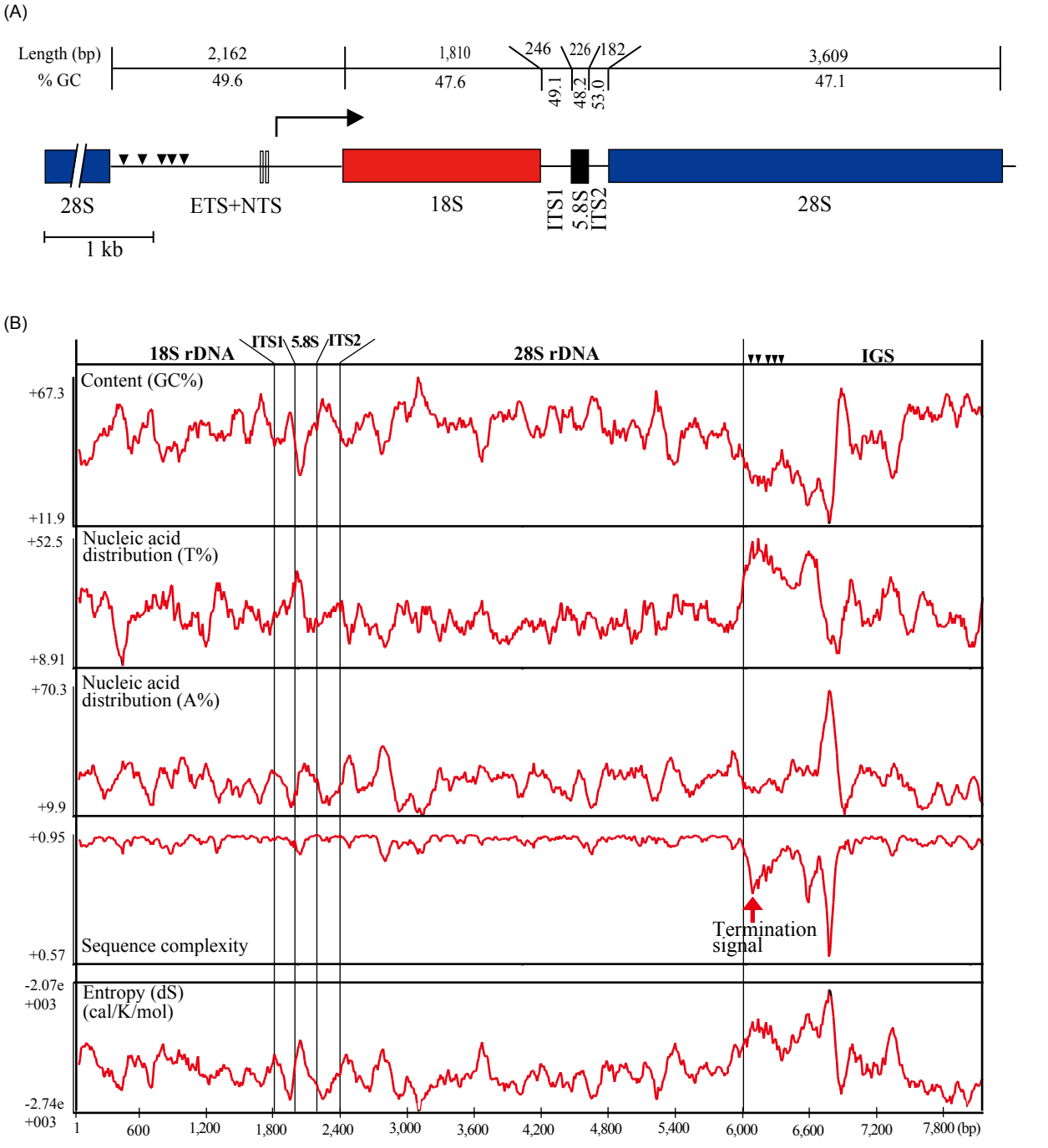

Fig. 2. A schematic representation of the single unit of rDNA (A), and GC content (%), nucleic acid distribution (% thymine), sequence complexity, and entropy (dS) in 100-bp windows across the entire rDNA nucleotides of *Chrysaora pacifica* (B). In the full rDNA (A), solid boxes indicate the ribosomal RNA genes and thin lines represent ITS or IGS. Nucleotide sequences in length and GC composition of each locus are represented near a line by calculation from a single unit of rDNA. The putative transcription start site is represented by an arrow; solid inverted-triangles represent sub-repeats in IGS.

the species were identical.

