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Comparison of genetic diversity and population structure of *Saccostrea mordax* lineages A, B, and C across South China Sea and Celebes Sea

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Saccostrea mordax is an ecologically important bivalve species and a potential genetic resource for oyster breeding that inhabit in the Indo-Western Pacific region, characterized by three distinct lineages: A, B, and C. However, limited information is available about its genetic variation and genetic structure. The present study was conducted to study genetic diversity and population structure of *S. mordax* lineages A, B, and C based on mitochondrial COI gene data across the South China Sea and Celebes Sea. The UPGMA phylogenetic tree among populations, BI phylogenetic tree among individuals and median network revealed three main clusters, corresponding to *S. mordax* lineages A, B, and C. The results of K2P sequence divergence, pairwise Φ_{st} , gene flow and AMOVA indicated clear genetic differences among the lineages, combined with sympatric in all 7 geographical populations, these suggests lineages A and B are transitional periods of new species formation. Moreover, different patterns of population subdivision were found within lineages A, B and C. In lineage A, significant genetic differentiation was only observed between YM (Celebes Sea) and other populations (South China Sea), with a significant correlation between geographical distance and genetic distance among populations. In lineages B and C, there was no genetic differentiation between YM (Celebes Sea) and other populations (South China Sea), and no significant correlation between genetic

distance and geographical distance among populations. The population history analyses indicated that populations of lineage A have experienced a recent sudden expansion, while populations of lineages B and C were large stable. These findings are essential for the conservation, sustainable utilization, and ecological restoration of *S. mordax*.

KEYWORDS

***Saccostrea mordax*, distinct lineage, genetic diversity, population structure, mitochondrial DNA (COI gene)**

1 Introduction

Oysters are of considerable economic value and play a pivotal role in the global aquaculture industry. In addition, they possess significant ecological importance (Klinbunga et al., 2003). Many oysters are important in aquaculture, with notable examples including the Hong Kong oyster (*Crassostrea hongkongensis*) (Langdon et al., 2003) and the Pacific oyster (*Crassostrea gigas*) (Hubert et al., 2009). As sessile organisms inhabiting estuarine and intertidal habitats, oysters play an integral role in the ecological dynamics of the shoreline. They offer a range of ecological benefits, including providing shelter, contributing to fisheries, facilitating water filtration, stabilizing shorelines, and acting as a coastal defence mechanism (Council, 2010). However, due to overfishing and human-induced habitat degradation, oyster populations have experienced significant declines worldwide (Beck et al., 2009). As a result, oyster reef ecosystems are considered among the most endangered and threatened marine ecosystems globally (Gillies et al., 2020).

Saccostrea mordax is a unique oyster species, characterized by three distinct lineages A, B and C (Lam and Morton, 2006; Sekino and Yamashita, 2013, 2016). Recent study suggests that lineage A and lineage B belong to the confirmed species *S. mordax*, while lineage C represents a new species of *Saccostrea* (Cui et al., 2021). *S. mordax* is widespread in the Indo-Western Pacific region, especially in southern China (Hainan, Guangxi, and Guangdong) and the intertidal and upper intertidal zones of rocky reefs in tropical Pacific island nations (Cui et al., 2021; Lu et al., 2024). It exhibits strong environmental tolerance, such as resistance to heat and desiccation, and can adapt well to oligotrophic environments. Despite its potential, *S. mordax* is not widely used in fisheries due to its small size, slow growth, and limited commercial value. Nevertheless, it holds significant aquaculture potential and ecological importance as a bivalve shellfish (Guo et al., 2024). Moreover, it is highly adaptable to island environments and is an important reef-building species for the protection of island coastlines.

Research on *S. mordax* remains limited. Some studies have preliminarily clarified the confusing taxonomic status of *Saccostrea* oysters, providing a foundation for biodiversity surveys,

conservation, ecological research, and aquaculture (Cui et al., 2021; Ghaffari et al., 2022; Lu et al., 2011). Research on the karyotype and chromosomal localization of its nuclear acid and major rRNA genes has also been conducted (Lu et al., 2011), and bidirectional sex change in *S. mordax* has been studied as well (Yasuoka and Yusa, 2017). Our team developed an artificial seedling technology using a one-step salinity reduction method and successfully achieved the first artificial breeding of *S. mordax*. To date, in *S. mordax*, the application of the mitochondrial COI gene has primarily focus on phylogeny studies and species identification. However, no information is available on the application of the mitochondrial COI gene in the genetic diversity and population structure of *S. mordax*. In fact, no molecular markers have been developed and used in the population genetics of wild *S. mordax* populations across South China Sea and Celebes Sea.

Studies of genetic diversity and population structure are important for evaluating the breeding potential and ecological restoration status of an organism. The mitochondrial DNA (mtDNA) genetic marker offers several advantages, including a fast evolutionary rate, maternal inheritance, and significant intraspecific polymorphism compared to other genetic markers (Avise et al., 1987). These features make mtDNA a valuable tool in studies analyzing population genetic structure (Emelianova et al., 2022; Liu et al., 2021; Sultana et al., 2022; Wei et al., 2023; Zhang et al., 2022). As more researchers use mitochondrial genes to study the population genetics of *Saccostrea* species, our understanding of these species continues to expand. For instance, based on the data from mitochondrial COI and SNP analysis, Nowland et al. (2019) found a widespread distribution of *S. echinata* in the tropical Indian-Pacific region and the existence of genetically distinct populations. In et al. (2016) concluded that after several generations of mass selection in *S. glomerata* using COI and microsatellite markers, there was a significant decline in genetic diversity across four distinct lines, although the combined genetic diversity across multiple mass selection lines may still converge with that of the wild population or ancestral line.

In the present study, we used mitochondrial COI gene to perform a comprehensive investigation of genetic diversity and population structure of *S. mordax* lineages A, B, and C across the South China Sea and Celebes Sea. The findings of this research

provide baseline information for the sustainable utilization, ecological restoration, and conservation of *S. mordax* in this region.

