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IDENTIFICATION OF *SACCOSTREA MORDAX* AND A NEW SPECIES *SACCOSTREA MORDOIDES* SP. NOV. (BIVALVIA: OSTREIDAE) FROM CHINA

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ABSTRACT The taxonomy of *Saccostrea* oysters is problematic because of their highly variable shells. Molecular studies have revealed diverse sequence lineages that do not correspond to valid species names including some *Saccostrea mordax*–like oysters. The *S. mordax*–like oysters ($n = 202$) were collected from southern China and classified into three lineages, *A*, *B*, and *C*, based on both shell morphology and mitochondrial *16S ribosomal RNA* (*16S*) and *cytochrome oxidase I* (*COI*) sequences. Molecular and morphological analyses indicate lineages *A* and *B* are *S. mordax*, and lineage *C* is a new species *Saccostrea mordoides* sp. nov. The shell morphology of the new species is variable, as in other *Saccostrea* oysters, but distinctive in that shell height is similar to shell length with the right valve much smaller than the left valve and having undulate laminae. The average Kimura's two-parameter distance between *S. mordoides* sp. nov. and other *Saccostrea* oysters is 0.046–0.120 for *16S* and 0.106–0.240 for *COI*, which are significantly higher than typical distances among closely related oyster species. This study shows that *S. mordoides* is a new species different from *S. mordax*, highlighting rich species diversity of *Saccostrea* oysters and the need for molecular taxonomy.

KEY WORDS: oyster taxonomy, molecular phylogeny, *16S ribosomal RNA*, *cytochrome oxidase I*, *Saccostrea mordax*, *Saccostrea mordoides*, Ostreidae

INTRODUCTION

Oysters are highly variable in shell morphology. Different authors identified species based on different ecotypes and created considerable confusion in oyster classification (Harry 1985, Guo et al. 2018). Among ostreid species, the taxonomy of rock oysters *Saccostrea* (Dollfus & Dautzenberg 1920) is most problematic because *Saccostrea* species have greater plasticity in shell morphology than species of other genera because of their close attachment to substrates (Hamaguchi et al. 2014).

Zhang and Lou (1956) described four *Saccostrea* species from rocky shores of China based on shell morphology: *Saccostrea glomerata*, *Saccostrea cucullata*, *Saccostrea mordax*, and *Saccostrea echinata*. Based on Mayr's (1963) definition of superspecies, Stenzel (1971) and Harry (1985) considered *S. cucullata* as the only valid *Saccostrea* species or superspecies in Indo-Pacific. Using both shell morphology and anatomy, Li and Qi (1994) recognized only two *Saccostrea* species, *S. cucullata* and *S. echinata*, in China, and regarded *S. glomerata*, *Saccostrea kegaki*, and *Saccostrea malabonensis* as synonyms of *S. echinata*, and *S. mordax* as synonym of *S. cucullata*. Xu and Zhang (2008) recorded five *Saccostrea* species (*S. glomerata*, *S. mytiloides*, *S. cucullata*, *S. mordax*, and *S. echinata*) in China. Lam and Morton (2009) described the shell characters of *S. cucullata* and *S. mordax* from Malaysia and Singapore. Huber (2010) suggested that *S. cucullata* was restricted to Central Western Africa, Natal, Red Sea, and to Arabia, and that the specimens from India, Andaman Sea, Gulf of Thailand, the Philippines, Australia, and Japan should be the small rock oyster *S. mordax*. Amaral and Simone (2016) described anatomical differences of five *Saccostrea* species (*S. cucullata*, *S. glomerata*, *S. echinata*, *Saccostrea palmula*, and *S. mordax*) from the Pacific Ocean. The taxonomy of *Saccostrea* remains confusing and unresolved, as many authors relied solely on shell characters which are highly variable depending on life stages and the environment (Lam & Morton 2006, Sekino & Yamashita 2016).

Analysis of DNA sequence data has contributed to resolving some questions about oyster identifications and taxonomic relationships (Guo et al. 2018). Multiplex genus- and species-specific polymerase chain reaction (PCR) markers were developed for the identification of oysters from China (Wang & Guo 2008), and *Crassostrea gigas angulata* was identified as a subspecies of *C. gigas* (Wang et al. 2010). Several new species of oysters have been identified based on mitochondrial DNA and morphology analysis (Wu et al. 2013, Xia et al. 2014, Li et al. 2017a, Hu et al. 2019). Lam and Morton (2006) using partial mitochondrial *16S* classified *Saccostrea* species of Indo-West Pacific into two clades, one consisted of *Saccostrea cucullata* A–G, *Saccostrea kegaki*, and *Saccostrea glomerata*, and the other consisted of *Saccostrea mordax A* and *B*. Hamaguchi et al. (2014) discovered the Indo-West Pacific rock oyster *S. cucullata* F in Kagoshima Bay, *S. cucullata C* and *S. cucullata F* in Wakayama Prefecture. These were the first records of Indo-West Pacific rock oysters in mainland Japan. According to the original description and type locality of *S. cucullata*, Sekino and Yamashita (2016) considered *S. cucullata* should not represent the Indo-West Pacific *Saccostrea* oysters, and refer to Lam and Morton's (2006) "*S. cucullata*" superspecies as "non-*mordax*" oysters. Furthermore, they identified additional *Saccostrea* lineages that were not reported by Lam and Morton (2006), including non-*mordax* lineage *H*, *I*, and *J*. Based on the original species description and type locality, Sekino and Yamashita (2016) considered that *Saccostrea malabonensis* might correspond to lineage *F* and *Saccostrea echinata*

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correspond to lineage *H*. Li et al. (2017a) identified *S. cucullata* lineage *F* and specimens from Myanmar as *S. malabonensis*. The species *Saccostrea palmula*, which is distinct from other species of *Saccostrea*, has variable morphologies within the Gulf of California but little sequence divergence (Raith et al. 2016). Guo et al. (2018) reviewed genetic data and recognized 21 *Saccostrea* species, including three possible species under *S. mordax*.

The species *Saccostrea mordax* is a widely distributed species and morphologically well recognized by its solid shells, with strong tooth-like margins, raised purple or black muscle scar, and purple interior margins (Huber 2010). The *S. mordax* and *S. mordax*–like oysters can be separated from the non-*mordax* oysters based on their mtDNA sequences and shell morphology, and further grouped into three lineages *A*, *B*, and *C* (Lam & Morton 2006, Sekino & Yamashita 2013). This study sampled and analyzed 202 *S. mordax*–like oysters from southern China. Morphological and phylogenetic analyses indicate that *S. mordax* lineages *A* and *B* correspond to the valid species *S. mordax,* and lineage *C* is a new species of *Saccostrea*.

