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DNA barcode assessment of Ceramiales (Rhodophyta) in the intertidal zone of the northwestern Yellow Sea*

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Abstract A total of 142 specimens of Ceramiales (Rhodophyta) were collected each month from October 2011 to November 2012 in the intertidal zone of the northwestern Yellow Sea. These specimens covered 21 species, 14 genera, and four families. Cluster analyses show that the specimens had a high diversity for the three DNA markers, namely, partial large subunit rRNA gene (LSU), universal plastid amplicon (UPA), and partial mitochondrial cytochrome c oxidase subunit I gene (COI). No intraspecific divergence was found in our collection for these markers, except for a 1–3 bp divergence in the COI of *Ceramium kondoi*, *Symphyocladia latiuscula*, and *Neosiphonia japonica*. Because short DNA markers were used, the phylogenetic relationships of higher taxonomic levels were hard to evaluate with poor branch support. More than half species of our collection failed to find their matched sequences owing to shortage information of DNA barcodes for macroalgae in GenBank or BOLD (Barcode of Life Data) Systems. Three specimens were presumed as *Heterosiphonia crispella* by cluster analyses on DNA barcodes assisted by morphological identification, which was the first record in the investigated area, implying that it might be a cryptic or invasive species in the coastal area of northwestern Yellow Sea. In the neighbor-joining trees of all three DNA markers, *Heterosiphonia japonica* converged with *Dasya* spp. and was distant from the other *Heterosiphonia* spp., implying that *H. japonica* had affinities to the genus *Dasya*. The LSU and UPA markers amplified and sequenced easier than the COI marker across the Ceramiales species, but the COI had a higher ability to discriminate between species.

Keyword: DNA barcoding; Ceramiales; red algae; large subunit rRNA gene (LSU); universal plastid amplicon (UPA); cytochrome c oxidase subunit I gene (COI)

1 INTRODUCTION

Ceramiales is one of the largest orders in Rhodophyta (red algae) with currently up to 10 families, 408 genera, and 2 480 species registered in AlgaeBase (Guiry, 2001; Guiry and Guiry, 2014). Similar to most marine algae, with simple morphologies, frequent convergence and high phenotypic variation in response to varying environmental conditions, Ceramiales are also difficult to identify with certainty (De Jone et al., 1998; Lee et al., 2001; Lin et al., 2001; Saunders, 2005, 2008; Clarkston and Saunders, 2010).

The DNA barcoding as a quick and accurate technique for species identification was first put forward in 1993 (Arnot et al., 1993). This method has been applied successfully in taxonomy and evolution research on animals, microbes, and terrestrial plants (Hebert et al., 2003a, b; Yoo et al., 2006; Yancy et al., 2008; Hollingsworth et al., 2009). However, for marine algae, using a single gene does not meet the requirements of a barcode in terms of discriminatory ability and universality of PCR primers (Moritz and Cicero, 2004). Until now, several markers for red

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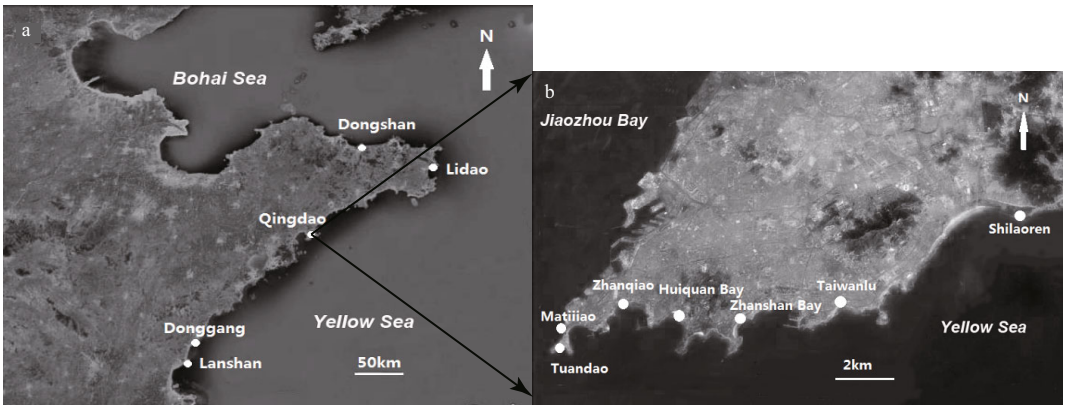

Fig.1 Sampling sites

a. northwestern coast of the Yellow Sea; b. coast of Qingdao.

macroalgae have been explored, including partial mitochondrial cytochrome c oxidase subunit I gene (COI), partial large-subunit rRNA gene (LSU), universal plastid amplicon (UPA), which is the domain V of the plastid large subunit 23S ribosomal gene, and partial large (*rbc*L) and small (*rbc*S) subunits of ribulose-1, 5-bisphosphate carboxylase (RuBisCO) gene, representing the cell nucleus, plastid, and mitochondrion genomes (Lee et al., 2001; Lin et al., 2001; Saunders, 2005; Robba et al., 2006; Sherwood, 2008; Sherwood et al., 2010b).