2 Materials and methods

2.1 Population sampling and DNA extraction

S. mordax specimens were randomly obtained from seven wild populations: Nanao, Shantou (SN), Dongchong, Shenzhen (SD), Wanshan Island, Zhuhai (ZW), Luhuitou, Sanya (SL), Yongxing Island, Xisha (XY), Meiji Reef, Nansha (NM), and Menado, Indonesia (YM) (Table 1, Figure 1). A total of 244 oyster samples (SL: 48, SD: 40, SN: 37, NM: 25, XY: 39, ZW: 33, YM: 22) were sequenced to detect the COI gene. All samples used in this study were divided into three lineages (A, B and C) of the *S. mordax* branch using COI as described by (Cui et al., 2021). The adductor muscles of fresh oysters were dissected and preserved in 75% ethanol at -20°C for future use. Genomic DNA was extracted from muscle tissue using the phenol-chloroform method (Li et al., 2006). DNA concentration and purity were determined using a NanoDrop2000 UV spectrophotometer, and DNA integrity was assessed by 1.0% agarose gel electrophoresis before storing at -20°C.

2.2 COI gene amplification and genotyping

Amplification of the COI gene segment was performed using the following primer pair: CF (COI) F/CF (COI) R: 5'-CGTCAGGATTATAGAGTTTATAGATGCC-3'/5'GAAGAA

TTAGCACATATACCTCAGGATG3', designed by primer 3, sequences were obtained from GenBank. PCR amplification was performed in a Veriti™ Thermal Cycler (Applied Biosystems, USA) with a 50 µL reaction volume. The PCR reaction mixture consisted of 2.0 mM MgCl₂, 1.5 µl of template DNA, 0.5 µM of each primer, 200 µM of each dNTP, 0.03 U/µl 2×Taq PCR StarMix (Dye) (Genstar, China). The PCR amplification was conducted using a 96-well thermal cycler under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 55°C for annealing, and 1 min at 72°C for extension, with a final 10 min extension at 72°C. The PCR products were analysed via 1% agarose gel electrophoresis and sequenced using the ABI3730xl DNA Analyzer sequencer (Invitrogen Biotechnology Co., Ltd, USA) in both forward and reverse directions. All sequences were confirmed through comparison with known GenBank sequences and manually checked.

2.3 Analysis of genetic diversity and population genetic structure

Nucleotide sequences were aligned using ClustalX 2.0 (Larkin et al., 2007) and manually adjusted. Sequences were trimmed using BioEdit (Hall, 1999) Version software to remove ambiguous or missing data, yielding a final fragment of 561 bp. The COI gene segment was validated through MEGA v11.0 software (Tamura et al., 2021). Haplotype diversity (Hd) and nucleotide diversity (π) were calculated using DnaSP (Librado and Rozas, 2009), while genetic differentiation between populations was assessed using pairwise Φ_{st} in Arlequin v3.5 (Excoffier and Lischer, 2010). The probability values were calculated using 10,000 random permutations. To access

TABLE 1 *Saccostrea mordax* sampling information, including geographic region, GPS positions and date, population code and sample size.

Code	Population	N	Location	Geographic region	Latitude	Longitude	Sampling date
SN	A B C	27 10 0	Nanao, Shantou	Guangdong province	23.4217N	117.0233E	June, 2023
SD	A B C	30 10 0	Dongchong, Shenzhen	Guangdong province	22.8862N	113.4579E	July, 2023
ZW	A B C	23 10 0	Wanshanda, Zhuhai	Guangdong province	21.9468N	113.7338E	June, 2023
SL	A B C	30 10 8	Luhuitou, Sanya	Hainan province	18.2160N	109.4948E	December, 2022
XY	A B C	29 10 0	Yongxingdao, Xisha	Hainan province	16.8328N	112.3432E	December, 2023
NM	A B C	10 5 10	Meijijiao, Nansha	Hainan province	9.9082N	115.5349E	January, 2024
YM	A B C	8 4 10	Manado, Sulawesi Utara	Indonesia	1.29N	124.50E	December, 2023



FIGURE 1
Map of sampling sites of *Saccostrea mordax* across South China Sea and Celebes Sea.

differentiation between geographical groups, analysis of molecular variance (AMOVA) was conducted in Arlequin v3.5. The relationship between genetic distance and geographical distance was tested using a Mantel test to confirm isolation by distance (IBD) (Mantel, 1967). Geographical distances were estimated as the shortest sea routes between locations, determined via Google Earth. The haplotype sequences have been submitted to GenBank under accession number PP989682-PP989794.

2.4 Neutral experiments and phylogenetic analyses

Historical demographic and spatial expansions were analysed using two statistical approaches in Arlequin v3.5. Specifically, Fu's F_s (Fu, 1997) and Tajima's D (Tajima, 1989a, 1989) tests were used to assess deviations from neutrality. Fu's F_s test, which is highly sensitive to population demographic expansion, yielded strong negative F_s values, suggested that the possibility of demographic growth. Additionally, the negative Tajima's D value, coupled with significant P values further supporting that the hypothesis of sudden population expansion could not be rejected. Moreover, the statistical analyses of mismatch distribution were also performed by Arlequin v3.5 with 10,000 permutations.

We used the Kimura (Kimura, 1980) two-parameter (K2P) model to calculate distances between populations and individuals. The phylogenetic relationship among individuals was examined by constructing a phylogenetic tree based on Bayesian inference (BI) in MrBayes with 1,000,000 Markov Chain Monte Carlo (MCMC) generations (Ronquist et al., 2012). A UPGMA phylogenetic tree

among populations was constructed using K2P distances (Nei and Kumar, 2000). A median joining haplotype network was constructed from the mitochondrial COI sequence data using the PopART (Bandelt et al., 1999; Leigh et al., 2015) with the default settings.

