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Species identity and spatial patterns of common oysters at oyster reefs of Guangdong, China

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Introduction: As concerns mount over the threats facing oyster reefs, awareness of the need to mitigate habitat loss and restore ecosystem services is increasing. However, challenges in identifying oysters have limited our understanding of their species- and population-level diversity, complicating efforts to establish effective marine protected areas. Therefore, this study aims to address these challenges by conducting a comprehensive survey along the coastline of Guangdong Province to assess the species composition and spatial distribution of oyster reefs across 51 intertidal sites, such as estuaries, islands, oyster farms, mudflats, and mangroves.

Methods: In total, we collected 742 oyster specimens by qualitative survey for oyster distribution and generated approximately 1,400 mitochondrial DNA sequences (N = 693 for 16S rRNA and N=706 for cytochrome c oxidase subunit I) to support genetic analysis. More than 30000 oysters sample collected by quantitative survey were applied to analyze the oyster assemblages.

Results: The analyses revealed 12 mitochondrial lineages representing three genera within the family Ostreidae. Phylogenetic analysis confirmed robust monophyletic groupings, confirming species identities and leading to the identification of two cryptic species within the genus *Saccostrea*. Based on DNA evidence, these two cryptic *Saccostrea* species were closely related to the known *S.* non-*mordax* D and H lineages. Quantitative analysis showed that *Crassostrea sikamea* was the most prevalent species in the study area, with an average abundance and biomass > 1,400 individuals/m^2 and 4,449 grams/m^2, respectively. Qualitative and quantitative assessment both revealed at least 6 species were identified at G07 (Jieyang), being the most biodiverse location of Guangdong Province.

Discussion: By mapping oyster distribution and updating the species inventory, our study provides a foundation for future research on oyster populations and informs conservation efforts aimed at preserving and restoring oyster habitats, thereby supporting marine biodiversity.

KEYWORDS

oyster reef, species identification, biodiversity, spatial pattern, cryptic species

1 Introduction

Oysters (Family Ostreidae)—a prominent group of marine bivalve mollusks—play a vital role in global aquaculture, providing food and livelihoods for millions of people while significantly contributing to local and national economies. In Guangdong Province, China, cupped and rock oysters are particularly important, with an annual production of 1.15 million metric tons supporting millions of residents (Guo et al., 2018; Ma et al., 2021a). Although oysters are common, accurately identifying oyster species remains challenging owing to their pronounced phenotypic plasticity and highly variable shell morphology (Wang et al., 2010; Salvi and Mariottini, 2017; Li et al., 2018). Early taxonomic efforts of China that relied on shell characteristics often misclassified ecotypes as distinct species, leading to confusion in species identification (Harry, 1985; Guo et al., 2018).

However, by virtue of the progress in molecular identification, DNA sequence analysis has significantly advanced Ostreidae taxonomy, revealing a few ecophenotypic variants and cryptic species within cupped oysters while refining our understanding of biodiversity and phylogeny. For example, in northern China, mitochondrial DNA markers, including cytochrome c oxidase subunit I (COI) and 16S rRNA, have confirmed *Crassostrea gigas* as the dominant oyster species, while *C. plicatula* and *C. talienwhanensis* have been identified as ecotypes of *C. gigas* (Wang et al., 2008). Similarly, the designated *C. rivularis* in China is now recognized as the red meat oyster (*C. ariakensis*) or the white meat oyster (*C. hongkongensis*) (Wang et al., 2004). Genetic analyses have also revealed several sympatric cryptic species, leading to the identification of new species such as *C. dianbaiensis* (Xia et al., 2014; Sekino et al., 2015), *C. zhanjiangensis* (Wu et al., 2013), and *Saccostrea mordoides* (Cui et al., 2021). Phylogenetic studies have also provided some grounds for supporting the validity of *Talonostrea talonata*, the reclassification of several commercially important *Crassostrea* species into the genus *Magallana*, and the conspecific relationship between *C. iredalei* and *C. bilineata* (Salvi and Mariottini, 2016; Li et al., 2017).

