

CLASSIFICATION OF COMMON OYSTERS FROM NORTH CHINA

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ABSTRACT Oysters are commonly found on rocky shores along China's northern coast, although there is considerable confusion as to what species they are. To determine the taxonomic status of these oysters, we collected specimens from nine locations north of the Yangtze River and conducted genetic identification using DNA sequences. Fragments from three genes, mitochondrial 16S rRNA, mitochondrial cytochrome oxidase I (COI), and nuclear 28S rRNA, were sequenced in six oysters from each of the nine sites. Phylogenetic analysis of all three gene fragments clearly demonstrated that the small oysters commonly found on intertidal rocks in north China are *Crassostrea gigas* (Thunberg, 1793), not *C. plicatula* (the zhe oyster) as widely assumed. Their small size and irregular shell characteristics are reflections of the stressful intertidal environment they live in and not reliable characters for classification. Our study confirms that the oysters from Weifang, referred to as Jinjiang oysters or *C. rivularis* (Gould, 1861), are *C. ariakensis* (Wakiya, 1929). We found no evidence for the existence of *C. talienwhanensis* (Crosse, 1862) and other *Crassostrea* species in north China. Our study highlights the need for reclassifying oysters of China with molecular data.

KEY WORDS: oyster, taxonomy, cytochrome oxidase I, 16S rRNA, 28S rRNA, *Crassostrea gigas*, *C. ariakensis*, *Alectryonella plicatula*, *Saccostrea cucullata*, Suminoe oyster

INTRODUCTION

There are several unresolved issues in oyster classification. The problems are caused by classification based on shell morphology, which is highly variable in oysters. One of the problems is the taxonomic status of oysters found along China's north coast. Oysters are common and abundant on the rocky shores of North China. They are found on rocks, concrete and other hard surfaces in almost all intertidal zones and harbors. These oysters are typically small in size and highly variable in shell morphology. There is considerable confusion and disagreement about the taxonomic identity of these oysters.

According to existing literature, eight oyster species occur in North China: the Pacific oyster (*Crassostrea gigas*), zhe or folded oyster (*C. plicatula* [Gmelin, 1791]), Sengmao or monk-hat oyster (*Saccostrea cucullata* [Born, 1778]), Dalianwan oyster (*C. talienwhanensis*), jinjiang or near-river oyster (*C. rivularis*, now *C. ariakensis*), Portuguese oyster (*C. angulata* [Lamarck, 1819]), milin or dense-scale oyster (*Ostrea denselamellosa* [Lischke, 1869]), and maozhua or cat's paw oyster (*Talonostrea talonata* [Hanley, 1864]) (Zhang & Lou 1956, Qi 1989, Li & Qi 1994, Xu & Huang 1993, Guo et al. 1999, Yu et al. 2003, Wang, et al. 2004, Lapegue et al. 2004). The latter two species are well defined, and there is no dispute about their taxonomic status. The dense-scale oysters are characterized by large (up to 15 cm), round and flat shells with densely populated layers of scales along the edge. The cat's paw oyster is small in size (2–3 cm), and its left shell has 5–8 ribs protruding out in the shape of cat's paws. These two species are relatively rare, but they can be easily identified and separated from other species.

Zhang & Lou (1956) classified the common oysters from North China as the following: (1) the monk-hat oyster (*O. cucullata*), a small sized oyster attached to rocks in shallow water between high and low tidal lines; (2) the Pacific oyster (*C. gigas*), a large sized and long shaped oyster found below the low tidal line in moderate and high salinity waters; (3) Dalianwan

oyster (*C. talienwhanensis*), a large, triangular shaped oyster with purple spots on shells found throughout north China; (4) the near-river oyster (*C. rivularis*) with large and highly variable shells, found mostly in low and intermediate (10‰ to 25‰) salinity waters. According to Zhang & Lou (1956), the oysters that are commonly found on intertidal rocks should be the monk-hat oyster.

Zhao et al. (1982) named the oyster commonly found on the rocks of Dalian coast as the folded oyster (*C. plicatula*). Since then, there has been no agreement whether the common intertidal oysters from north China is *O. cucullata* or *C. plicatula*, or both (Zhang & Lou 1956, Qi 1989, Guo et al. 1999, Yu et al. 2003, Xu & Huang 1993). Li & Qi (1994) considered that the *O. cucullata*, *C. rivularis*, and *C. talienwhanensis* of Zhang & Lou (1956) and *C. plicatula* of Zhao et al. (1982) were all *C. gigas*. They identified the monk-hat and folded oysters as *Saccostrea cucullata* and *Alectryonella plicatula*, respectively, two species of different genus found in southern China.

However, after analyzing genetic variation revealed by random amplified polymorphic DNA (RAPD), Liu & Dai (1998) concluded that *C. talienwhanensis*, *C. plicatula*, and *C. rivularis* were three different species. An analysis of 16S and COI sequences by Yu et al. (2003) indicated that *C. gigas* and *C. talienwhanensis* were the same species, and *C. plicatula* might be a morph of *C. ariakensis*. Further, Lapegue et al. (2004) reanalyzed the sequences of Yu et al. (2003) and showed that some of the *C. talienwhanensis* samples might be *C. angulata*.