MATERIALS AND METHODS

Sample Collection

Unknown oysters were collected at different times from Hainan, Guangdong, and Guangxi, China (Table 1 and Fig. 1). The samples were fixed in greater than 99% ethanol for subsequent DNA extraction.

Non-*mordax* oysters were identified based on mtDNA sequences and shell morphology (Lam & Morton 2006), and excluded from this study (n = 1,571). Only *Saccostrea mordax*–like oysters were subjected for further analyses (n = 202).

TABLE 1.

Sampling sites and sample size (n) for oysters collected and analyzed.

Site code	Sampling site	N
TGL	Tongguling, Wenchang, Hainan	19
ML	Mulantou, Wenchang, Hainan	5
JXJ	Jingxinjiao, Wenchang, Hainan	11
XH	Xinhai, Haikou, Hainan	2
CYW	Chunyuan Wan, Wanning, Hainan	2
HNB	Hainanbeigang, Wanning, Hainan	17
YLW	Yalang Wan, Sanya, Hainan	25
HTW	Haitang Wan, Sanya, Hainan	49
HT	Hongtang, Sanya, Hainan	2
DDH	Dadonghai, Sanya, Hainan	5
XDH	Xiaodonghai, Sanya, Hainan	4
TYH	Tianyahaijiao, Sanya, Hainan	24
GC	Ganchonggang, Danzhou, Hainan	2
XC	Xincun, Ledonglizu, Hainan	1
TMZ	Tiemaozai, Yangjiang, Guangdong	7
HBC	Hebeicun, Yangjiang, Guangdong	6
SBW	Shabawan, Yangjiang, Guangdong	4
NAD	Nanaodao, Shenzhen, Guangdong	8
ZL	Zhelang, Shanwei, Guangdong	3
WCT	Wucaitan, Beihai, Guangxi	6

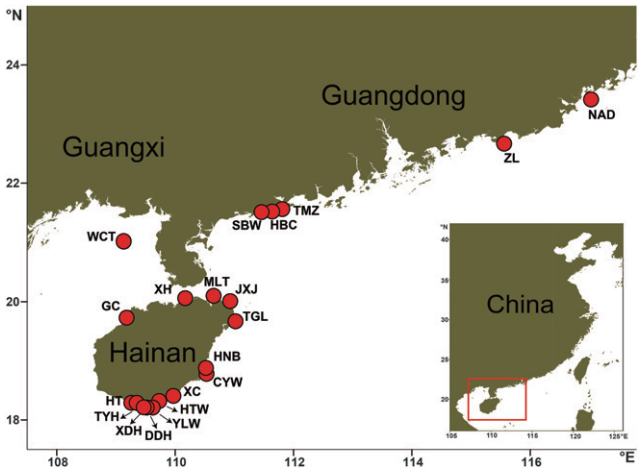

Figure 1. Sampling sites in southern China where oysters were collected.

DNA Extraction, PCR Amplification, and Sequencing

DNA was extracted from adductor muscle using the TIANamp Marine Animals DNA Kit (Tiangen, Beijing). Using primers 16SF (5′-GCCTGTTTATCAAAAACAT-3′) and 16SR (5′-CCGGTC TGAACTCAGATCACG-3′), a fragment of mitochondrial *16S* was amplified by PCR. A fragment of *cytochrome oxidase I* (*COI*) was amplified with primer pairs of LCO1490 (5′-GGTCAACA-AATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACT-TCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). Polymerase chain reaction was performed in 25 µL containing 9.5 µL ddH_2O, 12.5 µL 2 × TSINGKE Master Mix, 0.5 µL of each primer (10 µM), and 2 µL template DNA, on a BIO-RAD thermal cycler with the following parameters: pre-denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 48–50°C for 50 sec, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were checked with electrophoresis on a 1.0% agarose gel. Sequencing was performed on an ABI 3730xl DNA Analyzer. Sequences obtained in this study were submitted to the NCBI (http://www.ncbi.nlm.nih.gov/) under gene accession numbers MT298862–MT298891 (*16S*) and MT293806–MT293857 (*COI*).

Molecular Identification and Phylogenetic Analysis

DNA sequences were aligned using MEGA v. 7.0.26 (Kumar et al. 2016). Haplotypes were identified by DNASP v. 5.10.01 (Librado & Rozas 2009). Neighbor-joining (NJ) (Saitou & Nei 1987) and Bayesian inference (BI) approaches were used for phylogenetic analysis. Neighbor-joining analyses were performed with MEGA v. 7.0.26 using Kimura's two-parameter (K2P) distances (Kimura 1980). Before BI analyses, jModel Test v. 2.1.10 (Darriba et al. 2012) was used for searching the best-fit substitution model. MrBayes v. 3.2 (Ronquist et al. 2012) was used to calculate the BI tree. During BI analysis, Markov chain Monte Carlo simulation was run for 2×10^6 generations. Sequence divergence among groups was calculated with MEGA v. 7.0.26 using Kimura's 2-parameter model.

Description of Shell Characteristics

Conchological characteristics used to describe and identify species (Littlewood 1994) included plication of the margin,

ligament extent, the presence of chalky deposits on the inner surface of the valves, shape and attachment area of the left valve, the presence and pattern of chomata, and the color and shape of the adductor muscle scar.

RESULTS

Mitochondrial 16S Ribosomal RNA Gene

The *16S* fragment was sequenced for a total of 202 oysters (Table 1). Among the 202 sequences, 30 haplotypes were observed, and the common haplotype was found in 55.1% of the oysters and at all but two locations (Table 2). BLASTn was performed to search for related sequences in GenBank for each sequence examined in this study, identifying 28 closely related sequences (Table 3), representing all *Saccostrea* lineages or species proposed in previous studies. The length of the *16S* used for phylogenetic analysis was 495 bp. The *Hyotissa*, *Ostrea*, and *Crassostrea* species were used as out-groups.

The topological structures of the NJ and BI trees are congruent, although there were inconsistencies in the clustering patterns within major clades. Only the Bayesian tree is presented (Fig. 2). The *Saccostrea* oysters fell into *mordax* and non-*mordax* clades. All specimens from this study were found in three lineages (*A*, *B*, and *C*) of the *mordax* clade. Lineages *A* and *B* clustered together, although the position of some haplotypes varied within the cluster. Fifteen haplotypes representing 66.3% of all oysters studied and at all locations were clustered with *Saccostrea mordax A*. Lineage *B* contained 13 haplotypes representing 30.3% of oysters from 78.6% locations sampled. The mean sequence divergence between lineages *A* and *B* was 1.0% (Table 4). The pairwise sequence divergence between the haplotypes of *S. mordax A* and *B* was 0.4%–2.1%, suggesting that variation in both lineages is high and the relationship between the two lineages is complex. The other two haplotypes grouped with lineage *C* and were only found in Haitang Bay in Hainan Province. The mean sequence divergence between lineage *C* and lineages *A* and *B* were 4.2% and 4.1%, respectively, which are significantly higher than intraspecific divergence (Wang et al. 2010). Lineage *C* separated from other lineages with high supportive values.