On the other hand, within Saccostreinae, rock oysters were more complex led to multiple confusion and modification owing to their high morphological plasticity. Previous studies suggest a potential *Saccostrea* lineage that includes *S. mordax* (namely *S. scyphophilla* lineage A and B), as well as a "superspecies" of *S. cuccullata* which encompasses nine mitochondrial lineages (designated as lineages A–G along with *S. kegaki* and *S. glomerata*) (Lam and Morton, 2006). Based on this phylogenetic analysis, the nomenclature of *S. cuccullata* lineage C and *S. cuccullata* lineage F has been adopted and recorded in Malaysia and Singapore (Hamaguchi et al., 2014). In 2016, following the recognition that *S. cuccullata* is restricted to Central Western Africa and that its lineages should not represent the Indo-West Pacific *Saccostrea* oysters, *S. cuccullata* superspecies were redefined as "non-*mordax*" rock oysters (Sekino and Yamashita, 2016). As the misidentification revised, three additionally cryptic rock oyster species were confirmed and grouped into the *S.* non-*mordax* lineage, designated as lineage H, I, and J, clarifying the tentative lineage of Saccostreinae. Consequently, *Saccostrea* lineage F was recognized as *S. malabonensis*, while *S.* non-*mordax* B has been reclassified as *S. echinata* (Guo et al., 2018). More recently, *S.* non-*mordax* lineage J has been identified as *S. spathulata* (McDougall et al., 2024).

These identified cupped and rock oysters are keystone species in forming oyster reefs, which serve as vital ecosystems by providing services such as water filtration, habitat for various marine species, recreational opportunities, coastal storm protection and even potential candidates for genetic improvement (Tolley and Volety, 2005; Morris et al., 2021; Searles et al., 2021). However, 85% of the oyster reefs of the world have been lost, with most remaining reefs degraded and ecologically diminished (Jackson et al., 2001; Beck et al., 2011; Fitzsimons et al., 2019; Jiang et al., 2022). Although oyster reefs were once densely distributed in shallow marine environments of Guangdong, they have declined over the past decades owing to coastal development, overfishing, and uncontrolled nutrients and pollutants input, highlighting the need for improved conservation measures and more importantly effective restoration.

Therefore, understanding oyster species identity and their spatial distribution is essential as it informs monitoring reef health and restoration efforts. This knowledge enables more precise assessments of the oyster reef ecosystem, ultimately aiding in the conservation of oyster germplasm resources while maintaining its biodiversity (GuerraGarcía et al., 2008). Previous studies has defined the taxonomy and spatial distribution of cupped oysters (Wang et al., 2008, 2010; Guo et al., 2018), as well as

population genetic diversity and phylogeography of oysters in northern and southern China (Hu et al., 2018; Liu et al., 2021; Ma et al., 2021a, b). Most of these studies have been confined to specific regions and species though. As yet, the reference and detailed reports on oyster composition and distribution of Guangdong Province are relatively rare and outdated (Cheng et al., 2022). The 2007 national coastal survey by State Oceanic Administration in China (Wu et al., 2025) showed *C. ariakensis* as endemic to Guangdong but found no evidence of *C. hongkongensis* or *C. sikamea*—findings that appear inconsistent with current observation. As a result, this discrepancy highlights a critical gap in our understanding of oyster biodiversity and spatial distribution within the coastal ecosystems of Guangdong.

Therefore, this study aims to address these gaps by conducting the most extensive survey of oyster reefs in Guangdong to date, covering 51 stations along the coastline of the Guangdong Province. We seek to clarify species composition, resolve taxonomic ambiguities using phylogenetic analysis, and document spatial patterns of oyster abundance and distribution via quantitative research using conventional sample plot surveys. We hope to provide a baseline for future conservation planning and contribute to the sustainable management of oyster reef ecosystems.