The confusion about the taxonomic status of common oysters from north China calls for further analysis. In this study, we collected and sequenced oysters from 9 locations along China's northern coast for fragments of three genes: the mitochondrial 16S rRNA gene and cytochrome oxidase I (COI), and the nuclear 28S rRNA. Here we provide molecular evidence that the small oysters commonly found on rocky shore belong to one species—*C. gigas*, and the large oysters (referred to as the near-river oysters) collected from Weifang are the same species as *C. ariakensis* (Wakiya, 1929).

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MATERIALS AND METHODS

Sample Collection and Morphological Analyses

Oysters were collected from nine typical habitats along China's coast north of Yangtze River: Zhuanghe (ZH), Zhangzidao (ZZD), Dalian (DL) in Liaoning Province; Dongying (DY), Weifang (WF), Rongcheng (RC), Rushan (RS) and Qingdao (QD) in Shandong Province; and Lianyungang (LYG) in Jiangsu Province (Fig. 1). At Zhuanghe, Zhangzidao, Dongying, Rongcheng, Qingdao, and Lianyungang oysters were collected from rocks in intertidal zones. At Weifang, oysters were collected by divers from a depth of about eight meters. Oysters from Dalian and Rushan were cultured oysters derived from local wild seed.

The following shell characteristics were recorded: shell morphology, shell wall rigidity or thickness, valve lamellae, radial ribs, shell color, ligament channel, umbonal cavity volume, interior shell color, adductor muscle scar color and shape. Shell parameters were identified following definitions of Moore et al. (1971).

DNA Extraction, PCR Amplification and Sequencing

Six oysters from each site were selected for sequencing (Table 1). Oysters with variable shell morphology were chosen to cover all possible species. DNA was extracted from fresh adductor muscle tissue using phenol/chloroform extraction as described by Moore (1993). Primers (Invitrogen, USA), 16sar and 16sbr, were used to amplify a segment of the mitochondrial 16S ribosomal RNA gene (Palumbi et al. 1991). A segment of cytochrome oxidase subunit I (COI) was amplified using LCO1490 and HCO2198 primers (Folmer et al. 1994). A 28S

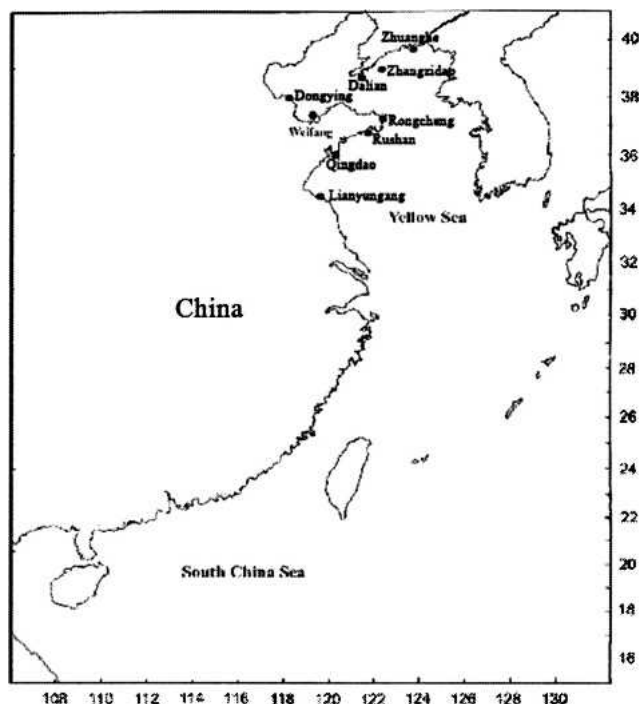


Figure 1. A map of China's coast showing collecting sites of oysters studied

TABLE 1.

Location and number of oysters sequenced.

Location	Code	No. Sequenced
Zhuanghe, Liaoning Province	ZH	6
Zhangzidao, Liaoning Province	ZZD	6
Dalian, Liaoning Province	DL	6
Dongying, Shandong Province	DY	6
Weifang, Shandong Province	WF	6
Rongcheng, Shandong Province	RC	6
Rushan, Shandong Province	RS	6
Qingdao, Shandong Province	QD	6
Lianyungang, Jiangsu Province	LYG	6