Mitochondrial COI Gene

A 561-bp fragment of the mitochondrial *COI* was sequenced for 79 of the 202 oysters, yielding 52 haplotypes. Phylogenetic analysis was conducted using all *COI* haplotypes obtained in this study and closely related sequences from GenBank

TABLE 2.

Distribution of haplotypes of the 16S rRNA gene observed in this study.

	Sampling site (as defined in Table 1)																			
Hap*	**TGL**	**HTW**	**CYW**	**YLW**	**MLT**	**HT**	**JXJ**	**HNB**	**GC**	**DDH**	**TYH**	**TMZ**	**HBC**	**SBW**	**XH**	**XC**	**XDH**	**ZL**	**NAD**	**WCT**
1(A)	13	24	1	16	4	2	7	5	2	2	13	3	4	2	1	0	4	0	4	3
2(B)	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
3(A)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
4(B)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
5(A)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
6(B)	0	6	0	4	0	0	1	1	0	2	3	2	0	0	0	0	0	0	2	0
7(B)	0	1	0	1	1	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0
8(B)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
9(A)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10(B)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
11(B)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
12(A)	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
13(B)	2	5	0	3	0	0	2	1	0	0	1	0	0	0	0	1	0	2	2	3
14(B)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0
15(B)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16(A)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17(A)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18(B)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
19(A)	0	2	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
20(A)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
21(A)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22(A)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
23(A)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24(A)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
25(C)	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26(C)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27(B)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
28(A)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
29(B)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
30(A)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

* Haplotypes *Saccostrea mordax A*, *B*, or *C*.

TABLE 3.

Details of *16S rRNA* and *cytochrome oxidase I* nucleotide sequences retrieved from GenBank.

Species	16S			CO1		
	Accession#	Source region	References	Accession#	Source region	References
Saccostrea kegaki	NC_030533	Taiwan	NA	NC_030533	Taiwan	NA
Saccostrea echinata	NC_036478	China	NA	KC683512	Guangxi, China	Li et al. 2013
Saccostrea glomerata	KX713252	Queensland Australia	Combosch et al. 2016	–	–	–
Saccostrea palmula	FJ768516	Sinaloa, Mazatlan, Mexico	Polson et al. 2009	KT317530	Baja California, Mexico	Raith et al. 2016
Saccostrea cucullata	NC_027724	Madagascar	Volatiana et al. 2016	NC_027724	Madagascar	Volatiana et al. 2016
Saccostrea circumsuta	LC002648	Amami, Misato, Japan	NA	LC002651	Amami, Japan	NA
Saccostrea malabonensis	LC005440	Okinawa, Japan	NA	LC005431	Okinawa, Japan	NA
Saccostrea scyphophilla	LM993883	NA	Salvi et al. 2014	–	–	–
Saccostrea sp.1	HQ660994	Shengshan, Zhejiang, China	Liu et al. 2011	–	–	–
Saccostrea non-*mordax A*	AY247352	Barrow I., Western Australia	Lam & Morton 2006	–	–	–
Saccostrea non-*mordax B*	AY247336	Cape d'Aguilar, Hong Kong	Lam & Morton 2006	–	–	–
Saccostrea non-*mordax C*	AY247380	Itoman, Okinawa, Japan	Lam & Morton 2006	LC110456	Kagoshima, Japan	Sekino & Yamashita 2013
Saccostrea non-*mordax D*	AY247391	Sanya, Hainan, China	Lam & Morton 2006	–	–	–
Saccostrea non-*mordax E*	AY247387	Shiman, Taiwan	Lam & Morton 2006	–	–	–
Saccostrea non-*mordax F*	AY247297	Kallang River, Singapore	Lam & Morton 2006	LC110452	Kagoshima, Japan	Sekino & Yamashita 2013
Saccostrea non-*mordax G*	AY247386	Shiman, Taiwan	Lam & Morton 2006	LC110519	Kagoshima, Japan	Sekino & Yamashita 2013
Saccostrea mordax A	AY247363	Quobba, Western Australia	Lam & Morton 2006	EU816062	South China Sea	NA
S. mordax B	AY247339	Cooee Bay, Australia	Lam & Morton 2006	EU816072	South China Sea	NA
Saccostrea non-*mordax H*	LC155015	Okinawa, Japan	Sekino & Yamashita 2016	LC110596	Okinawa, Japan	Sekino & Yamashita 2013
Saccostrea non-*mordax I*	LC111218	Okinawa, Japan	Sekino & Yamashita 2016	LC110579	Okinawa, Japan	Sekino & Yamashita 2013
Saccostrea non-*mordax J*	LC111260	Okinawa, Japan	Sekino & Yamashita 2016	LC110587	Okinawa, Japan	Sekino & Yamashita 2013
S. mordax C	AB748915	Okinawa, Japan	Sekino & Yamashita 2013	AB748839	Okinawa, Japan	Sekino & Yamashita 2013
Crassostrea ariakensis	KJ855254	Nantong, Jiangsu, China	Ren et al. 2014	KJ855254	Nantong, China	Ren et al. 2014
Crassostrea angulata	KJ855249	Dong'an, Fujian, China	Ren et al. 2014	KJ855249	Fujian, China	Ren et al. 2014
Hyotissa hyotis	LM993886	NA	Salvi et al. 2014	GQ166583	NA	Plazzi & Passamonti 2009
Hyotissa imbricata	–	–	–	AB076917	Okinawa, Japan	Matsumoto 2003
Hyotissa mcgintyi	AY376597	USA	Kirkendale et al. 2004	–	–	–
Ostrea denselamellosa	FJ743511	Jinju City, South Korea	Jung et al. 2008	HM015199	NA	Yu & Li 2011
Ostrea edulis	–	–	–	AF120651	NA	Giribet & Wheeler 1999
Ostrea stentina	AY376603	USA	Kirkendale et al. 2004	–	–	–

(Table 3), including two *Crassostrea* species, two *Ostrea* species, and two *Hyotissa* species that were used as out-groups.

