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# Mitochondrial DNA and morphological identification of *Crassostrea zhanjiangensis* sp. nov. (Bivalvia: Ostreidae): a new species in Zhanjiang, China

Xiangyun Wu, Shu Xiao and Ziniu Yu[a]

CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, P.R. China

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**Abstract –** Cupped oysters (Ostreidae, genus *Crassostrea*) occupy nearshore marine and estuarine habitats worldwide, providing many ecosystem goods and services as well as being a commercially important group of bivalves. In this study, the species identification of an "adulterant" oyster with small body size, which is often misidentified as a "young individual" of other sympatric species, including *C. sikamea* and *C. hongkongensis*, was determined for the first time, based on molecular markers (partial mitochondrial *cox1* and *rrnL* genes), phylogenetic analysis, and morphometric approaches. This novel species, *C. zhanjiangensis*, commonly known as the "cat ear oyster" in Guandu (a famous estuarine oyster farming region of Zhanjiang, Guangdong Province, China), appears to potentially influence the efficiency of *C. hongkongensis* spat collection due to niche competition on spat collection devices. Phylogenetic analyses confirm its status as the most basal taxon of the Indo-Pacific *Crassostrea.* A comparative study of the shell characteristics of *C. zhanjiangensis*, and other *Crassostrea* species revealed several distinctive morphological traits, including a generally smaller body size, a deeply cupped left valve, and a right valve that is convex in adults but flat in young individuals. Other distinctive features of the new species include life cycle traits that are unique compared with the sympatric *C. hongkongensis* and *C. sikamea* species, such as a higher growth rate in the fast growth phase after settlement, followed by a significantly slower growth rate and mass mortality during subsequent life stages. This study provides the basic information necessary for further ecological and population genetic studies on this new species.

**Keywords:** Mitochondrial gene / PCR / Molecular identification / Phylogeny / New species / Oyster / *crassostrea*

## 1 Introduction

Oysters (Family Ostreidae) occupy nearshore marine and estuarine habitats in temperate to tropical latitudes worldwide (Ruesink et al. 2005). Being ecosystem engineers, they can provide many ecosystem goods and services by creating habitat then used by other species, and can modify the physical and chemical environment, with major consequences for estuarine populations, communities, and food webs (Jackson et al. 2001; Ruesink et al. 2006). Oysters of the genus *Crassostrea* are a widespread and commercially important group of bivalves. The coastal areas along the South China Sea are species-rich regions of *Crassostrea* oysters Their cultivation is reputed to have several hundred years' history (Lam and Morton 2003) and is now becoming one of the largest coastal industries, with recent annual landings of around 3.5 million tons (nearly 78% of total world production) (Xia et al. 2009). Three oyster species dominate oyster aquaculture in the coastal waters of China; in order from north in Liaoning-Shandong, Fujian, to south in the Guangdong-Guangxi provinces these are: *C. gigas* (the Pacific oyster), *C. angulata* (the Portuguese oyster) and *C. hongkongensis* (the Hong Kong oyster) (Xia 2008; Xia et al. 2009; Wu et al. 2010; Li et al. 2013). In recent years, increasing pressure from local markets in Hong Kong, Macau, Taiwan, and mainland China have prompted expansion in the scale of oyster farming in China. Although oyster hatcheries are widely used for seed production, spat collection from the wild is still extensively practiced for Hong Kong and Portuguese oysters. Spat collection is economical, but both the quality and quantity of *C. hongkongensis* spat, from which is grown the most popularly cultured oyster in southern China, are affected by the variable flow of coastal rivers. Furthermore, oyster spat collected from different areas exhibit differences in production traits such as growth rate, meat flavour, and even disease resistance. Several areas still produce good quality oyster seed, including Huian and Putian in Fujian Province for *C. angulata*, and the Hengqin-Sanzhao area (Zhuhai), Beijin Harbour (Yangjiang) and Guandu (Zhanjiang) in Guangdong Province for the Hong Kong oyster (Xiao and Yu 2008; Li et al. 2013). Spat collected in these areas is usually cultured

[a] Corresponding author: carlzyu@scsio.ac.cn

**Table 1.** Species identification of *Crassostrea* oysters collected from oyster farms in different locations and at indicated dates.

| Location | Date | Number of individuals | Species (number; percentage) |
|---|---|---|---|
| Guandu, Zhanjiang | November, 2011 | 128 | *C. hongkongensis* (76; 59%)<br>*C. zhanjiangensis* (43; 34%)<br>*C. sikamea* (9; 7%) |
| Donghai Island, Zhanjiang | December, 2011 | 84 | *C. hongkongensis* (46; 55%)<br>*C. angulata* (38; 45%) |
| Guandu, Zhanjiang | May, 2012 | 187 | *C. hongkongensis* (83; 44%)<br>*C. zhanjiangensis* (89; 48%)<br>*C. sikamea* (15; 8%) |
| Henqing, Zhuhai | August, 2012 | 66 | *C. hongkongensis* (66; 100%) |

locally, but is also sold for cultivation elsewhere. Thus, efficiency of spat collection can potentially affect not only local oyster cultivation but also the oyster industry along the coast of the South China Sea. The most important factor influencing the efficiency of spat collection is niche competition on spat collection devices (e.g., concrete tiles) by sympatric species.