In addition, we compared the rDNA sequences of *Chrysaora pacifica* (GenBank No. KY212123) with the rDNA sequence of *Aurelia coerulea* (GenBank No. EU276014). High similarity and a small genetic distance were noted between the coding regions of these species; in contrast, the highest dissimilarity was identified in the rDNA IGS (67.3%), followed by ITS1 (45.6%). Of the coding regions, the 28S sequence (0.0651) manifested a greater genetic distance than did the others (18S, 0.0173; 5.8S, 0.0128) as calculated via the Kimura 2-parameter model. Dot plots of the complete rDNA regions of *C. pacifica* and *A. coerulea* graphically revealed sequence similarity (Fig. 4). It was determined by means of sliding

Fig. 3. Nucleotide sequences of the *Chrysaora pacifica* IGS rDNA. Bi-repeats are indicated by asterisks, and microsatellite-like nucleotides are marked by lines. Putative termination signals (poly(T) tract) are represented as Ts-1, Ts-2 with box. Blocks of both AT- and GC-rich regions are presented in separate boxes.

windows of 60 nucleotides along the entire rDNA region. As expected, high similarities were detected in the rDNA coding loci (*e.g.*, 18S, 5.8S, and 28S). By contrast, the dot plot showed no similarity between the loci within the noncoding rDNA regions.

General features of jellyfish 18S and 28S rDNA sequences were analyzed among 11 cnidarian species (Table 1). The comparative analyses showed that the transition:transversion ratio (Ts/Tv) was slightly higher in the 28S rDNA sequences. Parsimony-informative sites, in contrast, had much greater differences in 28S rDNA (514 sites, 15.8%) than in 18S rDNA (156 sites, 9.1%).

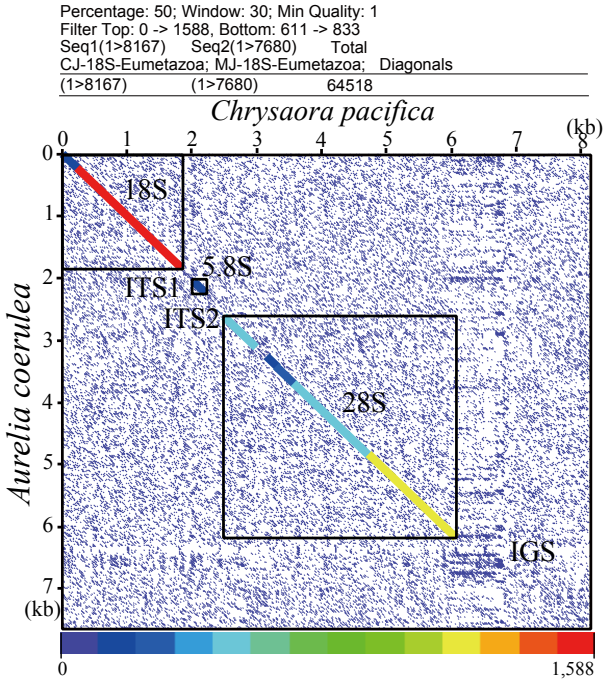

Fig. 4. A dot matrix comparison of rDNA sequences between *Chrysaora pacifica* (KY212123) and *Aurelia coerulea* (EU276014). Color scale bars represent consecutive sequence length of some regions detected similarly between the two sequence pairs. The open boxes in matrices indicate rDNA coding regions such as 18S, 5.8S, and 28S.