3 Results

3.1 Genetic diversity analysis

A total of 118 polymorphic sites and 113 haplotypes were detected from the 561 bp COI sequences of 244 specimens (Table 2). Haplotypes 1–41 were found exclusively in the *S. mordax* lineage B, haplotypes 42–98 were found exclusively in lineage A, and the remaining haplotypes 99–113 were discovered in lineage C. There were no shared haplotypes among the three genetic lineages (A, B, and C). Of the 113 unique haplotypes, only 19 (16.8%) were shared among more than one population, while the majority were population-specific. In lineage A, H44 was the most widely distributed, found in all seven populations, H64 was present in five populations, while H43, H76, and H92 were distributed in three populations. In lineage B, H11 and H18 were found in four populations, while H16 was distributed in three populations. In lineage C, H99 and H107 were shared among all populations, while H104 was found in two populations. Furthermore, the number of polymorphic loci ranged from 7 to 24, with lineage B across seven geographic locations exhibiting highest levels of polymorphic loci.

The haplotype diversity (H_d) and nucleotide diversity (π) across all populations were 0.881 and 0.02566, respectively (Table 2). The

TABLE 2 Genetic diversity for populations of *Saccostrea mordax* obtained from mitochondrial *cox1* gene.

Population	n	ns	np	Hd $\pm$ SD	π $\pm$ SD	k	Tajima'D	Fu's Fs
SLA	30	16	15	0.756 $\pm$ 0.086	0.00234 $\pm$ 0.00044	1.310	-2.29430*	-14.26735**
SDA	30	14	12	0.646 $\pm$ 0.101	0.00177 $\pm$ 0.00041	0.995	-2.39715*	-10.58119**
SNA	27	15	10	0.546 $\pm$ 0.115	0.00198 $\pm$ 0.00058	1.111	-2.46007*	-6.65348*
NMA	10	8	8	0.933 $\pm$ 0.077	0.00285 $\pm$ 0.00056	1.600	-1.87333*	-6.03172*
XYA	29	15	14	0.768 $\pm$ 0.082	0.00240 $\pm$ 0.00047	1.345	-2.19755*	-12.16230**
ZWA	23	11	10	0.688 $\pm$ 0.108	0.00185 $\pm$ 0.00044	1.036	-2.23309*	-7.74430*
YMA	8	7	7	0.964 $\pm$ 0.077	0.00369 $\pm$ 0.00070	2.071	-1.10748	-4.41788*
SLB	10	24	10	1.000 $\pm$ 0.045	0.01331 $\pm$ 0.00117	7.467	-0.56947	-4.40047*
SDB	10	15	9	0.978 $\pm$ 0.054	0.00848 $\pm$ 0.00117	4.756	-0.47494	-3.75895*
SNB	10	18	8	0.956 $\pm$ 0.059	0.00994 $\pm$ 0.00192	5.578	-0.57596	-1.69595
NMB	5	14	5	1.000 $\pm$ 0.126	0.01141 $\pm$ 0.00210	6.400	-0.34706	-0.96395
XYB	10	19	10	1.000 $\pm$ 0.045	0.01145 $\pm$ 0.00150	6.422	-0.20511	-4.93385*
ZWB	10	19	10	1.000 $\pm$ 0.045	0.00955 $\pm$ 0.00140	5.356	-0.94925	-5.63431*
YMB	4	10	4	1.000 $\pm$ 0.177	0.00951 $\pm$ 0.00236	5.333	-0.22234	-0.13907
SLC	8	14	6	0.952 $\pm$ 0.096	0.01120 $\pm$ 0.00187	6.286	-0.36426	-0.18273
NMC	10	15	6	0.889 $\pm$ 0.075	0.01093 $\pm$ 0.00162	6.133	-0.72192	-0.87643
YMC	10	19	8	0.933 $\pm$ 0.077	0.01256 $\pm$ 0.00165	7.044	-0.22897	-1.11130
Total	244	118	113	0.881 $\pm$ 0.020	0.02566 $\pm$ 0.00264	14.393	-0.95967	-4.96729*

Sample size (n), number of segregating sites (ns), number of haplotypes (np), haplotype diversity (Hd), nucleotide diversity (π), average number of nucleotide differences (k) and standard deviation (SD).

*Significant Tajima'D and Fu's Fs values ($P < 0.05$).

**Significant Tajima'D and Fu's Fs values ($P < 0.0001$).

haplotype diversity and nucleotide diversity in lineage B ($Hd = 0.991$, $\pi = 0.01052$) and lineage C ($Hd = 0.925$, $\pi = 0.01156$) were significantly higher than that in lineage A ($Hd = 0.757$, $\pi = 0.00241$). Moreover, the haplotype diversity and nucleotide diversity in lineages B and C ranged from 0.956 (SNB) to 1.000 (SLB, NMB, XYB, ZWB, YMB) and from 0.00848 (SDB) to 0.01331 (SLB), from 0.889 (NMC) to 0.952 (SLC) and from 0.01093 (NMC) to 0.01256 (YMC), respectively. The haplotype diversity and nucleotide diversity in the lineage A ranged from 0.546 (SNA) to 0.964 (YMA) and from 0.00177 (SDA) to 0.00369 (YMA).

3.2 Population structure

The variation in Φ_{st} values among populations was substantial, ranging from -0.1159 to 0.9797 (Table 3). Overall, populations of *S. mordax* lineages A, B, and C not only showed extremely significant differentiation but also exhibited a wide range of Φ_{st} values. In lineage A, significant genetic differentiation was found between YM (Celebes Sea) and other populations (South China Sea), while no genetic differentiation was observed among the other populations. In lineage B, significant genetic differentiation was found only between the SD and SN, SN and ZW populations, with no genetic differentiation among other populations. In lineage C, there was no

genetic differentiation among three populations. Correspondingly, the results of gene flow among populations also confirmed the differentiation among populations.