Each of these DNA markers has advantages and disadvantages in barcoding for red macroalgae. For instance, the UPA marker is easy to be amplified and sequenced for most red algae (Sherwood and Presting, 2007; Sherwood et al., 2010b), with appropriate interspecific variation (1.1%–6.7%) on distinguishing most species of red algae (Sherwood et al., 2010b; Zhao et al., 2012). The COI could be considered the most suitable DNA barcode for red algae because of its remarkable ability to discriminate between closely related species with large barcoding gaps (Saunders, 2005, 2008; Robba et al., 2006; Sherwood et al., 2010a, b). However**,** among the markers listed above, the COI is the most difficult to amplify and sequence across all red algae, even after seven different primer combinations were used (Clarkston and Saunders, 2010; Sherwood et al., 2010a). It was deduced that sequencing failures might be caused by the heterogeneity found within species at positions near the 3' ends of the primers (Saunders, 2008). Generally, rather than relying on a single marker, the combined use of two or more markers has been recommended (Lin et al., 2001; Hall et al., 2010; Saunders and Kucera, 2010).

In China, about 35 genera and 74 species of Ceramiales have been recorded, of which nearly 23 genera and 42 species have been reported to be distributed along the northwestern coast of the Yellow Sea (Tseng, 1984; Tseng et al., 2009). These records are sourced mostly from a taxonomic survey that was carried out in the 1980s, with identification based on morphological and structural characteristics, as well as reproductive features of Ceramiales. However, over the years since then, the biodiversity of macroalgae in coastal areas of China has decreased dramatically as a result of anthropogenic impacts, such as aquaculture and recreation (Liu et al., 1999). Until now, only a few published studies of coastal Rhodophyta from China have employed molecular approaches (Zhao et al., 2012). Basic studies and information about macroalgal barcodes in coastal macroalgae of China are needed to be established. This study aimed to get insights into the Ceramiales species of Rhodophyta, to generate baseline data of macroalgae, explore the potential cryptic or invasive species, and evaluate the biodiversity of coastal ecosystem in the northwestern Yellow Sea.

2 MATERIAL AND METHOD

2.1 Sampling

From October 2011 to November 2012, 142 specimens of the order Ceramiales were collected each month from the intertidal zone of the northwestern Yellow Sea during low tide (Fig.1, Table 1). Morphological microstructure characteristics were identified using an Olympus CX31 microscope (Olympus Co., Japan) following Tseng et al. (2009), Zheng (2001), and Xia (2011). The fresh collections were cleared of epiphytes, and samples for molecular analysis were frozen at -20°C. Representative specimens have been preserved in the Algal Herbarium of Ocean University of China.

Table 1 Ceramiales specimens collected from intertidal zones of the northwestern Yellow Sea from October 2011 to November 2012