2 Materials and methods

2.1 Survey site

A comprehensive survey of the oyster reef ecosystem along Guangdong coastlines was conducted from July 2022 to February 2023, covering 51 sites (Supplementary Table 1; Figure 1). Herein, to encompass the oyster's extensive natural distribution, we adopted a broad definition of oyster reefs for this study. These reefs constitute biogenic structures formed either by dense oysters' colonies with substantial vertical reliefs —commonly termed oyster reefs or beds—or secondary structures known as oyster aggregations, where adherent oysters settle upon either hard or soft substrates (Beck et al., 2009; Baggett et al., 2014). Site selection

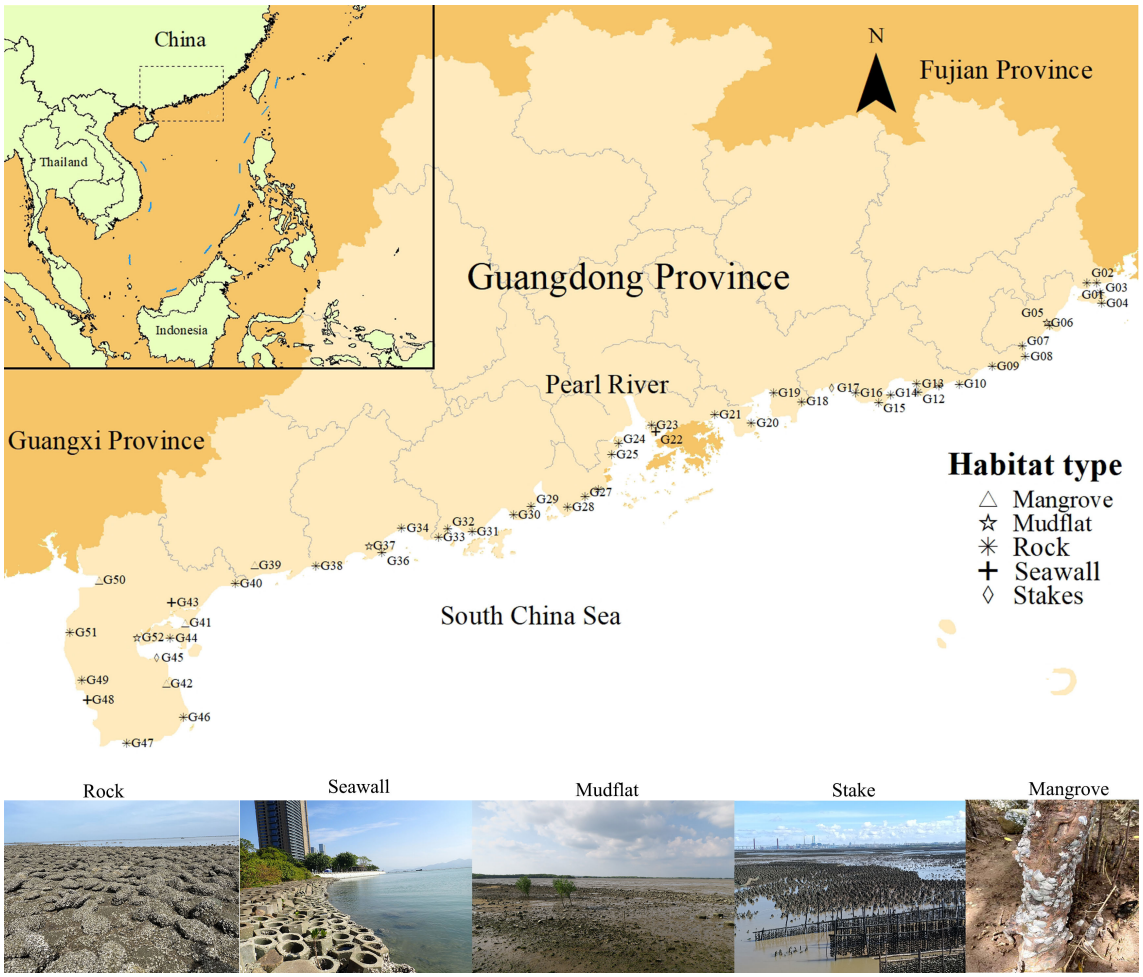

FIGURE 1
Survey sites of oyster reefs in Guangdong Province and their substrate types. The site numbers are numbered from east to west. Habitat distinctions are denoted by geometric markers: triangles indicating mangrove type, snowflakes indicating rock type, crosses indicating seawall type, and diamonds indicating Stakes. The frame in the upper-left corner designates the position of Guangdong Province.