Phylogeny of Semaeostomeae jellyfish, including *Chrysaora*

Phylogenetic relationships among the members of the order Semaeostomeae were investigated using ML trees inferred from the separate 18S and 28S rDNA sequences (Fig. 5). Additional Bayesian analyses generated nearly identical tree topologies as the ML trees. Hence, posterior probabilities (PP) from the Bayesian analyses were incorporated into the ML trees to support the strength of each branch. Comparisons of 18S and 28S trees indicated that branch topologies were generally identical, with a different position of the Cyaneidae family. According to expected changes per site, phylogenetic resolution of 18S rDNA was considerably lower (2.6-fold) than that of 28S rDNA. In a broad phylogenetic view, the order Semaeostomeae was clearly separated into four families (Cyaneidae, Drymonematidae, Pelagiidae, and Ulmaridae), forming unique monophyletic clades according to each taxonomic level. Among them, the family Drymonematidae diverged the earliest, and this finding was supported strongly by 100% bootstrap proportions (BP) and 1.00 PP. In addition, our 28S phylogeny suggested that Pelagiidae and Ulmaridae formed a sister clade (91% BP and 1.00 PP). As for the Pelagiidae relationships, our tree showed that the three tested genera-*Chrysaora*, *Pelagia*, and *Sanderia*-were not clearly separated into each genus group. For example, *Pelagia* and *Sanderia* ended up within clusters of *Chrysaora* in the 18S tree, but they were separated to form a unique clade in the 28S tree, with a sister relationship. On the other hand, *Chrysaora* formed a well-supported clade in the 18S tree (77% BP and 0.83 PP) and 28S tree (98% BP and 1.00 PP). After our analyses of these linages, *Chrysaora* formed a paraphyletic group according to both 18S and 28S phylogeny.

Table 1. Sequence characteristics of nearly complete 18S and 28S rDNAs among cnidarians (MA in Supplementary Table 1), including hydrozoans and scyphozoans. *p*-distances were calculated with the Kimura 2-parameter model

Locus	Nn	Nc	Nv	Ti	Ts	Tv	Ts/Tv	PI	%PI
18S rDNA	1,729	1498	227	1,621	53	36	1.46	156	9.1
28S rDNA	3,251	2,544	690	2893	170	112	1.51	514	15.8

Nn, total number of sites; Nc, total number of conserved sites; Nv, total number of variable sites; Ti, Total numbers of identical pairs; Ts, Total numbers of transitional pairs; Tv, Total numbers of transversional pairs; PI, parsimony-informative site.