rRNA fragment was amplified using primers D1F and D6R as described by Park and Ó Foighil (2000). PCR amplification was performed using a Biometra T1 thermal cycler. Reactions were performed in 50 μ L with final concentrations of: 2.0 mM MgCl₂, 150 μ M of each dNTP, 0.2 μ M each primer, 20 ng of template DNA, and 2 units of *Taq* polymerase (Promega, USA) in 5 μ L of $\times 10$ buffer. COI and 16S were amplified using the following protocol: initial denature at 95°C for 2 min, 30 cycles of 95°C for 1 min, 51°C (COI) or 57°C (16S) for 1 min, and 72°C for 1 min with a final extension at 72°C for 5 min. The 28S fragment was amplified by an initial denaturing at 94°C for 4 min, addition of *Taq* polymerase; 30 cycles of denaturing at 94°C for 40 s, annealing at 60°C for 40 s and extension at 72°C for 1.25 min; plus a final extension at 72°C for 10 min. A negative control (no template) was included during each PCR run. PCR products were purified using EZ Spin Column PCR Product Purification Kit (BBI, Canada). Sequencing was performed in both directions on an ABI PRISM 377XL DNA sequencer using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, USA).

Phylogenetic Analysis

The COI, 16S, and 28S sequences obtained in this study and those of other *Crassostrea*, *Ostrea* and *Saccostrea* species available from GenBank were used for phylogenetic analysis. Initial alignments were performed using CLUSTALW (Thompson et al. 1994). The sequences were trimmed to the same length as other published sequences after alignment. Maximum parsimony (MP) and neighbor-joining (NJ) analyses were performed using PHYLIP (Ver. 3.56C, Felsenstein 1989). Bootstrap analysis with 1,000 replications was performed using the SEQBOOT and CONSENSE programs. Consensus phylogenetic trees were drawn with DRAWGRAM program in the PHYLIP package. Pair-wise sequence divergences among haplotypes and reference species were calculated using the DNADIST program of PHYLIP according to Kimura's two-parameter model (Kimura 1980).

RESULTS

Distribution and Morphology of Oysters from North China

Oysters are common and abundant at all nine sites sampled in this study (Table 1). Shell morphology varies greatly within and among sites. In general, oysters collected below or at the

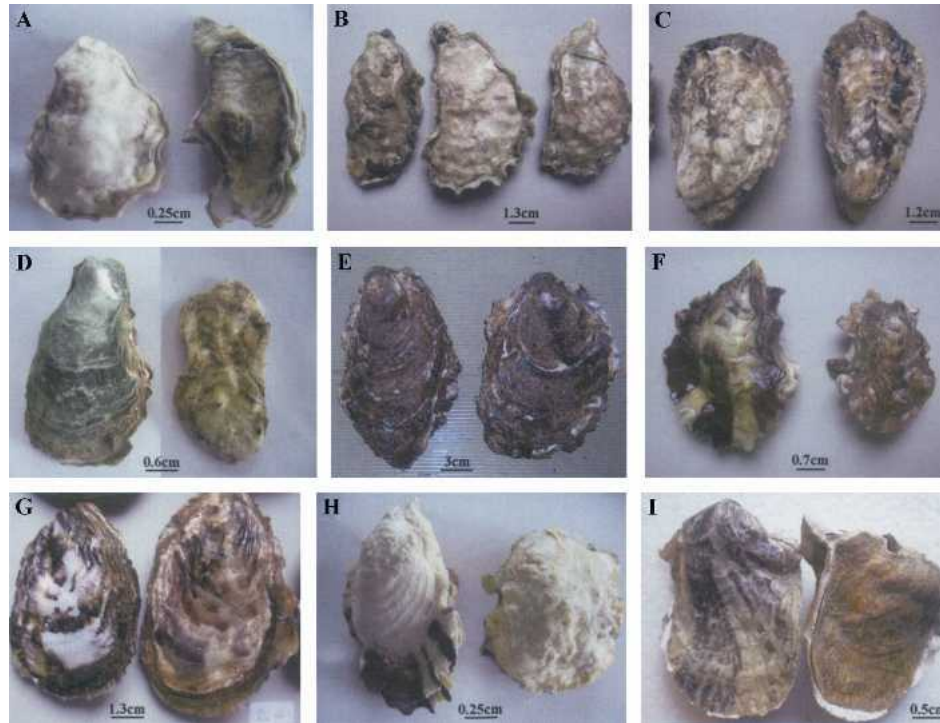


Figure 2. Shell morphology of representative oysters collected from 9 sites in North China: (A) Zhuanghe (ZH), Liaoning; (B) Zhangzidao (ZZD), Liaoning; (C) Dalian (DL), Liaoning; (D) Dongying (DY), Shandong; (E) Weifang (WF), Shandong; (F) Rongcheng (RC), Shandong; (G) Rushan (RS), Shandong; (H) Qingdao (QD), Shandong; (I) and Lianyungang (LYG), Jiangsu.

low tidal line are larger than oysters collected high above the low tidal line, so are the cultured oysters obtained from farms.

At most sites (ZH, ZZD, DY, RC, QD and LYG), oysters were collected from rocks at or above the low tidal line. These oysters are typically small (30–60 mm), flat and irregular in shape (Fig. 2). The color of the shells is variable ranging from white to yellow to brown. Some oysters especially those from Rongcheng have purple strips on the right valve. The shell's edge is highly variable: some smooth and some with waving plates protruding. Internally, the shells are milky white, sometimes with areas of gray, yellow, or brown. Muscle scars range from pink to dark purple. Ligament channels are mostly short with some exceptions.