TABLE 1 Sequences used for phylogenetic analysis downloaded in the GenBank.

Species	Accession number		Reference
	16S rRNA	COI	
Saccostrea spp.			
S. cuccullata	NC027724	NC027724	(Volatiana et al., 2015)
S. kegaki	NC030533	NC030533	(Cui et al., 2021)
S. malabonensis	ON649706	ON649706	(Cui et al., 2021)
S. echinata	NC036478	KC683513	(Cui et al., 2021)
S. non-mordax A (*S. echinata*)	AY247381	\	(Lam and Morton, 2006)
S. non-mordax B (*S. echinata*)	AY247336	KU947213	(Lam and Morton, 2006)
S. mordax A (*S. scyphophilla*)	AY247363	EU816062	(Lam and Morton, 2006; Cui et al., 2021)
S. mordax B (*S. scyphophilla*)	AY247339	EU816072	(Lam and Morton, 2006; Cui et al., 2021)
S.glomerata	KX713252 AY247343	\	(Sekino and Yamashita, 2016) (Lam and Morton, 2006)
S.palmula	FJ768516	\	(Sekino and Yamashita, 2016)
S. mordax C	AB748915	AB748839	(Sekino and Yamashita, 2016)
S. mordoides	MT298863	MT293849	(Cui et al., 2021)
S. scyphophilla	LM993883	MN608289	(Salvi et al., 2014)
S. non-mordax C	AY247380	LC110456	(Lam and Morton, 2006; Sekino and Yamashita, 2016)
S. non-mordax D (*S.* sp.1)	AY247391	\	(Lam and Morton, 2006)
S. non-mordax E	AY247387	\	(Lam and Morton, 2006)
S. non-mordax F (*S. malabonensis*)	AY247297	LC110452	(Lam and Morton, 2006)
S. non-mordax G	AY247386	LC110519	(Lam and Morton, 2006; Sekino and Yamashita, 2016)
S. non-mordax H (*S.* sp.2)	LC155015	LC110596	(Sekino and Yamashita, 2016)
S. non-mordax I	LC111218	LC110579	(Sekino and Yamashita, 2016)
S. non-mordax J (*S. spathulata*)	LC111260	LC110587	(Sekino and Yamashita, 2016)
Crassostrea spp.			
C. sikamea	NC012649	NC012649	(Wu et al., 2013)
C. virginica	\	AF152566	(Lam and Morton, 2006)
C. nippona	NC015248	NC015248	(Xia et al., 2014)
C. angulata	NC012648	NC012648	(Wu et al., 2013)
C. bilineata	NC013997	NC013997	(Wu et al., 2010)
C. hongkongensis	NC011518	AY632556 NC011518	(Wang et al., 2004; Xia et al., 2014)
C. ariakensis	NC012650	NC012650	(Wu et al., 2013)
C. zhanjiangensis	JX899654	JX899647	(Wu et al., 2013)
C. dianbaiensis	NC018763	NC018763 AB971935	(Xia et al., 2014) (Sekino et al., 2015)
C. talonata	KC847133	\	(Lu et al., 2024)
Other genera			
Hyotissa hyotis	LM993886	\	(Salvi et al., 2014)
Striostrea prismatica	\	KT317606	(Sekino et al., 2015)
Neopycnodonte cochlear	JF496758	\	(Cui et al., 2021)
Dendostrea cf. *crenulifera*	KC847121	KC683511	(Guo et al., 2018)

was based on historical distribution records in intertidal areas, including mangroves, mudflats, estuaries, and even aquaculture farms (Cheng et al., 2022). Adjustments to the initially selected sites were occasionally made when necessary to account for changes in the actual oyster reef distribution. Two forms of survey, namely qualitative and quantitative survey were conducted at each site during low tide.

