Acta Oceanol. Sin., 2014, Vol. 33, No. 4, P. 55–76
DOI: 10.1007/s13131-014-0415-8
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China

ZHENG Lianming[1,2,3]*, HE Jinru[1], LIN Yuanshao[1,2], CAO Wenqing[1,2], ZHANG Wenjing[1,2]

[1] College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China
[2] Marine Biodiversity and Global Change Research Center, Xiamen University, Xiamen 361005, China
[3] Fujian Provincial Key Laboratory for Coastal Ecology and Environmental Studies (CEES), Xiamen University, Xiamen 361005, China

Received 17 June 2013; accepted 20 October 2013

Abstract

Identification of hydrozoan species is challenging, even for taxonomic experts, due to the scarcity of distinct morphological characters and phenotypic plasticity. DNA barcoding provides an efficient method for species identification, however, the choice between mitochondrial cytochrome *c* oxidase subunit I (COI) and large subunit ribosomal RNA gene (16S) as a standard barcode for hydrozoans is subject to debate. Herein, we directly compared the barcode potential of COI and 16S in hydrozoans using 339 sequences from 47 pelagic hydrozoan species. Analysis of Kimura 2-parameter genetic distances (K2P) documented the mean intraspecific/interspecific variation for COI and 16S to be 0.004/0.204 and 0.003/0.223, respectively. An obvious "barcoding gap" was detected for all species in both markers and all individuals of a species clustered together in both the COI and 16S trees. These results suggested that the species within the studied taxa can be efficiently and accurately identified by COI and 16S. Furthermore, our results confirmed that 16S was a better phylogenetic marker for hydrozoans at the genus level, and in some cases at the family level. Considering the resolution and effectiveness for barcoding and phylogenetic analyses of Hydrozoa, we strongly recommend 16S as the standard barcode for hydrozoans.

Key words: DNA barcoding, hydrozoan, COI, 16S rRNA

Citation: Zheng Lianming, He Jinru, Lin Yuanshao, Cao Wenqing, Zhang Wenjing. 2014. 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. Acta Oceanologica Sinica, 33(4): 55–76, doi: 10.1007/s13131-014-0415-8

1 Introduction

Marine hydrozoans encompass a diversity of forms, including benthic and pelagic life history stages with many species having both phases within their life cycle. Hydrozoans, especially the pelagic hydrozoans, play an important role in the coastal and deep-sea ecosystem, as some of the most important predators and competitors (Mills, 1995). However, identification of hydrozoan species is challenging, due to their paucity of phylogenetically informative characters and phenotypic plasticity (Bouillon and Boero, 2000). In addition, problems have been aggravated because the cryptic species in many groups cannot be revealed due to the lack of sufficient morphological data (Moura et al., 2008, 2011a; Cantero et al., 2010; Pontin and Cruickshank, 2012). Consequently, although there are molecular phylogenetic studies for many members in Hydrozoa (Collins, 2000, 2002; Collins et al., 2005, 2006, 2008; Dunn et al., 2005; Cartwright et al., 2008; Leclère et al., 2009; Martinez et al., 2010), the relationships within the main hydrozoan group, especially in the lower taxonomic (e.g., intrageneric) levels, are unresolved. It is important to note that the problems in species identification have become one of the major obstacles to phylogenetic studies and future revisions for hydrozoans.

DNA barcoding has made tagging of species identifications possible (Hebert et al., 2003, 2004a). The 5′ region of mitochondrial cytochrome *c* oxidase subunit I (COI) gene is recommended as the universal and standard barcoding marker for most animals (Hebert et al., 2004b; Ward et al., 2005; Hajibabaei et al., 2006; Ratnasingham and Hebert, 2007). However, its applicability to cnidarians is controversial. Slow evolutionary rates of COI and other mitochondrial genes (including the mitochondrial large subunit ribosomal RNA gene, 16S) have been detected in most anthozoans (McFadden et al., 2000, 2011; Shearer et al., 2002; Hellberg, 2006; Huang et al., 2008), and therefore, COI has been assumed to be useless for DNA barcoding in these taxa. However, Sinniger et al. (2008) recommended that both COI and 16S could be useful as DNA barcodes for species of the order Zoantharia, and the same case was detected in Ceriantharia (Stampar et al., 2012). For hydrozoans, the use of COI for DNA barcoding is also controversial. Shearer et al. (2002) demonstrated that the mutational rate of COI in one hydrozoan species was low, and caution was also advised for its barcode efficiency in Hydrozoa by Huang et al. (2008). However, Govindarajan et al. (2005a) found a high substitution rate in COI for *Obelia geniculata* (Linnaeus, 1758) (6.54×10^{-9} substitutions site^{-1} year^{-1}, 3.5 million years ago) and suggested that COI should be a useful tool for studying hydrozoan phylogeography. Recently, Ortman et al. (2010) and Bucklin et al. (2011) reported that COI could be used as DNA barcoding of species across the Medusozoa, al-

Foundation item: The National Natural Science Foundation of China under contract No. 41006078; the Fundamental Research Funds for the Central Universities under contract No. 2010121037; the Public Science and Technology Research Funds Projects of Ocean under contract Nos 201005012-3 and 201005015-5; the Natural Science Foundation of Fujian Province of China under contract No. 2011J05116.
*Corresponding author, E-mail: zhlm@xmu.edu.cn

though their works included only a few sequences of other subclasses of Hydrozoa except for Siphonophore.

Compared to COI, 16S has been found to be much easier to amplify, particularly in the Anthoathecata and Leptothecata (Miglietta et al., 2009; Moura et al., 2011a, b). 16S has been widely employed to resolve phylogenetic questions within Hydrozoa from the taxonomic levels of family to population (Collins et al., 2005; Govindarajan et al., 2005a, b, 2006; Schuchert, 2005a, b; Leclère et al., 2007, 2009; Miglietta et al., 2007, 2009; Nawrocki et al., 2010; Moura et al., 2012), whereas the nuclear 18S rRNA and 28S rRNA genes have been used for evolutionary studies at higher taxonomic levels (Bridge et al., 1995; Collins, 2000; Collins et al., 2006; Govindarajan et al., 2006). Moreover, 16S has also been considered a valuable marker for differentiating morphologically undistinguishable, nominal species, including undescribed taxa (Schuchert and Reiswig, 2006; Moura et al., 2008, 2011a, b; Miranda et al., 2010). Impressively, Schuchert (2006, 2007, 2008a, b, 2009) used 16S as the supplement of species descriptions to review the European athecate hydroids and their medusae.

The ideal barcoding gene should have an observable gap between intra- and interspecific levels of divergence and, most important, correctly identify species (Hebert et al., 2004b; Meyer and Paulay, 2005; Köhler, 2007). No study has directly compared the barcode potential of COI and 16S in the same group of hydrozoans, so it is difficult to determine the superiority of one marker over the other. In this study, we barcoded pelagic hydrozoans from Chinese coastal waters using both COI and 16S, and compared their utility and efficiency for the DNA barcoding of hydrozoans.

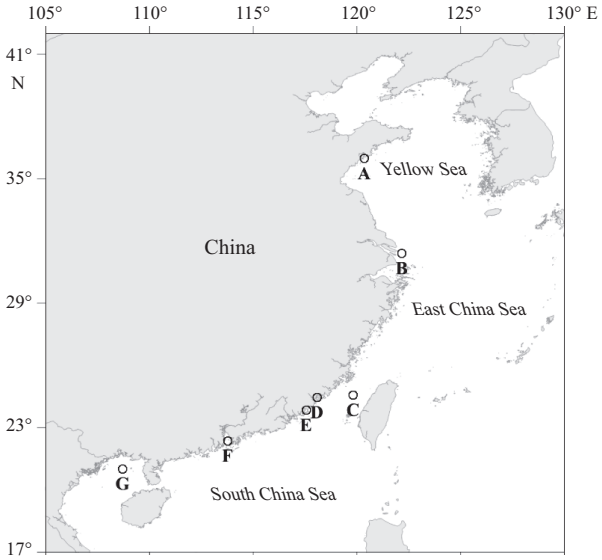

Fig.1. Sampling site distribution for this study. Symbols A–G stand for Jiaozhou Bay, Changjiang River Estuary, Taiwan Strait, Xiamen Bay, Dongshan Bay, Zhujiang River Estuary, and Beibu Gulf, respectively.

2 Materials and methods

2.1 *Sample preparation*

Specimens of pelagic hydrozoans were collected using a plankton net (mesh size: 500 μm) along the Chinese coast from May 2005 to August 2012, mainly in the Yellow Sea (Jiaozhou Bay), East China Sea (Changjiang River Estuary, Taiwan Strait, Xiamen Bay, and Dongshan Bay), and South China Sea (Zhujiang River Estuary and Beibu Gulf) (Fig. 1, Table S1 Supporting Information). To avoid DNA contamination by undigested food, all individuals were separated and acclimated in the filtered sea water at least 24 h before preservation. Specimens were accurately identified with the help of expert taxonomists, Professors Xu Zhenzu and Huang Jiaqi of Xiamen University, and then were preserved in 95% ethanol.

2.2 *Molecular methods*

Total DNA was extracted from either the whole individual or a part of the umbrella of the medusae by the SDS-proteinase K/phenol-chloroform extraction method (Zheng et al., 2009). DNA was preserved in TE buffer and stored at –20°C.

A partial region of COI gene was amplified using universal primers (LCO-1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO-2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al., 1994). 16S was partially amplified using the published primers (16S-L 5′-GAC TGT TTA CCA AAA ACA TA-3′ and 16S-H 5′-CAT AAT TCA ACA TCG AGG-3′) (Ender and Schierwater, 2003). Polymerase Chain Reactions (PCR) were carried out on Bio-Rad S1000™ Thermal Cycler using TaKaRa Ex Taq™ Kit by 25 μL reaction system. Protocols for COI amplification were 94°C for 4 min, 33 cycles (94°C for 40 s, 50°C for 1 min, 72°C for 90 s); finally, fragments were elongated at 72°C for 5 min. Amplification for 16S was achieved with 5 cycles (94°C for 1 min, 45°C for 50 s, 72°C for 1 min), followed by 30 cycles (94°C for 50 s, 50°C for 1 min, 72°C for 1 min); finally, fragments were elongated at 72°C for 5 min. DNA sequencing was performed directly from PCR amplification products on an ABI PRISM 3730 Genetic Analyzer using BigDye® Terminator v3.1 by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd, China.

Sequences for multiple individuals per species were generated when possible. Raw sequences were initially matched to their corresponding chromatogram files to ensure sequencing quality. All COI and 16S sequences were aligned using CLUSTALX V2 (Larkin et al., 2007), and were subsequently edited with the help of EditSeq V7.1 to ensure correct alignment and placement of insertion/deletion events. GenBank BLAST searches were performed to confirm the accuracy and validity of all sequences, and detect artifactual sequences and any potential pseudogenes. All sequences were deposited in GenBank with the accession numbers JQ715881–JQ716211 (three sequences, JQ716075, JQ716076, and JQ716087, were not included in this analysis) and JX965906–JX965916 (Table S1 Supporting Information).

Sequence divergence was determined by the Kimura 2-Parameter (K2P) model (Kimura, 1980) using the software MEGA V5 (Tamura et al., 2011). Pairwise K2P distances between any two sequences, including intraspecies and interspecies, were calculated and classified to different taxonomic levels to assess the variation among all taxonomic groups. The nucleotide variance rate in the same genus was also compared between COI and 16S to evaluate their potential to determine species boundaries in hydrozoans. Neighbor-Joining (NJ, based on K2P model with both transition and transversion substitutions included; pairwise deletion was chosen when dealing with gaps and miss-

ing data) and Maximum-Likelihood (ML, based on GTR model with Gamma Distributed (G) selection in rates and sites option with number of discrete gamma categories set automatically as 5; partial deletion with 95% site coverage cutoff) trees were created by MEGA V5. Node support for the two approaches was inferred with bootstrap analysis (1 000 replicates). The COI and 16S trees were constructed by the sequences from the same species included in this analysis, along with the sequences of other species in the same genus that were already deposited in GenBank (Table S2 Supporting Information). Outgroups were chosen from a range of major cnidarian groups: *Aurelia aurita* (Linnaeus, 1758) and *A. limbata* (Brandt, 1835) (Scyphozoa), *Craterolophus convolvulus* (Johnston, 1835) (Staurozoa), and *Siderastrea radians* (Pallas, 1766) (Anthozoa).

3 Results

In total, 47 species representing two major Hydrozoan classes, Hydroidolina and Trachylinae, including 18 families and 29 genera were sequenced. COI was successfully sequenced from 159 of 227 individuals (70.0%), while 16S was successfully sequenced from 180 samples (79.3%). Forty-three species with COI gene fragment and 40 species with 16S gene fragment were submitted to the NCBI GenBank. Sequence length ranged from 643–712 base pairs for COI, and 499–560 base pairs for 16S. Partial sequences of COI for 23 species and 16S for 16 species were reported for the first time, which could be used for further studies.