The gene flow between lineages A, B, and C was less than 0.4. In lineage A, the gene flow between YM and other populations ranged from 2.6289-6.5976, while the gene flow among other populations ranged from 29.4867 to infinity. In lineage B, the gene flow between SD and SN populations, and SN and ZW populations, was 1.8739 and 1.6803, respectively, while the gene flow among other populations ranged from 3.2404 to infinity. In lineage C, the gene flow among three populations ranged from 14.0594 to infinity. The results of the AMOVA analysis on the sample groups (Table 4) indicate a high genetic variation between the three groups (88.05%), whereas the variation within the populations is lower at 11.45%. The result showed highly significant differences among groups ($F_{CT} = 0.8805$, $P = 0.0000$) and within subpopulations relative to the total population ($F_{ST} = 0.8855$, $P = 0.0000$), but no differences among subpopulations relative to the groups ($F_{SC} = 0.0419$, $P = 0.1975$).

The Mantel test revealed a significant correlation between genetic distance and geographical distance among populations of lineage A ($r = 0.8807$, $P = 0.0016$), but there was no significant correlation among populations of lineage B ($r = -0.3012$, $P = 0.8935$) and C ($r = 0.6188$, $P = 0.1708$).

TABLE 3 The pairwise Φ_{st} (below the diagonal) and gene flow (above the diagonal) between populations of *Saccostrea mordax*..

Population	SLA	SDA	SNA	NMA	XYA	ZWA	YMA	SLB	SDB	SNB	NMB	XYB	ZWB	YMB	SLC	NMC	YMC
SLA		1029.0779	Inf	31.4730	Inf	Inf	4.9054	0.2121	0.1513	0.1675	0.1412	0.2014	0.1538	0.1526	0.0116	0.0128	0.0136
SDA	0.0005		Inf	45.0990	97.5942	Inf	2.6289	0.1930	0.1345	0.1493	0.1209	0.1823	0.1375	0.1255	0.0103	0.0117	0.0126
SNA	−0.0061	−0.0033		76.7622	Inf	Inf	3.2114	0.2113	0.1470	0.1629	0.1340	0.1991	0.1503	0.1405	0.0112	0.0126	0.0135
NMA	0.0156	0.0110	0.0065		129.2796	29.4867	4.4681	0.3593	0.2302	0.2622	0.2336	0.3287	0.2407	0.2347	0.0184	0.0205	0.0222
XYA	−0.0023	0.0051	−0.0029	0.0039		Inf	6.5976	0.2146	0.1542	0.1690	0.1444	0.2041	0.1560	0.1550	0.0120	0.0133	0.0141
ZWA	−0.0051	−0.0020	−0.0053	0.0167	−0.0110		4.1174	0.2250	0.1522	0.1699	0.1382	0.2095	0.1572	0.1409	0.0116	0.0132	0.0142
YMA	0.0925*	0.1598**	0.1347**	0.1006*	0.0705*	0.1083*		0.3903	0.2530	0.2905	0.2693	0.3618	0.2628	0.2735	0.0204	0.0223	0.0242
SLB	0.7022**	0.7215**	0.7029**	0.5819**	0.6997**	0.6897**	0.5616**		Inf	9.1532	Inf	Inf	43.3614	Inf	0.0305	0.0301	0.0329
SDB	0.7677**	0.7880**	0.7728**	0.6847**	0.7643**	0.7666**	0.6641**	−0.0047		1.8739	Inf	Inf	Inf	51.0070	0.0252	0.0260	0.0286
SNB	0.7491**	0.7700**	0.7542**	0.6560**	0.7473**	0.7464**	0.6326**	0.0518	0.2106*		3.2404	12.8755	1.6803	41.9676	0.0253	0.0255	0.0284
NMB	0.7798**	0.8052**	0.7887**	0.6816**	0.7760**	0.7834**	0.6499**	−0.0782	−0.0871	0.1337		Inf	Inf	Inf	0.0286	0.0290	0.0328
XYB	0.7128**	0.7328**	0.7152**	0.6033**	0.7102**	0.7047**	0.5802**	−0.0351	−0.0029	0.0374	−0.0500		17.8266	Inf	0.0297	0.0297	0.0326
ZWB	0.7648**	0.7844**	0.7689**	0.6750**	0.7622**	0.7608**	0.6555**	0.0114	−0.0605	0.2293**	−0.0684	0.0273		Inf	0.0255	0.0259	0.0286
YMB	0.7661**	0.7994**	0.7806**	0.6805**	0.7634**	0.7802**	0.6464**	−0.0610	0.0097	0.0118	−0.0710	−0.0578	−0.0137		0.0270	0.0279	0.0319
SLC	0.9774**	0.9797**	0.9781**	0.9645**	0.9766**	0.9774**	0.9608**	0.9425**	0.9520**	0.9519**	0.9459**	0.9440**	0.9515**	0.9489**		Inf	15.9854
NMC	0.9750**	0.9771**	0.9754**	0.9607**	0.9741**	0.9743**	0.9574**	0.9432**	0.9507**	0.9515**	0.9451**	0.9439**	0.9507**	0.9472**	−0.1159		14.0594
YMC	0.9735**	0.9755**	0.9737**	0.9575**	0.9726**	0.9724**	0.9538**	0.9383**	0.9459**	0.9463**	0.9384**	0.9388**	0.9460**	0.9400**	0.0303	0.0343	

P is the probability that any random value obtained with 10,000 permutations is > observed value.
*indicates significant differences (*P*<0.05), ** indicates extremely significant differences (*P*<0.01).