Family	Genus	Species	Voucher	Number of specimens represented	Collection details of the representative specimen	GenBank accession No.		
						LSU	UPA	COI
Ceramiaceae	*Callithamnion*	*C. corymbosum*	QDTD20120604-R7	4	Tuandao Bay, Qingdao, Jun. 2012	KC795866	KC795892	-
	Ceramium	*C. diaphanum*	QDSLR20111028-R8	9	Shilaoren, Qingdao, Oct. 2011	KC795860	KC795886	KC795911
		C. kondoi	QDSLR20111028-R11	15	Shilaoren, Qingdao, Oct. 2011	KC795845	KC795871	KC795897
		C. kondoi	QDHQW20120309-R8	1	Huiquan Bay, Qingdao, Mar. 2012	KC795846	KC795873	KC795898
		C. kondoi	QDSLR20111128-R18	1	Shilaoren, Qingdao, Nov. 2011	KC795848	KC795874	KC795899
		C. kondoi	QDHQW20111124-R21	1	Huiquan Bay, Qingdao, Nov. 2011	KC795847	KC795872	KC795900
		C. japonicum	QDHQW20121115-R5	3	Huiquan Bay, Qingdao, Nov. 2012	KC795870	KC795896	KC795912
		C. tenerrimum	QDHQW20120309-R14	2	Huiquan Bay, Qingdao, Mar. 2012	KC795867	KC795893	-
		Species 1 of Ceramiaceae[a]	QDHQW20121115-R1	1	Huiquan Bay, Qingdao, Nov. 2012			
		Species 2 of Ceramiaceae[a]	QDHQW20121115-R3	1	Huiquan Bay, Qingdao, Nov. 2012			-
	Pterothamnion	*P. yezoense*	QDHQW20120309-R25	2	Huiquan Bay, Qingdao, Mar. 2012	KC795865	KC795891	-
Dasyaceae	*Heterosiphonia*	*H. japonica*	QDMTJ20111029-R22	10	Matijiao, Qingdao , Oct. 2011	KC795855	KC795881	KC795907
		H. crispella	QDHQW20111124-R23	3	Huiquan Bay, Qingdao, Nov. 2011	KC795857	KC795883	KC795909
Delesseriaceae	*Acrosorium*	*A. yendoi*	QDHQW20120407-R7	5	Huiquan Bay, Qingdao, Apr. 2012	KC795858	KC795884	-
	Phycodrys	*P. radicosa*	QDHQW20111124-R17	6	Huiquan Bay, Qingdao, Nov. 2011	KC795861	KC795887	-
	Tsengiella	*T. spinulosa*	QDMTJ20111029-R20	5	Matijiao, Qingdao, Oct. 2011	KC795856	KC795882	KC795908
Rhodomelaceae	*Polysiphonia*	*P. senticulosa*	QDHQW20120110-R24	7	Huiquan Bay, Qingdao, Jan. 2012	KC795862	KC795888	-
	Neosiphonia	*N. japonica*	QDMTJ20111126-R17	9	Matijiao, Qingdao, Nov. 2011	KC795853	KC795877	KC795905
		N. japonica	QDSLR20111028-R4	1	Shilaoren, Qingdao, Oct. 2011	KC795852	KC795878	KC795903
		N. japonica	QDSLR20120308-R21	1	Shilaoren, Qingdao, Mar. 2012	KC795851	KC795879	KC795904
		N. japonica	QDMTJ20111029-R21	1	Matijiao, Qingdao, Oct. 2011	KC795854	KC795880	KC795906
	Symphyocladia	*S. latiuscula*	QDHQW20111124-R12	38	Huiquan Bay, Qingdao, Nov. 2011	KC795849	KC795876	KC795902
		S. latiuscula	QDMTJ20111126-R18	3	Matijiao, Qingdao, Nov. 2011	KC795850	KC795875	KC795901
	Neorhodomela	*N. munita*	QDHQW20120110-R4	5	Huiquan Bay, Qingdao, Jan. 2012	KC795863	KC795889	-
	Chondria	*C. crassicaulis*	QDHQW20111027-R9	2	Huiquan Bay, Qingdao, Oct. 2011	KC795859	KC795885	KC795910
	Chondrophycus	*C. intermedius*	QDHQW20120821-R1	1	Huiquan Bay, Qingdao, Aug. 2012	KC795868	KC795894	-
	Laurencia	*L. nipponica*	QDHQW20120309-R13	2	Huiquan Bay, Qingdao, Mar. 2012	KC795864	KC795890	-
		L. obtusa	QDHQW20121115-R2	3	Huiquan Bay, Qingdao, Nov. 2012	KC795869	KC795895	-

- indicates that no sequence was obtained. [a] sequences from species 1 and 2 of Ceramiaceae have not been submitted to GenBank.

2.2 Molecular analyses

DNA was extracted using a DNeasy Plant Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's recommendation. LSU, UPA, and COI were amplified using the primers and amplification protocols described by Sherwood and Presting (2007), Sherwood et al. (2010b), and Saunders (2005). The LSU sequence was amplified using the following primers from Sherwood et al. (2010b): forward nu28SF, 5'-GGAATCCGCYAAG-GAGTGTG-3' (T_m=56.9°C) and reverse nu28SR, 5'-TGCCGACTT-CCCTTACCTGC-3' (T_m=59.7°C). The PCR cycle was: 94°C for 2 min, 40 cycles at 94°C for 20 s, 55°C for 30 s, 72°C for 50 s, and a final extension at 72°C for 5 min (Sherwood et al., 2010b). The UPA sequence was amplified using the following primer pair: p23SrV_f1, 5'-GGACAGAAAGACCCT-ATGAA-3' and p23SrV_r1, 5'-TCAGCCTGTTATCCCTAGAG-3' (Sherwood and Presting, 2007) The PCR cycle was: initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 20 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The COI sequence was amplified using the following primers: GazF1, 5'-TCAACAAATCATA-AAGATATTGG-3' (Saunders, 2005) and R686, 5'-CCACCWGMAGGA-TCAA-3' (Sherwood et al., 2010b). The PCR cycle was: 94°C for 1.5 min, followed by 40 cycles at 94°C for 30 s, 47°C for 40 s, 72°C for 40 s, and a final extension at 72°C for 5 min. The PCR amplifications were performed with a Mycylcer thermal cycler (Bio-Rad, USA). Each 20 μL PCR solution consisted of 10 μL PCR Mix, 0.2 μL Taq (5 U/μL), 1 μL of each primer (10 mmol/L), and 5.8 μL ddH_2O. The sequencing was performed by the BGI Biotech Co. Ltd. (Shenzhen, China).