3.1 *Genetic divergences*

Genetic distances calculated in reference to the Kimura 2-Parameter model were statistically arranged in relation to their taxonomic levels (Table 1 and Table S3 Supporting Information). For both COI and 16S, variation within a species, even for those of the same genus, was always less than, and did not overlap with, variation between species (Fig. 2). These results indicated an obvious "barcoding gap" (Meyer and Paulay, 2005) that was observed for all taxa. The mean intraspecific divergence of COI for all species was 0.004 (ranging from 0–0.033), and 99.50% were less than 0.030 (Table 1 and Table S3 Supporting Information). However, a distance of 0.033 was discovered within *Aequorea conica* (Browne, 1905), between the individu-

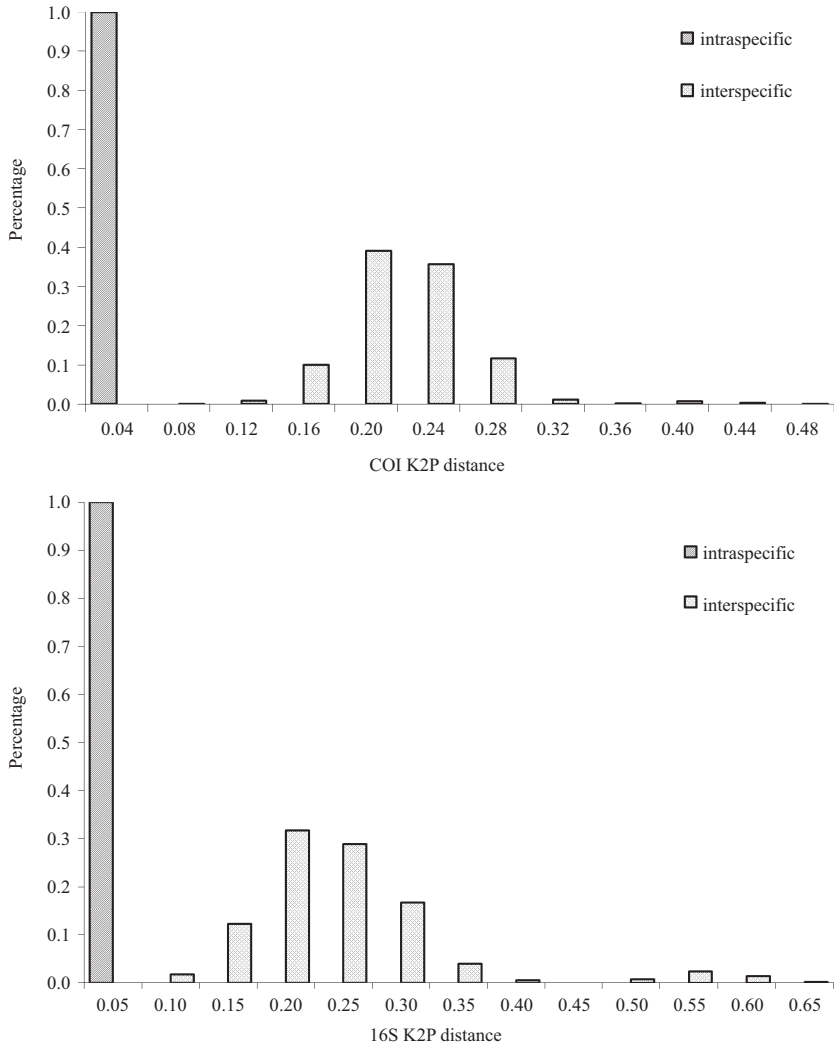

Fig.2. Distribution of the intraspecific and interspecific genetic variabilities (Kimura 2-parameter distance) of COI and 16S.

Table 1. Genetic distance between different taxonomic levels for both COI and 16S sequences. Columns are taxonomic groups, number of comparisons within a taxonomic group, and minimum, maximum, mean, and standard deviation (S.D.) of Kimura 2-Parameter (K2P) distance analyzed for this study

Taxonomic group	COI					16S				
	N	Min	Mean	Max	S.D.	N	Min	Mean	Max	S.D.
Intraspecies	406	0.000	0.004	0.033	0.005	623	0.000	0.003	0.016	0.003
Interspecies	12 155	0.045	0.204	0.475	0.041	15 487	0.062	0.223	0.642	0.087
Intra-genus	613	0.045	0.166	0.243	0.036	689	0.062	0.133	0.236	0.040
Aequorea	197	0.128	0.167	0.243	0.024	299	0.070	0.114	0.149	0.022
Blackfordia	15	0.142	0.147	0.153	0.003	24	0.164	0.166	0.169	0.002
Bougainvillia	2	0.067	0.070	0.073	0.005	/	/	/	/	/
Clytia	53	0.099	0.120	0.132	0.010	115	0.062	0.103	0.136	0.030
Eirene	323	0.092	0.176	0.223	0.034	220	0.068	0.169	0.236	0.036
Eutima	6	0.203	0.208	0.212	0.003	15	0.185	0.187	0.190	0.002
Helgicirrha	6	0.126	0.127	0.130	0.001	9	0.099	0.099	0.099	0.000
Malagazzia	/	/	/	/	/	6	0.069	0.069	0.069	0.000
Turritopsis	11	0.045	0.119	0.226	0.082	1	0.129	0.129	0.129	/
Intra-family	731	0.104	0.192	0.248	0.024	678	0.073	0.198	0.287	0.052
Aequoreidae	23	0.127	0.157	0.200	0.019	28	0.107	0.120	0.140	0.010
Bougainvillidae	3	0.104	0.116	0.136	0.017	40	0.073	0.075	0.077	0.001
Campanulariidae	28	0.128	0.165	0.174	0.011	/	/	/	/	/
Eirenidae	667	0.153	0.195	0.248	0.022	592	0.126	0.211	0.287	0.039
Laodiceidae	4	0.239	0.240	0.241	0.001	4	0.157	0.159	0.160	0.002
Lovenellidae	/	/	/	/	/	6	0.183	0.183	0.183	0.000
Malagazziidae	3	0.174	0.175	0.176	0.001	5	0.123	0.132	0.137	0.008
Pandeidae	3	0.211	0.216	0.221	0.005	3	0.199	0.204	0.212	0.007
Intra-suborder	4 257	0.129	0.187	0.311	0.030	6 111	0.080	0.181	0.359	0.038
Capitata	20	0.238	0.246	0.253	0.004	/	/	/	/	/
Conica	4 103	0.129	0.185	0.311	0.029	5 930	0.080	0.179	0.303	0.036
Filifera	134	0.184	0.226	0.287	0.023	181	0.179	0.246	0.359	0.039
Intra-order	1 883	0.087	0.189	0.305	0.032	2 524	0.086	0.191	0.344	0.035
Anthoathecata	171	0.183	0.239	0.305	0.027	168	0.233	0.265	0.344	0.025
Leptothecata	1 712	0.087	0.184	0.239	0.028	2 356	0.086	0.186	0.261	0.029
Intra-subclass	4 051	0.157	0.224	0.426	0.025	5 129	0.189	0.296	0.642	0.100
Hydroidolina	4 048	0.157	0.224	0.326	0.025	5 129	0.189	0.296	0.642	0.100
Trachylinae	3	0.426	0.426	0.426	0.000	/	/	/	/	/
Intra-class (Hydrozoa)	620	0.203	0.286	0.475	0.067	356	0.257	0.324	0.582	0.046

als collected from the coastal bays (Xiamen Bay: JQ716176 and Dongshan Bay: JQ716177) and Taiwan Strait (JQ716175). Interspecific distance values ranged from 0.045–0.475 (mean 0.204). Indeed, genetic divergence of 16S was very similar to COI with mean intraspecific and interspecific genetic distances of 0.003 and 0.223, respectively (Table 1).

The average K2P distance for COI between species within the same genus was 0.166 (ranging from 0.045–0.243), while the average distance within families was 0.192 (ranging from 0.104–0.248). *Turritopsis* sp. and *T. lata* contributed to the lowest congeneric divergence (0.045), while the largest K2P distance was found between *Aequorea* sp. and *A. taiwanensis* (0.243) (Zheng et al., 2009). Within genera, the K2P distances for 16S sequences were the lowest in the genus *Clytia* (*Clytia* sp. XM and *Clytia* sp. KC; 0.062) and highest in the genus *Eirene* (*Eirene brevistylus* (Huang and Xu, 1994) and *E. hexanemali* (Goette, 1886); 0.236) with a mean value of 0.133, while the average distance within families was 0.198 (ranging from 0.073–0.287).

The level of divergence of COI among congeneric species was about 40 times higher than intraspecific genetic distance, and the divergence among confamilial taxa was a little higher than that between congenerics (Table 1). In the case of 16S, the mean divergence among congeneric species was more than 40 times higher than intraspecific genetic distance, but the divergence among confamilial taxa was obviously higher than that of congenerics. Meanwhile, the nucleotide variance rate of COI in the same genus was just a little higher than that of 16S (Table 2).

3.2 *Tree-based identification*

All species formed distinct clusters with high support in the COI and 16S trees (Figs 3 and 4), which indicated that both markers correctly identified all species. Two species in the genus *Clytia*, *Clytia* sp. XM. and *Clytia* sp. KC. (we proposed them as two new and valid species based on morphology; Zhou et al., 2013), formed strongly supported lineages in both the COI and 16S trees, and were clearly separated from the other species of *Clytia*. We also found obvious intraspecific divergences within *Liriope tetraphylla* (Chamisso and Eysenhardt, 1821), which was comprised of two strongly supported lineages in the 16S tree. Although COI and 16S trees illustrated genetic divergence among intra- and interspecific hierarchical units, the systematic relationships within most families were not clearly resolved. However, both the COI and 16S trees supported the monophyly of the Bougainvillidae (Figs 3 and 4), and the 16S tree suggested

Table 2. Comparison of nucleotide variance rate of COI and 16S sequences in the same genus

Genus	Species No.	Sequence No.	COI			16S		
			V.S.	Length/bp	V.R./%	V.S.	Length/bp	V.R./%
Aequorea	5	26	139	537	27.37	113	504	22.42
Blackfordia	2	8	98	665	14.74	81	538	15.06
Clytia	7	20	152	492	30.89	132	478	27.02
Eirene	6	22	223	673	33.14	156	483	32.30
Eutima	2	5	121	642	18.85	89	521	17.08
Helgicirrha	2	5	80	675	11.85	46	506	9.09
Turritopsis	2	2	99	590	16.78	72	503	14.31

Notes: Sequence No. is the number of COI or 16S sequences in one genus (each species has the same number of sequences for COI and 16S). V.S. represents variance site and V.R. variance rate.

that the Laodiceidae and Aequoreidae formed a monophyletic clade (Fig. 4). Most genera appeared monophyletic in the 16S tree, but only three clades—*Helgicirrha*, *Turritopsis*, and *Proboscidactyla*—had high bootstrap values. *Blackfordia* and *Eirene* were polyphyletic on the basis of 16S data (Fig. 4). In contrast to the 16S tree, in this analysis, most genera that included two or more species did not form monophyletic clades in the COI tree (Fig. 3): *Eugymnanthea* and *Eutima*, and *Leuckartiara* and *Turritopsis* comprised two distinct clades, respectively; and *Aequorea australis* (Uchida, 1947), *Clytia gracilis* (Sars, 1850) (AY789899), and *C. linearis* (Thorneley, 1900) (AY789897) did not cluster with their respective groups but rather grouped with other species.

4 Discussion

4.1 *Intra- and interspecific genetic divergences*

DNA barcoding provides an opportunity to identify species rapidly and effectively. The extent of intraspecific variation and interspecific divergence might confirm the species delineation (Meyer and Paulay, 2005), especially given that genetic divergences are ordinarily lower among individuals of a species than between closely related species (del-Prado et al., 2010). In contrast, when genetic variation within species and divergence among species overlaps, DNA barcoding becomes less effective. Ideally, DNA barcodes should have a "barcoding gap", which means there is no overlap between levels of intra- and interspecific genetic distance. A number of studies have illustrated the potential for COI to identify intra- and interspecific variability (Zemlak et al., 2009; Ortman et al., 2010; Sun et al., 2012). In our study, for both COI and 16S, the intraspecific variation was much lower than the interspecific variation (Fig. 2), which demonstrated the efficacy of both COI and 16S for barcoding hydrozoans. Hebert et al. (2004a) proposed that interspecific divergence should be about ten times higher than intraspecific divergence. In our data, the level of divergence of COI or 16S among congeneric species was more than 40 times higher than intraspecific genetic distance (Table 1). Hence, these results showed that both gene fragment sequences could diagnose the species of hydrozoans efficiently and accurately.

4.2 *Evaluating the barcode potential of COI and 16S in hydrozoans*

DNA barcoding could help bring about a resurgence of interest in taxonomy (Hebert and Gregory, 2005). For hydrozoans, DNA barcoding can be used as part of a species description (Moura et al., 2008, 2011b). However, the choice between COI and 16S as a standard barcode is controversial due to the lack of enough data that can guide selection and validate the results (Pontin and Cruickshank, 2012). An ideal application of barcoding would be a system in which the sequence variants found within a species group together excluded all other species in a cluster diagram based on genetic distance (Ortman et al., 2010). In our study, the "barcoding gap" was obviously detected for all taxa based on COI or 16S sequences, and the same species were clustered under the same nodes by high bootstrap values (Figs 3 and 4), which indicated both COI and 16S could be useful as a biological barcoding tool for distinguishing species within the studied taxa. However, according to our result, one of the advantages of using 16S data for barcoding hydrozoans is that, unlike COI, the sequences are easier to amplify and sequence because they are relatively more conservative, which was consistent with the results of other research (Miglietta et al., 2009; Moura et al., 2011a, b).