This study was initiated following a series of field investigations of the Hong Kong oyster in the farming regions of Zhanjiang, Guangdong Province (Li et al. 2013). Our original intention was to collect specimens of cultured young *C. hongkongensis* individuals for population genetic analysis. As the shell morphology of this species is phenoplastic and greatly affected by habitat, we employed partial mitochondrial *cox1* gene sequencing as a molecular marker for species identification. Unexpectedly, a very high proportion of the randomly sampled specimens was found to represent oyster species other than *C. hongkongensis*. According to descriptions from experienced local oyster farmers, most non-Hong Kong oysters are considered to be *C. sikamea* (the Kumamoto oyster), a species sympatric with *C. hongkongensis* and commonly considered as an "adulterant oyster" in Hong Kong oyster aquaculture. Our initial molecular data supported this claim; however, further studies unexpectedly identified some *cox1* sequences that match neither those of *C. sikamea*, nor those of any other known species of the genus *Crassostrea*. Therefore, it appears these individuals represent a hitherto undescribed species from this region.

The objective of this study was to determine the specific identity of this Zhanjiang oyster species using molecular markers (partial mitochondrial *cox1* and *rrnL* genes), phylogenetic analysis, and morphometric approaches. Morphological descriptions of shell characters and a brief record of the oyster life history obtained in this study will provide the basic information necessary for further ecological and population genetic studies of this new species.

## 2 Materials and methods

### 2.1 Sample collection

Samples of the unidentified species of oyster were collected from oyster farms in different locations at different times (Table 1). For each collection, individuals were removed from randomly selected strings holding concrete spat collectors ($10 \times 10 \times 1.5$ cm) or oyster shells and transported to the laboratory alive.

### 2.2 DNA extraction, PCR amplification and sequencing

Whole genomic and mitochondrial DNA of each individual was extracted from adductor muscle or visceral mass using the TIANamp Marine Animals DNA kit (Tiangen, Beijing). A partial *cox1* segment was amplified by polymerase chain reaction (PCR) with primer pairs of COIL1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and COIH2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). The primer pair of 16sar (5′-CGCCTGTTTATCAAAAACAT-3′) and 16sbr (5′-CCGGTCTGAACTCAGA TCACGT-3′) was used to amplify the partial *rrnL* segment. PCR reactions were performed in a 25 $\mu$l volume with 0.5 $\mu$l template DNA (approximately 30 ng), 0.5 $\mu$l 10 mM dNTP mix, 2.5 $\mu$l 10 × buffer ($Mg^{2+}$ plus), 1 $\mu$l of each primer (10 $\mu$M), and 0.25 $\mu$l (1 U) Ex*Taq* polymerase (Takara, Dalian, China). The PCR reactions were performed on an ABI Veriti thermal cycler (Applied Biosystems, California, USA) with the following parameters: pre-denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 20 sec, 45−55 °C annealing temperature (52° for *cox1* and 50 °C for *rrnL*) for 20 sec, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. PCR products were separated by electrophoresis on a 1% agarose gel, purified with QIAquick PCR Purification kit (QIAGEN, California, USA) and bi-directionally sequenced on an ABI 3730xl DNA Sequencer (Applied Biosystems, California, USA).

### 2.3 Molecular identification and phylogenetic analysis

A basic local alignment search (nucleotide blast) was performed to find regions of local similarity between sequences in Genbank for each cytochrome c oxidase subunit I (*cox1*) and large mitochondrial ribosomal RNA subunit (*rrnL)* sequence examined in this study. To confirm the taxonomic status of the morphologically novel species obtained in this study, *cox1* and *rrnL* sequences of the new species and those of other species of *Crassostrea*, *Ostrea* and *Saccostrea* available from Genbank were subjected to phylogenetic analysis (Table 2). It is common to find several sequences representing different isolates of a species in Genbank. Thus, in order to simplify analysis and to