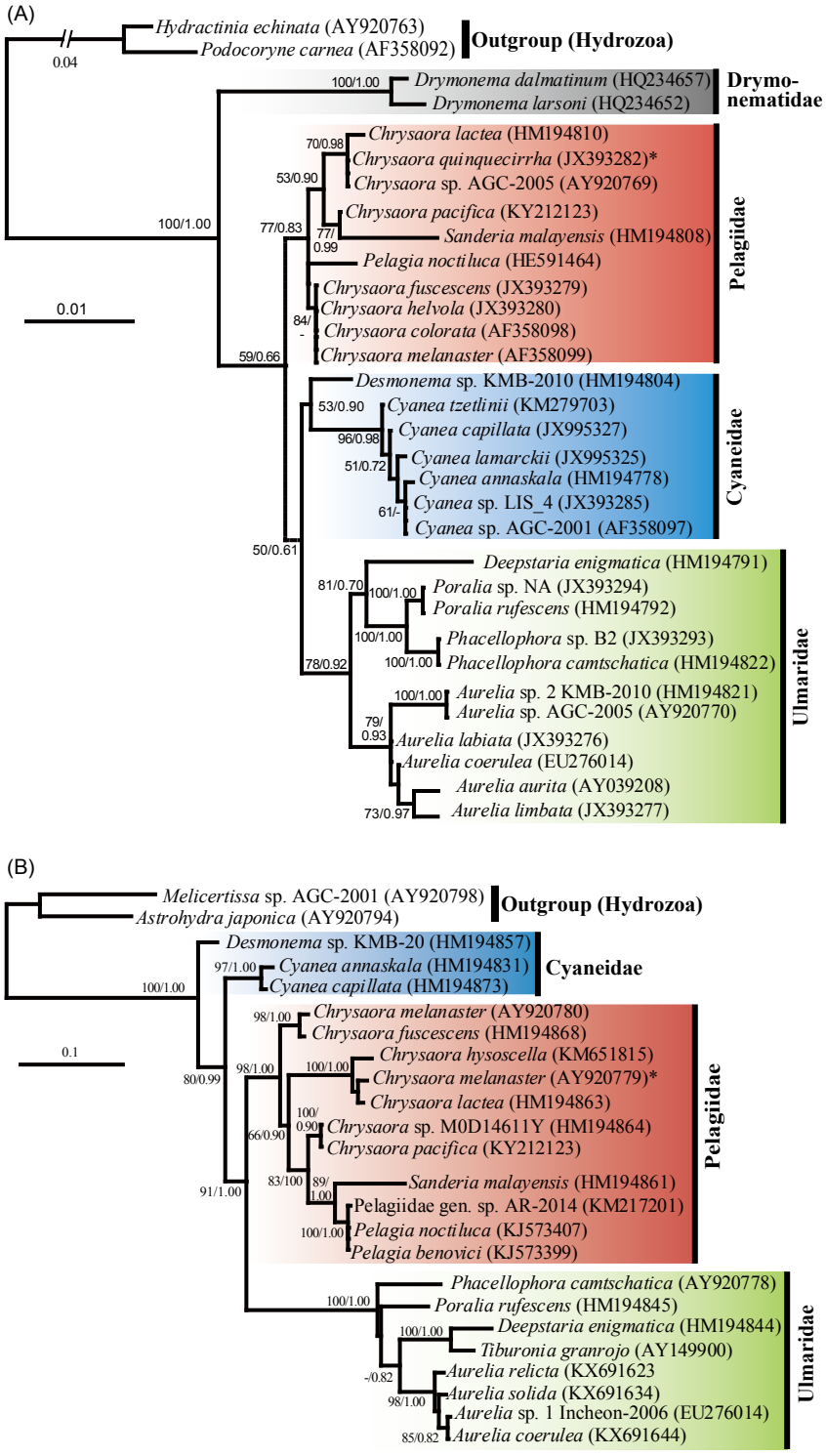

Fig. 5. Phylogenetic relationships between jellyfishes within the order Semaeostomeae inferred from nearly complete 18S rDNA (A) and partial 28S rDNA sequences (B) with maximum-likelihood (ML) algorithms. ML analyses of 18S and 28S were used as the nucleotide substitution model of GTR+G. Two hydrozoans (*Hydractinia echinata* and *Podocoryne carnea* for 18S rDNA; *Astrohydra japonica* and *Melicertissa* sp. for 28S) were included as the outgroups. Additional Bayesian analysis generated similar topology of the tree compared with the ML tree. Posterior probabilities (PP) from the analyses were incorporated into the ML tree to support the strength of each branch. The first and second numbers at the nodes display bootstrap proportions (BP) (> 50%) in ML and PP (> 0.50) in Bayesian, respectively. Branch lengths are proportional to the scale given. *Represents controversial species names, because they were suspected as different species by Bayha et al. (2017).

Chrysaora pacifica was clearly separated from the morphologically close relatives, *C. melanaster* and *C. quinquecirrha*, as evident by its high BP (98%) and PP (1.00) values in the 28S tree.

Additional phylogenetic analyses of the rDNA sequences of the family Pelagiidae showed that *Chrysaora* was separated into two clades (Fig. 6A, B): one included *C. colorata* (Russell, 1964); *C. fuscescens* Brandt, 1835; *C. melanaster*; and *C. helvola* Brandt, 1838, while the other included *C. pacifica*: *C. lactea* Eschscholtz, 1829; and *C. quinquecirrha*. Overall phylogenetic lineages of *Chrysaora* were comparable between 18S and 28S trees. Nonetheless, *Pelagia noctiluca* (Forsskål, 1775) and *Sanderia malayensis* Goette, 1886 were separated into different clades of *Chrysaora* (100% BP supporting) in the 18S tree but formed a clade in the 28S tree (89% BP, 1.00 PP). Our molecular lineage of *Chrysaora* generally matched those of *C. melanaster*, *C. fuscescens*, and *C. hysoscella*