Oysters collected from deep waters at Weifang are noticeably different from oysters from other sites. They are large, up to 200 mm, and some are round and horse-shoe shaped. The shells are thick and relatively smooth with multiple layers of overlapping and concentric plates on the right valve. Radiant ribs are absent or weak. Shell color is variable, ranging from yellow to brown (Fig. 2E). Ligament channels are short and shallow. The umbo cavity is shallow to medium. The internal side of shells is milky white with areas chalky or lacking of the nacre layer. The adductor muscle scar has no coloration, which is unique for the subtidal oysters from Weifang.

The cultured oysters from Dalian and Rushan are large (100–200 mm), elongated or ovate. The left valve is convex with obvious radial ribs. The right valve is flat. Shell color ranges from white, yellow to brown, and often with purple strips. The internal sides of the shells are white with pink to purple muscle scars. Ligament channels are shallow, and the umbo cavity is deep (Fig. 2C, G).

Mitochondrial 16S rRNA Sequence

A 497 bp fragment of the mitochondrial 16S rRNA gene was sequenced for 54 individuals collected from nine sites (Table 1). Five haplotypes (Haplotype 1, 2, 3, 4, 5) were identified among the 54 sequences. Oysters from Weifang had two haplotypes (Haplotype 4, 5), with five oysters having Haplotype 4 and one oyster having Haplotype 5 (Table 2). Oysters from the other eight sites had 3 haplotypes (Haplotype 1, 2, 3). Haplotype 1 is the common haplotype and shared by 46 of the 48 oysters. One oyster from Dongying has Haplotype 2, and one oyster from Qingdao has Haplotype 3 (Table 2).

TABLE 2.
Distribution of the five 16S rRNA haplotypes obtained in this study.

Site	Haplotype				
	1	2	3	4	5
ZH	6	0	0	0	0
ZZD	6	0	0	0	0
DL	6	0	0	0	0
DY	5	1	0	0	0
WF	0	0	0	5	1
RC	6	0	0	0	0
RS	6	0	0	0	0
QD	5	0	1	0	0
LYG	6	0	0	0	0

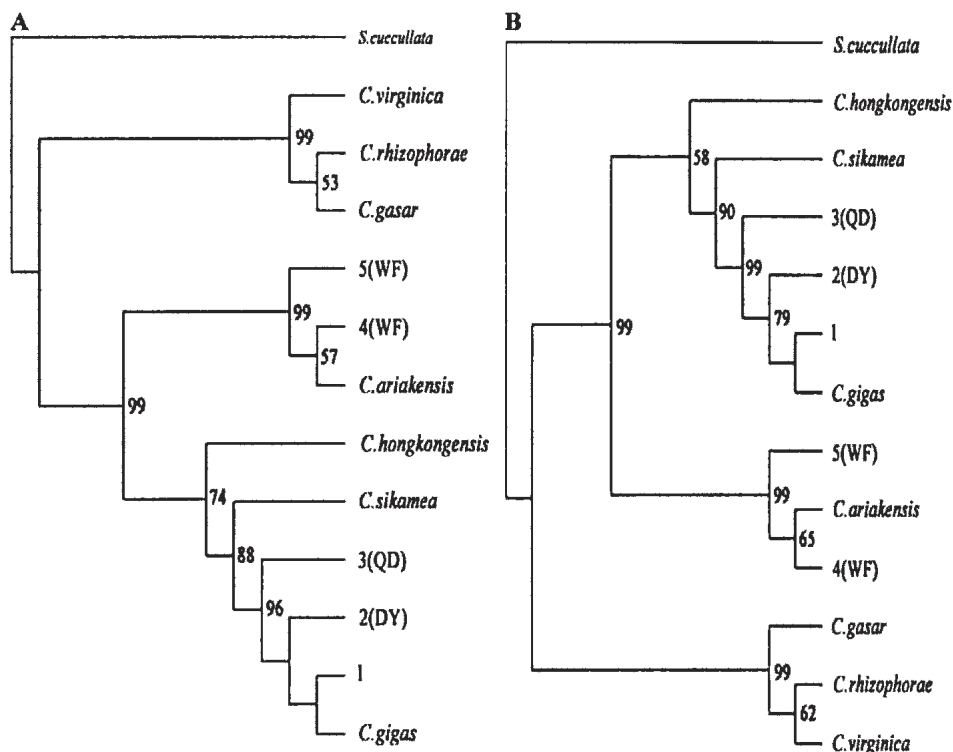


Figure 3. Phylogenetic trees based on parsimony (A) and neighbor-joining (B) analyses of partial mt16S sequence using Kimura two-parameter distances with 1,000 bootstrap. *Saccostrea cucullata* was used as an outgroup. Haplotypes 1 ~ 5 were obtained in this study. Number at each step refers to the bootstrap values when larger than 50.