**Table 2.** List of the samples and sequences from Genbank used in this study.

| Species | Locality | *cox1* | *rrnL* |
|---|---|---|---|
| *Crassostrea angulata* | Taiwan, China | NC_012648 | NC_012648 |
| *Crassostrea ariakensis* | Yingkou, Liaonning Province, China | NC_012650 | NC_012650 |
| *Crassostrea belcheri* | Surat Thani, Thailand | EU007466 | |
| *Crassostrea brasiliana* | Santa Catarina State, Brazil | FJ717642 | FJ478029 |
| *Crassostrea gasar* | Unknown | FJ717611 | JF808179 |
| *Crassostrea gigas* | Unknown | NC_001276 | NC_001276 |
| *Crassostrea gryphoides* | Goa, India | EU007489 | |
| *Crassostrea hongkongensis* | Hainan Province, China | NC_011518 | NC_011518 |
| *Crassostrea iredalei* | Wenchang, Hainan Province, China | NC_013997 | NC_013997 |
| *Crassostrea madrasensis* | Goa, India | EU007460 | |
| *Crassostrea nippona* | Ogatsu Bay, Japan | NC_015248 | NC_015248 |
| *Crassostrea rhizophorae* | Santa Catarina State, Brazil | | FJ478030 |
| *Crassostrea sikamea* | Nantong, Jiangsu Prov., China | NC_012649 | NC_012649 |
| *Crassostrea* sp. 1 | Wenchang, Hainan Province, China | HQ661024 | HQ660983 |
| *Crassostrea* sp. 2 | Beihai, Guangxi Prov., China | | HQ660984 |
| *Crassostrea* sp. Canela 1 | Bragança (Canela Island) | HM003525 | |
| *Crassostrea* sp. Canela 2 | Bragança (Furo do Café) | HM003526 | |
| *Crassostrea virginica* | Delaware Bay, USA | NC_007175 | NC_007175 |
| *Crassostrea zhanjiangensis* | Zhanjiang, Guangdong, China | JX899646-52 | JX899653-55 |
| *Saccostrea commercialis* | Moreton Island, Queensland, Australia | | AF353100 |
| *Saccostrea cucullata* | Hoi HaWan, Hong Kong, China | | AF458905 |
| *Saccostrea glomerata* | Hobart, Tasmania, Australia | EU007483 | |
| *Saccostrea mordax* | South China Sea | NC_013998 | NC_013998 |
| *Saccostrea palmula* | Mazatlan, Estero de La, Mexico | | FJ768525 |
| *Ostrea denselamellosa* | Qingdao, Shandong Prov., China | NC_015231 | NC_015231 |
| *Ostrea edulis* | Unknown | NC_016180 | NC_016180 |
| *Ostrea stentina* | Unknown | | JF808189 |
| *Ostrea lurida* | Willapa Bay, USA | | FJ768566 |

avoid using incorrect sequences caused by misidentifications, RefSeq sequences, namely complete mitochondrial genomes proofed by Genbank staff, were used for several species for which they were available.

MEGA 5 (Tamura et al. 2012) was used for sequence alignments. Maximum likelihood (ML), neighbour-joining (NJ) and maximum parsimony (MP) were employed for phylogenetic reconstructions. MP analyses were performed using Phylogenetic Analysis Using Parsimony, PAUP 4.0b10 (Swofford 2002), with a total of 1000 random addition searches using TBR (a tree-rearrangement: tree bisection-reconnection). Bootstrap (BP) values were calculated from 1000 bootstrap replicates with 10 random additions per replicate in PAUP. NJ and ML analyses were performed in MEGA 5, and ML analyses were also performed using PhyML 3.0 online execution (http://www.atgc-montpellier.fr/phyml). We initially used the pearl oyster *Pinctada maxima* (Pteriidae; GQ452847) as an outgroup to investigate the relationship between the three oyster genera *Crassostrea*, *Ostrea* and *Saccostrea*. All analyses yielded topology indicating that *Crassostrea* is a clade separate from *Ostrea* and *Saccostrea* (data not shown). Thus, in order to make maximum usage of alignment characters, we performed phylogenetic inferences for *Crassostrea* species using *Ostrea* spp. and *Saccostrea* spp. as multiple outgroups.

### 2.4 Description of shell characters

Conchological distinctions used for the identification of other *Crassostrea* oysters were employed for the description of shell features and characters of the new species (Lam and Morton 2003; Qi 2004; Huber 2010), including shape and surface sculpture, external and internal shell colour, attachment area of valves, and position, colour, and relative size of the adductor muscle scar.