Forward and reverse sequences of each sample were aligned to obtain a complete and accurate sequence for each DNA barcode. The LSU, UPA, and COI sequences were then aligned using Clustal X1.83 (UCD, Dublin, Ireland) (Thompson et al., 1994). After alignment, one voucher was selected to represent a group of specimens with identical LSU, UPA, or COI sequences for further cluster analyses. Specimens that had identical LSU or UPA sequences but different COI sequences were also selected for further analyses. In addition, LSU, UPA, and COI sequences of Ceramiales species were downloaded from GenBank based on the results of BLAST (Basic Local Alignment Search Tool) performing on NCBI (U.S. National Center for Biotechnology Information, http://blast.ncbi.nlm.nih.gov/Blast.cgi; David and Medha, 2007) against the nucleotide sequence database. Neighbor-joining (NJ) trees were constructed based on the maximum-likelihood composite model in MEGA v.4 (Tamura et al., 2007) using the Kimura 2-parameter method that computes evolutionary distances (Kimura, 1980). One thousand bootstrap replicates were used to estimate the reliability of the branches.

3 RESULT

3.1 Analyses of DNA markers

The LSU and UPA markers from all 142 specimens were amplified, and 137 sequences were obtained for each of the markers. Only 83 of the COI markers could be amplified, out of which 39 sequences were obtained. The amplification and sequencing success rate for LSU, UPA, and COI was 100% and 96.5%, 100% and 96.5%, and 58.4% and 50.0%, respectively. The LSU and UPA sequences were obtained for all the collected taxa, but no COI sequences were obtained for 11 species (Table 1). Clustering trees based on the specimen sequences and the sequences from GenBank were constructed to illustrate the levels of divergence within and between species (Figs.2–4). The maximum intraspecific divergence (pairwise distances) was 0.025 for LSU, 0.018 for UPA, and 0.152 3 for COI, all of which were in sequences from *Heterosiphonia crispella*. The minimum interspecific divergence (pairwise distances) within genus was 0 for LSU in *Pterothamnion* and *Ceramium*, between *P. yazoense* and *P. villosum*, and among *C. diaphanum*, *C. tenerrimum* and *C. womersleyi*, 0.003 for UPA between *Laurencia* sp. and *Laurencia nidifica*, and 0.029 6 for COI between *Neosiphonia harveyi* and *Neosiphonia japonica*. The maximum interspecific divergence within genus was 0.041 for LSU between *Polysiphonia howei* and *Polysiphonia senticulosa*, 0.057 for UPA between *Polysiphonia* sp. and *P. senticulosa*, and 0.259 9 for COI between *Ceramium kondoi* and *Ceramium japonicum*. When the overall pairwise distances were compared it was clear that the COI had the highest values among the three markers.

3.2 DNA barcoding analyses

Our collection covers 21 species, 14 genera, and four families of Ceramiales. Eleven of the species belong to 11 genera (Table 1); four were assigned to genus *Ceramium*, two were *Heterosiphonia*, and two were *Laurencia*. The remaining two species (species 1 and 2) were assigned only to the family Ceramiaceae