DNA barcoding can be used not only for species diagnosis, but would also be helpful to facilitate the identification of cryptic species. Only the genes that evolve rapidly enough to efficiently detect species boundaries could be used as a DNA barcode. COI has been described as a useful marker to determine species boundaries in Hydrozoa (Huang et al., 2008; Ortman et al., 2010). Moreover, it also has been used previously as supporting evidence for the existence of cryptic species in Hydrozoa (Folino-Rorem et al., 2008; Pontin and Cruickshank, 2012) and other taxa of Cnidaria (Dawson and Jacobs, 2001; Dawson and Martin, 2001; Holland et al., 2004). A great variability of congeneric divergence (ranging from 0.045–0.243) was detected in our results. These levels were very similar to the interspecific distances in *Turritopsis* (0.361–12.11; Miglietta et al., 2007), Campaunlaridae (0.085–0.202; Govindarajan et al., 2006), *Cordylophora* (0.078–0.153; Folino-Rorem et al., 2008), *Aurelia* (0.235; Dawson, 2003), *Cassioper* (0.234; Holland et al., 2004), *Cyanea* (0.153; Dawson, 2005), and Medusozoa (0.056–0.381; Ortman et al., 2010), which indicated that COI should be a useful marker to determine species boundaries within the studied taxa. In the case of 16S, there was also considerable variability between species within a genus (mean variation ranged from 0.062–0.236), which was very similar to COI in the present study (Table 1). These levels were consistent with levels of 16S variability found in *Dendrophyllia* (0.079) and *Lophelia* (0.070; Le Goff-Vitry et al., 2004), *Eugymnanthea* (0.119–0.126; Govindarajan et al., 2005b), *Coryne* (0.037–0.092; Schuchert, 2005a), *Cordylophora* (0.035–0.078; Folino-Rorem et al., 2008), *Lytocarpia* (0.100–0.110), and *Streptocaulus* (0.230–0.260; Moura et al., 2012). Few differences were detected in the nucleotide variance

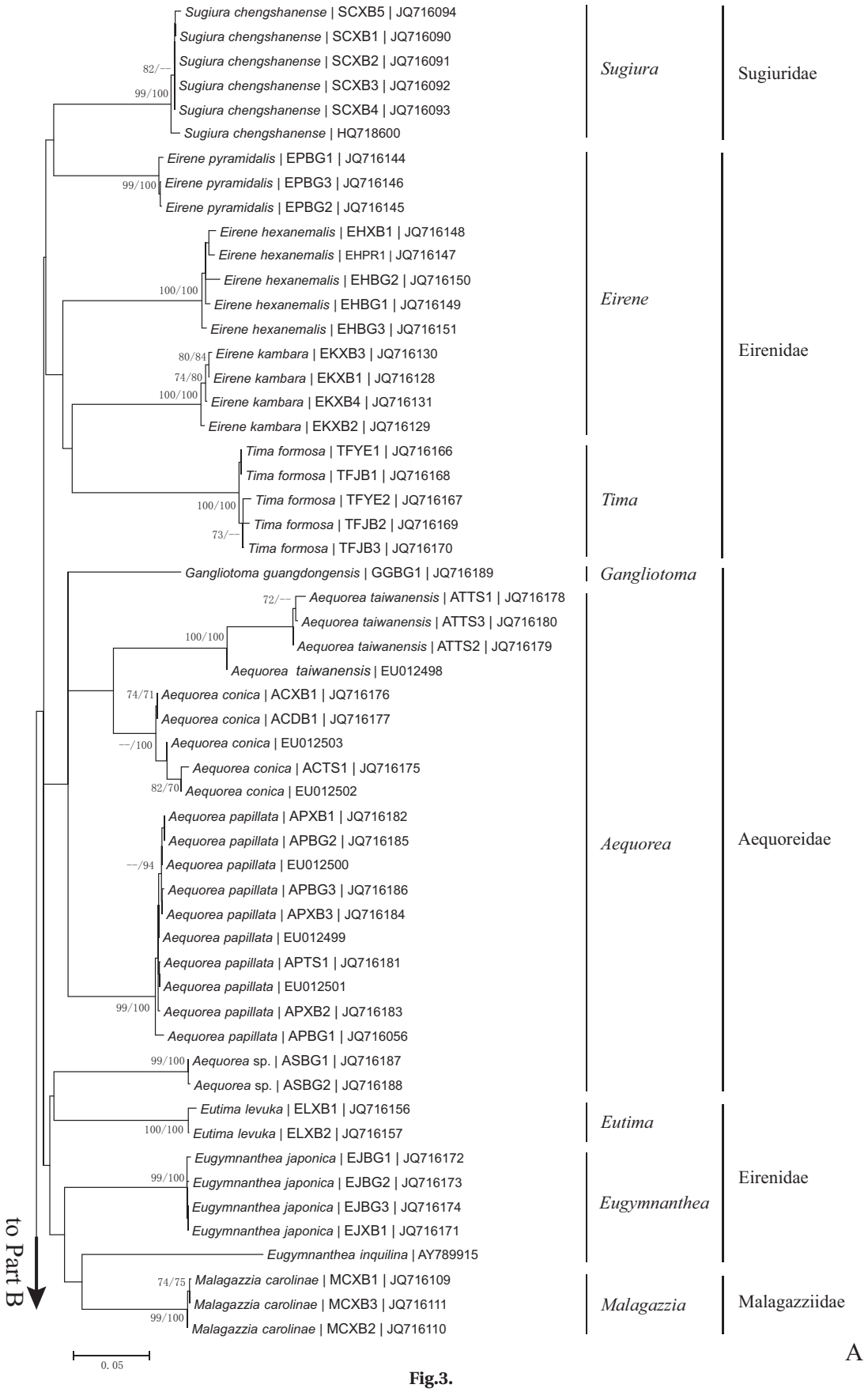
Sugiura chengshanense | SCXB5 | JQ716094
Sugiura chengshanense | SCXB1 | JQ716090
Sugiura chengshanense | SCXB2 | JQ716091
Sugiura chengshanense | SCXB3 | JQ716092
Sugiura chengshanense | SCXB4 | JQ716093
Sugiura chengshanense | HQ718600
Sugiura
Sugiuridae
Eirene pyramidalis | EPBG1 | JQ716144
Eirene pyramidalis | EPBG3 | JQ716146
Eirene pyramidalis | EPBG2 | JQ716145
Eirene hexanemalis | EHXB1 | JQ716148
Eirene hexanemalis | EHPR1 | JQ716147
Eirene hexanemalis | EHBG2 | JQ716150
Eirene hexanemalis | EHBG1 | JQ716149
Eirene hexanemalis | EHBG3 | JQ716151
Eirene kambara | EKXB3 | JQ716130
Eirene kambara | EKXB1 | JQ716128
Eirene kambara | EKXB4 | JQ716131
Eirene kambara | EKXB2 | JQ716129
Eirene
Tima formosa | TFYE1 | JQ716166
Tima formosa | TFJB1 | JQ716168
Tima formosa | TFYE2 | JQ716167
Tima formosa | TFJB2 | JQ716169
Tima formosa | TFJB3 | JQ716170
Tima
Eirenidae
Gangliotoma guangdongensis | GGBG1 | JQ716189
Gangliotoma
Aequorea taiwanensis | ATTS1 | JQ716178
Aequorea taiwanensis | ATTS3 | JQ716180
Aequorea taiwanensis | ATTS2 | JQ716179
Aequorea taiwanensis | EU012498
Aequorea conica | ACXB1 | JQ716176
Aequorea conica | ACDB1 | JQ716177
Aequorea conica | EU012503
Aequorea conica | ACTS1 | JQ716175
Aequorea conica | EU012502
Aequorea papillata | APXB1 | JQ716182
Aequorea papillata | APBG2 | JQ716185
Aequorea papillata | EU012500
Aequorea papillata | APBG3 | JQ716186
Aequorea papillata | APXB3 | JQ716184
Aequorea papillata | EU012499
Aequorea papillata | APTS1 | JQ716181
Aequorea papillata | EU012501
Aequorea papillata | APXB2 | JQ716183
Aequorea papillata | APBG1 | JQ716056
Aequorea sp. | ASBG1 | JQ716187
Aequorea sp. | ASBG2 | JQ716188
Aequorea
Aequoreidae
Eutima levuka | ELXB1 | JQ716156
Eutima levuka | ELXB2 | JQ716157
Eutima
Eugymnanthea japonica | EJBG1 | JQ716172
Eugymnanthea japonica | EJBG2 | JQ716173
Eugymnanthea japonica | EJBG3 | JQ716174
Eugymnanthea japonica | EJXB1 | JQ716171
Eugymnanthea inquilina | AY789915
Eugymnanthea
Eirenidae
Malagazzia carolinae | MCXB1 | JQ716109
Malagazzia carolinae | MCXB3 | JQ716111
Malagazzia carolinae | MCXB2 | JQ716110
Malagazzia
Malagazziidae
82/--
99/100
100/100
80/84
74/80
100/100
100/100
73/--
72/--
100/100
74/71
--/100
82/70
--/94
99/100
99/100
100/100
99/100
74/75
99/100
to Part B
0. 05
A

Fig.3.

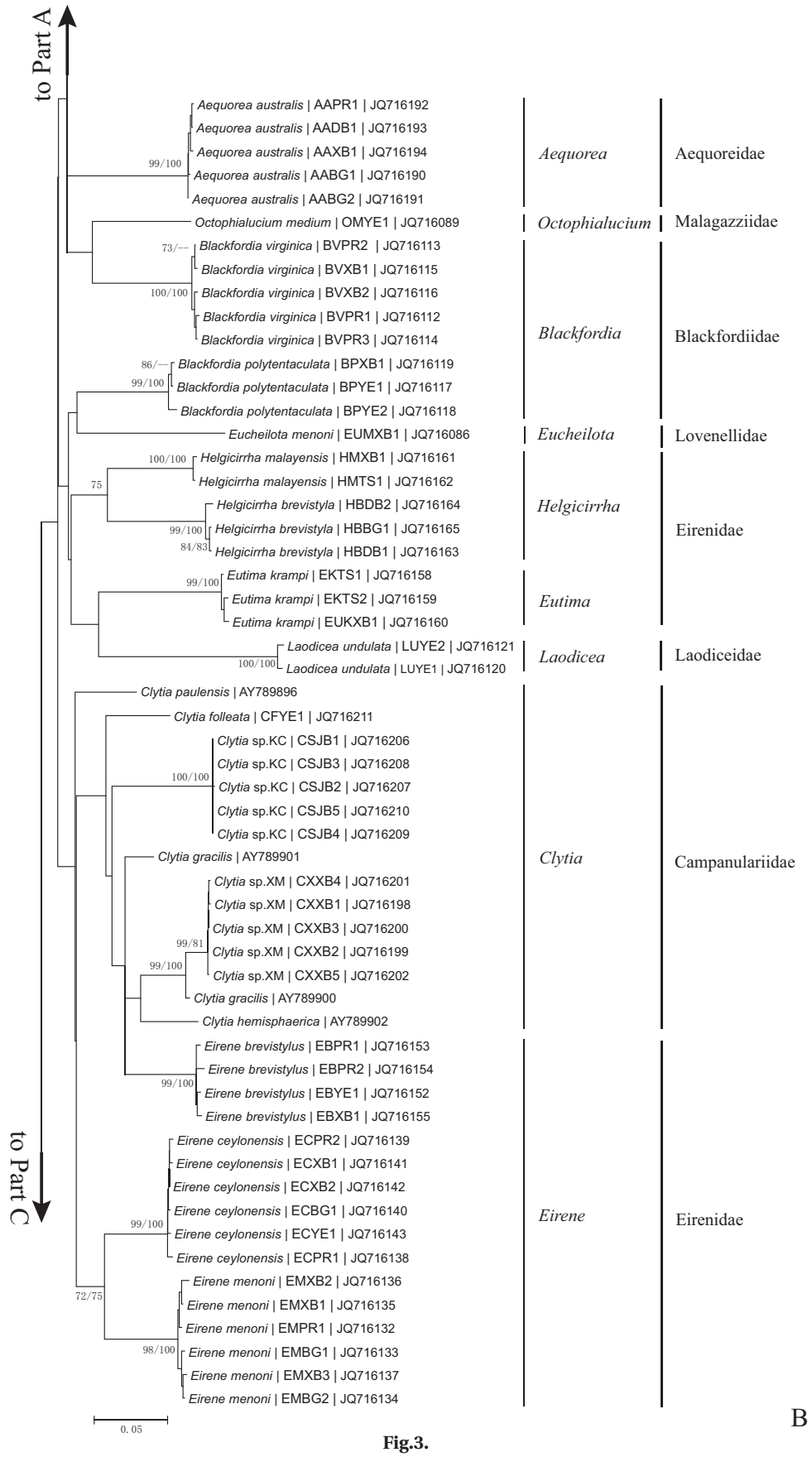
to Part A
to Part C
Aequorea australis | AAPR1 | JQ716192
Aequorea australis | AADB1 | JQ716193
Aequorea australis | AAXB1 | JQ716194
Aequorea australis | AABG1 | JQ716190
Aequorea australis | AABG2 | JQ716191
99/100
Aequorea
Aequoreidae
Octophialucium medium | OMYE1 | JQ716089
Octophialucium
Malagazziidae
Blackfordia virginica | BVPR2 | JQ716113
Blackfordia virginica | BVXB1 | JQ716115
Blackfordia virginica | BVXB2 | JQ716116
Blackfordia virginica | BVPR1 | JQ716112
Blackfordia virginica | BVPR3 | JQ716114
73/--
100/100
Blackfordia
Blackfordiidae
Blackfordia polytentaculata | BPXB1 | JQ716119
Blackfordia polytentaculata | BPYE1 | JQ716117
Blackfordia polytentaculata | BPYE2 | JQ716118
86/--
99/100
Eucheilota menoni | EUMXB1 | JQ716086
Eucheilota
Lovenellidae
Helgicirrha malayensis | HMXB1 | JQ716161
Helgicirrha malayensis | HMTS1 | JQ716162
Helgicirrha brevistyla | HBDB2 | JQ716164
Helgicirrha brevistyla | HBBG1 | JQ716165
Helgicirrha brevistyla | HBDB1 | JQ716163
100/100
75
99/100
84/83
Helgicirrha
Eutima krampi | EKTS1 | JQ716158
Eutima krampi | EKTS2 | JQ716159
Eutima krampi | EUKXB1 | JQ716160
99/100
Eutima
Eirenidae
Laodicea undulata | LUYE2 | JQ716121
Laodicea undulata | LUYE1 | JQ716120
100/100
Laodicea
Laodiceidae
Clytia paulensis | AY789896
Clytia folleata | CFYE1 | JQ716211
Clytia sp.KC | CSJB1 | JQ716206
Clytia sp.KC | CSJB3 | JQ716208
Clytia sp.KC | CSJB2 | JQ716207
Clytia sp.KC | CSJB5 | JQ716210
Clytia sp.KC | CSJB4 | JQ716209
Clytia gracilis | AY789901
Clytia sp.XM | CXXB4 | JQ716201
Clytia sp.XM | CXXB1 | JQ716198
Clytia sp.XM | CXXB3 | JQ716200
Clytia sp.XM | CXXB2 | JQ716199
Clytia sp.XM | CXXB5 | JQ716202
Clytia gracilis | AY789900
Clytia hemisphaerica | AY789902
100/100
99/81
99/100
Clytia
Campanulariidae
Eirene brevistylus | EBPR1 | JQ716153
Eirene brevistylus | EBPR2 | JQ716154
Eirene brevistylus | EBYE1 | JQ716152
Eirene brevistylus | EBXB1 | JQ716155
99/100
Eirene ceylonensis | ECPR2 | JQ716139
Eirene ceylonensis | ECXB1 | JQ716141
Eirene ceylonensis | ECXB2 | JQ716142
Eirene ceylonensis | ECBG1 | JQ716140
Eirene ceylonensis | ECYE1 | JQ716143
Eirene ceylonensis | ECPR1 | JQ716138
99/100
Eirene menoni | EMXB2 | JQ716136
Eirene menoni | EMXB1 | JQ716135
Eirene menoni | EMPR1 | JQ716132
Eirene menoni | EMBG1 | JQ716133
Eirene menoni | EMXB3 | JQ716137
Eirene menoni | EMBG2 | JQ716134
72/75
98/100
Eirene
Eirenidae
0. 05
B

Fig.3.