## 3 Results

### 3.1 Molecular identification and phylogeny

The *cox1* gene was sequenced from a total of 465 oyster specimens and used to identify the oyster species by comparison with sequences available in Genbank (Table 2). Using a Blastn search, 271 sequences were identified as originating

from *C. hongkongensis*, 24 from *C. sikamea* and 38 from *C. angulata*; the remaining 132 sequences were nearly identical to each other, but did not precisely match any sequences in the database (Table 1). These sequences are thought to identify a hitherto undescribed species, now termed *Crassostrea zhanjiangensis*. The partial *rrnL* gene was also sequenced from the mtDNA of these 132 individuals and used for phylogenetic analysis. Among the 132 sequences of each gene, we found three intact haplotypes of *rrnL* (hap 1−3) and seven (hap 1−7) haplotypes of *cox1* (without indels), a variation pattern expected for bivalve mt genes. Sequences representing different haplotypes obtained in this study have been deposited in the Genbank under accession numbers JX899646–JX899655.

The data set based on *cox1* and *rrnL* genes contains a large number of informative characters for phylogenetic analysis. The *cox1* sequences include 574 aligned nucleotide positions, with 222 parsimony-informative characters, while 122 characters are informative in the *rrnL* dataset (of 406 bp total). Although tree topologies and nodal supports are not completely identical between phylogenies based on the *rrnL* and *cox1* datasets, several important similarities can be seen, including 1) monophyly of the three oyster genera (*Crassostrea*, *Ostrea* and *Saccostrea*); 2) separation of the American oysters (*C. virginica*, *C. rhizophorae*, *C. gasar* and *C. brasiliana*) from the Indo-Pacific oyster group; and 3) resolution of the new species, *C. zhanjiangensis*, as the most basal taxon in the Indo-Pacific oyster group (Fig. 1).

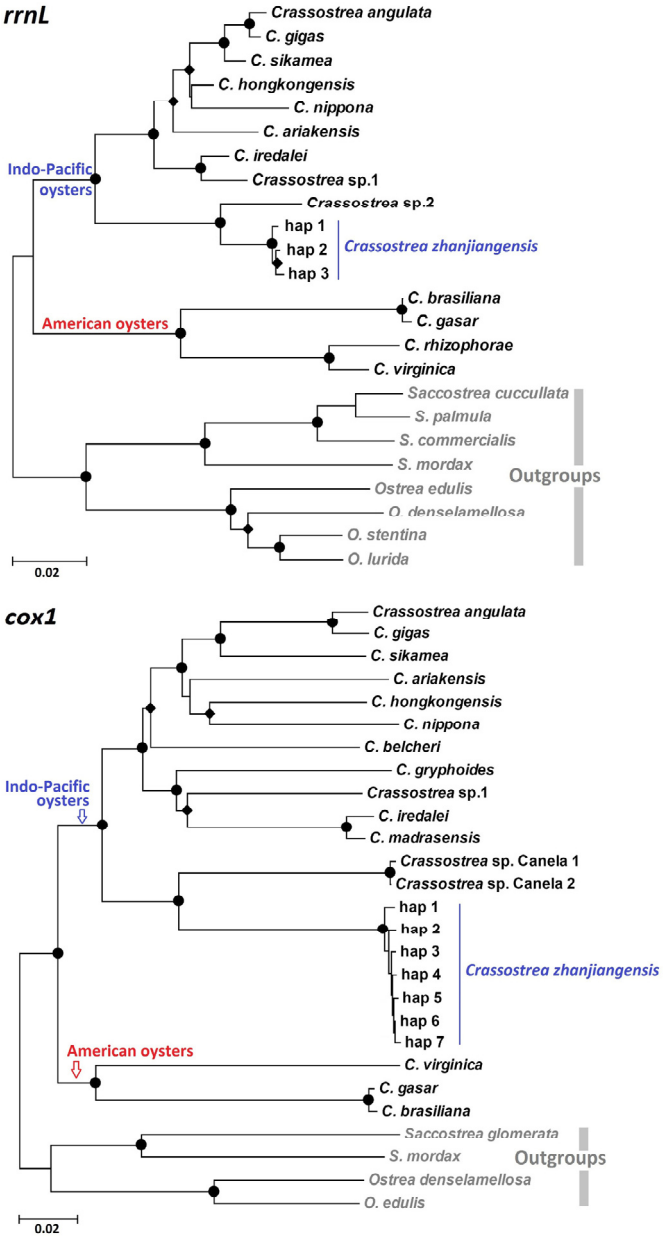


**Fig. 1.** Phylogenetic reconstruction of *Crassostrea* oysters using partial mitochondrial *rrnL* and *cox1* gene sequences. The neighbour-joining (NJ) tree is shown. Bootstrap (BP) values were calculated for maximum likelihood (ML), NJ and maximum parsimony (MP) analyses. Nodes with high statistical support (BP $\geqslant$ 75) from all three phylogenetic methods (i.e. NJ, ML and MP) are marked with a solid circle while moderate supports (50 $\leqq$ BP < 75) are marked with a rhombus. Three haplotypes of *rrnL* (hap 1−3) and seven (hap 1−7) haplotypes of *cox1* of *C. zhanjiangensis* were presented.