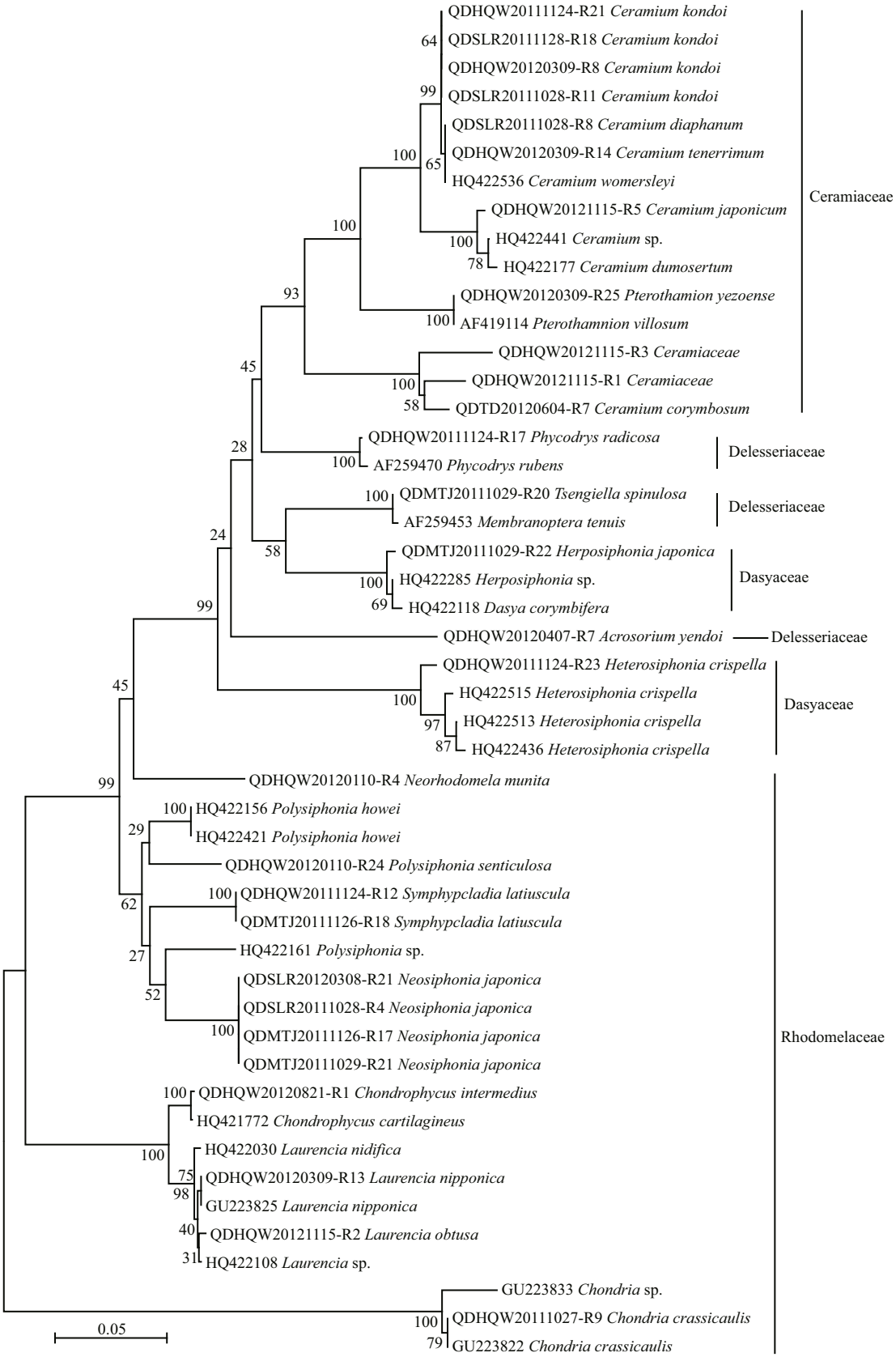

Fig.2 Neighbor-joining tree of LSU sequences from Ceramiales

The sequences from the specimens collected for this study are those serial numbers with "-Rxx"; the other sequences are from GenBank. Bootstrap values were obtained from 1 000 replications (values below 50% are not shown).