2.2 Qualitative survey

At each site, GPS coordinates were recorded to map the distribution of oyster populations within a defined survey polygon. To comprehensively document diversity of oysters in the region, varying morphologies of oysters based on shell features, coloration and diagnostic pattern were collected. Totally 742 samples by qualitative survey were collected from 51 survey sites. In order to describe the original features of oysters' specimen, specimens were photographed *in situ* before being removed from their substrate and individually stored in labeled plastic containers. Ultimately, the flesh was separated by sterilized scalpel and then stored at -20°C for subsequent laboratory analysis. All samples of qualitative survey were employed to molecular identification, and haplotypes separation as well as genetic diversity. Shells of all specimens were deposited in the South China Sea Monitoring Center, State Oceanic Administration.

2.3 Quantitative survey

Each oyster reef site was classified into upper, middle, and lower tidal zones based on natural tidal patterns. In the upper and lower

tidal zones, three quadrats of almost 0.1 m × 0.1 m were laid out by quadrats placement methodology at random, adhering to the same guideline of intertidal zone research (Lu et al., 2024), while in the middle tidal zone, nine quadrats were collected in same method. The number of quadrats was based on the ground that nine quadrats informed by species accumulation curve was sufficient to collect all species of each site based on previous research (Supplementary Figure 1). All macrobenthic organisms, including oysters and algae within the quadrats, were removed using a brush and screwdriver and fixed in 95% ethanol. A 15 cm ruler was included in each quadrat and photographed to provide a scale reference, and each photograph was analyzed to calculate exact sample area of quadrats using ZEISS Axio Vision image processing software.

Owing to huge amounts of oysters detected by quadrats layout, tentative morphological identification was performed via the quantitative survey. *Crassostrea* species were identified based on established shell morphology descriptions (Huber, 2010; Wang et al., 2010). Within the genus *Saccostrea*, species of *S. echinata*, *S. malabonensis*, and *S. scyphophilla* were distinguishable. For example, *S. echinata* displays dense hyote (tubular) spines on its right valve and *S. malabonensis* exhibits a curved shell margin coupled with strong ribs forming a distinctive plicated structure, while *S. scyphophilla* is characterized by an orderly and jagged shell margin and purple spots on right valves. Despite this, owing to vague morphological distinction of other *Saccostrea*, we were unable to accurately identify them to species-level. Consequently, to ensure consistency, unresolved *Saccostrea* species were provisionally classified as *S.* non-*mordax*. Finally, an integrated approach was employed, wherein morphological results were guided and further validated by molecular results in the qualitative survey.

Oyster abundances were determined based on the number and weight of individuals, which were then transformed into density (ind/m^2) and biomass (g/m^2) metrics (Baggett et al., 2014). Biomass were measured by whole specimen with shells in wet weight after removing surface attachment and blotting excess moisture. Spatial distribution maps were generated using ArcGIS 10.7 to visualize these data.

2.4 Description of shell forms

Description of distinct shell characteristics for unresolved oysters included external and internal features. External features examined included the area of left valve attachment, the pattern of radial ribs, grooves, marginal plication, and growth lamellae, alongside the occurrence of growth lines and hyote spines (Supplementary File Figure 4). Internal features considered included the presence or absence of chalky deposits and chomata, the shape of adductor muscle scar and ligament (Littlewood, 1994; Sekino and Yamashita, 2016). The shell height was measured by vernier calipers with 0.1 mm accuracy, and the whole weight was measured by electronic scales with 0.01 g accuracy (Zhang et al., 2017). These characteristics facilitated the preliminary identification of oysters during qualitative surveys and were conducive to the discovery of new taxa within the reefs.