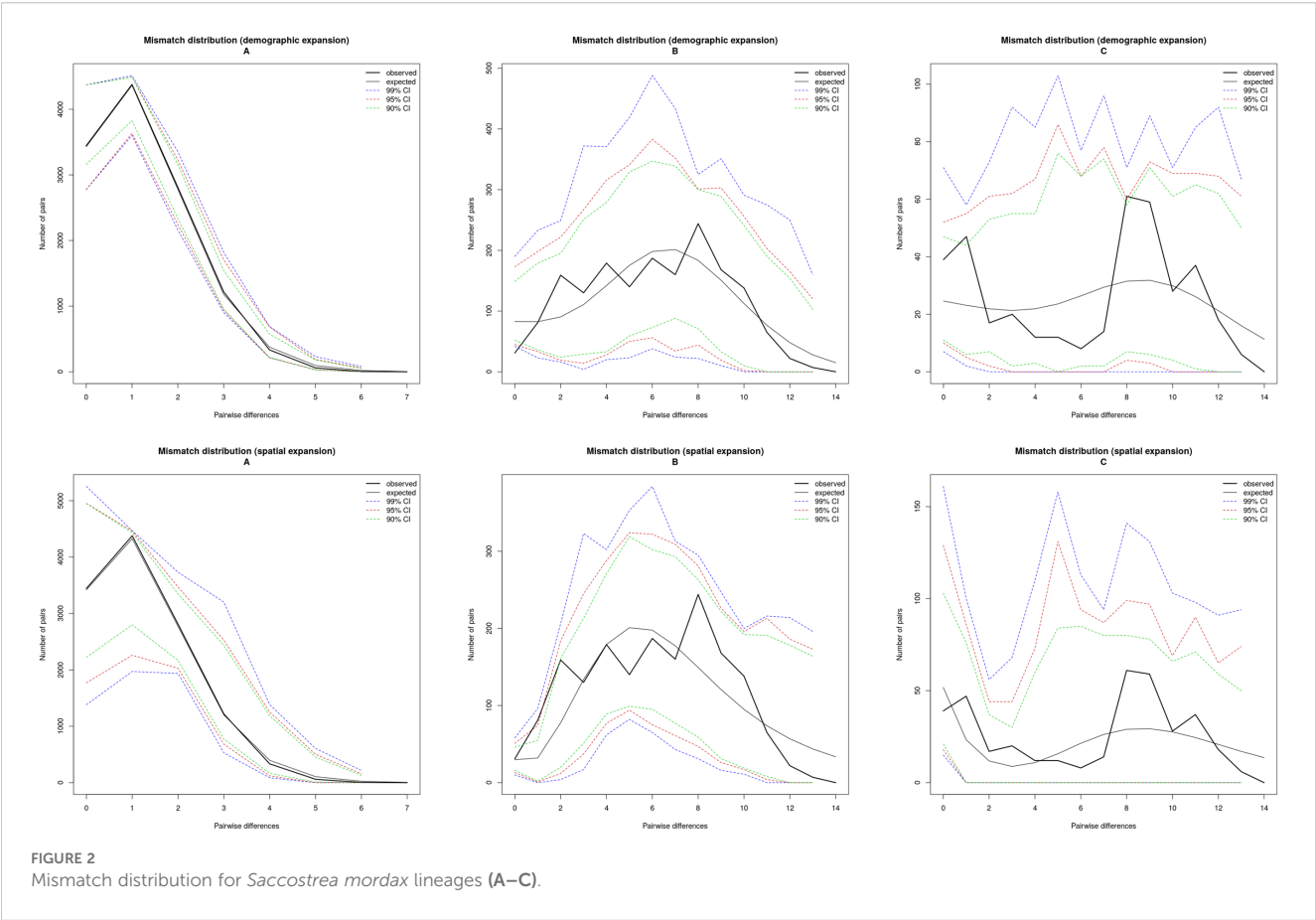
TABLE 4 Results of the analysis of molecular variance (AMOVA) for *Saccostrea mordax* populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P
Geographic regions(SLA,SNA,SDA,NMA,YMA,ZWA,XYA/SLB,SNB,SDB,NMB,YMB,ZWB,XYB/SLC, NMC,YMC)					
Among groups	2	1390.083	11.04120 Va	88.05	0.00000
within groups	14	32.633	0.06279 Vb	0.50	0.19746
Within populations	227	325.997	1.43611 Vc	11.45	0.00000
Total	243	1748.713	12.54010	100	

3.3 Neutrality tests and phylogenetic analysis

Tajima's D, which measures the difference between the number of segregating sites and pairwise genetic distances, produced negative values for all populations combined. Tajima's D is significantly negative in all populations except YM of lineage A, but no significantly in all populations of lineages B and C. Fu's *F_s* statistic was significantly negative for 11 of the 17 populations (Table 2), indicating non-equilibrium dynamics. Among them, all populations of lineage A were found to be significant, as were same populations (SL, SD, XY, and ZW) of lineage B. However, all populations of lineage C and the remaining populations (SN, NM and YM) of lineage B were not significant. Correspondingly, the mismatch distribution of lineage A was unimodal, supporting the

hypothesis of the recent sudden expansion model. The mismatch distribution of lineage B was unimodal of normal distribution, supporting the hypothesis of the large stable after expansion in history. And, the mismatch distribution of lineage C was bimodal, supporting the hypothesis of the large stable population (Figure 2). The UPGMA phylogenetic tree revealed that the 17 populations clustered into three main branches, corresponding to *S. mordax* lineages A, B, and C (Figure 3). All seven populations of lineage B formed a sister branch with the populations of lineage A, while the three populations of lineage C formed a single branch. Within branches A and C, the populations of the South China Sea grouped together first, and then with the YM population of the Celebes Sea. The mean sequence divergence between lineages A and B was 2.0%, with pairwise divergence between their haplotypes ranging from 1.1% to 2.7%. Within lineage A, haplotype divergence ranged from