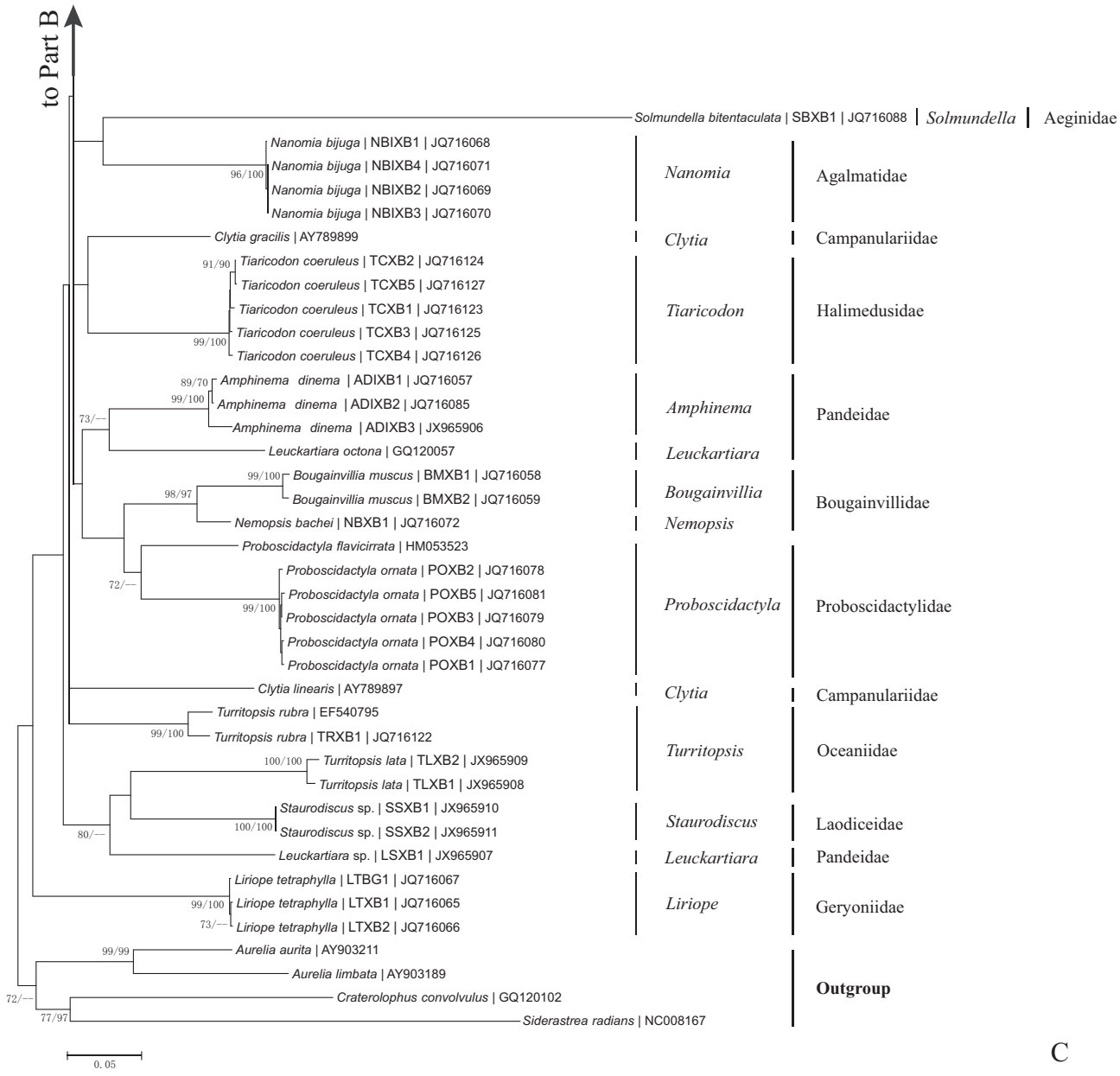

Fig.3. Maximum-Likelihood cluster diagram based on mtDNA cytochrome *c* oxidase subunit I (COI), with the tree split into three sections: A–C. Bootstrap values higher than 60 are shown above the branches. The first number along the branches refers to ML bootstrap values, and the second number refers to Neighbor-Joining bootstrap values. Genera and family lineages are indicated.

rate within the same genus between COI and 16S sequences (Table 2), which indicated that 16S should have similar potential as COI to determine the species boundaries in Hydrozoa. In fact, 16S has been successfully used to reveal cryptic diversity in marine hydroids (Miglietta et al., 2007, 2009; Moura et al., 2008, 2011a, b). In this study, high 16S divergence was found among the individuals of *Liriope tetraphylla* from coastal waters of China, California, and the Caribbean (Panama) (0.087–0.119), which likely indicated there were some crypsis in this cosmopolitan species. According to our study, both COI and 16S supported that *Clytia* sp. XM., which was considered as conspecific to two nearly cosmopolitan species, *C. hemisphaerica* and *C. gracilis*, is a new and valid species in genus *Clytia* (Zhou et al., 2013). These results demonstrated that species previously considered common and widely distributed are in fact species complexes, and these species need careful revision.

The most significant contribution of DNA barcoding to biodiversity conservation efforts is its useful role in improving and speeding up the assessments about phylogenetic diversity (Faith and Williams, 2005). Although COI is recognized as a primary gene choice for DNA barcoding in a wide range of taxa and has been shown to contain some phylogenetic information be-

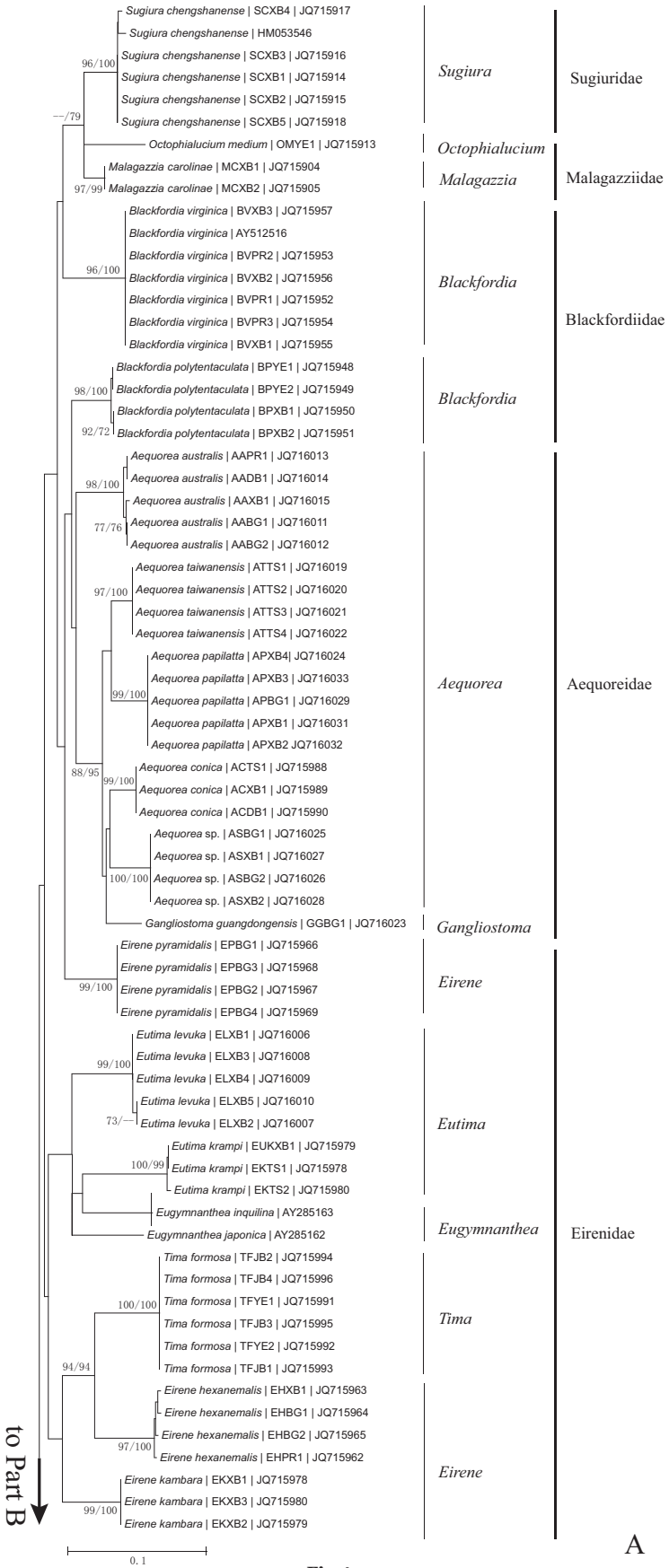
Sugiura chengshanense | SCXB4 | JQ715917
Sugiura chengshanense | HM053546
Sugiura chengshanense | SCXB3 | JQ715916
Sugiura chengshanense | SCXB1 | JQ715914
Sugiura chengshanense | SCXB2 | JQ715915
Sugiura chengshanense | SCXB5 | JQ715918
Octophialucium medium | OMYE1 | JQ715913
Malagazzia carolinae | MCXB1 | JQ715904
Malagazzia carolinae | MCXB2 | JQ715905
Blackfordia virginica | BVXB3 | JQ715957
Blackfordia virginica | AY512516
Blackfordia virginica | BVPR2 | JQ715953
Blackfordia virginica | BVXB2 | JQ715956
Blackfordia virginica | BVPR1 | JQ715952
Blackfordia virginica | BVPR3 | JQ715954
Blackfordia virginica | BVXB1 | JQ715955
Blackfordia polytentaculata | BPYE1 | JQ715948
Blackfordia polytentaculata | BPYE2 | JQ715949
Blackfordia polytentaculata | BPXB1 | JQ715950
Blackfordia polytentaculata | BPXB2 | JQ715951
Aequorea australis | AAPR1 | JQ716013
Aequorea australis | AADB1 | JQ716014
Aequorea australis | AAXB1 | JQ716015
Aequorea australis | AABG1 | JQ716011
Aequorea australis | AABG2 | JQ716012
Aequorea taiwanensis | ATTS1 | JQ716019
Aequorea taiwanensis | ATTS2 | JQ716020
Aequorea taiwanensis | ATTS3 | JQ716021
Aequorea taiwanensis | ATTS4 | JQ716022
Aequorea papilatta | APXB4| JQ716024
Aequorea papilatta | APXB3 | JQ716033
Aequorea papilatta | APBG1 | JQ716029
Aequorea papilatta | APXB1 | JQ716031
Aequorea papilatta | APXB2 JQ716032
Aequorea conica | ACTS1 | JQ715988
Aequorea conica | ACXB1 | JQ715989
Aequorea conica | ACDB1 | JQ715990
Aequorea sp. | ASBG1 | JQ716025
Aequorea sp. | ASXB1 | JQ716027
Aequorea sp. | ASBG2 | JQ716026
Aequorea sp. | ASXB2 | JQ716028
Gangliostoma guangdongensis | GGBG1 | JQ716023
Eirene pyramidalis | EPBG1 | JQ715966
Eirene pyramidalis | EPBG3 | JQ715968
Eirene pyramidalis | EPBG2 | JQ715967
Eirene pyramidalis | EPBG4 | JQ715969
Eutima levuka | ELXB1 | JQ716006
Eutima levuka | ELXB3 | JQ716008
Eutima levuka | ELXB4 | JQ716009
Eutima levuka | ELXB5 | JQ716010
Eutima levuka | ELXB2 | JQ716007
Eutima krampi | EUKXB1 | JQ715979
Eutima krampi | EKTS1 | JQ715978
Eutima krampi | EKTS2 | JQ715980
Eugymnanthea inquilina | AY285163
Eugymnanthea japonica | AY285162
Tima formosa | TFJB2 | JQ715994
Tima formosa | TFJB4 | JQ715996
Tima formosa | TFYE1 | JQ715991
Tima formosa | TFJB3 | JQ715995
Tima formosa | TFYE2 | JQ715992
Tima formosa | TFJB1 | JQ715993
Eirene hexanemalis | EHXB1 | JQ715963
Eirene hexanemalis | EHBG1 | JQ715964
Eirene hexanemalis | EHBG2 | JQ715965
Eirene hexanemalis | EHPR1 | JQ715962
Eirene kambara | EKXB1 | JQ715978
Eirene kambara | EKXB3 | JQ715980
Eirene kambara | EKXB2 | JQ715979
96/100
--/79
97/99
96/100
98/100
92/72
98/100
77/76
97/100
99/100
88/95
99/100
100/100
99/100
99/100
73/--
100/99
100/100
94/94
97/100
99/100
0. 1
to Part B
Sugiura
Octophialucium
Malagazzia
Blackfordia
Blackfordia
Aequorea
Gangliostoma
Eirene
Eutima
Eugymnanthea
Tima
Eirene
Sugiuridae
Malagazziidae
Blackfordiidae
Aequoreidae
Eirenidae
A

Fig.4.