2.5 Molecular and morphological identification

For samples collected by qualitative survey, total genomic DNA was extracted from the adductor muscle using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. Mitochondrial DNA fragments of 16S rRNA and COI were amplified using the primer pairs ArL(F) (5'-CGCCTGTTT ATCAAAAACAT-3') and BrH(R) (5'-CCGGTCTGAACT CAGAT CACG-3') (Palumbi et al., 1996), as well as LCO1490 (5'-GGTCAACAAATCATAA AGATATTGG-3') and HCO2198 (5'-TAAACT TCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). Polymerase chain reactions were performed in a 25 μl reaction mixture containing 13.38 μl ddH_2O, 2.5 μl *Ex Taq* Buffer (TaKaRa HS), 1.5 μl of each primer (10 mM), 2 μL template DNA, and 0.12 μl *Ex Taq* HS, using a BIO-Metro thermal cycler. The PCR conditions for 16S rRNA included an initial denaturation at 94°C for 3 min, followed by 40 cycles of 94°C, 45°C, and 72°C for 40, 40, and 45 s, respectively, with a final extension at 72°C for 7 min. For COI, the PCR protocol included an initial denaturation at 94°C for 3 min, followed by 5 cycles of 94°C, 45°C, and 72°C for 40, 40, and 50 s, respectively. This was followed by 35 cycles of 94°C, 51°C, and 72°C for 40, 40, and 50 s, respectively, with a final extension at 72°C for 7 min. PCR products were confirmed using 1% agarose gel electrophoresis and sequenced by Sangon Biotech Company (Guangzhou, China). The obtained sequences were initially analyzed using BioEdit 7.0. Molecular identification was conducted based on BLAST searches against the NCBI database. More importantly, the identification was further confirmed by phylogenetic tree constructed by correct and non-local reference sequence (Table 1). Haplotypes were identified using DNASP v. 5.10.01 (Librado and Rozas, 2009) and used to calculate haplotype diversity. Each identified haplotype was designated following a standardized format, combining "Hap number" with "ML+serial numbers" for qualitative surveys. The compiled sequences of *S.* sp.1 were deposited in NCBI under accession numbers PQ452111 and PQ459553–PQ459559 for the COI and 16S rRNA genes, respectively. Sequences of *S.* sp.2 were assigned under accession numbers PQ455391–PQ455392 and PQ459552 for the COI and 16S rRNA genes, respectively.

2.6 Phylogenetic analysis

DNA sequences from this study, combined with reference sequences downloaded from NCBI, were analyzed using Phylosuite v. 1.2.3 and MEGA v. 7.0.26 (Kumar et al., 2016; Zhang et al., 2020) (Table 1). Several plug-in programs were utilized, including MAFFT for sequence alignment, TrimAI for trimming redundant sequence at both ends, Gblocks for removing

10.3389/fmars.2025.1602823

TABLE 2 Species identification and distribution of oysters at oyster reefs along coastline of Guangdong Province by qualitative survey.

Sites	A	B	C	D	E	F	G	H	I	J	K	L	Number of samples	Number of species
G01	3					6		6	3			1	19	5
G02	2							1					3	2
G03	15					6		14	3			2	40	5
G04	2					1		2				2	7	4
G05	1					6		5	1				13	4
G06	4	1				13		3	9				30	5
G07	1		2			5	2	4	7				21	6
G08	1							4		5	1		11	4
G09	1							1	3				5	3
G10	6					1		2	2	1			12	5
G11	1					6		7	5	6		1	26	6
G12	7					3			4	1			15	4
G13	1					1		6	2			6	16	5
G14						1		5	1	5			12	4
G15	1					2		11	1	5		1	21	6
G16	3					14			1				18	3
G17	2			5		10		1	4				22	5
G18	3			2		4		3	3				15	5
G19	1					7		2				3	13	4
G21								3	3			4	10	3
G22	3			4		19		3					29	4
G23				1		11							12	2
G24				10		4							14	2
G25				4		10							14	2
G26				3		10							13	2
G27				1		10		1					12	3
G28				3		15		1					19	3
G29				6		9		9	1				25	4
G30						8		3	2				13	3
G31				1		2		1					4	3
G32						8		2	1				11	3
G34				2		8			1				11	3
G36							2	6		1			9	3
G37									2				2	1
G38	6					5	2	7	1	1			22	6
G39						15			4				19	2
G40	5					9		5	1	3			23	5
G41	2					5			3				10	3