Tree scale: 0.01

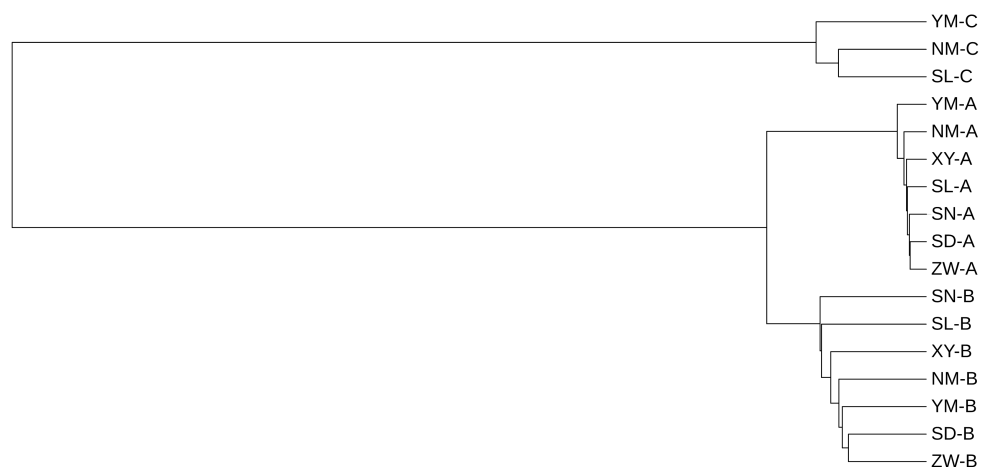


FIGURE 3
UPGMA phylogenetic tree among 17 populations of *Saccostrea mordax* based on K2P distances matrix of mitochondrial COI sequences.

0.2% to 1.1%, while in lineage B, it ranged from 0.2% to 2.4%. Furthermore, the mean sequence divergence between lineage C and the other lineages was 9.8% and 10.4%, exceeding the divergence typically seen between two oyster species (Supplementary Table S1) (Guo et al., 2018).

The median network based on nucleotide divergences among the haplotypes reveals distinct phylogenetic structures among populations, forming three main haplotype clusters (Figure 4). Dominant haplotypes such as H44, H18, H99, and H107 were identified, with H44 serving as the central radiation point within lineage A, radiating numerous haplotypes in a star-like pattern and separating from the other two lineages. H18 is a shared haplotype among SDB, SNB, NMB, and XYB populations, while haplotypes H107 and H99 are shared among SL, NM, and YM of lineage C. Haplotypes within lineage A are more clustered, whereas those in lineages B and C are more dispersed, with minimal overlap between C- and B-type radiations, consistent with the clustering pattern observed in the individual clustering diagram (Figure 5).

4 Discussion

Genetic confirmation of the geographic range and distinct lineages of *S. mordax* is important for conservation, sustainable utilization, and ecological restoration. This is particularly relevant for artificial breeding in aquaculture, as different species require different culture conditions and techniques (Jobling, 2012). Through COI analysis and identification in this study, it was found that *S. mordax* lineages A and B were widely distributed in the South China Sea and Celebes Sea. This result is consistent with those of (Cui et al., 2021; Morton, 2004), which support a broad distribution across the tropical and subtropical regions of the Western Pacific, spanning both the Northern and Southern Hemispheres. Prior to this study, the *S. mordax* lineages C was only known to be naturally distributed in Haitang Wan, Sanya,

Hainan of China (Cui et al., 2021) and Okinawa Island, Yakata of Japan (Sekino and Yamashita, 2013). In this study, *S. mordax* lineage C has been newly discovered in Luhuitou, Sanya and Meijijiao, Nansha, Hainan of China, as well as Manado, Sulawesi Utara of Indonesia, significantly expanding the known natural distribution area of *S. mordax* lineages C. In addition, the proportion of lineage C individuals increased significantly from north to south (SLC = 13%, NMC = 55%, YMC = 71%). Other studies have utilized genetic data to explore the distribution patterns of tropical oyster species within the Pacific region, including *S. echinata* (Nowland et al., 2019) and *C. iredalei* (Zainal Abidin et al., 2016).

Genetic diversity forms the foundation of a species' ability to adapt and evolve. There is a positive linear relationship exists between genetic diversity within a species and its capacity to adapt to environmental changes (Cruz et al., 2013). In this study, completely different patterns of genetic diversity were observed between lineage A and lineage B/C. Populations of lineage A exhibited low nucleotide diversity and high haplotype diversity, which was concordant with previous reports for oyster species, such as *C. hongkongensis* ($H_d = 0.814$, $\pi = 0.00236$) (Li et al., 2013). However, populations of lineages B and C exhibited both high nucleotide diversity and haplotype diversity, similar to findings for *S. echinata* ($H_d = 0.949$, $\pi = 0.006$) (Nowland et al., 2019). Previous studies on *C. sikamea*, reported a genetic diversity pattern where Japanese populations showed high haplotype diversity and low nucleotide diversity, while Chinese populations exhibited high haplotype and nucleotide diversities (Hu et al., 2018). It was speculated that the observed pattern may be attributed to population instability, habitat discontinuities, and evolutionary forces. With evolutionary forces likely being the primary factor in this study.