## 3.2 Species description

Systematics

Order Ostreoida Ferussac, 1822

Suborder Ostreina Ferussac, 1822

Superfamily Ostreoidea Rafinesque, 1815

Family Ostreidae Rafinesque, 1815

*Crassostrea zhanjiangensis* new species

### 3.2.1 Type measurements and deposition

The holotype specimen and one paratype specimen comprised of dry shells and tissues preserved in 95% ethanol have been deposited in the Marine Biodiversity Collection of South China Sea, Guangzhou. The holotype and all paratype specimens were genetically identified. Shell measurements of the type materials are shown in Table 3.

### 3.2.2 Description of the holotype

The shell of *C. zhanjiangensis* elongates dorso-ventrally with a spatulate form, being broader toward the ventral margin and tapering dorsally (Fig. 2). The left valve is deeply cupped and the right valve is slightly convex. The margins of both valves are slightly fluted. The attachment area is small and appears as a patch at the umbone, with the rest of the left valve surface unattached. Concentric growth increment lines spreading from the umbone of the right valve give rise to yellow lamellae. The direction of growth of the left valve is counter to that of the right, producing concentric and slightly erect layers of old ventral margins during each season of faster growth.

**Table 3.** Shell measurements of the type materials.

| Accession number | Height (mm) | Length (mm) | Depth (mm) | Notes (mm) |
|---|---|---|---|---|
| Holotype SCSMBM 001617 | 25.7 | 30.5 | 16.5 | Dark purple adductor muscle scar, adult from an oyster farm |
| Paratype 1 SCSMBM 001618 | 16.3 | 12.4 | 4.6 | White adductor muscle scar, young individual from an oyster farm |
| Paratype 2 SCSMBM 001619 | 18.3 | 12.8 | 10.1 | Dark purple adductor muscle scar, adult from rocky shore next to an oyster farm |
| Paratype 3 SCSMBM 001620 | 14.5 | 13.6 | 5.1 | White adductor muscle scar, young individual from an oyster farm |