Fig. 6. Phylogenetic relationships of the family Pelagiidae, including the genera *Chrysaora*, *Pelagia* and *Sanderia*, inferred from 18S rDNA (A), 28S rDNA (B) and morphological characters (C), which were redrawn from Fig. 95 in Morandini and Marques (2010). Phylogenetic trees of the rDNAs were constructed using the maximum-likelihood (ML) algorithms with the GTR+G model. A jellyfish *Cyanea capillata* (the family Cyaneidae) was used as the outgroup. Additional Bayesian trees generated similar branch patterns. The first and second numbers at the nodes display bootstrap proportions (BP) and posterior probabilities (PP) obtained in the ML and Bayesian analyses, respectively. Branch lengths are proportional to the scale given. Thick lines represent congruent branches between 18S and 28S, and morphological systematics.

(Linnaeus, 1767) (Fig. 6C) reported by Morandini and Marques (2010), but the others were mostly incompatible.

DISCUSSION

The taxonomy of the genus *Chrysaora* has a complicated history. Nevertheless, it was recently revised on the basis of morphology and geographical distribution reported in studies conducted mainly on fixed specimens preserved in museums of Brazil, Europe, and the USA (Morandini and Marques 2010). Their morphological identification; however, is still often confusing because of the variation in morphology observed in different life stages and regions. Morphological obscurity among species has also been a cause for concern. For example, Korean *Chrysaora pacifica* was misidentified as *Dactylometra quinquecirrha* Agassiz, 1862 by Park (2002), and has been classified this way for the last 15 years owing to their morphological similarity. Recently, we redescribed the Korean jellyfish as *C. pacifica* by means of morphological characteristics and partial rDNA comparisons (Lee et al. 2016). Using these key characteristics of our specimens, we confirmed that our present jellyfish should belong morphologically to *C. pacifica*.

A recent study on *Chrysaora* uncovered the existence of 20 valid species (Collins et al. 2018). In addition, we found that 13 species and their DNA sequences (645 sequences) matched the *Chrysaora* sequences in the NCBI database, (https://www.ncbi.nlm.nih.gov/; GenBank, accessed 20 Mar. 2018); the nuclear rDNA loci (18S, ITS, and/or 28S) of these 13 species were identified. Nonetheless, as in most cases, only partial sequences of the rDNA regions were available in the NCBI database, and a complete comparison could not be made. With our present complete rDNA data, we were able to broadly compare individual sequences according to each rDNA component. As for interspecific variation, the 18S and 28S rDNA sequences were analyzed using available data. Via BLASTn searches, we detected seven sequences of *Chrysaora* for 18S rDNA (GenBank Nos. HM194811, KY249594, AF358098, AF358099, AY920769, HM194810 and HM194815), and two sequences for the 28S locus (AY920780 and AY920779). Comparative analyses indicated that our 18S rDNA shares 100% similarity with that of *C. pacifica* Sc01 collected on the Namildae Beach, Korea (Lee et al. 2016), followed by 99.1% similarity with *C. colorata* from the United States (Collins 2002). Besides, our 28S sequence turned out to share 97.2% similarity with that of *C. melanaster* (Collins et al. 2006), followed by 93.6% similarity with *A. coerulea* 28S rDNA (Ki et al. 2009).

In case of ITS rDNA, BLASTn searches showed that our sequence matched that of *Pelagiidae* sp. (KM036431) but did not match *Chrysaora* ITS rDNA, whereas *Chrysaora* ITS sequences (*e.g.*, *C. hysoscella*, KM651820; *C. fulgida* (Reynaud, 1830), HM348773; and *C. chinensis* Vanhöffen, 1888, JN202955) are recorded in GenBank (accessed 20 Mar. 2018). These results suggest that the rDNA ITS sequence of *C. pacifica* is quite different from those of other *Chrysaora*. With such high variation, the ITS locus can be considered a useful molecular marker for defining intraspecific differences because it is less subject to functional constraints and evolves more rapidly (Bena et al. 1998). This finding indicates that the *Chrysaora* rDNA ITSs may be suitable markers of population differences for determining biogeographic distribution patterns of the species.