It has been suggested that the population history of marine species can be divided into four groups based on different values of haplotype diversity and nucleotide diversity derived from

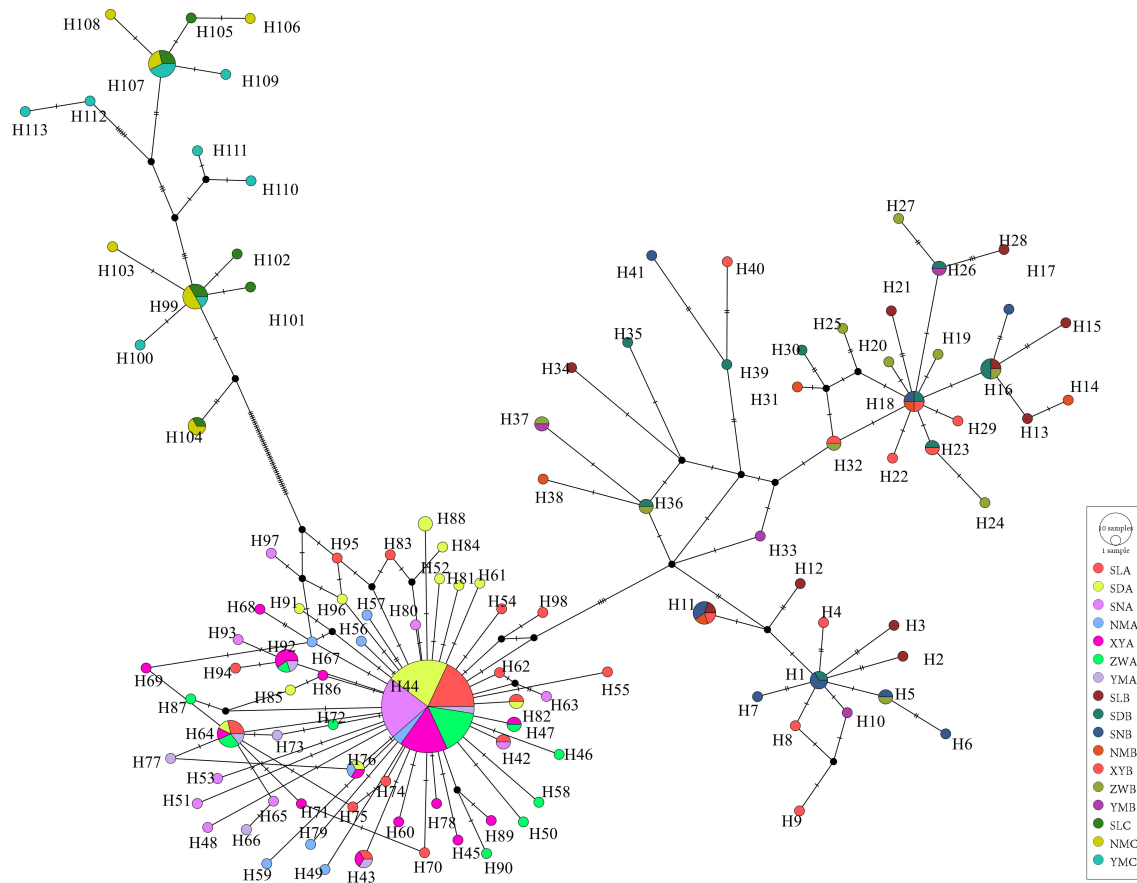


FIGURE 4

A median joining haplotype network of *Saccostrea mordax* mitochondrial COI sequence data. Each bar on the branch corresponds to a single nucleotide substitution. Larger circles contain specific frequency size.

mitochondrial gene sequences (Grant and Bowen, 1998). The populations of lineage A belong to the second group (high Hd, low π), indicating a recent population expansion from a small effective population size in their histories. The significant negative values of Fu's F_s /Tajima's D and mismatch distribution further support the occurrence of recent population expansion in lineage A populations. It is suggested that populations of lineage A might have experienced a population bottleneck and expansion caused by climatic oscillations during the Pleistocene period (Arnaud et al., 2000; Dynesius and Jansson, 2000). In comparison, populations of lineages B and C belong to the fourth group (high Hd, high π), which suggests either a large stable population with a long evolutionary history or the result of secondary contact between differentiated lineages. This is also supported by the lack of significant negative Tajima's D values and mismatch distribution.

Current studies on the genetic structure and differentiation of *S. mordax* populations clearly reveal the present of three genetically distinct clusters, which include lineage A (SLA, SDA, SNA, NMA, XYA, ZWA), lineage B (SLB, SDB, SNB, NMB, XYB, ZWB) and lineage C (SLC, NMC, YMC). This pattern of genetic structure aligns well with the distribution of *S. mordax* lineage A, *S. mordax* lineage B, and *S. mordax* sp. nov., as suggested by previous study

(Cui et al., 2021). Previous study also found that the level of divergence between lineages A and B was considered intraspecific or at subspecies levels using K2P sequence divergence (Cui et al., 2021). In this study, the average K2P sequence divergence between lineages A and B was also 2.0% in COI, consistent with (Cui et al., 2021). This value was higher than the intraspecific divergence of most species (Guo et al., 2018; Wang et al., 2010). Meanwhile, the pairwise sequence divergences between haplotypes of lineages A and B were higher (1.1%–2.7%) than those within lineage A (0.2%–1.1%). Moreover, pair-wise Φ_{st} values between populations of lineages A and B not only showed extremely significant differentiation, but also exhibited a high degree (0.5616–0.8052), and gene flow between populations of lineages A and B was less than 0.4. Combined with the results of AMOVA and sympatric in all 7 geographical populations, this suggests lineages A and B are transitional periods of new species formation.

The hypothesis suggests that the high dispersal capacities of marine bivalves within extended larval stages should result in widespread distribution across large geographical areas. The interaction between their biological traits and ocean currents is expected to lead to low geographical differentiation (Arnaud et al., 2000). Some studies on bivalve population differentiation (Kusnadi