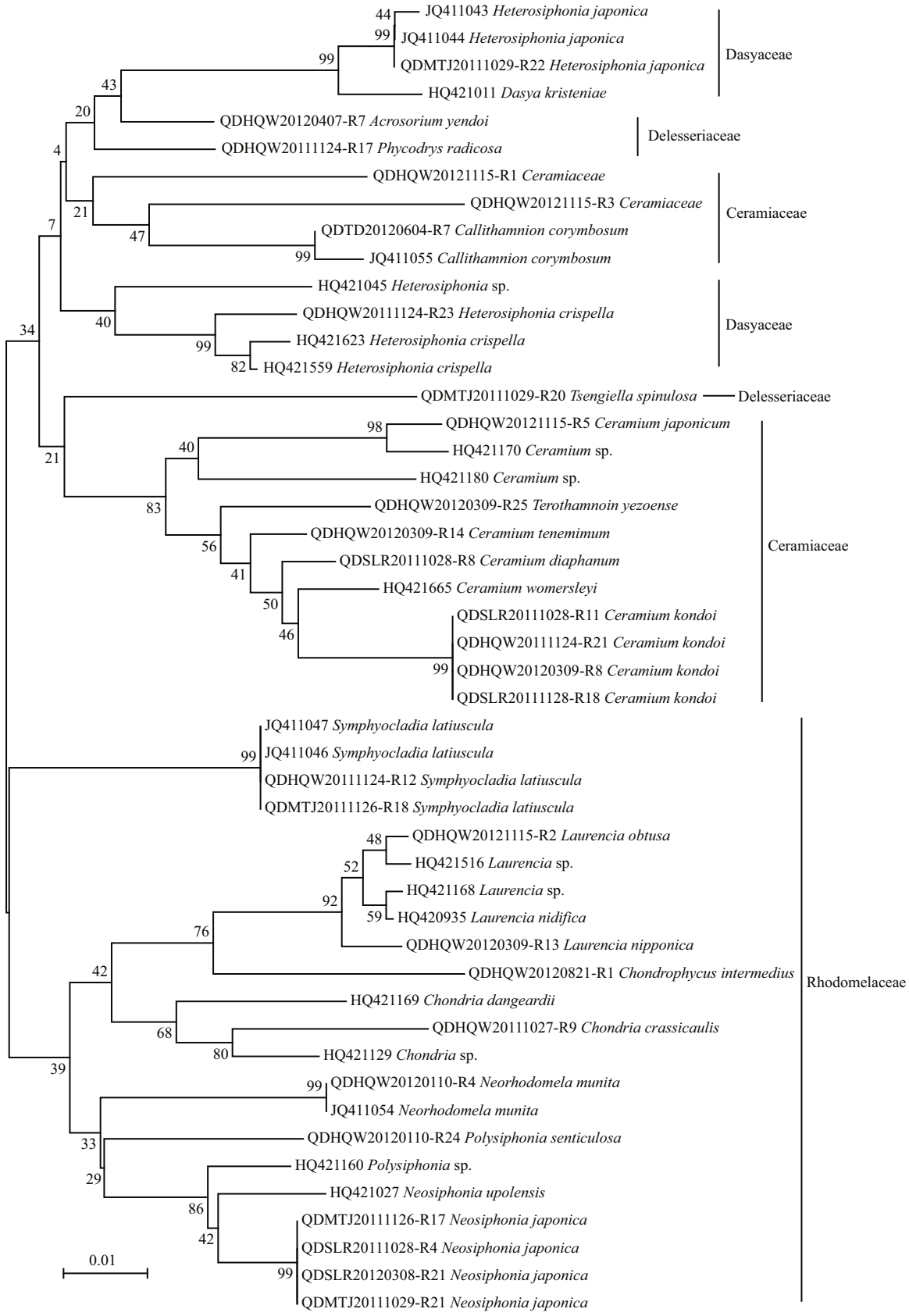

Fig.3 Neighbor-joining tree of UPA sequences from Ceramiales

The sequences from the specimens collected for this study are those serial numbers with "-Rxx"; the other sequences are from GenBank. Bootstrap values were obtained from 1 000 replications (values below 50% are not shown).