The BI and NJ trees from *COI* have the same topology that is consistent with the *16S* trees, except that lineages *A* and *B* were separated more clearly in the *16S* tree. Only the BI tree for *COI* is presented (Fig. 3). Most haplotypes are clustered with *Saccostrea mordax A* or *B*, whereas five haplotypes were clustered with the reference *S. mordax* C (AB748839) and clearly separated from other lineages. The *COI* sequences are more variable than the *16S* sequences. The mean sequence divergence between lineages *A* and *B* was 2.0% (Table 5), with the pairwise sequence divergence between the haplotypes of *A* and *B* ranging from 0.9% to 3.0%. The mean sequence divergence between lineage *C* and other lineages was 10.4%–27.7%, which is well above the divergence between two oyster species (Guo et al. 2018). The high divergence in both genes suggests that

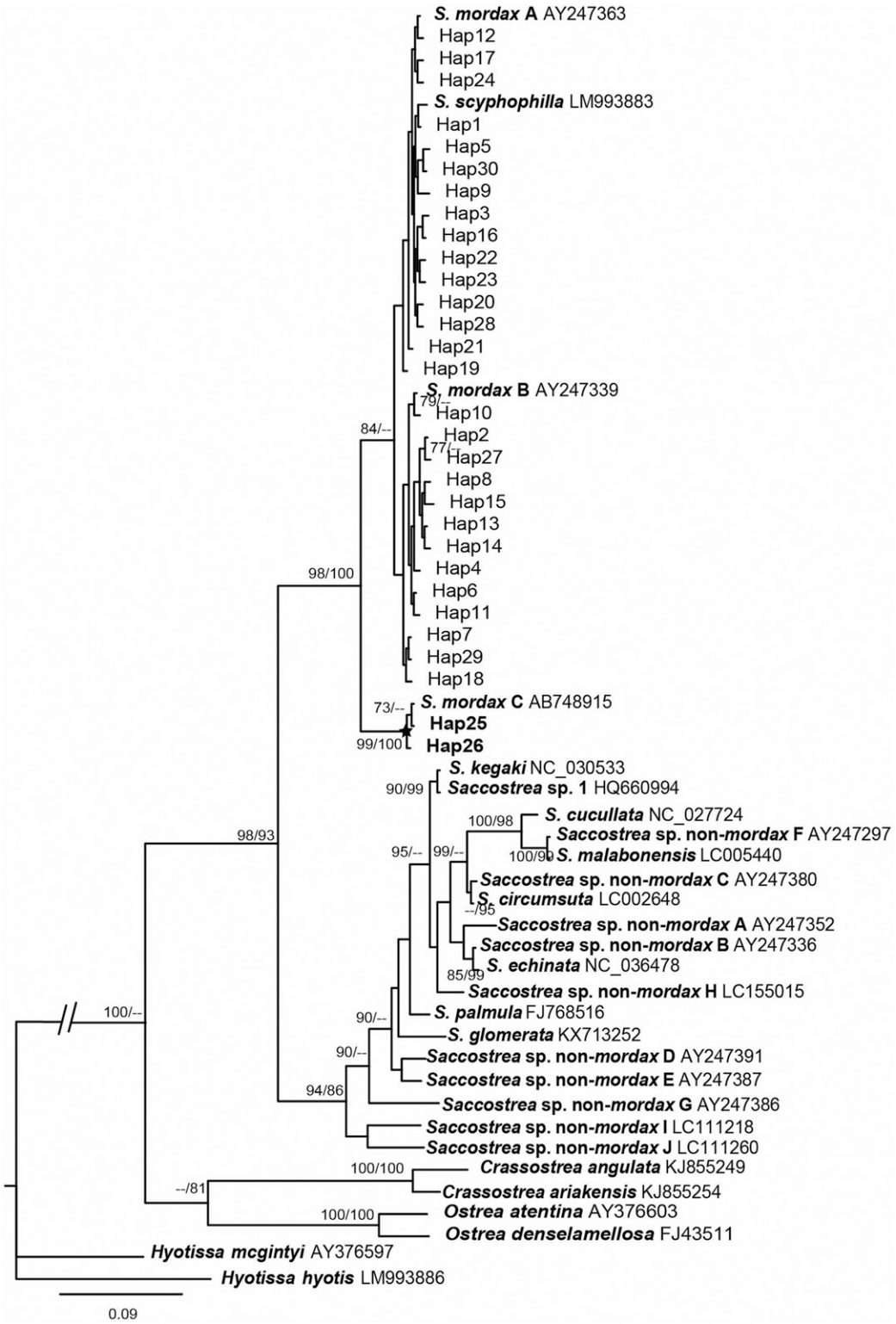

Figure 2. Bayesian phylogenetic tree of *Saccostrea* oysters reconstructed based on partial 16S rRNA sequence. Numbers at nodes are posterior probability/bootstrap values (≥70).

TABLE 4.

Mean sequence divergence of 16S rRNA between species and lineages of *Saccostrea*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. *Saccostrea kegaki*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. *Saccostrea glomerata*	0.042	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3. *Saccostrea palmula*	0.022	0.044	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4. *Saccostrea cucullata*	0.056	0.059	0.051	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5. *Saccostrea circumsuta*	0.020	0.044	0.032	0.039	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6. *Saccostrea malabonesis*	0.061	0.067	0.061	0.022	0.046	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7. *Saccostrea echinata*	0.022	0.055	0.039	0.056	0.020	0.061	–	–	–	–	–	–	–	–	–	–	–	–	–
8. *Saccostrea* non-*mordax A*	0.032	0.052	0.032	0.056	0.029	0.064	0.025	–	–	–	–	–	–	–	–	–	–	–	–
9. *Saccostrea* non-*mordax B*	0.020	0.052	0.037	0.054	0.018	0.059	0.002	0.022	–	–	–	–	–	–	–	–	–	–	–
10. *Saccostrea* non-*mordax C*	0.022	0.047	0.034	0.041	0.002	0.049	0.022	0.032	0.020	–	–	–	–	–	–	–	–	–	–
11. *Saccostrea* non-*mordax D*	0.037	0.042	0.029	0.059	0.044	0.072	0.044	0.042	0.047	0.047	–	–	–	–	–	–	–	–	–
12. *Saccostrea* non-*mordax E*	0.032	0.042	0.029	0.048	0.034	0.059	0.032	0.029	0.029	0.037	0.025	–	–	–	–	–	–	–	–
13. *Saccostrea* non-*mordax F*	0.061	0.067	0.061	0.022	0.046	0.000	0.061	0.064	0.059	0.049	0.072	0.059	–	–	–	–	–	–	–
14. *Saccostrea* non-*mordax G*	0.070	0.078	0.062	0.061	0.067	0.075	0.065	0.067	0.062	0.070	0.062	0.049	0.075	–	–	–	–	–	–
15. *Saccostrea* non-*mordax H*	0.018	0.051	0.032	0.061	0.029	0.061	0.032	0.032	0.029	0.032	0.042	0.037	0.061	0.078	–	–	–	–	–
16. *Saccostrea* non-*mordax I*	0.063	0.066	0.055	0.071	0.063	0.076	0.068	0.077	0.066	0.061	0.066	0.063	0.076	0.080	0.076	–	–	–	–
17. *Saccostrea* non-*mordax J*	0.058	0.066	0.056	0.079	0.058	0.082	0.066	0.061	0.064	0.061	0.059	0.061	0.082	0.084	0.066	0.056	–	–	–
18. *Saccostrea mordax A*	0.123	0.110	0.096	0.112	0.123	0.117	0.130	0.115	0.126	0.120	0.100	0.113	0.117	0.110	0.119	0.108	0.097	–	–
19. *Saccostrea mordax B*	0.119	0.108	0.098	0.116	0.128	0.123	0.125	0.115	0.122	0.125	0.101	0.109	0.123	0.107	0.116	0.110	0.099	0.010	–
20. *Saccostrea mordax C*	0.113	0.104	0.095	0.110	0.116	0.113	0.119	0.110	0.116	0.119	0.104	0.110	0.113	0.103	0.109	0.119	0.104	0.042	0.041