Phylogenetic analysis was conducted with the five 16S haplotypes identified in this study and the following reference sequences from GenBank: *C. gigas* (S66183), *C. ariakensis* (Kim AY007427), *S. cucullata* (AF498507), *C. virginica* (AF092285), *C. rhizophorae* (AJ312938), *C. gasar* (AJ312937), *C. hongkongensis* (AY160756), and *C. sikamea* (AY632551). *S. cucullata* was used as an outgroup. Including the outgroup, 26 nucleotide positions were variable in the 16S data set. Phylogenetic

analysis using NJ and MP procedures produced almost identical results (Fig. 3A, B). As expected, all five haplotypes identified in this study were clustered with members of *Crassostrea*, away from *S. cucullata*. The two haplotypes from Weifang were closely clustered with *C. ariakensis*, and formed one clade. The two haplotypes differed from the reference sequence by only one base. A closer examination indicated that Haplotype 4 was the same sequence as that of *C. ariakensis* from Korea (Kim

TABLE 3.
Pair-wise divergence among five mt16S rRNA haplotypes obtained in this study (1–5) and reference species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 (all)	0													
2 (DY)	0.0021	0												
3 (QD)	0.0021	0.0043	0											
<i>C. gigas</i>	0.0000	0.0021	0.0021	0										
<i>C. sikamea</i>	0.0173	0.0195	0.0195	0.0173	0									
4 (WF)	0.0488	0.0510	0.0510	0.0488	0.0442	0								
5 (WF)	0.0465	0.0488	0.0488	0.0465	0.0419	0.0021	0							
<i>C. ariakensis</i>	0.0465	0.0488	0.0488	0.0465	0.0419	0.0000	0.0036	0						
<i>C. hongkongensis</i>	0.0239	0.0261	0.0261	0.0239	0.0239	0.0285	0.0263	0.0307	0					
<i>C. gasar</i>	0.1967	0.1967	0.1995	0.1967	0.1908	0.1995	0.1995	0.1967	0.1967	0				
<i>C. rhizophorae</i>	0.1859	0.1859	0.1831	0.1859	0.1884	0.2034	0.2034	0.2005	0.1943	0.1181	0			
<i>C. virginica</i>	0.2027	0.206	0.1993	0.2027	0.2156	0.2305	0.2271	0.2271	0.2194	0.2349	0.1446	0		
<i>S. cucullata</i>	0.1772	0.1799	0.1744	0.1772	0.1747	0.1744	0.1717	0.1717	0.1690	0.2567	0.2335	0.2913	0	
<i>Alectryonella plicatula</i>	0.2165	0.2195	0.2135	0.2165	0.2139	0.1980	0.1980	0.1951	0.2046	0.2960	0.2576	0.3308	0.1744	0

TABLE 4.
Distribution of 13 mt COI haplotypes obtained in this study.

Site	Haplotype												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ZH	4	0	0	0	1	1	0	0	0	0	0	0	0
ZZD	4	0	0	0	0	0	0	0	1	1	0	0	0
DL	4	2	0	0	0	0	0	0	0	0	0	0	0
DY	5	0	1	0	0	0	0	0	0	0	0	0	0
WF	0	0	0	0	0	0	0	0	0	0	4	1	1
RC	5	0	0	0	0	0	1	0	0	0	0	0	0
RS	5	0	0	0	0	0	0	1	0	0	0	0	0
QD	5	0	0	1	0	0	0	0	0	0	0	0	0
LYG	6	0	0	0	0	0	0	0	0	0	0	0	0

et al. 2000). The three haplotypes from all other eight sites were closely clustered with *C. gigas*. Haplotype 1 is identical with the reference sequence from *C. gigas*. Haplotype 2 and Haplotype 3 differed from the *C. gigas* sequence by one base. Sequence divergence between Haplotypes 1–3 and Haplotypes 4–5 was about 4.65% to 5.10%, which was higher than that between *C. gigas* and *C. sikamea* (1.73%) (Table 3). No divergence was observed between Haplotype 1 and *C. gigas*, the divergence between Haplotype 2/3 and *C. gigas* was only 0.21%. There was no divergence between Haplotype 4 and *C. ariakensis* from Korea, and the divergence between Haplotype 5 and *C. ariakensis* was only 0.36%, which is much lower than that between *C. gigas* and *C. sikamea* (1.73%). These data show that oysters from Weifang are *C. ariakensis*, and oysters from all other sites are *C. gigas*. The haplotypes obtained from this study are neither *S. cucullata* nor *A. plicatula*, as evidenced by the high divergences, 17.44% to 21.95% (Table 3).