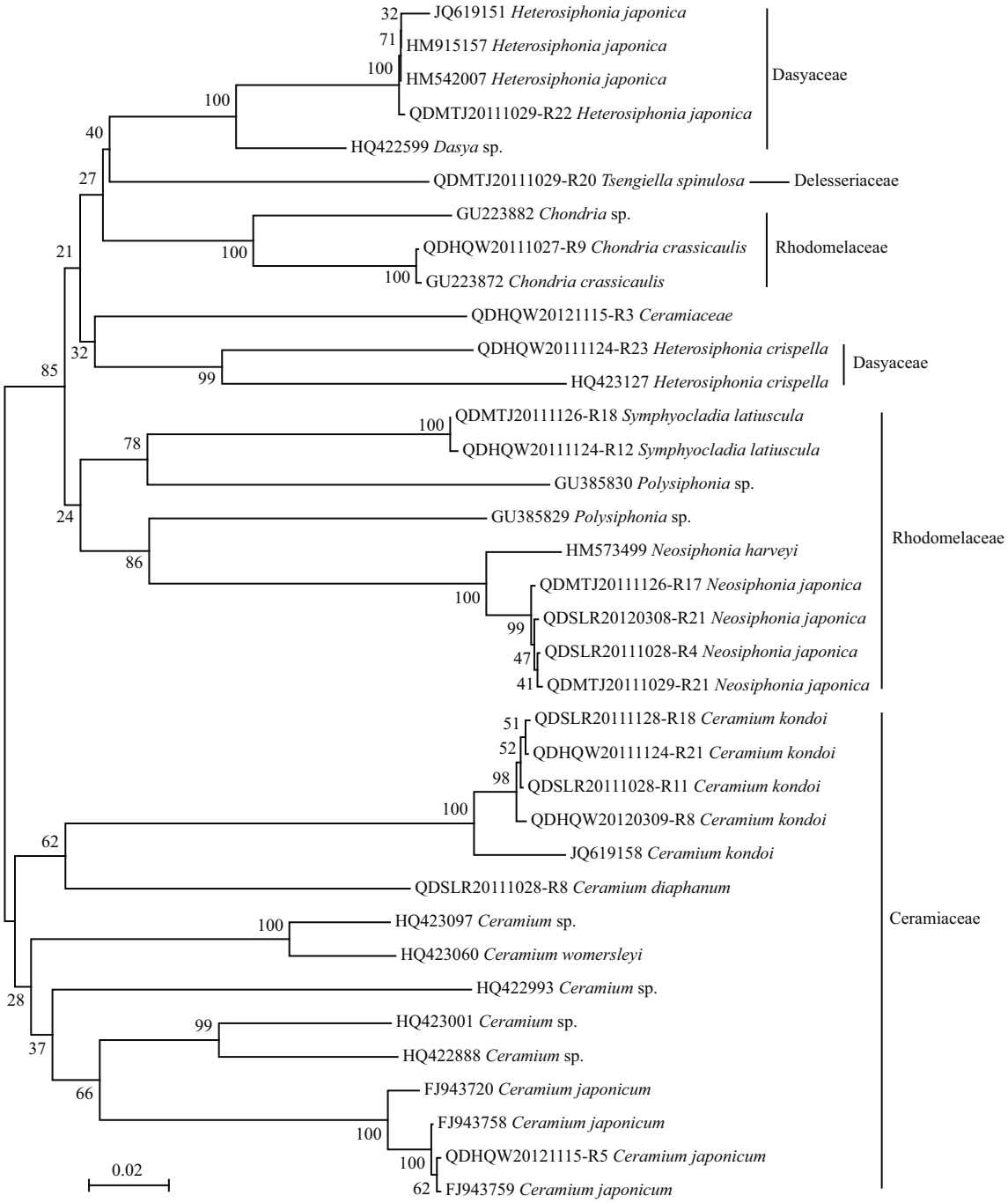

Fig.4 Neighbor-joining tree of COI sequences from Ceramiales

The sequences from the specimens collected for this study are those serial numbers with "-Rxx"; the other sequences are from GenBank. Bootstrap values were obtained from 1 000 replications (values below 50% are not shown).

(Table 1). No discrepancies were detected in the three marker sequences within most of the species; the exceptions were for three species *C. kondoi*, *Symphyocladia latiuscula*, and *N. japonica* in which a 1–3 bp divergence was found in the COI sequences.

In the NJ trees for LSU, UPA, and COI, a proportion of the branch supports (23%, 41%, and 36%, respectively) were lower than 50%. Not all the genera from same family were clustered together, except for Ceramiaceae and Rhodomelaceae in LSU Tree, Rhodomelaceae in UPA tree, and Ceramiaceae in COI tree (Figs.2–4). In all the NJ trees of LSU, UPA, and COI, *Heterosiphonia japonica* first converged with *Dasya* spp. and were distant from the other *Heterosiphonia* spp. At the genus and species levels, the LSU marker exhibited low resolution, especially

between *Polysiphonia* and *Neosiphonia*, but also among *Ceramium diaphanum*, *Ceramium tenerrimum*, and *Ceramium womersleyi*. On the contrary, the UPA and COI trees could discriminate among genera and species, and the COI tree also showed sequence divergence within species such as *C. japonicum*, *C. kondoi*, and *N. japonica* (Fig.4).

Nine of the species in our collection had little or no sequence divergence with their corresponding sequences from GenBank; for example, the *H. japonica* were identical in both UPA and COI, the *Chondria crassicaulis* were consistent in LSU and COI, *S. latiuscula* congruent in UPA and only had 1bp difference on COI. The *C. japonicum* presented little distance of 0.001 with GenBank sequence FJ943759 on COI, the *Neorhodomela munita* UPA had no distance with GenBank sequence JQ411054, and also the *Laurencia nipponica* LSU had no difference with GenBank sequence GU223825. The *Callithamnion corymbosum* had a distance of 0.045 on UPA from GenBank sequence JQ411055. The sequences of *C. kondoi* diverged from GenBank COI sequence JQ619158 by 0.033–0.037, and *H. crispella* had sequence distances to same species from GenBank by 0.019, 0.018, and 0.157 on LSU (HQ422515), UPA (HQ421559) and COI (HQ423127), respectively, with strong support by 99 and 100 bootstraps in their clustering trees (Figs.2–4).

Ten species and species1 and species 2 of Ceramiaceae failed to find matches by sequence alignment, due to lack of corresponding gene sequences in GenBank or BOLD, respectively.

4 DISCUSSION

The LSU, UPA, and COI markers identified in the present study showed consistent applicability and capability of species discrimination with the results from previous studies (Saunders, 2005, 2008; Robba et al., 2006; Sherwood and Presting, 2007; Sherwood et al., 2010a, b). That is, LSU and UPA were amplified and sequenced easier than COI across all the Ceramiales species, but the LSU marker could not be used to discriminate at the species level. LSU, for example, could not distinguish the *Ceramium* species *C. womersleyi*, *C. diaphanum*, and *C. tenerrimum* because there was no sequence divergence among them. On the other hand, COI had better discriminating ability than LSU and UPA, with the least conserved indicated by large divergences or long branches. In this study, three species confirmed their classification by molecular accordance on COI. Besides, the COI sequence diversity was found at intraspecific level in *C. kondoi*, *N. japonica* and *S. latiuscula*, supporting the ability of this gene in identifying genetic diversity within species (Kim et al., 2010).