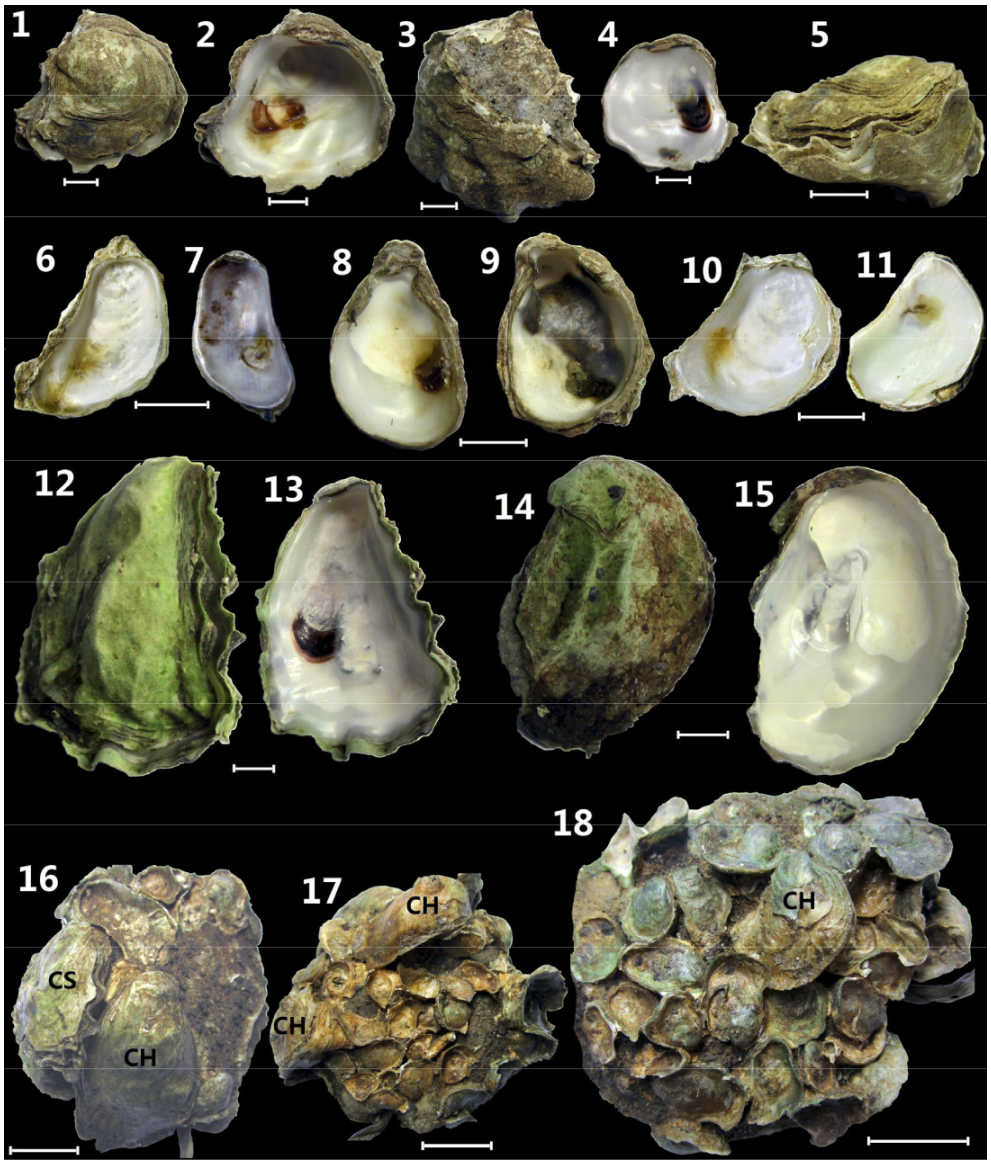


**Fig. 2.** Types of *Crassostrea zhanjiangensis* collected from Guandu, Zhanjiang. (1) external view of the right valve of the holotype; (2) internal view of the left valve of the holotype; (3) external view of the left valve of the holotype; (4) internal view of the right valve of the holotype; (5) anterior view of the holotype; (6) internal view of the left valve of paratype 1; (7) internal view of the right valve of paratype 1; (8) internal view of the left valve of paratype 2; (9) internal view of the right valve of paratype 2; (10) internal view of the left valve of paratype 3; (11) internal view of the right valve of paratype 3; (12) external view of the left valve of the *C. sikamea*; (13) internal view of the right valve of the *C. sikamea*; (14) external view of the left valve of the *C. hongkongensis*; (15) internal view of the right valve of the *C. hongkongensis*; and (16-18) oysters on concrete tiles collected from an oyster farm; CH: *C. hongkongensis*; CS: *C. sikamea*. Bar scale: 1-15, 5 mm; 16-18, 25 mm.

No commissural projections are present on either inner shell. The umbonal cavity is very deep on the left valve. The outer shell surface is chartreuse while the interior of the shell is translucent white. The adductor muscle scar is large, located toward the posterior side between the middle and ventral third of the pallial area, and is light purple and oval-shaped in the left valve and dark purple and D-shaped in the right valve.

#### 3.2.3 Variability in shell characters

*Crassostrea zhanjiangensis* generally has a small body size compared to other members of the genus. The shell morphology of this species is phenoplastic and greatly affected by niche, as seen in congeneric oysters. The left valve is usually deeply cupped as seen in the holotype and paratype 2 specimens (Fig. 2). The right valve is usually convex in adults and flat in young individuals. The attachment area is variably small, usually not more than half the shell height. The adductor muscle scars varied in colour from dark purple to white with light purple growth lines.

#### 3.2.4 Anatomy

The anatomy of *C. zhanjiangensis* is indistinguishable from that of other identified *Crassostrea* species as described by Torigoe (1981).

#### 3.2.5 Ecology

*C. zhanjiangensis* occurs both on oyster farms and in the wild, where individuals can be found on intertidal and subtidal rocks along the shoreline of Guandu, Zhanjiang, that is, under estuarine conditions. Animals with mature gonads are present during the period from April to September with spawning occurring primarily in summer (June-August). In Guandu, three species, *C. hongkongensis*, *C. zhanjiangensis* and *C. sikamea*, appear to have strong niche overlap; in particular, the mass spawning time of *C. hongkongensis* and *C. zhanjiangensis* is almost identical.

The life history strategies of *C. hongkongensis* and *C. zhanjiangensis* may be distinguished in two ways in stages after larval attachment. *C. zhanjiangensis* uses a fast-growth strategy during the first growth phase, and hence is likely to prevail in areas where it settles (e.g., on concrete tiles) (Fig. 2: 17-18). Compared with the Hong Kong and Kumamoto oysters, the growth rate of *C. zhanjiangensis* slows down after its first fast growth phase. At this juvenile stage, *C. zhanjiangensis* exhibits mass mortality, a life history trait that is also unique.

#### 3.2.6 Etymology

This is the first new species of the Ostreidae recorded from Zhanjiang (21°11′N, 110°24′E), Guangdong Province, China, and is thus named after the locality in which the oyster was originally found. In Guandu, this oyster is known as the "cat ear oyster" because the shells of juvenile individuals on concrete tiles are usually shaped like cat's ears.