Mitochondrial COI DNA Sequence

A 607 bp fragment of the COI sequences was sequenced for the 54 oysters selected in this study, generating 13 haplotypes (Table 4). Three (Haplotype 11, 12, 13) of these haplotypes occurred in Weifang oysters, four oysters had Haplotype 11, and the other two oysters had Haplotype 12 and 13, respectively. The majority of oysters from the other eight sites (38 of 48) shared a common haplotype—Haplotype 1. Haplotype 2 was shared by two oysters from Dalian. Haplotypes 3–10 were represented by one oyster each from variable sites (Table 4).

The partial COI sequences from the 13 haplotypes obtained in this study were subjected to phylogenetic analysis along with the following reference sequences from GenBank: *C. gigas* (AF152565), *C. iredalei* (AY038078), *C. belcheri* (AY038077), *C. nippona* (AF300616), *C. hongkongensis* (AY160746), *C. virginica* (AF152566), *S. cucullata* (AY038076), and *C. sikamea* (AY632568). *S. cucullata* was used as an outgroup. Including the outgroup, 121 nucleotide positions were variable in the COI data set. The NJ and MP trees were nearly identical, and both supported the same general patterns: (1) the oysters in Weifang clustered together with *C. ariakensis*; (2) the oysters from other sites clustered together with *C. gigas* (Fig. 4). Haplotype 1 is identical to the *C. gigas* sequence, and Haplotype 11 is identical to *C. ariakensis*.

Sequence divergence between Haplotypes 1–10 and Haplotypes 11–13 ranged from 16% to 16.7%. There was no divergence between Haplotype 1 and *C. gigas*, and between Haplotype 11 and *C. ariakensis*. The divergence between Haplotypes 2 ~ 10 and *C. gigas* was about 0.2% ~ 0.4%. The divergence between Haplotype 12 ~ 13 and *C. ariakensis* was 0.2%. These levels of divergence were consistent with intraspecific variation and considerably smaller than that

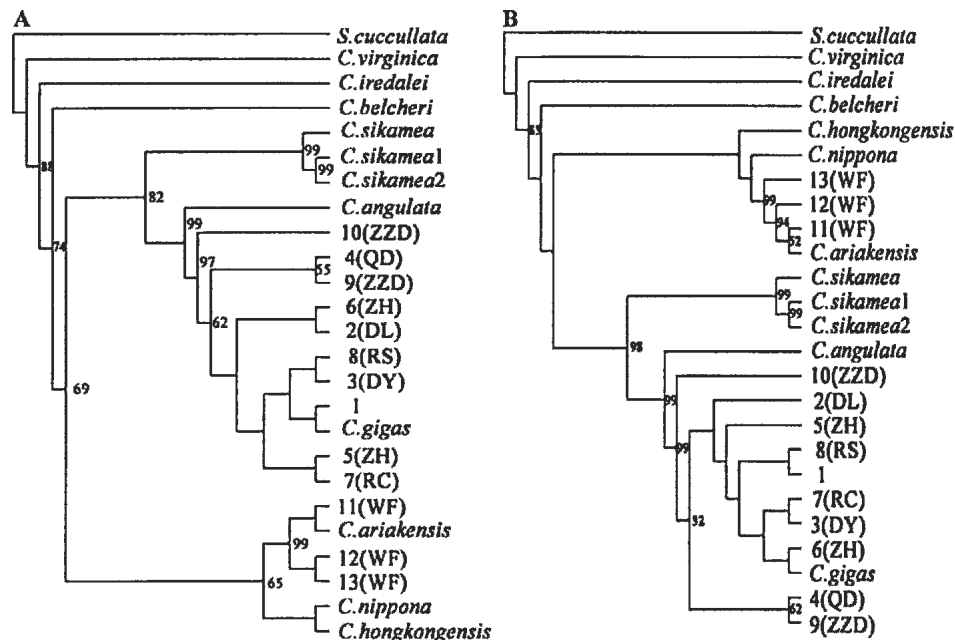


Figure 4. Phylogenetic trees based on parsimony (A) and neighbor-joining (B) analyses of partial COI sequence using Kimura two-parameter distances with 1,000 bootstrap. *Saccostrea cucullata* was used as an outgroup. Haplotypes 1 ~ 13 are obtained in this study. Number at each step refers to the bootstrap values when larger than 50.

between *C. gigas* and *C. sikamea* (9.74%) and that between *C. gigas* and *C. angulata* (2.46%). Divergences between the 13 haplotypes of this study and that of *C. sikamea*, *C. ariakensis*, *S. cucullata* were too high (9.74% to 31.70%) to be conspecific (Table 5).

Nuclear 28S rRNA Sequence

A 945 bp fragment of the nuclear 28S rRNA gene was sequenced for all 54 oysters collected in this study. Only two haplotypes were identified: one (Haplotype 1) for the 48 oysters from ZH, ZZD, DL, DY, RC, RS, QD, and LYG and the other (Haplotype 2) for the six oysters from Weifang.