As stated above, the rDNA cistron consists of coding (18S, 5.8S, and 28S) and noncoding loci (ITS and IGS), which evolve differently, and thus each rDNA has different degrees of sequence variability. These data give taxonomists various options regarding molecular markers, depending on the taxonomic level (*e.g.*, order, family, genus, species and subspecies) (Ki et al. 2009). Recently, we characterized this sequence variability in the jellyfish, by comparing the complete rDNA sequence of the moon jelly with that of other invertebrates, like nematodes and insects (Ki et al. 2009). Here, we determined another complete rDNA sequence, from *C. pacifica*, and compared the two jellyfish rDNA sequences. As expected, coding rDNA loci were found to be similar in length and DNA sequence (*e.g.*, 98.0% for 18S, 98.7% for 5.8S, and 93.3% for 28S); however, length variation was greater between the noncoding regions, such as ITS and IGS (52.1% similarity for ITS1, 37.0% for ITS2, and 34.6% for IGS), whereas some regions completely lacked similarity. This finding is also well supported by the dot plot analysis in this study.

The noncoding IGS locus of jellyfishes contains many structural motifs, such as poly(T) tracts, minisatellites, AT- and GC-rich sequences, and bi-repeat patterns. Particularly, the secondary structure of poly(T) suggests that upstream sequences of both tracts can form hairpin

structures. Among them, Ts-1 forms a more stable structure than the others do in Mfold predictions (http://unafold.rna.albany.edu/?q=mfold, accessed 10 Mar. 2018) with default settings. This finding reveals that nucleotides between 28S and the termination signal sequences may form a stem-and-loop structure. This kind of folding pattern is in agreement with our previous results, namely that the moon jelly *Aurelia coerulea* (EU276014) and anthozoan *Junceella fragilis* (Ridley, 1884) (AF154670) have the same poly(T) tract on the 5' side of the IGS (Ki et al. 2008). These observations suggest that the putative termination signal may be generally present in the IGS of cnidarians and jellyfishes as well. On the other hand, we detected 5-repeat minisatellites (5'-CTA ACC CTA GCC CTA ACC-3') in the IGS of *A. coerulea* (Ki et al. 2009); this kind of minisatellite was also found in *Chrysaora pacifica*; however, their nucleotide sequences turned out to be completely different. These data indicate that jellyfishes may have common minisatellite patterns in the IGS, and this signature IGS may serve as a potential genetic marker for studies on jellyfish populations.

In general, the 18S rDNA sequences are relatively conserved when compared to 28S rDNA, and thus the former have been employed for phylogenetic and systematic analyses of higher taxonomic levels, such as the phylum Cnidaria and class Scyphozoa (Ki et al. 2009; Bayha et al. 2010; Rizman-Idid et al. 2016). In the present study, the 18S rDNA phylogeny of members of the order Semaeostomeae uncovered clear-cut separation between each family (*e.g.*, Cyaneidae, Drymonematidae, Pelagiidae and Ulmaridae). In contrast, the phylogenetic tree of closely related taxa (*e.g.*, *Chrysaora*) showed polytomic branch patterns owing to sequence similarities among species. These unresolved relationships among jellyfishes have generally been reflected in the results of other phylogenetic studies involving genus level comparisons (Collins et al. 2006). Even recent phylogenetic analyses of the family Pelagiidae by means of several genes (*COI*, 16S rRNA, partial 28S rRNA, and their combination) suggest that *Chrysaora* is paraphyletic with respect to species of *Sanderia*, *Pelagia* and *Mawia* (Bayha et al. 2017). In our 28S phylogeny, family relationships within the order Semaeostomeae were not completely consistent with those of the 18S tree: Pelagiidae formed a sister relationship with Cyaneidae in the 18S tree but formed a clade with Ulmaridae in the 28S tree. At the species and genus levels, 28S rDNA considerably resolved the species of *Chrysaora*, as compared to the 18S data. Consequently, we examined molecular divergence of the two rDNA molecules among 12 species of cnidarians, mainly jellyfishes. Parsimony-informative sites were much stronger in 28S rDNA sequences than in the 18S rDNA sequences. Judging by the parsimony-informative values, the 28S rDNA of the tested jellyfishes may evolve 1.736-fold more rapidly than the 18S rDNA. In addition, *p*-distance comparisons of the jellyfishes (Fig. 7) showed that 28S nucleotide variations are significantly different from those of 18S rDNA (Student's *t* test, $P < 0.01$). Although our phylogenetic tree did not include all the members of *Chrysaora*, our 28S phylogeny is comparable to a morphological phylogeny (Morandini and Marques 2010) and is more clearly resolved than 18S rDNA. Thus, the 28S rDNA sequences manifest greater genetic divergence (*i.e.*, lower similarity) than do the 18S rDNA sequences in jellyfishes. In addition, our phylogenetic and comparative data suggest that 28S rDNA sequences may be more suitable for discriminating species of *Chrysaora* than 18S rDNA ones.