## 4 Discussion

The majority of known oyster species were described on the basis of shell morphology. However, phenotypic plasticity means that shell characters alone may not be adequate to identify species of *Crassostrea*, particularly for young individuals. Instead, both morphological and genetic characteristics should be considered when identifying species. The most recently described new species, *C. hongkongensis*, was the first commercially important taxon with plastic morphology to be identified using genetic data (Lam and Morton 2003). Subsequently, both nuclear and mitochondrial genes have been used to distinguish species and to infer phylogenetic relationships (Boudry et al. 2003; Wang et al. 2004; Camara et al. 2008; Reece et al. 2008; Xia et al. 2009; Wu et al. 2010; de Melo 2010; Liu et al. 2011). We note that the phenotypic plasticity of *C. zhanjiangensis* shell morphology and its comparatively small body size prevented researchers from identifying this species previously. Instead, specimens of *C. zhanjiangensis* were often misidentified as young individuals of other sympatric species, including *C. sikamea* and *C. hongkongensis*.

We identified *C. zhanjiangensis* as a new species based on evidence from the following sources. First, its shell shape is similar to that of other *Crassostrea* species. In particular, both inner shells have no commissural projections; this is a primary synapomorphy of the genus *Crassostrea*, not shared by species from closely related genera such as *Saccostrea* and *Ostrea*. Secondly, mtDNA sequence-based phylogenetic analyses unambiguously confirm its taxonomic status as a member of the Indo-Pacific *Crassostrea*. Our inference is reasonable because 1) the molecular markers (i.e., partial *cox1* and *rrnL* genes) used in this study have been widely and successfully used in phylogenetic reconstructions of marine bivalves including scallops (Mahidol et al. 2007; Puslednik and Serb 2008), mussels (Wood et al. 2007) and clams (Kappner and Bieler 2006; Chen et al. 2011); and 2) molecular phylogeny inferred in this study unambiguously agrees with the morphological taxonomy of true oysters (i.e., Ostreidae family), particularly the well-supported monophyly of Indo-Pacific *Crassostrea*, American *Crassostrea*, *Saccostrea* and *Ostrea*. Additionally, studies of the morphological characteristics of the shells of *C. zhanjiangensis* and other Indo-Pacific *Crassostrea* species have shown that there are several distinctive traits that distinguish the new species from other known species. In China, at least nine *Crassostrea* oysters have already been described based on morphological characters, including *C. gigas*, *C. angulata*, *C. sikamea*, *C. ariakensis*, *C. hongkongensis*, *C. belcheri*, *C. nippona*, *C. iredalei* and *C. vitrefacta* (Xia 2008). DNA sequence data are available for most *Crassostrea* oysters in China, with the exception of *C. vitrefacta.* In this case, the morphological features of the *C. vitrefacta* type specimen are distinguishable from those of *C. zhanjiangensis* in having strong chomata along the margins of both valves and radiating colour bands on the right valve (Lam and Morton 2003).

In order to determine whether *C. zhanjiangensis* is a synonym of other non-Indo-Pacific *Crassostrea* species, we reviewed all species of this genus listed in WoRMS (World Register of Marine Species, http://www.marinespecies.org), WMSDB (Worldwide Mollusc Species Data Base, http://www.bagniliggia.it/WMSD/WMSDhome.htm), eoL (Encyclopedia