Phylogenetic analysis was conducted with the two haplotypes identified in this study and reference sequences from GenBank: *C. gigas* (AF137051), *C. ariakensis* (AF137052), *C. virginica* (AF137050), *C. rhizophorae* (AF137049), *C. belcheri* (Z29545), *S. cucullata* (Z29553), *S. commercialis* (Z29552), and *C. sikamea* (AY632554). *S. cucullata* was used as an outgroup. Including the outgroup, 33 nucleotide positions were variable in the 28S data set. In NJ and MP trees, oysters from Weifang formed one clade with *C. ariakensis*, and oysters from other sites were identified with *C. gigas* (Fig. 5).

No divergence was observed between Haplotype 1 and *C. gigas* and between Haplotype 2 and *C. ariakensis*, and the oysters collected in this study were clearly different from *C. sikamea*, *S. cucullata*, and *A. plicatula* (Table 6).

DISCUSSION

Classification of the Oysters in North China

The taxonomic status of the oysters in north China has been the subject of much confusion. Because the oysters are typically

found on intertidal rocks, their morphological characteristics vary greatly because of the stressful and variable environment they live in. Consequently, classification based on shell characteristics has been difficult, and different studies have reached different conclusions (Zhang & Lou 1956, Qi 1989, Li & Qi 1994, Xu & Huang 1993). The existence of *C. gigas* and *C. rivularis* in north China is well accepted, although the two species have often been misidentified by different authors (Zhang & Lou 1956, Li & Qi 1994). It has been shown that the *C. rivularis* from Bohai Sea in north China is the same species as *C. ariakensis* (Wang et al. 2004). Two questions remain unresolved: (1) what species are the small oysters that are commonly found in intertidal zones along the northern coast? and (2) is *C. talienwhanensis* an independent species?

The Small Intertidal Oysters

The small intertidal oysters from north China were first named the monk-hat oyster (*O. cucullata*) by Zhang & Lou (1956) and then the folded oyster *C. plicatula* by Zhao et al. (1982). After analyzing anatomic characteristics, Li & Qi (1994) concluded that neither classification was correct, and the small intertidal oysters were actually *C. gigas*. On the other hand, a genetic analysis suggested that *C. plicatula* was an independent species (Liu & Dai 1998).

In this study, we collected the common oysters from nine sites and sequenced three gene fragments for phylogenetic analysis. Our data support Li & Qi's (1994) classification that the small intertidal oysters from all sites (except Weifang) are *C. gigas*. This is clear from all three genes studied. At the mt16S gene, the common haplotype shared by 96% of the oysters from the eight sites (excluding Weifang) are identical to that of *C. gigas*. The remaining two haplotypes are minor variants of *C. gigas* haplotypes, as shown by the small sequence divergences (Table 3). For the mtCOI gene,

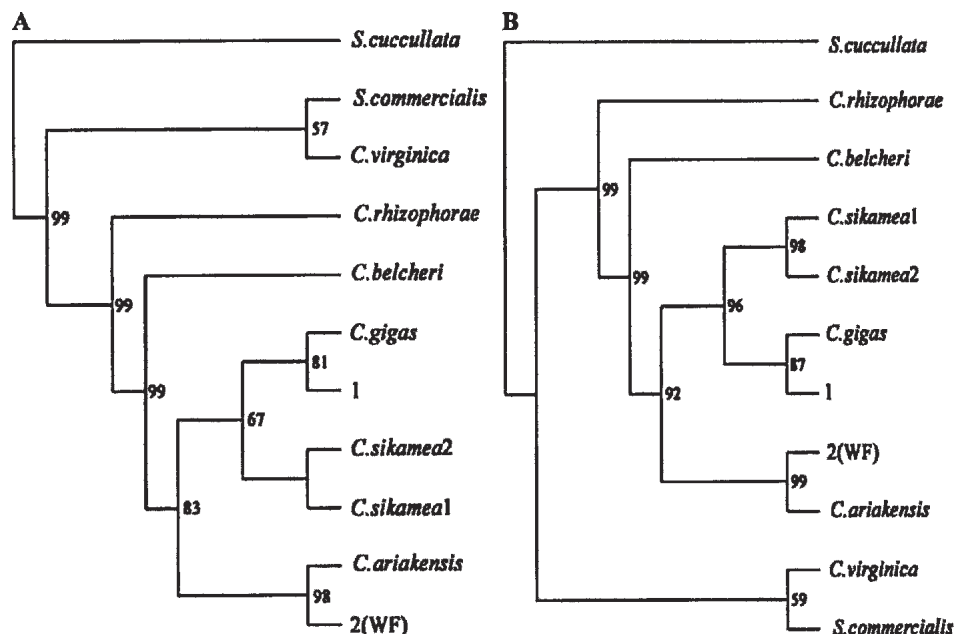


Figure 5. Phylogenetic trees based on parsimony (A) and neighbor-joining (B) analyses of partial 28SrRNA sequence using Kimura two-parameter distances with 1,000 bootstrap. *Saccostrea cucullata* was used as an outgroup. Haplotypes 1 and 2 were obtained from this study. Numbers at each step refers to the bootstrap values when larger than 50.