(Continued)

TABLE 2 Continued

Sites	A	B	C	D	E	F	G	H	I	J	K	L	Number of samples	Number of species
G42	3					6		1					10	3
G43	1			15				3					19	3
G44	6					9							15	2
G45	3					20		1			1		25	4
G46	4					2		5	4	1			16	5
G47	7					3		9	1				20	4
G48	4					4							8	2
G49						3							3	1
G50						8			2				10	2
G51	11				1	11			1			1	25	5
Total	110	1	2	57	1	300	6	137	76	29	2	21	742	12

r; A, *C. angulata*; B, *C. ariakensis*; C, *C. dianbaiensis*; D, *C. hongkongensis*; E, *C. bilineata*; F, C. sikamea; G, *D.* cf *crenulifera*; H, *S. echinata*; I, *S. scyphophilla*; J, *S. malabonensis*; K, *S.* sp. 2; L, *S.* sp. 1.

divergent and ambiguously aligned blocks, and ModelFinder for model selection (Gerard and Jose, 2007; Capella-Gutierrez et al., 2009; Standley, 2013). Following sequence procession, phylogenetic analysis was conducted using Bayesian inference (BI) and ML methods. A Bayesian tree was constructed with a Markov Chain Monte Carlo simulation run for 2×10^6 generations, while ML analysis was performed using IQ-Tree with bootstrap support based on 5,000 replications (Huelsenbeck, 2012). Sequence divergence among haplotype groups was calculated in MEGA v. 7.0.26 using Kimura's two-parameter (K2P) model (Kimura, 1980).

3 Results

3.1 Species identities of oysters in Guangdong

Molecular analyses and phylogenetic assessments were conducted on representative individuals from oyster samples of distinct shell features. In total, 706 COI sequences and 693 16S rRNA sequences were generated from 742 individuals (Table 2), yielding 240 COI haplotypes and 67 16S rRNA haplotypes (Figures 2, 3).

Since the topological structures of the IQ-Tree and BI tree were generally congruent at the upper branches, with only minor difference observed at the lower branches at the interspecies level (e.g., the position of *S. kegaki* and *S.* non-*mordax* H) for both mitochondrial DNA, the BI tree was selected for further analysis. The corresponding IQ-Trees are provided in the Supplementary Figures 2, 3. Using *Striostrea prismatica* as the outgroup for BI and ML trees based on COI sequences, 12 oyster species across three genera were identified. The COI fragment used for phylogenetic analysis was 550 bp in length. The BI tree topology revealed that the representative species belonged to three subfamilies. At the species level, the following oysters formed a robust clade: *C. sikamea*, *C. hongkongensis*, *C. angulata*, *C. ariakensis*, *C. dianbaiensis*, *C. bilineata*, *S. malabonensis*, *S. echinata*, *S. scyphophilla*, *S.* sp. 1, *S.* sp. 2, and *D.* cf. *crenulifera* (Figures 4A–L).

Regarding BI and IQ trees based on 16S rRNA, *Neopycnodonte cochlearo* and *Hyotissa hyotis* were selected as outgroup species. In total, 12 oyster species across three genera were identified using the 16S rRNA genetic marker. The 16S rRNA fragment used for phylogenetic analysis was 495 bp in length. *S.* sp. 1 was closely related to *S.* non-*mordax* D, forming a sister group with *S.* non-*mordax* E, while *S.* sp. 2 grouped with S. non-*mordax* H in the 16S rRNA tree. Consequently, the 16S rRNA tree topology was largely congruent with that of the COI tree, except for variations in the interspecies relationships among *S.* non-*mordax* lineages within Saccostreinae.

3.2 Genetic structure and molecular diversity

Among the COI sequences, *C. sikamea* exhibited 112 haplotypes, accounting for 45.71% of the total haplotypes identified in this study. *Saccostrea echinata* exhibited 47 haplotypes, contributing 19.18% of the total haplotypes. Similarly, *S. scyphophilla*, with 23 haplotypes, exhibited the highest nucleotide and haplotype diversities with values of 0.0144 and 0.993, respectively. This pattern was consistent in genetic divergence analyses, with *S. scyphophilla* showing a greater pairwise distance compared to that of other rock oyster species (Tables 3, 4).