Saccostrea mordax A: AY247363; haplotypes 1, 5, 3, 9, 12, 16, 17, 19, 20, 21, 22, 23, 24, 28 and 30.
Saccostrea mordax B: AY247339, HQ660993, haplotypes 2, 4, 6, 7, 8, 10, 11, 13, 14, 15, 18, 27 and 29.
Saccostrea mordax C: AB748915, LC005441, EU815980, haplotypes 25 and 26.

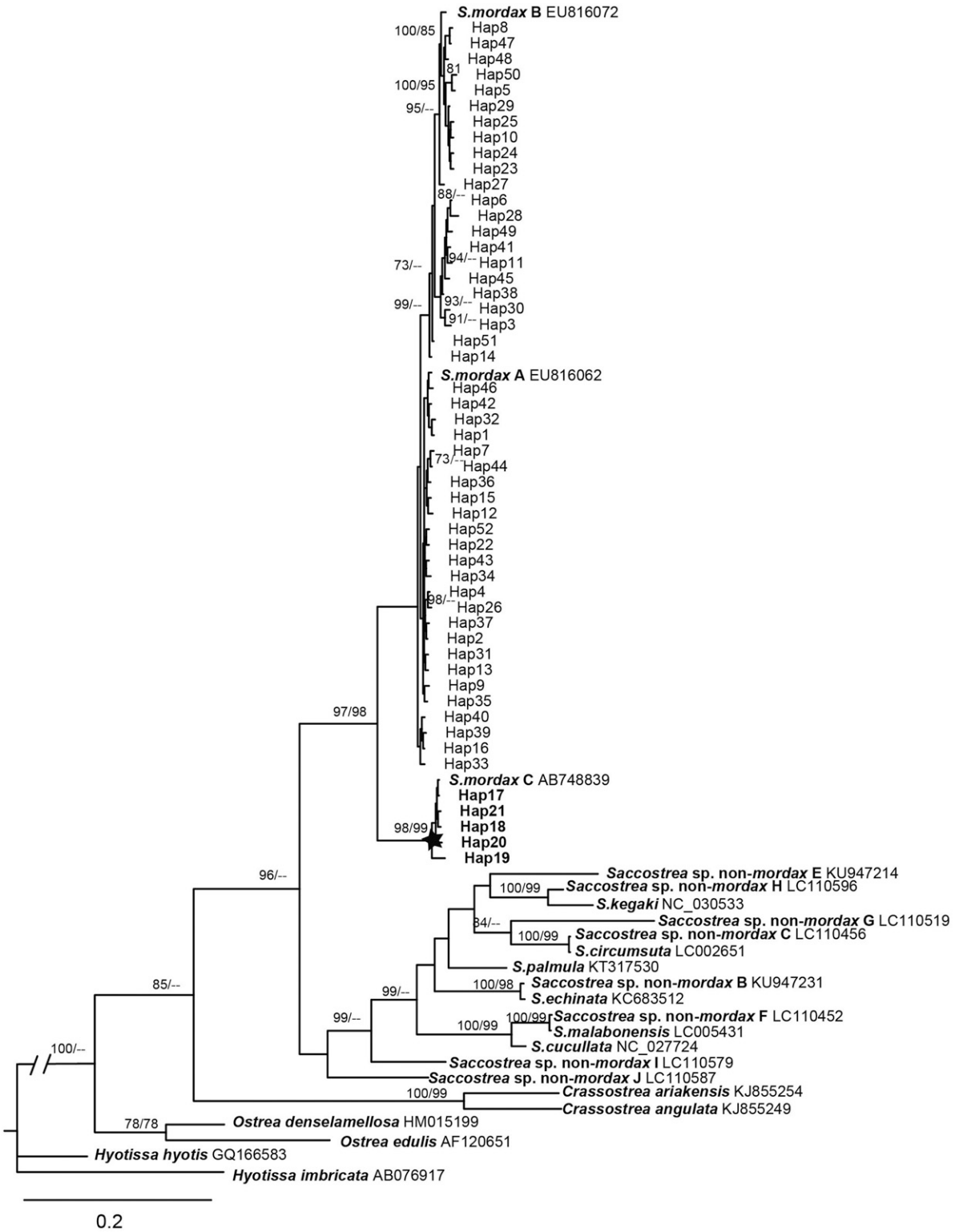

Figure 3. Bayesian phylogenetic tree of *Saccostrea* oysters based on partial *cytochrome oxidase I* DNA sequences. Numbers on nodes are posterior probability/bootstrap values (≥70).

TABLE 5.

Mean sequence divergence of *cytochrome oxidase I* gene between species and lineages of *Saccostrea*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *Saccostrea kegaki*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. *Saccostrea palmula*	0.184	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3. *Saccostrea cucullata*	0.214	0.158	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4. *Saccostrea circumsuta*	0.162	0.177	0.176	–	–	–	–	–	–	–	–	–	–	–	–	–
5. *Saccostrea malabonensis*	0.197	0.186	0.053	0.179	–	–	–	–	–	–	–	–	–	–	–	–
6. *Saccostrea echinata*	0.176	0.143	0.189	0.157	0.202	–	–	–	–	–	–	–	–	–	–	–
7. *Saccostrea* non-*mordax B*	0.173	0.146	0.186	0.154	0.206	0.005	–	–	–	–	–	–	–	–	–	–
8. *Saccostrea* non-*mordax C*	0.162	0.177	0.176	0.000	0.179	0.157	0.154	–	–	–	–	–	–	–	–	–
9. *Saccostrea* non-*mordax E*	0.192	0.183	0.186	0.213	0.213	0.223	0.227	0.213	–	–	–	–	–	–	–	–
10. *Saccostrea* non-*mordax F*	0.194	0.189	0.055	0.182	0.002	0.199	0.202	0.182	0.216	–	–	–	–	–	–	–
11. *Saccostrea* non-*mordax G*	0.232	0.231	0.231	0.184	0.231	0.224	0.221	0.184	0.237	0.228	–	–	–	–	–	–
12. *Saccostrea* non-*mordax H*	0.064	0.159	0.172	0.162	0.168	0.155	0.152	0.162	0.186	0.172	0.211	–	–	–	–	–
13. *Saccostrea* non-*mordax I*	0.213	0.161	0.195	0.199	0.201	0.181	0.184	0.199	0.206	0.204	0.200	0.203	–	–	–	–
14. *Saccostrea* non-*mordax J*	0.243	0.227	0.214	0.204	0.220	0.220	0.217	0.204	0.282	0.224	0.278	0.247	0.211	–	–	–
15. *Saccostrea mordax A*	0.239	0.206	0.196	0.231	0.228	0.186	0.180	0.231	0.234	0.232	0.237	0.218	0.176	0.224	–	–
16. *S. mordax B*	0.257	0.207	0.206	0.241	0.236	0.183	0.179	0.241	0.246	0.240	0.241	0.222	0.190	0.222	0.020	–
17. *S. mordax C*	0.277	0.242	0.214	0.248	0.227	0.194	0.194	0.248	0.252	0.224	0.259	0.250	0.218	0.202	0.104	0.115