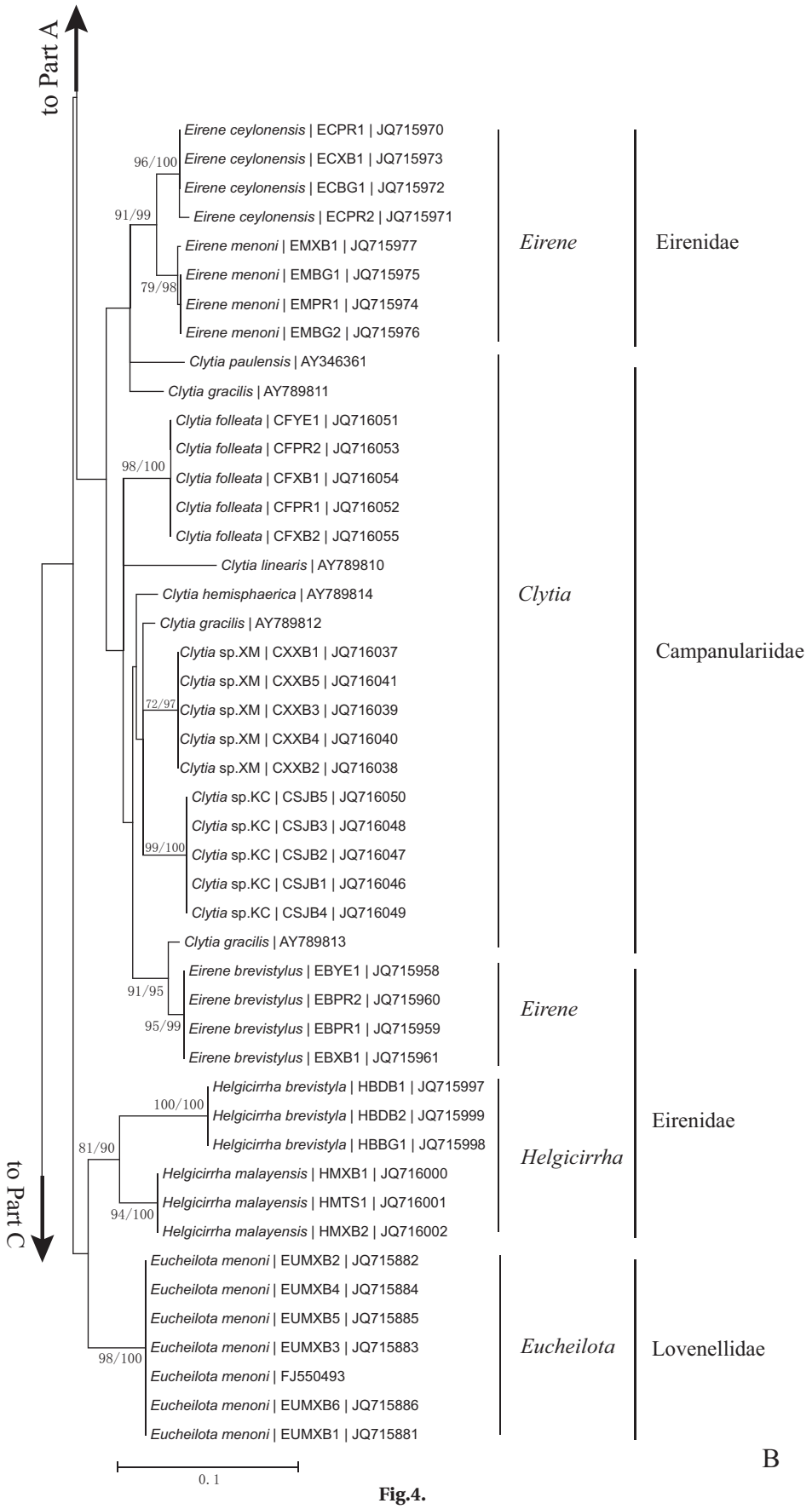
to Part A
to Part C
Eirene ceylonensis | ECPR1 | JQ715970
Eirene ceylonensis | ECXB1 | JQ715973
Eirene ceylonensis | ECBG1 | JQ715972
Eirene ceylonensis | ECPR2 | JQ715971
Eirene menoni | EMXB1 | JQ715977
Eirene menoni | EMBG1 | JQ715975
Eirene menoni | EMPR1 | JQ715974
Eirene menoni | EMBG2 | JQ715976
Clytia paulensis | AY346361
Clytia gracilis | AY789811
Clytia folleata | CFYE1 | JQ716051
Clytia folleata | CFPR2 | JQ716053
Clytia folleata | CFXB1 | JQ716054
Clytia folleata | CFPR1 | JQ716052
Clytia folleata | CFXB2 | JQ716055
Clytia linearis | AY789810
Clytia hemisphaerica | AY789814
Clytia gracilis | AY789812
Clytia sp.XM | CXXB1 | JQ716037
Clytia sp.XM | CXXB5 | JQ716041
Clytia sp.XM | CXXB3 | JQ716039
Clytia sp.XM | CXXB4 | JQ716040
Clytia sp.XM | CXXB2 | JQ716038
Clytia sp.KC | CSJB5 | JQ716050
Clytia sp.KC | CSJB3 | JQ716048
Clytia sp.KC | CSJB2 | JQ716047
Clytia sp.KC | CSJB1 | JQ716046
Clytia sp.KC | CSJB4 | JQ716049
Clytia gracilis | AY789813
Eirene brevistylus | EBYE1 | JQ715958
Eirene brevistylus | EBPR2 | JQ715960
Eirene brevistylus | EBPR1 | JQ715959
Eirene brevistylus | EBXB1 | JQ715961
Helgicirrha brevistyla | HBDB1 | JQ715997
Helgicirrha brevistyla | HBDB2 | JQ715999
Helgicirrha brevistyla | HBBG1 | JQ715998
Helgicirrha malayensis | HMXB1 | JQ716000
Helgicirrha malayensis | HMTS1 | JQ716001
Helgicirrha malayensis | HMXB2 | JQ716002
Eucheilota menoni | EUMXB2 | JQ715882
Eucheilota menoni | EUMXB4 | JQ715884
Eucheilota menoni | EUMXB5 | JQ715885
Eucheilota menoni | EUMXB3 | JQ715883
Eucheilota menoni | FJ550493
Eucheilota menoni | EUMXB6 | JQ715886
Eucheilota menoni | EUMXB1 | JQ715881
96/100
91/99
79/98
98/100
72/97
99/100
91/95
95/99
100/100
81/90
94/100
98/100
Eirene
Clytia
Eirene
Helgicirrha
Eucheilota
Eirenidae
Campanulariidae
Eirenidae
Lovenellidae
0. 1
B

Fig.4.

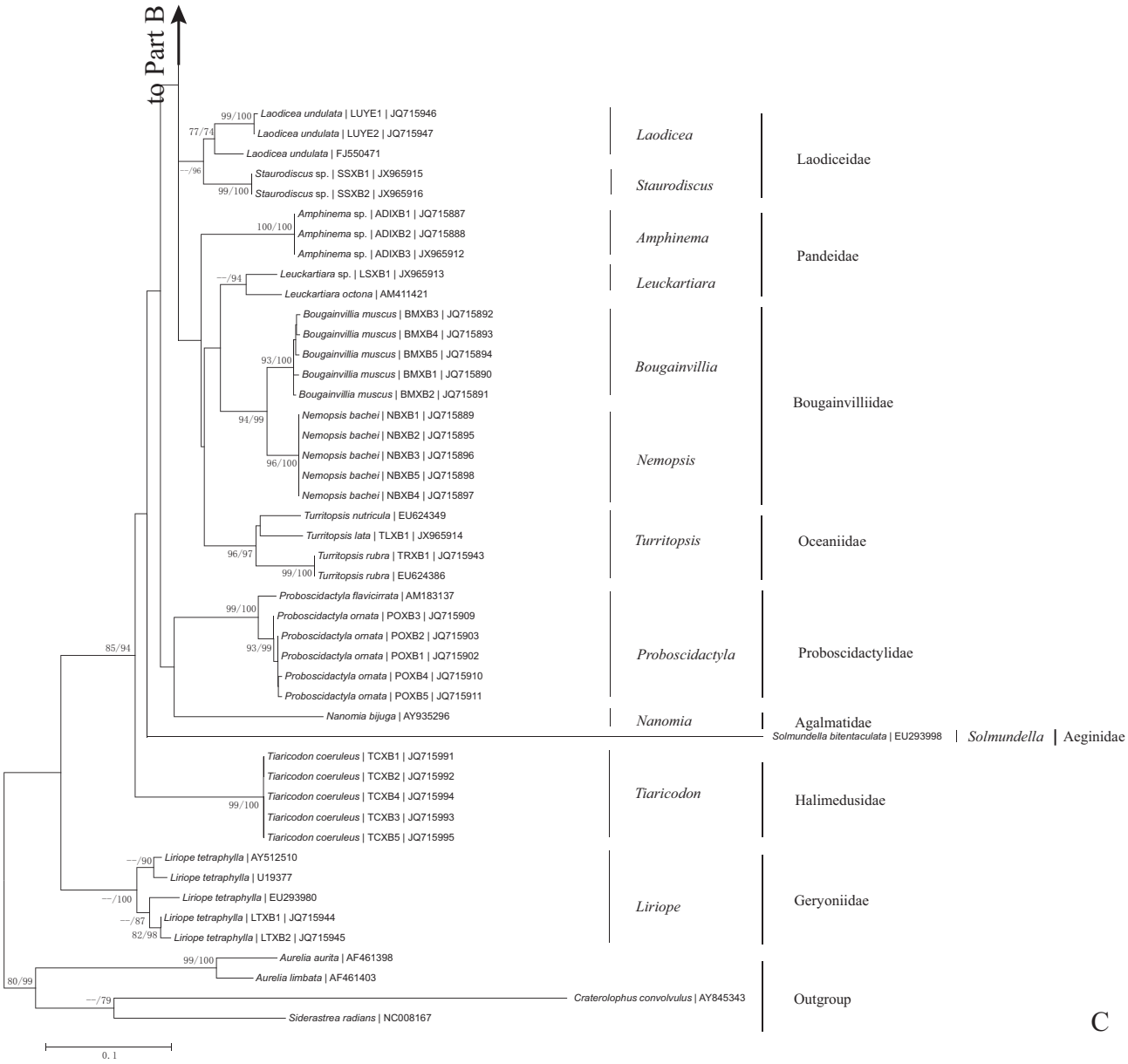

Fig.4. Maximum-Likelihood cluster diagram based on mtDNA 16S, with the tree split into three sections: A–C. Bootstrap values higher than 60 are shown above the branches. The first number along the branches refers to ML bootstrap values, and the second number refers to Neighbor-Joining bootstrap values. Genera and family lineages are indicated.

tween closely related taxa (Bucklin et al., 2010; Xia et al., 2012), gene saturation results in low phylogenetic signal at deeper levels (Hajibabaei et al., 2007; Ortman et al., 2010). In our analysis, the ML tree based on COI did not clearly diagnose most genera that included two or more species. For example, the species of *Aequorea*, *Clytia*, *Eugymnanthea*, *Eutima*, *Leuckartiara*, and *Turritopsis* did not constitute monophyletic groups; the genera *Helgicirrha* and *Proboscidactyla* appeared monophyletic without high bootstrap support (Fig. 3); and almost all families included in this analysis did not constitute monophyletic groups except for Bougainvillidae. A similar result was also found in the DNA barcoding studies of Medusozoan (Ortman et al., 2010), which demonstrated that COI sequence data lack sufficient phylogenetic signal to reconstruct genera-level and higher relationships of medusozoans.

In contrast to the COI tree, the 16S tree provided interesting insights. The species of the following four genera formed well-supported monophyletic clades: *Helgicirrha*, *Leuckartiara*, *Proboscidactyla*, and *Turritopsis*. The genera *Aequorea*, *Clytia*, *Eugymnanthea*, and *Eutima* appeared monophyletic, though without high bootstrap support in the 16S tree (Fig. 4). The genus *Eirene* was not monophyletic according to our data from the analysis (Fig. 4), which was consistent with the results of the phylogenetic analysis about the genus *Eirene* using two nuclear

(18S and ITS1) and two partial mitochondrial gene (COI and 16S) sequences. The mean sequence divergence between the species of genus *Eirene* reached 0.169, which suggested a rapid evolution of the 16S gene within this genus. At the family level, the 16S tree also showed some positive results corresponding to the current taxonomic arrangements. On one hand, Bougainvillidae and Laodiceidae obtained firm support from 16S gene data, forming strongly supported monophyletic clades. On the other hand, the genera within Malagazziidae and Aequoreidae clustered together with low bootstrap values. Furthermore, our results agreed with the statements by Kubota (1983, 2000), who indicated that the genera *Eutima* and *Eugymnanthea* have a close relationship and could be merged into a single genus (Fig. 4). These results suggested that 16S should be a useful phylogenetic marker for the hydrozoans at the genus level, and in some cases at the family level, for which it has been shown that COI did not work within these studied taxa. The same results were obtained in the study of endemic Antarctic benthic hydroids by Cantero et al. (2010).

Recently, Ortman et al. (2010) and Bucklin et al. (2011) extrapolated that COI was "broadly useful" for DNA barcoding of species across the Medusozoa, but was not phylogenetically informative for higher taxonomic ranks. As an alternative "DNA barcode", the existence of the barcoding gap and the monophyletic character strongly supported 16S as an effective and efficient DNA barcode for the hydrozoans in the present analysis. Secondly, 16S was shown to be more successful than COI in extrapolating phylogenetic relations of hydrozoans at the genus level, and in some cases at the family level. Finally, as a result of the resolution and effectiveness for barcoding and phylogenetic analyses of Hydrozoa (see the review in our introduction, and our results), 1 566 16S sequences representing 596 hydrozoan species were deposited in GenBank at the present time, compared to only 621 COI sequences for 169 species. For these reasons, and because 16S is easily amplified and sequenced across hydrozoan taxa (Moura et al., 2011b), we strongly recommend 16S as the standard barcode for hydrozoans.