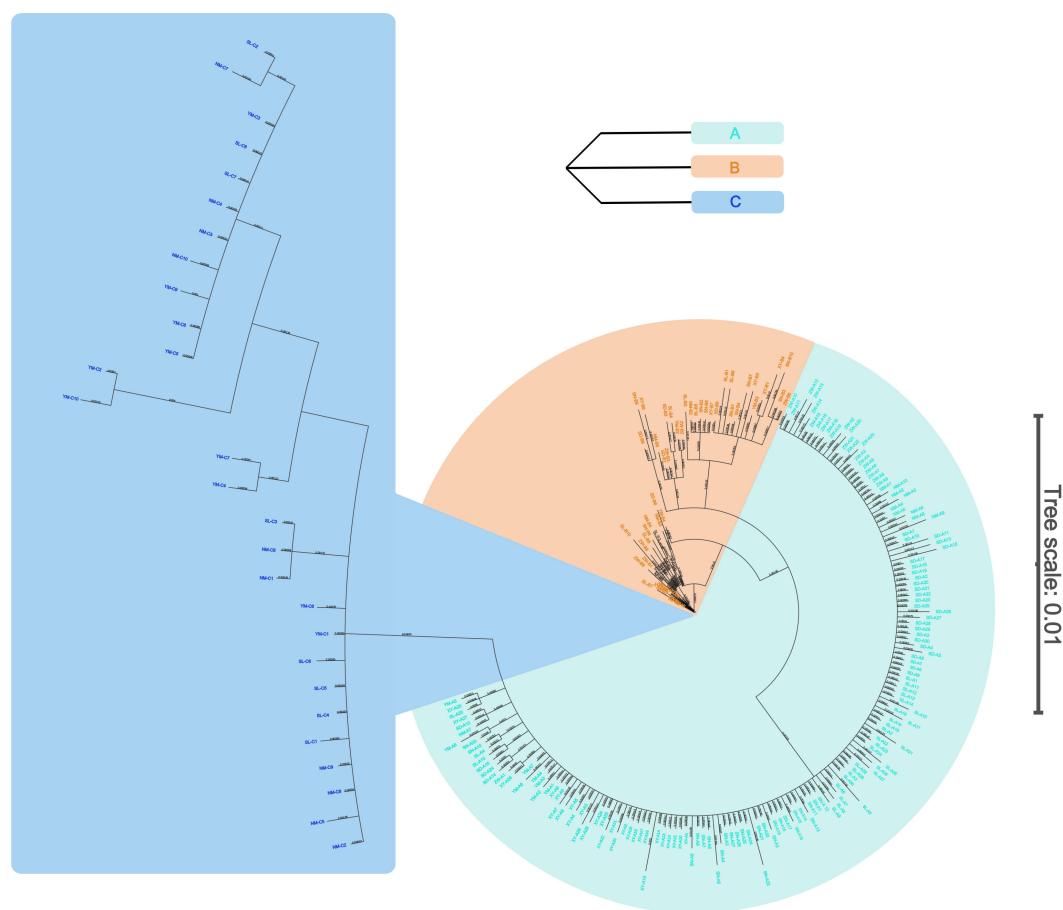


FIGURE 5
Phylogenetic tree among individuals of *Saccostrea mordax* based on Bayesian inference.

et al., 2022; Vadopalas et al., 2004) support this idea; however, other research contradicts this hypothesis (Nowland et al., 2019; Zhan et al., 2009), indicating that additional factors may influence population structure and dispersal. In this study, different types of population subdivision within lineages A, B and C were found across the South China Sea and Celebes Sea. In lineage A, significant genetic differentiation was only observed between YM (Celebes Sea) and other populations (South China Sea), with a significant correlation between geographical distance and genetic distance among populations. This population subdivision was mainly caused by ocean currents (McManus, 1994), geographic distance, and climatic oscillations during the Pleistocene period. The lack of genetic differentiation among populations in the South China Sea could be attributed to ocean currents (Fang et al., 2012) and the long planktonic larvae stage (Ma et al., 2021). In lineages B and C, there was no genetic differentiation between YM (Celebes Sea) and other populations (South China Sea), and no significant correlation between genetic distance and geographical distance among populations of lineages B and C. Moreover, the SD and SN, SN and ZW populations in lineage B also exhibited significant genetic differentiation, possibly due to complex hydrology factors, including numerous bays, vortices, and runoff of the Pearl River.

5 Conclusion

Current studies performed a comprehensive investigation of genetic diversity and population structure of *S. mordax* lineages A, B, and C across the South China Sea and Celebes Sea. The UPGMA phylogenetic tree among populations, BI phylogenetic tree among individuals and median network revealed three main clusters, corresponding to *S. mordax* lineages A, B, and C. The results of K2P sequence divergence, pairwise Φ_{st} , gene flow and AMOVA indicate clear genetic differences among the lineages, combined with sympatric in all 7 geographical populations, these suggests lineages A and B are transitional periods of new species formation. Moreover, different patterns of population subdivision were found within lineages A, B and C across the South China Sea and Celebes Sea. The population history analyses indicated that populations of lineage A have experienced a recent sudden expansion, while populations of lineages B and C were large stable. In the future, it is necessary to collect more geographical populations of *S. mordax* lineages A, B and C to verify the results of this study. Since only one genetic marker was used in this study, further analysis using more advanced genomic markers, such as SNPs detected through genome scans, would be beneficial to support our findings. Furthermore,

this study significantly expanded the known natural distribution area and the proportion of individuals in each geographical population of *S. mordax* lineages A, B and C. This information is essential for the conservation, sustainable utilization, and ecological restoration of *S. mordax*.

Data availability statement

The haplotype sequences were submitted to the NCBI, with the accession number PP989682-PP989794.

Author contributions

YJZ: Software, Formal analysis, Writing – original draft, Data curation, Visualization. HM: Data curation, Validation, Project administration, Conceptualization, Writing – review & editing. CH: Formal analysis, Writing – original draft, Resources. ZZ: Resources, Writing – original draft, Visualization. ZW: Writing – original draft, Investigation, Formal analysis. XD: Software, Writing – original draft. JW: Writing – original draft, Data curation, Validation. YQ: Writing – original draft, Resources, Project administration. JuL: Project administration, Writing – original draft, Validation. ZY: Conceptualization, Writing – original draft, Validation. YP: Supervision, Project administration, Conceptualization, Writing – review & editing. YHZ: Methodology, Conceptualization, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2025.1593382/full#supplementary-material>

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