S. mordax A: EU816062, haplotypes 1, 2, 4, 7, 9, 12, 13, 15, 16, 22, 31, 32, 33, 34, 35, 36, 37, 39, 40, 42, 43, 44, 46, and 52.
S. mordax B: EU816072, haplotypes 3, 5, 6, 8, 10, 11, 14, 23, 24, 25, 28, 27, 29, 30, 38, 41, 45, 47, 48, 49, 50, and 51.
S. mordax C: AB748839, haplotypes 17, 18, 19, 20, and 21.

lineage *C* is a new species, independent of the other two *S. mordax* lineages.

Systematics of the New Species

Order Ostreida Férussac (1822)
  Superfamily Ostreoidea Rafinesque (1815)
    Family Ostreidae Rafinesque (1815)
      Subfamily Saccostreinae Li and Wang (2013)
        Saccostrea mordoides sp. nov. new species

Type Measurements and Deposition

The holotype and two paratypes comprising empty dry shells and ethanol-preserved tissues have been deposited in the Marine Biological Museum of the Chinese Academy of Sciences, Institute of Oceanology, Qingdao. Shell measurements of the type materials are given in Table 6.

Description of the Holotype

The shell of the holotype is moderately sized with triangular outline (Fig. 4A). The shell height is about the same as the shell length, whereas the shell height is usually much longer than the shell length in most *Saccostrea mordax* (Fig. 5). The left valve is slightly cupped and completely attached. The margin of the left valve is thick and steep with evenly spaced ribs ending as marginal crenulations. The right valve is slightly convex with brownish patches and much smaller than the left valve. The right valve is only slightly smaller than the left valve in *S. mordax* (Fig. 5). Concentric growth lines spreading from the umbo of the right valve give rise to brown lamellae. The hinge line is straight and short. The interior of the shell is mostly glossy and white (Fig. 4A). The right valve has purplish-brown margin with elongated chomata distributed unevenly in a single line. The adductor muscle scar on the right valve is located toward the posterior ventral half of the pallial area, circular and deep purple with light purple bands.

TABLE 6.

Shell measurements and characteristics of the type materials of *Saccostrea mordoides* sp. nov.

Accession number	Height (mm)	Length (mm)	Depth (mm)	Notes
Holotype MBM286093	11.8	45.5	35.1	Deep purple adductor muscle scar, strained with light purple band on the right valve, and white adductor muscle scar with light purple patch on the left valve
Paratype 1 MBM286094	8.2	28.3	20.9	Deep purple inner surface with light purple adductor muscle scar on the right valve, and white adductor muscle scar on the left valve
Paratype 2 MBM286095	11.9	23.5	15.0	White inner surface with deep purple adductor muscle scar on the right valve, and light purple adductor muscle scar on the left valve