In conclusion, this study determined for the first time the complete sequence of a single rDNA unit of *Chrysaora pacifica* and characterized the different rDNA loci regarding their utility as

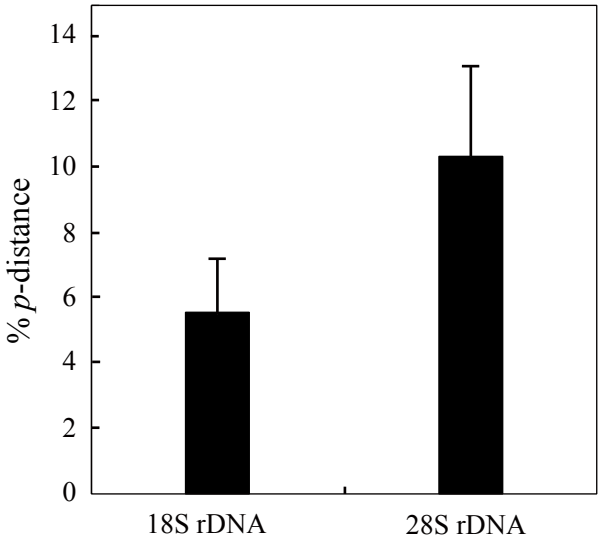

Fig. 7. Nucleotide divergences of the cnidarians 18S and 28S rDNAs (datasets used in Table 1) based on corrected *p*-distances. Genetic distances between each paired sequence were calculated by the Kimura 2-parameter model, where a total of 16 cnidarian species were compared. Statistical analysis showed that the 18S rDNA divergences were significantly different from those of 28S rDNA (Student *t*-test, $P < 0.05$, $N = 66$).

phylogenetic and taxonomic identification markers. Genetic-distance analysis indicates that 28S rDNA is more informative and yields highly resolved relationships of *Chrysaora* species than 18S data do. On the other hand, the ITS and IGS are quite variable, suggesting that they cannot serve as phylogenetic markers but can be potentially used to discriminate subspecies and/or populations. In addition, our results suggest that DNA-based tools are effective at discriminating between species of jellyfishes (Ki et al. 2010). The present molecular approaches improved the understanding of deep relationships among the members of the family Pelagiidae (*e.g.*, *Chrysaora*, *Sanderia* and *Pelagia*); however, these phylogenetic lineages (see Fig. 6) are not congruent with the latest morphological taxonomy proposed by Morandini and Marques (2010). Perhaps this discrepancy is due to inconsistency in the morphological characteristics and/or low taxon sampling. In the future, redescription of the morphological characteristics via reverse taxonomy (Markmann and Tautz 2005) and additional DNA data from different taxa need to be included for a better comparison.

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Authors' contributions: JC analyzed the data and wrote the manuscript. WDY coordinated the research. HYL and SJC provided specimens and analyzed morphology. YS and WBY performed the experiments and analyzed the data. JSK conceived and designed project, analyzed the data, and wrote the manuscript. All the authors read and approved the final manuscript.

Availability of data and materials: The DNA sequences are accessible from GenBank by accession numbers. The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethics approval consent to participate: Not applicable.

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Supplementary Material

Table S1. Taxa examined and GenBank accession numbers for nuclear 18S, and 28S rDNAs. (download)