5 Conclusions

DNA barcoding offers great help to understand the extent of biodiversity by providing a simple and quick way to identify species (Hebert et al., 2003; Hebert and Gregory, 2005). A challenge remains, however, in its accuracy and efficiency (Krishnamurthy and Francis, 2012). Another point of contention is that the regions of DNA used for barcoding often present limited information for higher phylogenetic resolution (Moritz and Cicero, 2004). For hydrozoans, the choice between COI and 16S as a standard barcode is subject to debate. The results from our study established two facts indicating the barcode potential of COI and 16S for hydrozoans. First, both COI and 16S could identify all species efficiently and accurately using the distance-based (K2P distance) approach. Second, 16S was shown to be a better phylogenetic marker for hydrozoans at the genus level, and in some cases at the family level, and we believed that increased taxa sampling will increase the understanding of the phylogenetic relationships of the members of hydrozoans in the coastal waters of China based on 16S data. Considering these reasons and the fact that 16S is easily amplified and sequenced across hydrozoan taxa (Moura et al., 2011b), we strongly recommend 16S to be the standard barcode for hydrozoans.

Acknowledgements

Specimen identification would not have been possible without the support of Professors Huang Jiaqi and Xu Zhenzu of Xiamen University. This work also received support from the "908 special" marine biological sample libraries. Additionally, thanks to the Open and Sharing Platform of Equipment and Technology (OSPET) of COE of Xiamen University for providing equipment and technical support.

References

Bouillon J, Boero F. 2000. The Hydrozoa: A new classification in the light of old knowledge. Thalassia Salentina, 24: 1–45

Bridge D, Cunningham C W, DeSalle R, et al. 1995. Class-level relationships in the Phylum Cnidaria: molecular and morphological evidence. Molecular Biology and Evolution, 12: 679–689

Bucklin A, Ortman B D, Jennings R M, et al. 2010. A "Rosetta Stone" for zooplankton: DNA barcode analysis of holozooplankton diversity of the Sargasso Sea (NW Atlantic Ocean). Deep-Sea Research II, 57: 2234–2247

Bucklin A, Steinke D, Blanco-Bercial L. 2011. DNA barcoding of marine metazoa. Annual Review of Marine Science, 3: 471–508

Cantero A L P, Sentandreu V, Latorre A. 2010. Phylogenetic relationships of the endemic Antarctic benthic hydroids (Cnidaria, Hydrozoa): what does the mitochondrial 16S rRNA tell us about it? Polar Biology, 33: 41–57

Cartwright P, Evans N M, Dunn C W, et al. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). Journal of the Marine Biological Association of the United Kingdom, 88: 1663–1672

Collins A G. 2000. Towards understanding the phylogenetic history of Hydrozoa: hypothesis testing with 18S gene sequence data. Scientia Marina, 64: 5–22

Collins A G. 2002. Phylogeny of Medusozoa and the evolution of cnidarian life cycles. Journal of Evolutionary Biology, 15: 418–432

Collins A G, Bentlage B, Lindner A, et al. 2008. Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematic taxa. Journal of the Marine Biological Association of the United Kingdom, 88: 1673–1685

Collins A G, Schuchert P, Marques A C, et al. 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. Systematic Biology, 55: 97–115

Collins A G, Winkelmann S, Hadrys H, et al. 2005. Phylogeny of Capitata and Corynidae (Cnidaria, Hydrozoa) in light of mitochondrial 16S rDNA data. Zoologica Scripta, 34: 91–99

del-Prado R, Cubas P, Lumbsch H T, et al. 2010. Genetic distances within and among species in monophyletic lineages of Parmeliceae (Ascomycota) as a tool for taxon delimitation. Molecular Phylogenetics and Evolution, 56: 125–133

Dawson M N. 2003. Macro-morphological variation mong cryptic specie of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). Marine Biology, 143: 369–379

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

Dawson M N, Jacobs D K. 2001. Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). Biological Bulletin, 200: 92–96

Dawson M N, Martin L E. 2001. Geographic variation and ecological adaptation in *Aurelia* (Scyphozoa, Semaeostomeae): some implications from molecular phylogenetics. Hydrobiologia, 451: 259–273

Dunn C W, Pugh P R, Haddock S H D. 2005. Molecular phylogenetics of the siphonophora (Cnidaria), with implications for the evolution of functional specialization. Systematic Biology, 54: 916–935

Ender A, Schierwater B. 2003. Placozoa are not derived cnidarians: evidence from molecular morphology. Molecuar Biology Evolution,

20: 130–134
Faith D P, Williams K J. 2005. How large-scale DNA barcoding programs can boost biodiversity conservation planning: linking phylogenetic diversity (PD) analyses to the barcode of life database (BoLD). Abstract. In: Australian entomological society's 36th AGM and scientific conference/7th invertebrate biodiversity and conservation conference/Australian systematics society, Canberra, Australia, 4–9 December 2005
Folino-Rorem N C, Darling J A, D'Ausilio C A. 2008. Genetic analysis reveals multiple cryptic invasive species of the hydrozoan genus Cordylophora. Biological Invasions, 11: 1869–1882
Folmer O, Black M, Hoeh W, et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294–299
Govindarajan A F, Boero F, Halanych K M. 2006. Phylogenetic analysis with multiple markers indicates repeated loss of the adult medusa stage in Campanulariidae (Hydrozoa, Cnidaria). Molecular Phylogenetics and Evolution, 38: 820–834
Govindarajan A F, Halanych K M, Cunningham C W. 2005a. Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). Marine Biology, 146: 213–222
Govindarajan A F, Piraino S, Gravili C, et al. 2005b. Species identification of bivalve-inhabiting marine hydrozoans of the genus *Eugymnanthea*. Invertebrate Biology, 124: 1–10
Hajibabaei M, Janzen D H, Burns J M, et al. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America, 103: 968–971
Hajibabaei M, Singer G A C, Hebert P D N, et al. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics, 23: 167–172
Hebert P D N, Gregory T R. 2005. The promise of DNA barcoding for taxonomy. Systematic Biology, 54: 852–859
Hebert P D N, Penton E H, Burns J M, et al. 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Science of the United States of America, 101: 14812–14817
Hebert P D N, Ratnasingham S, deWaard J R. 2003. Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. Proceedings of the Royal Society B-Biological Sciences, 270: S96–S99
Hebert P D N, Stoeckle M Y, Zemlak T S, et al. 2004b. Identification of birds through DNA barcodes. PloS Biology, 2: 1657–1663
Hellberg M E. 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evolutionary Biology, 6: 1–8
Holland B S, Dawson M N, Crow G L, et al. 2004. Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. Marine Biology, 145: 1119–1128
Huang D, Meier R, Todd P A, et al. 2008. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. Journal of Molecular Evolution, 66: 167–174
Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111–120
Köhler F. 2007. From DNA taxonomy to barcoding: how a vague idea evolved into a biosystematic tool. Mitteilungen aus dem Museum für Naturkunde Berlin Zoologische Reihe, 83: 44–51
Krishnamurthy P K, Francis R A. 2012. A critical review on the utility of DNA barcoding in biodiversity conservation. Biodiversity and Conservation, 21: 1901–1919
Kubota S. 1983. Studies on life history and systematics of the Japanese commensal hydroids living in bivalves, with some reference to their evolution. Journal of the Faculty of Science Hokkaido University Series VI Zoology, 23: 296–402
Kubota S. 2000. Parallel, paedomorphic evolutionary processes of the bivalve-inhabiting hydrozoans (Leptomedusae, Eirenidae) deduced from morphology, life cycle and biogeography, with special reference to taxonomic treatment of *Eugymnanthea*. Scientia Marina, 64: 241–247
Larkin M A, Blackshields G, Brown N P, et al. 2007. Clustal W and clustal X version 2.0. Bioinformatics, 23: 2947–2948
Leclère L, Schuchert P, Cruaud C, et al. 2009. Molecular phylogenetics of Thecata (Hydrozoa, Cnidaria) reveals long-term maintenance of life history traits despite high frequency of recent character changes. Systematic Biology, 58: 509–526
Leclère L, Schuchert P, Manuel M. 2007. Phylogeny of the Plumularioidea (Hydrozoa, Leptothecata): evolution of colonial organization and life cycle. Zoologica Scripta, 36: 371–394
Le Goff-Vitry M C, Rogers A D, Baglow D. 2004. A deep sea slant on the molecular phylogeny of the *Scleractinia*. Molecular Phylogenetics and Evolution, 30: 167–177
Martinez D E, Iniguez A R, Percell K M, et al. 2010. Phylogeny and biogeography of *Hydra* (Cnidaria: Hydridae) using mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution, 57: 403–410
McFadden C S, Benayahu Y, Pante E, et al. 2011. Limitations of mitochondrial gene barcoding in Octocorallia. Molecular Ecology Resources, 11: 19–31
McFadden C S, Tullis I, Hutchinson M B, et al. 2000. Rates of evolution of cnidarian mitochondrial genes. American Zoologist, 40: 1124–1124
Meyer C P, Paulay G. 2005. DNA barcoding: error rates based on comprehensive sampling. PLoS Biolog, 3: 2229–2238
Miglietta M P, Piraino S, Kubota S, et al. 2007. Species in the genus *Turritopsis* (Cnidaria, Hydrozoa): a molecular evaluation. Journal of Zoological Systematics and Evolutionary Research, 45: 11–19
Miglietta M P, Schuchert P, Cunningham C W. 2009. Reconciling genealogical and morphological species in a worldwide study of the Family Hydractiniidae (Cnidaria, Hydrozoa). Zoologica Scripta, 38: 403–430
Mills C E. 1995. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystems. ICES Journal of Marine Science, 52: 575–581
Miranda L S, Collins A G, Marques A C. 2010. Molecules clarify a Cnidarian life cycle — the "Hydrozoan" *Microhydrula limopsicola* is an early life stage of the Staurozoan *Haliclystus antarcticus*. PLoS One, 5: e10182
Moritz C, Cicero C. 2004. DNA barcoding: promise and pitfalls. Plos Biology, 2: 1529–1531
Moura C J, Cunha M R, Porteiro F M, et al. 2011a. Polyphyly and cryptic diversity in the hydrozoan families Lafoeidae and Hebellidae (Cnidaria:Hydrozoa). Invertebrate Systematics, 25: 454–470
Moura C J, Cunha M R, Porteiro F M, et al. 2011b. The use of the DNA barcode gene 16S mRNA for the clarification of taxonomic problems within the family Sertulariidae (Cnidaria, Hydrozoa). Zoologica Scripta, 40: 520–537
Moura C J, Cunha M R, Porteiro F M, et al. 2012. A molecular phylogenetic appraisal of the systematics of the Aglaopheniidae (Cnidaria: Hydrozoa, Leptothecata) from the north-east Atlantic and west Mediterranean. Zoological Journal of the Linnean Society, 164: 717–727
Moura C J, Harris D J, Cunha M R, et al. 2008. DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. Zoologica Scripta, 37: 93–108
Nawrocki A M, Schuchert P, Cartwright P. 2010. Phylogenetics and evolution of Capitata (Cnidaria: Hydrozoa), and the status of the family Corynidae. Zoological Scripta, 39: 290–304
Ortman B D, Bucklin A, Pagès F, et al. 2010. DNA barcoding the Medusozoa using mtCOI. Deep-Sea Research II: Topical Studies in Oceanography, 57: 2148–2156
Pontin D R, Cruickshank R H. 2012. Molecular phylogenetics of the genus *Physalia* (Cnidaria: Siphonophora) in New Zealand coastal waters reveals cryptic diversity. Hydrobiologia, 686: 91–105

Ratnasingham S, Hebert P D N. 2007. BOLD: the barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Notes, 7: 355–364

Schuchert P. 2005a. Species boundaries in the hydrozoan genus *Coryne*. Molecular Phylogenetics and Evolution, 36: 194–199

Schuchert P. 2005b. Rediscovery of *Coryne fucicola* (de Filippi, 1866) (Cnidaria: Hydrozoa). Cahiers de Biologie Marine, 46: 305–310

Schuchert P. 2006. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Capitata Part 1. Revue Suisse de Zoologie, 113: 325–410

Schuchert P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. Revue Suisse de Zoologie, 114: 195–396

Schuchert P. 2008a. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 3. Revue Suisse de Zoologie, 115: 221–302

Schuchert P. 2008b. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 4. Revue Suisse de Zoologie, 115: 677–757

Schuchert P. 2009. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 5. Revue Suisse de Zoologie, 116: 441–507

Schuchert P, Reiswig H M. 2006. *Brinckmannia hexactinellidophila*, n.genera, n. sp. a hydroid living in tissues of glass sponges of the reefs, fjords, and seamounts of Pacific Canada and Alaska. Canadian Journal of Zoology, 84: 564–572

Shearer T L, van Oppen M J H, Romano S L, et al. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Molecular Ecology, 11: 2475–2487

Sinniger F, Reimer J D, Pawlowski J. 2008. Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia). Zoological Science, 25: 1253–1260

Stampar S N, Maronna M M, Vermeij M J A, et al. 2012. Evolutionary diversification of banded tube-dwelling anemones (Cnidaria; Ceriantharia; Isarachnanthus) in the Atlantic Ocean. PLoS One, 7: e41091

Sun Yan, Li Qi, Kong Lingfeng, et al. 2012. DNA barcoding of Caenogastropoda along coast of China based on the COI gene. Molecular Ecology Resources, 12: 209–218

Tamura K, Peterson D, Peterson N, et al. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28: 2731–2739

Ward R D, Zemlak T S, Innes B H, et al. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B—Biological Sciences, 360: 1847–1857

Xia Yun, Gu Haifeng, Peng Rui, et al. 2012. COI is better than 16S rRNA for DNA barcoding Asiatic salamanders (Amphibia: Caudata: Hynobiidae). Molecular Ecology Resources, 12: 48–56

Zemlak T S, Ward R D, Connell A D, et al. 2009. DNA barcoding reveals overlooked marine fishes. Molecular Ecology Resources, 9: 237–242