**Table 4.** List of *Crassostrea* species in different databases.

| Species | WoRMS (18) | WMSDB (22) | Huber M. 2010 (12) | eoL (11) | NCBI (14) | Distributions |
|---|---|---|---|---|---|---|
| *C. aequatorialis* (d'Orbigny, 1846) | √ | – | – | – | – | Tropical West America |
| *C. angulata* (Lamarck, 1819) | × | √ | – | – | √ | France |
| *C. ariakensis* (Fujita, 1913) | √ | √ | √ | – | √ | Indo-Pacific |
| *C. belcheri* (G.B. Sowerby II, 1871) | √ | √ | √ | – | √ | Philippines |
| *C. bilineata* (Röding, 1798) | √ | √ | √ | – | – | Madagascar |
| *C. brasiliana* (Lamarck, 1819) | √ | – | √ | – | √ | North America |
| *C. columbiensis* (Hanley, 1846) | √ | √ | – | √ | – | Columbia |
| *C. corteziensis* (Hertlein, 1951) | √ | – | – | – | – | Tropical West America |
| *C. dactylena* (Iredale, 1939) | √ | √ | √ | – | – | Australia |
| *C. denticulate* Born, 1778 | – | √ | – | – | – | Netherlands |
| *C. gasar* (Dautzenberg, 1891) | √ | √ | – | – | √ | Nigeria |
| *C. gigas* (Thunberg, 1793) | √ | √ | √ | – | √ | Yellow Sea, China |
| *C. guyanensis* Ranson, 1967 | – | √ | – | – | – | Trinidad and Tobago; Trinidad; French Guiana,Surinam, Brazil |
| *C. gryphoides* (Schlotheim 1813) | – | – | – | √ | √ | Yemen; Iran; South Africa; Miocene of Austria; Egypt; France |
| *C. hongkongensis* Lam and Morton, 2003 | √ | √ | √ | – | √ | South China Sea |
| *C. hatchery* Ihering, 1899 | – | – | – | √ | – | Argentina; Chile |
| *C. inequivalvis* Sowerby, 1871 | – | √ | – | – | – | Indo-West Pacific |
| *C. iredalei* (Faustino, 1932) | – | – | – | – | √ | South China Sea; Philippines; Thailand |
| *C. kawauchidensis* Tamura, 1977 | – | – | – | √ | – | Cretaceous of Japan |
| *C. lineate* Röding, 1798 | – | √ | – | – | – | Japan; Taiwan |
| *C. lugubris* (Sowerby, 1871) | – | √ | – | – | – | Venezuela |
| *C. madrasensis* (Preston) | – | – | – | – | √ | Eocene; Gulf Coast |
| *C. nippona* (Seki, 1934) | √ | √ | √ | – | √ | Philippines, Pandanan Island |
| *C. paraibanensis* Singarajah, 1980 | √ | √ | – | √ | – | Brazil |
| *C. patagonica* d'Orbigny, 1842 | – | – | – | √ | – | Rio Negro, Miocene of Argentina |
| *C. raincourti* Deshayes, 1858 | – | – | – | √ | – | Cenozoic; W Europe |
| *C. rhizophorae* (Guilding, 1828) | √ | √ | √ | – | √ | Venezuela, Margarita Island |
| *C. rivularis* (Gould, 1861) | √ | √ | – | – | – | Indo-Pacific |
| *C. sikamea* (Amemiya, 1928) | √ | √ | √ | – | √ | Japan; South China Sea |
| *C. titan* Conrad, 1853 | – | – | – | √ | – | California |
| *C. transitoria* Hupé, 1854 | – | – | – | √ | – | Chile; Argentina |
| *C. tulipa* (Lamarck, 1819) | √ | √ | √ | – | – | Senegal |
| *C. vaquerosensis* Loel and Corey, 1932 | – | – | – | √ | – | California |
| *C. virginica* (Gmelin, 1791) | √ | √ | √ | √ | √ | Easten America |
| *C. vitrefacta* Thunberg, 1793 | – | √ | – | – | – | Indo-West Pacific |

of Life, http://eol.org/), in Huber (2010), and NCBI (National Center for Biotechnology Information). All species listed in these databases have been confirmed by professional taxonomists and hence have reference value for *Crassostrea* species worldwide. A total of 35 species are recognized in these sources (Table 4) and 14 of these species have DNA sequences available in Genbank. For the remaining 21 species, morphological descriptions, photographs, and other information (e.g., habitat and distribution) are available. These identifying characteristics of each species were carefully compared with those of the new taxon. The results showed that *C. zhanjiangensis* differs in many aspects from each of these known *Crassostrea* species.

The description of *C. zhanjiangensis* provides valuable information for wild spat collection in the Hong Kong oyster industry in Guandu, Zhanjiang. The mass mortality of juveniles

(commonly thought to be Hong Kong oysters) on concrete tiles was previously presumed to be caused by nutrient limitation due to high density spat settlement. We have now learned that the seed on concrete tiles that suffer high mortality are primarily those of *C. zhanjiangensis* rather than Hong Kong oysters. No further study has been made, ecologically or physiologically, regarding the mechanism that triggers mass mortality of this species after the first fast growth season. However, we can conclude that the Hong Kong oyster seed quality in Guandu is still good and is not encountering germ degeneration, as argued by local farmers (Xiao and Yu 2008). According to our preliminary investigation, the niche occupancy rate of *C. zhanjiangensis* can reach as high as 100%. During the Hong Kong oyster seed collection season, good timing of deployment of seed collection devices will be vital for seed collection efficiency and for reducing spat attachment of unwanted (adulterated) species (primarily *C. zhanjiangensis* and *C. sikamea*). The optimal seed collection time, when the spawning peak for the Hong Kong oyster does not overlap with the spawning peak of *C. zhanjiangensis*, could be very short. Therefore, precise forecasting of spawning time of both species is necessary and could be determined using molecular markers.

This study provides the basis for future population genetics and ecological studies of *C. zhanjiangensis*, a new member of the "true oysters" that plays a significant role in nearshore ecosystems. Furthermore, our findings reveal that the species diversity of Indo-Pacific oysters was hitherto underestimated and that mitochondrial DNA based molecular diagnosis should be a powerful tool for future taxonomic work on this challenging group.

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