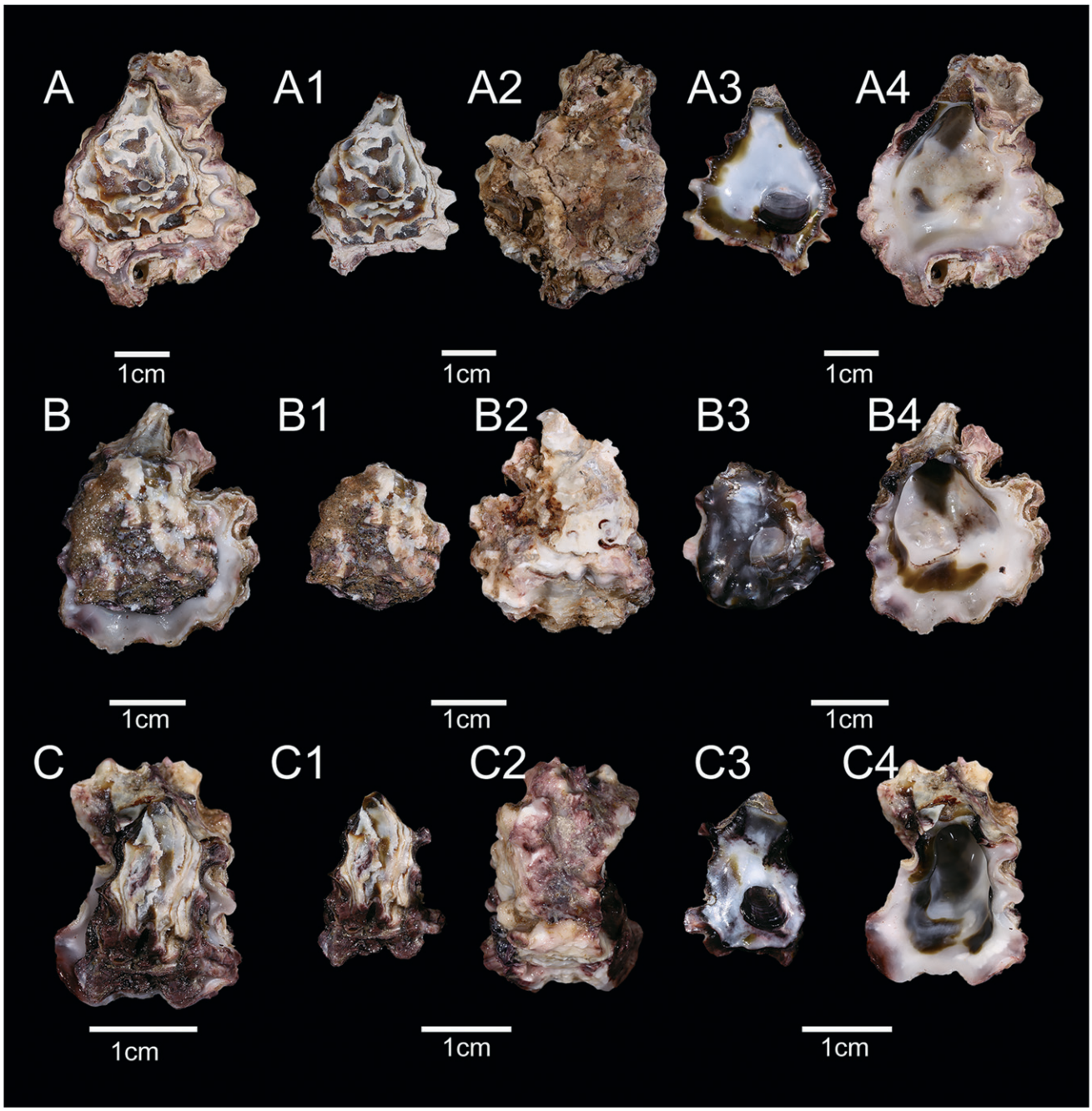

Figure 4. Shell morphology of *Saccostrea mordoides* sp. nov. from Haitang Bay, Hainan, China. The holotype (A): external view of the right (A1) and left valves (A2), internal view of the right (A3) and left valves (A4); Paratype 1 (B): external view of the right (B1) and left (B2) valves, and internal view of the right (B3) and left (B4) valves; Paratype 2 (C): external view of the right (C1) and left (C2) valves, and internal view of the right (C3) and left (C4) valves.

The muscle scar on the left valve is white with a brownish patch.

Variability in Shell Characters

Like other *Saccostrea* species, shells of *Saccostrea mordoides* sp. nov. are highly variable as demonstrated by the holotype and two paratypes (Fig. 4). The outline is triangular or elongated oval, and the attachment area is variable, depending on substratum and space. The left valve of juveniles is thin and cupped. The inner surface of the right valve is usually white or dark purple (Fig. 4). The adductor muscle scar varied in shape from circular to elongated oval and in color from light to dark purple. Shell characteristics and muscle scars are highly variable in *Saccostrea mordax* (Fig. 5).

Habitat

All five specimens of *Saccostrea mordoides* sp. nov. were collected from rocky shores in Haitang Bay, Hainan Province, China (Fig. 1). The salinity was 25.9, and the water temperature was 29°C at the time of collection on May 22, 2013. The five

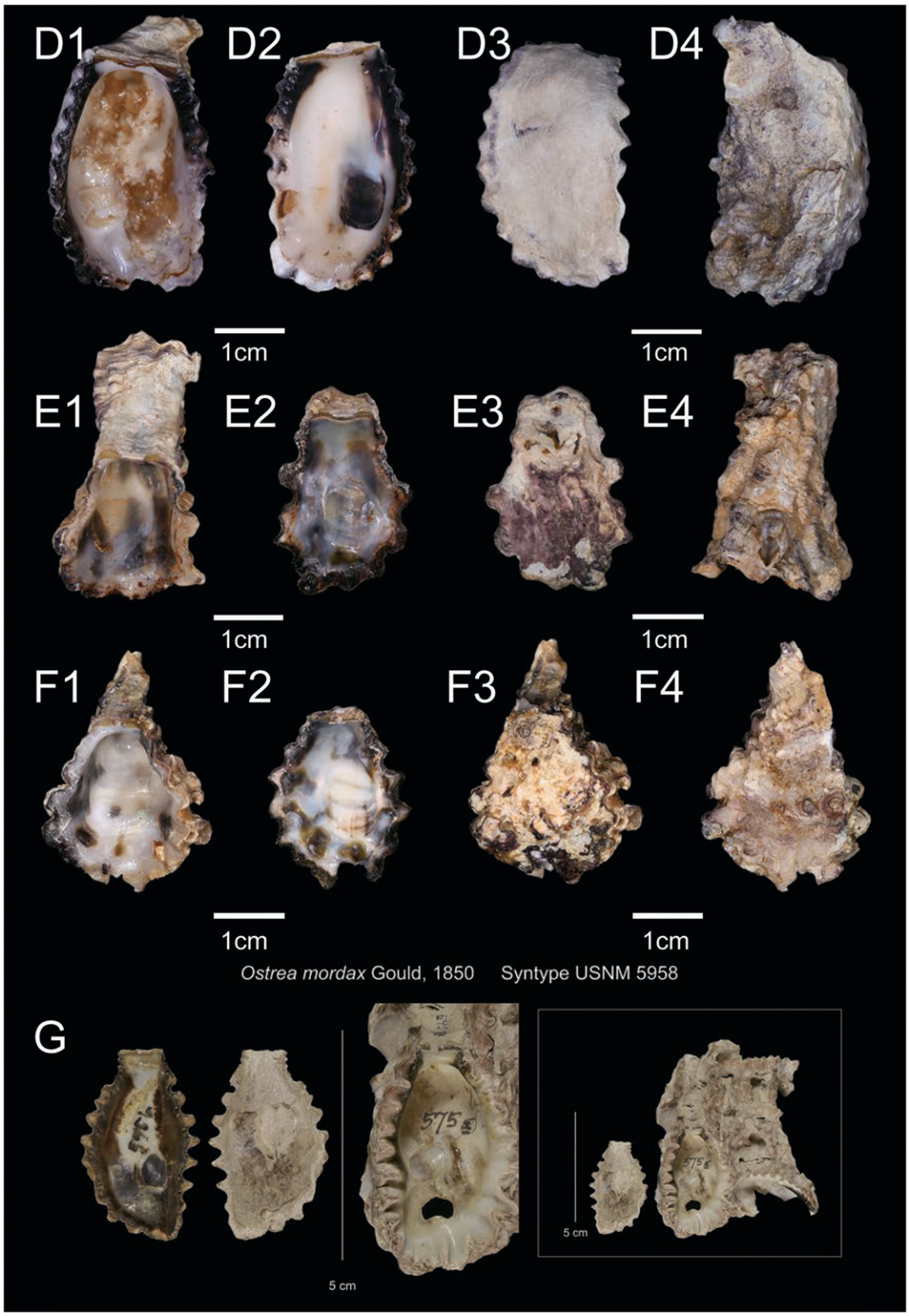

Figure 5. Shell morphology of *Saccostrea mordax* from southern China and syntype. D1–D4, elongated D-shape of *S. mordax A*; E1–E4, cup shape of *S. mordax A*; F1–F4, triangular shape of *S. mordax B*; and *G*, syntype of *S. mordax* Gould (1850) from Fiji (Smithsonian National Museum of Natural History Collections USNM 5958).

specimens of *S. mordoides* sp. nov. were among 47 oysters collected from the Haitang Bay site, and the rest were *Saccostrea mordax A* (29) and *B* (13). Within the sampling range, *S. mordax A* and *B* are also sympatric (Table 2).

Etymology

Shell morphology of the new species is similar to that of *Saccostrea mordax*, and hence named *Saccostrea mordoides* sp. nov.