Zheng Lianming, Lin Yuanshao, Li Shaojing, et al. 2009. *Aequorea taiwanensis* n. sp (Hydrozoa, Leptomedusae) and mtCOI sequence analysis for the genus *Aequorea*. Acta Oceanologica Sinica, 28: 109–115

Zhou Konglin, Zheng Lianming, He Jinru, et al. 2013. Detection of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) with DNA barcoding and life cycle analyses. Journal of the Marine Biological Association of the United Kingdom, 93: 2075–2088

Table S1. Species information of Hydrozoan in this study. DNA vouchers deposited at College of Ocean and Earth Sciences, Xiamen University, China

Species	Sample No.	Locality	Position	Date	Collector	Accession No.	
						COI	16S
Aequorea australis	AABG1	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716190	JQ716011
Aequorea australis	AABG2	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716191	JQ716012
Aequorea australis	AAPR1	Zhujiang River Estuary	22.510 3°N, 113.689 4°E	Apr. 2006	Zheng Lianming	JQ716192	JQ716013
Aequorea australis	AADB1	Dongshan Bay	23.818 3°N, 117.562 6°E	Jul. 2010	Zheng Lianming	JQ716193	JQ716014
Aequorea australis	AAXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716194	JQ716015
Aequorea australis	AAXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716195	JQ716016
Aequorea australis	AAXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716196	JQ716017
Aequorea australis	AAXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	He Jinru	JQ716197	JQ716018
Aequorea conica	ACTS1	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716175	JQ715988
Aequorea conica	ACXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	JQ716176	JQ715989
Aequorea conica	ACDB1	Dongshan Bay	23.818 3°N, 117.562 6°E	Jul. 2010	Zheng Lianming	JQ716177	JQ715990
Aequorea papilatta	APXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ716024
Aequorea papilatta	APBG1	Beibu Gulf	20.852 4°N, 109.257 6°E	Nov. 2006	Zheng Lianming	JQ716056	JQ716029
Aequorea papilatta	APTS1	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716181	JQ716030
Aequorea papilatta	APXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	JQ716182	JQ716031
Aequorea papilatta	APXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	JQ716183	JQ716032
Aequorea papilatta	APXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	JQ716184	JQ716033
Aequorea papilatta	APBG2	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716185	JQ716034
Aequorea papilatta	APBG3	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716186	JQ716035
Aequorea papilatta	APBG4	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	/	JQ716036
Aequorea sp.	ASBG1	Beibu Gulf	20.852 4°N, 109.257 6°E	Nov. 2006	Zheng Lianming	JQ716187	JQ716025
Aequorea sp.	ASBG2	Beibu Gulf	20.852 4°N, 109.257 6°E	Nov. 2006	Zheng Lianming	JQ716188	JQ716026
Aequorea sp.	ASXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	/	JQ716027
Aequorea sp.	ASXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	/	JQ716028
Aequorea taiwanensis	ATTS1	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716178	JQ716019
Aequorea taiwanensis	ATTS2	Taiwan Strait	21.666 7°–23.8500°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716179	JQ716020
Aequorea taiwanensis	ATTS3	Taiwan Strait	21.666 7°–23.8500°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716180	JQ716021
Aequorea taiwanensis	ATTS4	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	/	JQ716022
Amphinema dinema	ADIXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716057	JQ715887
Amphinema dinema	ADIXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716085	JQ715888
Amphinema dinema	ADIXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Aug. 2012	He Jinru	JX965906	JX965912
Blackfordia polytentaculata	BPYE1	Changjiang River Estuary	31.532 4°N, 122.153 7°E	May 2005	Zheng Lianming	JQ716117	JQ715948
Blackfordia polytentaculata	BPYE2	Changjiang River Estuary	31.532 4°N, 122.153 7°E	May 2005	Zheng Lianming	JQ716118	JQ715949

to be continued

Continued from Table S1

Species	Sample No.	Locality	Position	Date	Collector	Accession No.	
						COI	16S
Blackfordia polytentaculata	BPXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	JQ716119	JQ715950
Blackfordia polytentaculata	BPXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	/	JQ715951
Blackfordia virginica	BVPR1	Zhujiang River Estuary	22.510 3°N, 113.689 4°E	Apr. 2006	Zheng Lianming	JQ716112	JQ715952
Blackfordia virginica	BVPR2	Zhujiang River Estuary	22.510 3°N, 113.689 4°E	Apr. 2006	Zheng Lianming	JQ716113	JQ715953
Blackfordia virginica	BVPR3	Zhujiang River Estuary	22.510 3°N, 113.689 4°E	Apr. 2006	Zheng Lianming	JQ716114	JQ715954
Blackfordia virginica	BVXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	JQ716115	JQ715955
Blackfordia virginica	BVXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	JQ716116	JQ715956
Blackfordia virginica	BVXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	/	JQ715957
Bougainvillia muscus	BMXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716058	JQ715890
Bougainvillia muscus	BMXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716059	JQ715891
Bougainvillia muscus	BMXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715892
Bougainvillia muscus	BMXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715893
Bougainvillia muscus	BMXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715894
Bougainvillia vervoorti	BSXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716060	/
Clytia folleata	CFYE1	Changjiang River Estuary	31.532 4°N, 122.153 7°E	May 2005	Zheng Lianming	JQ716211	JQ716051
Clytia folleata	CFPR1	Zhujiang River Estuary	22.510 3°N, 113.689 4°E	Apr. 2006	Zheng Lianming	/	JQ716052
Clytia folleata	CFPR2	Zhujiang River Estuary	22.510 3°N, 113.689 4°E	Apr. 2006	Zheng Lianming	/	JQ716053
Clytia folleata	CFXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	/	JQ716054
Clytia folleata	CFXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	/	JQ716055
Clytia sp.*KC*	CSJB1	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716206	JQ716046
Clytia sp.*KC*	CSJB2	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716207	JQ716047
Clytia sp.*KC*	CSJB3	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716208	JQ716048
Clytia sp.*KC*	CSJB4	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716209	JQ716049
Clytia sp.*KC*	CSJB5	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716210	JQ716050
Clytia sp.*XM*	CXXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716198	JQ716037
Clytia sp.*XM*	CXXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716199	JQ716038
Clytia sp.*XM*	CXXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716200	JQ716039
Clytia sp.*XM*	CXXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716201	JQ716040
Clytia sp.*XM*	CXXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716202	JQ716041
Clytia sp.*XM*	CXXB6	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716203	JQ716042
Clytia sp.*XM*	CXXB7	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716204	JQ716043
Clytia sp.*XM*	CXXB8	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	JQ716205	JQ716044
Clytia sp.*XM*	CXXB9	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2011	He Jinru	/	JQ716045
Corymorpha verrucosa	CVXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716061	/
Corymorpha verrucosa	CVXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716062	/

to be continued

Continued from Table S1

Species	Sample No.	Locality	Position	Date	Collector	Accession No.	
						COI	16S
Corymorpha verrucosa	CVXB3	Xiamen Bay	24.3871°N, 118.1430°E	Oct. 2011	He Jinru	JQ716063	/
Corymorpha verrucosa	CVXB4	Xiamen Bay	24.3871°N, 118.1430°E	Oct. 2011	He Jinru	JQ716064	/
Diphyes chamissonis	DCXB1	Xiamen Bay	24.3871°N, 118.1430°E	Jul. 2010	He Jinru	/	JQ715939
Diphyes chamissonis	DCXB2	Xiamen Bay	24.3871°N, 118.1430°E	Jul. 2010	He Jinru	/	JQ715940
Diphyes chamissonis	DCXB3	Xiamen Bay	24.3871°N, 118.1430°E	Aug. 2010	He Jinru	/	JQ715941
Diphyes chamissonis	DCXB4	Xiamen Bay	24.3871°N, 118.1430°E	Aug. 2010	He Jinru	/	JQ715942
Eirene brevistylus	EBYE1	Changjiang River Estuary	31.5324°N, 122.1537°E	May 2005	Zheng Lianming	JQ716152	JQ715958
Eirene brevistylus	EBPR1	Zhujiang River Estuary	22.5103°N, 113.6894°E	Oct. 2006	Zheng Lianming	JQ716153	JQ715959
Eirene brevistylus	EBPR2	Zhujiang River Estuary	22.5103°N, 113.6894°E	Oct. 2006	Zheng Lianming	JQ716154	JQ715960
Eirene brevistylus	EBXB1	Xiamen Bay	24.3871°N, 118.1430°E	Jul. 2011	He Jinru	JQ716155	JQ715961
Eirene ceylonensis	ECXB2	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2008	Zheng Lianming	JQ716142	/
Eirene ceylonensis	ECYE1	Changjiang River Estuary	31.5324°N, 122.1537°E	May 2005	Zheng Lianming	JQ716143	/
Eirene ceylonensis	ECPR1	Zhujiang River Estuary	22.5103°N, 113.6894°E	Oct. 2006	Zheng Lianming	JQ716138	JQ715970
Eirene ceylonensis	ECPR2	Zhujiang River Estuary	22.5103°N, 113.6894°E	Oct. 2006	Zheng Lianming	JQ716139	JQ715971
Eirene ceylonensis	ECBG1	Beibu Gulf	20.8524°N, 109.2576°E	Nov. 2006	Zheng Lianming	JQ716140	JQ715972
Eirene ceylonensis	ECXB1	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2008	Zheng Lianming	JQ716141	JQ715973
Eirene hexanemalis	EHBG3	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	JQ716151	/
Eirene hexanemalis	EHPR1	Zhujiang River Estuary	22.5103°N, 113.6894°E	Oct. 2006	Zheng Lianming	JQ716147	JQ715962
Eirene hexanemalis	EHXB1	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2008	Zheng Lianming	JQ716148	JQ715963
Eirene hexanemalis	EHBG1	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	JQ716149	JQ715964
Eirene hexanemalis	EHBG2	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	JQ716150	JQ715965
Eirene kambara	EKXB4	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2007	Zheng Lianming	JQ716131	/
Eirene kambara	EKXB1	Xiamen Bay	24.3871°N, 118.1430°E	Mar. 2007	Zheng Lianming	JQ716128	JQ715978
Eirene kambara	EKXB2	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2007	Zheng Lianming	JQ716129	JQ715979
Eirene kambara	EKXB3	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2007	Zheng Lianming	JQ716130	JQ715980
Eirene menoni	EMXB2	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2008	Zheng Lianming	JQ716136	/
Eirene menoni	EMXB3	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2008	Zheng Lianming	JQ716137	/
Eirene menoni	EMPR1	Zhujiang River Estuary	22.5103°N, 113.6894°E	Oct. 2006	Zheng Lianming	JQ716132	JQ715974
Eirene menoni	EMBG1	Beibu Gulf	20.8524°N, 109.2576°E	Nov. 2006	Zheng Lianming	JQ716133	JQ715975
Eirene menoni	EMBG2	Beibu Gulf	20.8524°N, 109.2576°E	Nov. 2006	Zheng Lianming	JQ716134	JQ715976
Eirene menoni	EMXB1	Xiamen Bay	24.3871°N, 118.1430°E	Apr. 2008	Zheng Lianming	JQ716135	JQ715977
Eirene pyramidalis	EPBG1	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	JQ716144	JQ715966
Eirene pyramidalis	EPBG2	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	JQ716145	JQ715967
Eirene pyramidalis	EPBG3	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	JQ716146	JQ715968
Eirene pyramidalis	EPBG4	Beibu Gulf	20.9549°N, 108.7576°E	Dec. 2007	Zheng Lianming	/	JQ715969
Eucheilota menoni	EUMXB1	Xiamen Bay	24.3871°N, 118.1430°E	Jul. 2011	He Jinru	JQ716086	JQ715881

to be continued

Continued from Table S1

Species	Sample No.	Locality	Position	Date	Collector	Accession No.	
						COI	16S
Eucheilota menoni	EUMXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715882
Eucheilota menoni	EUMXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715883
Eucheilota menoni	EUMXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715884
Eucheilota menoni	EUMXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715885
Eucheilota menoni	EUMXB6	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715886
Eugymnanthea japonica	EJXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2008	Zheng Lianming	JQ716171	/
Eugymnanthea japonica	EJBG1	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716172	/
Eugymnanthea japonica	EJBG2	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716173	/
Eugymnanthea japonica	EJBG3	Beibu Gulf	20.954 9°N, 108.757 6°E	Dec. 2007	Zheng Lianming	JQ716174	/
Eutima krampi	EKTS1	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716158	JQ716003
Eutima krampi	EKTS2	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716159	JQ716004
Eutima krampi	EUKXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716160	JQ716005
Eutima levuka	ELXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2007	Zheng Lianming	JQ716156	JQ716006
Eutima levuka	ELXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	Zheng Lianming	JQ716157	JQ716007
Eutima levuka	ELXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	He Jinru	/	JQ716008
Eutima levuka	ELXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ716009
Eutima levuka	ELXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Nov. 2011	He Jinru	/	JQ716010
Gangliostoma guangdongensis	GGBG1	Beibu Gulf	20.852 4°N, 109.257 6°E	Nov. 2006	Zheng Lianming	JQ716189	JQ716023
Helgicirrha brevistyla	HBDB1	Dongshan Bay	23.818 3°N, 117.562 6°E	Jul. 2010	Zheng Lianming	JQ716163	JQ715997
Helgicirrha brevistyla	HBDB2	Dongshan Bay	23.818 3°N, 117.562 6°E	Jul. 2010	Zheng Lianming	JQ716164	JQ715998
Helgicirrha brevistyla	HBBG1	Beibu Gulf	20.852 4°N, 109.257 6°E	Nov. 2006	Zheng Lianming	JQ716165	JQ715999
Helgicirrha malayensis	HMXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	Zheng Lianming	JQ716161	JQ716000
Helgicirrha malayensis	HMTS1	Taiwan Strait	21.666 7°–23.850 0°N, 116.783 3°–118.933 3°E	Jun. 2006	Zheng Lianming	JQ716162	JQ716001
Helgicirrha malayensis	HMXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Mar. 2007	Zheng Lianming	/	JQ716002
Laodicea undulate	LUYE1	Changjiang River Estuary	31.532 4°N, 122.153 7°E	Apr. 2006	Zheng Lianming	JQ716120	JQ715946
Laodicea undulate	LUYE2	Changjiang River Estuary	31.532 4°N, 122.153 7°E	Apr. 2006	Zheng Lianming	JQ716121	JQ715947
Leuckartiara sp.	LSXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Aug. 2012	He Jinru	JX965907	JX965913
Liriope tetraphylla	LTBG1	Beibu Gulf	20.852 4°N, 109.257 6°E	Nov. 2006	Zheng Lianming	JQ716067	/
Liriope tetraphylla	LTXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716065	JQ715944
Liriope tetraphylla	LTXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	He Jinru	JQ716066	JQ715945
Lovenella haichangensis	LHYE1	Changjiang River Estuary	31.532 4°N, 122.153 7°E	Apr. 2006	Zheng Lianming	/	JQ715912
Malagazzia carolinae	MCXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716111	/
Malagazzia carolinae	MCXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716109	JQ715904
Malagazzia carolinae	MCXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716110	JQ715905
Malagazzia condensum	MCOXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715906