TABLE 6.
Pair-wise divergence among two 28S rRNA sequences obtained from this study and reference species.

	1	2	3	4	5	6	7	8	9	10	11
1 (all)	0										
<i>C. gigas</i>	0.0000	0									
2 (WF)	0.0111	0.0111	0								
<i>C. ariakensis</i>	0.0111	0.0111	0.0000	0							
<i>C. sikamea1</i>	0.0033	0.0033	0.0100	0.0100	0						
<i>C. sikamea2</i>	0.0022	0.0022	0.0089	0.0089	0.0000	0					
<i>C. belcheri</i>	0.0271	0.0271	0.0294	0.0294	0.0260	0.0249	0				
<i>C. rhizophorae</i>	0.0798	0.0798	0.0797	0.0797	0.0835	0.0824	0.0853	0			
<i>C. virginica</i>	0.0684	0.0684	0.0707	0.0707	0.0709	0.0697	0.0774	0.0848	0		
<i>S. cucullata</i>	0.0685	0.0685	0.0709	0.0709	0.0711	0.0699	0.0776	0.0876	0.0090	0	
<i>Alectryone plicatula</i>	0.0957	0.0957	0.0983	0.0983	0.0970	0.0959	0.1130	0.1222	0.0807	0.0835	0

although many haplotypes are observed, the common haplotypes are identical with that of *C. gigas*, and all other haplotypes are minor variants and closely clustered with *C. gigas* (Fig. 4). The highly conserved 28S rRNA genes show that all intertidal oysters have the same haplotypes as *C. gigas*.

The small intertidal oysters are clearly not *O. cucullata* or *C. plicatula*, two species now known as *S. cucullata* and *A. plicatula*, respectively (Li & Qi 1994). This is evident from both morphological and molecular data. The molecular data show that the divergence between the oysters sampled in this study and *S. cucullata* is larger than that between any sister-species within *Crassostrea*. Similarly, oysters from this study are clearly not the folded oyster (*A. plicatula*) as indicated by large divergences in mt16S and 28S sequences (Table 3 and 6).

Except for the small size, the intertidal oysters collected in this study have similar characteristics as *C. gigas* (Li & Qi 1994, Torigoe 1981, Okutani 2000), albeit more variable. The small size is probably caused by limitations in food as a consequence of living in the intertidal zone. The strong wave action may also force oysters to stick close to the substrate, causing the shells to be flat and irregular in shape.

The Large Subtidal Oysters from Weifang

In shell morphology and DNA sequences, oysters collected from deep waters at Weifang are different from oysters from other sites. In morphology, the Weifang oysters have distinctive smooth shells with layered concentric plates and a white adductor muscle scar. In DNA sequence, all three genes show that the Weifang oysters are different from oysters from other sites (shared no haplotypes). Phylogenetic analysis clearly indicates that the Weifang oysters, which are referred to as jinjiang or near-river oyster *C. rivularis* locally, are indeed *C. ariakensis*. This finding is in agreement with our previous studies (Wang et al. 2004, Wang 2004). The shell morphology of the Weifang oyster is almost identical to that of *C. ariakensis* from Ariake Bay in Japan (Okutani 2000).

Status of the Dalianwan Oyster (*C. talienwhanensis*)

Some believe *C. talienwhanensis* is an independent species (Zhang & Lou 1956, Liu & Dai 1988), whereas others consider it

a synonym of *C. gigas* (Li & Qi 1994). We consciously collected oysters from three sites in and around Dalian. Some of the oysters we collected, especially those from Dalian and Dongying had obvious radial ribs and purple strips that are characteristic of *C. talienwhanensis*. However, the molecular data indicate that they are the same species as *C. gigas*. It is possible that our sampling missed true *C. talienwhanensis*, but available data from this study do not support the existence of the Dalianwan oyster (*C. talienwhanensis*) as an independent species.

Lapegue et al. (2004) reanalyzed the sequences of Yu et al. (2003) and showed that some of the *C. talienwhanensis* sequences are possibly *C. angulata*. Our analysis finds no *C. angulata* from Dalian or any of the nine sites in north China. The COI haplotypes obtained from this study are clearly different from that of *C. angulata* (Table 5). In another analysis of thousands of oysters from China, we observed *C. angulata* in southern China but never north of Yangtze River (Guo et al. 2006). It is possible that our sampling missed *C. angulata* from north China. It is also possible that the *C. angulata* samples of Yu et al. (2003) may not be native to Dalian as there is considerable cross-regional transportation of oyster seed in China. Anyway, further research is needed to determine if there is *C. angulata* in north China.

In summary, this study provides clear molecular evidence that the common oysters from north China belong to two species: the small intertidal oysters are *C. gigas* and the large subtidal oysters from Weifang are *C. ariakensis*. We see no evidence for the existence of *C. angulata* and *C. talienwhanensis* in Dalian, and the latter is probably synonymous with *C. gigas*.

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