Shell Morphology of Saccostrea mordax *A and B*

Shells of *Saccostrea mordax A* and *B* are variable and mostly found in three ecotypes: elongated D-shape, cup shape, and triangular (Fig. 5). The shell morphology of the elongated D-shape ecotype (Fig. 5, D1–D4) is consistent with the syntype of *S. mordax* (Fig. 5, G), which also has elongated D-shaped shells with rigid tooth-like crenulations (Gould 1850). The syntype has a right valve that is smaller than the left valve and has a dark purple band on the interior along plicated shell margin. The surface of the right valve is white and smooth. The inner edge of the right valve has round chomatas producing corresponding pits on the left valve. The adductor muscle scar is round and deep purple on both shells, whereas it is only purple on the right shell on the specimens from this study. The right valve fits tightly into the left valve with tooth-like crenulations, making it nearly impossible to separate the shells without damage. The syntype of *S. mordax* was collected from Fiji Islands and also found in Japan, Hainan of China, and Australia (Lam & Morton 2006).

The cup-shaped ecotype has a well-developed umbonal cavity and mostly white adductor muscle scar. The size of the right valve is small with purple patches along the interior margin. The exterior surface of the right valve has radial ribs and purple patches (Fig. 5, E1–E4). The triangular ecotype is similar to *Saccostrea mordax* described by Lam and Morton (2004). It has thin and fragile shells. The left valve is completely attached to the substrate with margin steeply raised. The right valve is slightly convex, with parallel grooves extending from halfway along the dorsoventral axis to the ventral shell margin. The margin of the right valve is round and has evenly spaced crenulations (Lam & Morton 2004). The interior of the shells is white with dark purple patches along the margin. The adductor muscle scar is oval and white, with faint purple stripes (Fig. 5, F1–F4).

DISCUSSION

The New Species Saccostrea mordoides *sp. Nov.*

Oysters of genus *Saccostrea* are generally grouped into *Saccostrea mordax* and non-*mordax* superspecies based on shell morphology. The taxonomy of each superspecies remains problematic. Genetic analysis indicates that each superspecies contains multiple genetic lineages that have not been properly assigned to species. Lam and Morton (2006) discovered two groups of *S. mordax*. Sekino and Yamashita (2013) suggested that at least two *S. mordax* lineages inhabited the coasts of Okinawa Island including lineage *C*.

This study shows that *Saccostrea mordax* lineage *C* is a new species with high genetic divergence from *S. mordax* lineages *A* and *B*. The new species status of *Saccostrea mordoides* sp. nov. is supported by phylogenetic analyses of both mitochondrial *16S* and *COI*. Whereas *S. mordoides* sp. nov. is clustered with *S. mordax A* and *B* on the phylogenetic trees, the K2P sequence divergence between the haplotypes of *S. mordoides* sp. nov. and *mordax A*/*B* is 4.1%–5.3% in *16S* and 8.9%–12.3% in *COI*, which are higher than most closely related oyster species (Guo et al. 2018). For example, the sequence divergence between *Crassostrea gigas* and *C. sikamea*, two well-recognized species, is 2.1%–2.4% in 16S and 10% in *COI* (Wang et al. 2010). As a unique taxonomic unit, *S. mordoides* sp. nov. is separated from other lineages of *Saccostrea* in both *16S* and *COI* trees with high support values (Figs. 2 and 3).

Despite variations, *Saccostrea mordoides* sp. nov. has some morphological characteristics that are different from those of sympatric *Saccostrea mordax* and other species of *Saccostrea*. According to the original species description and the photo of the syntype (Fig. 5, G), *S. mordax* is a medium-sized oyster (SH 50 mm) with concave left valve and the brown margin erected and profoundly plicated (Gould 1850). Compared with *S. mordax*, *S. mordoides* sp. nov. does not have strongly plicated margin, and its hinge line is much shorter.

To confirm *Saccostrea mordoides* sp. nov. is not a synonym of other *Saccostrea* species, the new species is compared with all species of this genus listed in the World Register of Marine Species (http://www.marinespecies.org/), Encyclopedia of Life (http://eol.org/), Worldwide Mollusc Species Database (http://www.bagniliggia.it/WMSD/WMSDhome.htm), Inaba and Torigoe (2004) and Huber (2010) in morphological description, habitat, and distribution. The *S. mordoides* sp. nov. is clearly different from known *Saccostrea* species.

This study indicates that *Saccostrea mordoides* sp. nov. is distributed in Hainan Province of China and Yakata of Japan (Sekino & Yamashita 2013). Data on the new species remain limited, and further studies are needed to determine its full geographic distribution. Furthermore, most sequence lineages of *Saccostrea* do not correspond to valid species names yet, highlighting abundant challenges in the taxonomy of *Saccostrea*. More studies are needed to study and assign the lineages to specific species.

The Saccostrea mordax *Lineages A and B*

The average K2P sequence divergence between *Saccostrea mordax* lineages *A* and *B* is about 1.0% in *16S* and 2.0% in *COI*, similar to that observed between two subspecies *Crassostrea gigas gigas* and *C. gigas angulata*, 1.05%–1.32% in *16S* and 2.22%–3.37% in *COI* (Wang et al. 2010). It is higher than intraspecific divergence in most species (Wang et al. 2010, Guo et al. 2018). The pairwise sequence divergence between some haplotypes of *S. mordax A* and *B* was even higher or close to interspecific level, although haplotypes of *16S* and *COI* were not consistently separated into two clades in the phylogenetic trees. These results indicate that considerable genetic divergence exists between some *S. mordax A* and *B* oysters, but the level of divergence seems to be at intraspecific or subspecies levels.

Although the shell morphology of *Saccostrea mordax A* and *B* is variable and appears in three different ecotypes, one of the ecotypes, the elongated D-type, closely resembles the syntype of *S. mordax* (Gould 1850), suggesting that the species identification is correct. Although the other two ecotypes show different shell characteristics, they are genetically similar and should also be classified as *S. mordax*. There is no clear association between genetic lineages (*S. mordax A* and *B*) and ecotypes, suggesting that the variation in shell morphology is mostly due to environmental factors. The two genetic lineages are sympatric in southern China, as expected for the same species *S. mordax* (Table 2). Huber (2010) considered *S. mordax* was synonym of *Saccostrea scyphophilla*. Without molecular data and detailed morphological analyses, it is prudent to retain *S. mordax* as the species name. Although the type locality of *S. mordax* is Fiji Islands, *S. mordax A* and *B* have a wide distribution in the tropical and subtropical Western Pacific and in both Northern and Southern Hemispheres. Wide distributions have been reported for some other oyster species such as

Crassostrea talonata (Li et al. 2017b), *Ostrea equestris*, and *Ostrea neostentina* (Hu et al. 2019). A comprehensive survey of all *mordax*-like oysters across its reported range of distribution may reveal additional variation or species.

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