to be continued

Continued from Table S1

Species	Sample No.	Locality	Position	Date	Collector	Accession No.	
						COI	16S
Malagazzia condensum	MCOXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715907
Malagazzia condensum	MCOXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715908
Nanomia bijuga	NBIXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716068	/
Nanomia bijuga	NBIXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716069	/
Nanomia bijuga	NBIXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716070	/
Nanomia bijuga	NBIXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716071	/
Nemopsis bachei	NBXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716072	JQ715889
Nemopsis bachei	NBXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715895
Nemopsis bachei	NBXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	/	JQ715896
Nemopsis bachei	NBXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715897
Nemopsis bachei	NBXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715898
Nemopsis bachei	NBXB6	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715899
Nemopsis bachei	NBXB7	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715900
Nemopsis bachei	NBXB8	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715901
Obelia sp.	OSBG1	Beibu Gulf	21.248 4°N, 108.804 9°E	May 2011	Zheng Lianming	JQ716073	/
Obelia sp.	OSBG2	Beibu Gulf	21.248 4°N, 108.804 9°E	May 2011	Zheng Lianming	JQ716074	/
Octophialucium medium	OMYE1	Changjiang River Estuary	31.532 4°N, 122.153 7°E	Apr. 2006	Zheng Lianming	JQ716089	JQ715913
Proboscidactyla ornata	POXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716077	JQ715902
Proboscidactyla ornata	POXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716078	JQ715903
Proboscidactyla ornata	POXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716079	JQ715909
Proboscidactyla ornata	POXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716080	JQ715910
Proboscidactyla ornata	POXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716081	JQ715911
Solmundella bitentaculata	SBXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2011	He Jinru	JQ716088	/
Staurodiscus sp.	SSXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Aug. 2012	He Jinru	JX965910	JX965915
Staurodiscus sp.	SSXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Aug. 2012	He Jinru	JX965911	JX965916
Sugiura chengshanense	SCXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716090	JQ715914
Sugiura chengshanense	SCXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716091	JQ715915
Sugiura chengshanense	SCXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716092	JQ715916
Sugiura chengshanense	SCXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716093	JQ715917
Sugiura chengshanense	SCXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716094	JQ715918
Sugiura chengshanense	SCXB6	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716095	JQ715919
Sugiura chengshanense	SCXB7	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716096	JQ715920
Sugiura chengshanense	SCXB8	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716097	JQ715921
Sugiura chengshanense	SCXB9	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716098	JQ715922
Sugiura chengshanense	SCXB10	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716099	JQ715923

to be continued

Continued from Table S1

Species	Sample No.	Locality	Position	Date	Collector	Accession No.	
						COI	16S
Sugiura chengshanense	SCXB11	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716100	JQ715924
Sugiura chengshanense	SCXB12	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716101	JQ715925
Sugiura chengshanense	SCXB13	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716102	JQ715926
Sugiura chengshanense	SCXB14	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716103	JQ715927
Sugiura chengshanense	SCXB15	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716104	JQ715928
Sugiura chengshanense	SCXB16	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716105	JQ715929
Sugiura chengshanense	SCXB17	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716106	JQ715930
Sugiura chengshanense	SCXB18	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716107	JQ715931
Sugiura chengshanense	SCXB19	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	JQ716108	JQ715932
Sugiura chengshanense	SCXB20	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715933
Sugiura chengshanense	SCXB21	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715934
Sugiura chengshanense	SCXB22	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715935
Sugiura chengshanense	SCXB23	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715936
Sugiura chengshanense	SCXB24	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715937
Sugiura chengshanense	SCXB25	Xiamen Bay	24.387 1°N, 118.143 0°E	Jul. 2010	He Jinru	/	JQ715938
Tiaricodon coeruleus	TCXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	He Jinru	JQ716123	JQ715981
Tiaricodon coeruleus	TCXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	He Jinru	JQ716124	JQ715982
Tiaricodon coeruleus	TCXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Apr. 2011	He Jinru	JQ716125	JQ715983
Tiaricodon coeruleus	TCXB4	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716126	JQ715984
Tiaricodon coeruleus	TCXB5	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716127	JQ715985
Tiaricodon coeruleus	TCXB6	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715986
Tiaricodon coeruleus	TCXB7	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	/	JQ715987
Tima formosa	TFYE1	Changjiang River Estuary	31.532 4°N, 122.153 7°E	May 2005	Zheng Lianming	JQ716166	JQ715991
Tima formosa	TFYE2	Changjiang River Estuary	31.532 4°N, 122.153 7°E	May 2005	Zheng Lianming	JQ716167	JQ715992
Tima formosa	TFJB1	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716168	JQ715993
Tima formosa	TFJB2	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716169	JQ715994
Tima formosa	TFJB3	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	JQ716170	JQ715995
Tima formosa	TFJB4	Jiaozhou Bay	36.120 1°N, 120.252 6°E	Jun. 2010	Zheng Lianming	/	JQ715996
Turritopsis lata	TLXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Aug. 2012	He Jinru	JX965909	/
Turritopsis lata	TLXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Aug. 2012	He Jinru	JX965908	JX965914
Turritopsis rubra	TRXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	May 2011	He Jinru	JQ716122	JQ715943
Turritopsis sp.	TNXB1	Xiamen Bay	24.387 1°N, 118.143 0°E	Oct. 2011	He Jinru	JQ716082	/
Turritopsis sp.	TNXB2	Xiamen Bay	24.387 1°N, 118.143 0°E	Nov. 2011	He Jinru	JQ716083	/
Turritopsis sp.	TNXB3	Xiamen Bay	24.387 1°N, 118.143 0°E	Nov. 2011	He Jinru	JQ716084	/

Table S2. The information of the homologous sequences available from the GenBank database

Species	mt COI Accession No.	mt 16S Accession No.
Aequorea conica	EU012502	/
Aequorea conica	EU012503	/
Aequorea papillata	EU012499	/
Aequorea papillata	EU012500	/
Aequorea papillata	EU012501	/
Aequorea sp.	EU012498	/
Aurelia aurita	AY903211	AF461398
Aurelia limbata	AY903189	AF461403
Blackfordia virginica	/	AY512516
Clytia gracilis	AY789900	AY789812
Clytia gracilis	AY789901	AY789813
Clytia gracilis	AY789899	AY789811
Clytia hemisphaerica	AY789902	AY789814
Clytia linearis	AY789897	AY789810
Clytia paulensis	AY789896	AY346361
Craterolophus convolvulus	GQ120102	AY845343
Eucheilota menoni	/	FJ550493
Eugymnanthea inquilina	AY789915	AY285163
Eugymnanthea japonica	/	AY285162
Laodicea undulata	/	FJ550471
Leuckartiara octona	GQ120057	AM411421
Liriope tetraphylla	/	AY512510
Liriope tetraphylla	/	EU293980
Liriope tetraphylla	/	U19377
Nanomia bijuga	/	AY935296
Proboscidactyla flavicirrata	HM053523	AM183137
Siderastrea radians	NC008167	NC008167
Solmundella bitentaculata	/	EU293998
Sugiura chengshanense	HQ718600	HM053546
Turritopsis nutricula	/	EU624349
Turritopsis rubra	EF540795	EU624386

Table S3. Species information, number of individuals, mean and standard deviation of intra-specific Kimura 2-Parameter (K2P) genetic distance, and GenBank Accession numbers for 47 species of Hydrozoa analyzed in this study

Taxon	mtCOI				mt16S			
	N	Mean	S.D.	Accession No.	N	Mean	S.D.	Accession No.
Anthomedusae								
Bougainvillidae								
Bougainvillia muscus	2	0.012	/	JQ716058, 6059	5	0.007	0.002	JQ715890-5894
Bougainvillia staurogaster	1	/	/	JQ716060	/	/	/	/
Nemopsis bachei	1	/	/	JQ716072	8	0.001	0.001	JQ715889-5901
Corymorphidae								
Euphysora verrucosa	4	0.002	0.002	JQ716061-6064	/	/	/	/
Halimedusidae								
Tiaricodon coeruleus	5	0.007	0.002	JQ716123-6127	7	0.002	0.002	JQ715981-5987
Clavidae								
Turritopsis lata	1	/	/	JQ716122	1	/	/	JQ715943
Turritopsis nutricola	3	0.002	0.001	JQ716082-6084	/			/
Pandeidae								
Amphinema dinema	2	0.005	/	JQ716057, 6085	2	0.002	/	JQ715887-5888
Proboscidactylidae								
Proboscidactyla ornata	5	0.004	0.002	JQ716077-6081	5	0.002	0.001	JQ715902, 5903, JQ715909-5911
Leptomedusae								

to be continued

Continued from Table S3

Taxon	mtCOI				mt16S			
	N	Mean	S.D.	Accession No.	N	Mean	S.D.	Accession No.
Aequoreidae								
Aequorea australis	8	0.009	0.010	JQ716190-6197	8	0.005	0.003	JQ716011-6018
Aequorea conica	3	0.022	0.019	JQ716175-6177	3	0.001	0.001	JQ715988-5990
Aequorea papillata	7	0.004	0.003	JQ716056, JQ716181-6186	9	0.006	0.005	JQ716024, JQ716029-6036
Aequorea sp.	2	0.001	/	JQ716187, 6188	4	0.001	0.001	JQ716025-6028
Aequorea taiwanensis	3	0.008	0.004	JQ716178-6180	4	0.001	0.001	JQ716019-6022
Gangliotoma guangdongensis	1	/	/	JQ716189	1	/	/	JQ716023
Blackfordiidae								
Blackfordia polytentaculata	3	0.008	0.003	JQ716117-6119	4	0.005	0.002	JQ715948-5951
Blackfordia virginica	5	0.006	0.002	JQ716112-6116	6	0.001	0.002	JQ715952-5957
Campanulariidae								
Clytia folleata	1	/	/	JQ716211	5	0.001	0.001	JQ716051-6055
Clytia sp.*KC*	5	0.001	0.000	JQ716206-6210	5	0.003	0.002	JQ716046-6050
Clytia xiamenensis sp. nov.	8	0.001	0.002	JQ716198-6205	9	0.002	0.002	JQ716037-6045
Obelia sp.one	2	0.001	/	JQ716073, 6074	/	/	/	/
Obelia sp.two	2	0.001	/	JQ716075, 6076	/	/	/	/
Eirenidae								
Eirene brevistylus	4	0.009	0.001	JQ716152-6155	4	0.004	0.004	JQ715958-5961
Eirene ceylonensis	6	0.006	0.002	JQ716138-6143	4	0.004	0.002	JQ715970-5973
Eirene hexanemalis	5	0.013	0.004	JQ716147-6151	4	0.006	0.001	JQ715962-5965
Eirene kambara	4	0.006	0.003	JQ716128-6131	3	0.004	0.002	JQ715978-5980
Eirene menoni	6	0.010	0.004	JQ716132-6137	4	0.004	0.001	JQ715974-5977
Eirene pyramidalis	3	0.004	0.002	JQ716144-6146	4	0.007	0.001	JQ715966-5969
Eugymnanthea japonica	4	0.002	0.002	JQ716171-6174	/	/	/	/
Eutima krampi	3	0.007	0.001	JQ716158-6160	3	0.006	0.001	JQ716003-6015
Eutima levuka	2	0.006	/	JQ716156, 6157	5	0.008	0.004	JQ716006-6010
Helgicirrha brevistyla	3	0.006	0.004	JQ716163-6165	3	0.001	0.001	JQ715997-5999
Helgicirrha malayensis	2	0.006	/	JQ716161, 6162	3	0.005	0.002	JQ716000-6002
Tima formosa	5	0.006	0.003	JQ716166-6170	6	0.005	0.002	JQ715991-5943
Laodiceidae								
Laodicea undulata	2	0.009	/	JQ716120, 6121	2	0.006	/	JQ715946, 5947
Lovenellidae								
Eucheilota menoni	1	/	/	JQ716086	6	0.001	0.002	JQ715881-5886
Eucheilota multicirrs	1	/	/	JQ716087	/	/	/	/
Lovenella haichangensis	/	/	/	/	1	/	/	JQ715912
Malagazziidae								
Malagazzia carolinae	3	0.002	0.001	JQ716109-6111	2	0.002	/	JQ715904, 5905
Malagazzia condensum	/	/	/	/	3	0.000	0.000	JQ715906-5908
Octophialucium medium	1	/	/	JQ716089	1	/	/	JQ715913
Sugiuridae								
Sugiura chengshanense	19	0.001	0.002	JQ716090-6108	25	0.002	0.002	JQ715914-5938
Siphonophorae								
Diphyidae								
Diphyes chamissonis	/	/	/	/	4	0.008	0.004	JQ715939-5942
Agalmatidae								
Nanomia bijuga	4	0.001	0.001	JQ716068-6071	/	/	/	/
Narcomedusae								
Aeginidae								
Solmundella bitentaculata	1	/	/	JQ716088	/			/
Trachymedusae								
Geryoniidae								
Liriope tetraphylla	3	0.003	0.000	JQ716065-6067	2	0.013	/	JQ715944, 5945