Theoretically, DNA barcoding has obvious advantages for species identification and for genetic diversity evaluation, not only because of its independence from morphological features, which usually need accumulative experience in distinguishing species with intricate convergence and plastic phenotype, but also because it does not rely on reproductive structures or life cycles (Xiao et al., 2004; Saunders 2005, 2008; Evans et al., 2007; Sherwood et al., 2010a, b). Even within a species, high diversity and various phylogenetic relationships have been discovered through DNA barcoding (Sherwood et al., 2011; Kim et al., 2012). In the present study, *Heterosiphonia* species were separated by other family and failed to cluster together in all three NJ trees, suggesting that this genus has large divergence in the nucleotide sequences. In all three NJ trees, *H. japonica* converged first with *Dasya* spp. and was distant from other *Heterosiphonia* spp., corroborating previous deductions (de Jong et al., 1998; Choi, 2001; Choi et al., 2002) that the Dasyaceae family is polyphyletic and that *H. japonica* has affinities to the genus *Dasya* indicated by phylogenetic analyses of anatomical and nuclear SSU rDNA sequence. In our study, only six species (three by COI and four by UPA) could be confirmed by sequence matching. On the other hand, because short DNA markers were used, the phylogenetic relationships and branch support are not suitable to be assessed (Sherwood et al., 2010b). In our study, because of low sequences achievement for COI, the phylogenetic analysis of concatenated three markers failed to be carried out. The analysis based on the concatenated LSU and UPA (less than 1 000 bp) also showed low support with bootstrap values that were mostly less than 70% (data no shown). Therefore, only the patterns of diversity for species and groups of closely related species could be evaluated.

Cryptic or invasive species have often been revealed by DNA barcoding (Robba et al., 2006; Saunders, 2008, 2009; Rueness, 2010; Sherwood et al., 2010a, b). Three specimens (represented by QDHQW20111124-R23) that were hard to identify without reproductive structures, even their morphological structure are similar to *H. crispella*. By analyses of three NJ trees, we presumed that they might belong to *H. crispella* based on the strong

support of their sequence clustered together with GenBank sequences of *H. crispella*, although the sequence distance is large on COI as 0.157, but closer on UPA as 0.018. This species has not been recorded previously in China (Tseng, 1983; Zheng, 2001; Tseng et al., 2009; Xia, 2011), which implies that it might be a cryptic or invasive species in the investigated region.

On the other hand, the shortage of related sequences in the available databases seriously impeded the application of DNA barcoding for species identification. In this study, 12 species failed to find matched sequences from GenBank or BOLD. At the meantime, it should be noted that using GenBank sequences to identify species should be treated with caution for two reasons: one, accurate identification of species cannot be guaranteed (Harris, 2003; Vilgalys, 2003); and two, the quality assurance of the sequences in GenBank is less rigorous. And some sequences may contain large gaps or many ambiguous sites that decrease the strength of multiple sequence analyses for the purposes of species identification and may inflate the perceived levels of species diversity (Le Gall and Saunders, 2010).

Therefore, simply to depend on molecular identification is not feasible, and DNA barcoding should base on solidly traditional morphological identification (Bensasson et al., 2001; Hebert et al., 2003a; Schindel and Miller, 2005; Witt et al., 2006; Maggs et al., 2007). At present, a standard barcode marker that is suitable for all orders or families has not been established, and a combination of several DNA markers is still necessary for species identification (Saunders, 2008; Sherwood et al., 2010a). For DNA barcoding of red algae, such as Ceramiales, we suggest that UPA and COI could be applied complementarily, as recommended by Sherwood et al. (2010b).

A total of 14 genera and 21 species of Ceramiales were collected in our 1-year monthly survey. In their, books, Tseng (1984) and Tseng et al. (2009) have recorded nearly 23 genera and 42 species that are distributed along the northwestern coast of the Yellow Sea. Based on investigations conducted in May 1998 and May 1999, Liu et al. (1999) reported 23 genera and 36 species of red algae (but only 12 species of Ceramiales) in the Qingdao intertidal zone. From August 2004 to May 2005, Fu et al. (2009) carried out monthly monitoring on benthic macroalgae in rocky intertidal zones of Qingdao and reported a total 13 species of Ceramiales. The species diversity explored in the present study is lower than in the records of Tseng (1984) and Tseng et al. (2009), but higher than the records of Liu et al. (1999) and Fu et al. (2009). The investigated area and period in our study are relatively less than those of Tseng (1984) and Tseng et al. (2009), but more expansive than those of Liu et al. (1999) and Fu et al. (2009). Thus, it is hard to deduce that the biodiversity in these regions has decreased dramatically because of the anthropogenic impact as concluded by Liu et al. (1999). Further studies need to be carried out to fully investigate the biodiversity of Ceramiales and other macroalgae species in this region.

5 CONCLUSION

In this study, 142 specimens that covered 21 species, 14 genera, and four families were assessed by DNA barcoding. The results indicated that in the intertidal zone of the northwestern Yellow Sea, there was high diversity between the Ceramiales species, while, within a species, were relatively conservative. This study also corroborated previous reports that DNA barcoding is a quick and helpful technique for taxonomy and for uncovering invasive or cryptic species. However, because short DNA markers were used, it was hard to evaluate phylogenetic relationships at higher taxa levels. Considering the shortage or inaccurate information of DNA barcodes for macroalgae that is available, at present, DNA barcoding for species identification still needs to be used in combination with traditional morphological methods.

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