Regarding the 16S rRNA genetic locus, *S. echinata* and *C. sikamea* comprised 13 haplotypes each, representing 20.31% of the total haplotypes. *S. echinata* exhibited the highest nucleotide and haplotype diversities with values of 0.0028 and 0.710, respectively. A similar pattern was observed in genetic divergence

10.3389/fmars.2025.1602823

TABLE 3 Genetic diversity of common oysters in Guangdong Province based on cytochrome c oxidase subunit I (COI) and 16SrRNA.

Species	Barcode	Haplotypes (n)	Haplotype diversity (h)	Nucleotide diversity (Π)
C. sikamea	COI	112	0.929	0.0059
	16S rRNA	13	0.107	0.0003
C. angulata	COI	20	0.853	0.0056
	16S rRNA	6	0.103	0.0003
C. hongkongensis	COI	10	0.441	0.0010
	16S rRNA	4	0.103	0.0003
C. dianbaiensis	COI	2	1.000	0.0058
	16S rRNA	1	0.000	0.0000
C. ariakensis	COI	1	0.000	\
	16S rRNA	1	0.000	\
C. bilineata	COI	1	0.000	\
	16S rRNA	1	0.000	\
S. malabonensis (*S. non-mordax* F)	COI	20	0.573	0.0060
	16S rRNA	9	0.214	0.0006
S. mordax A&B (*S. scyphophilla*)	COI	23	0.991	0.0144
	16S rRNA	5	0.630	0.0025
S. sp.1 (*S. non-mordax* D)	COI	2	0.222	0.0004
	16S rRNA	7	0.700	0.0052
S. non-*mordax* A&B (*S. echinata*)	COI	47	0.759	0.0074
	16S rRNA	13	0.710	0.0028
S. sp.2 (*S. non-mordax* H)	COI	2	1.000	0.0019
	16S rRNA	1	0.000	0.0000
D. cf. *crenulifera*	COI	5	0.933	0.0053
	16S rRNA	2	0.500	0.0013
Total sequence	COI	245	0.968	0.1427
	16S rRNA	64	0.800	0.0750

COI, Cytochrome c oxidase subunit I; cf, *confer*.

analyses, with *S. echinata* and *S. scyphophilla* displaying greater pairwise distances compared to that of other rock oysters (Tables 1, 4).

3.3 *Saccostrea* sp. 1 and sp. 2

In this study, we described the shell morphology of the unidentified species, *Saccostrea* sp. 1 and sp. 2, to tentatively distinguish them from other oyster species. 9 and 23 individuals for COI sequences and 16S rRNA, respectively, were designated to *Saccostrea* sp. 1. These specimens were collected from rocky shores in Shantou (G3 and G4), Shanwei (G11, G13 and G15), Shenzhen (G21), Chaozhou (G01) and Xuwen (G51). Hap 41 of *S.* sp.1 for 16S rRNA was identified in all those regions (except Xuwen), making it the most common haplotype of *Saccostrea* sp. 1. Conversely, two individuals were identified as *S.* sp. 2, representing 2 and 1 haplotype for COI and 16S rRNA, respectively, via molecular analysis (Table 3). Specimens of *S.* sp. 2 were sourced from the rocky shore of Jieyang (G08) and Zhanjiang (G45).

The shell morphology of *Saccostrea* sp. 1 was characterized by its relatively small size compared to that of other rock oysters, with shell heights typically ranging from 20–40 mm (Figure 4G). The shell shape was usually elongated and elliptical, tapering towards the posterior end. The left valve was slightly cupped and primarily attached to the substrate. The left valve featured prominent, well-defined ribs that extended to the dorsal shell margin, forming distinct plications. The internal margin of the left valve possessed clearly visible chomata, and chalky deposits were present on the inner surface. The right valve, which featured brownish patches on

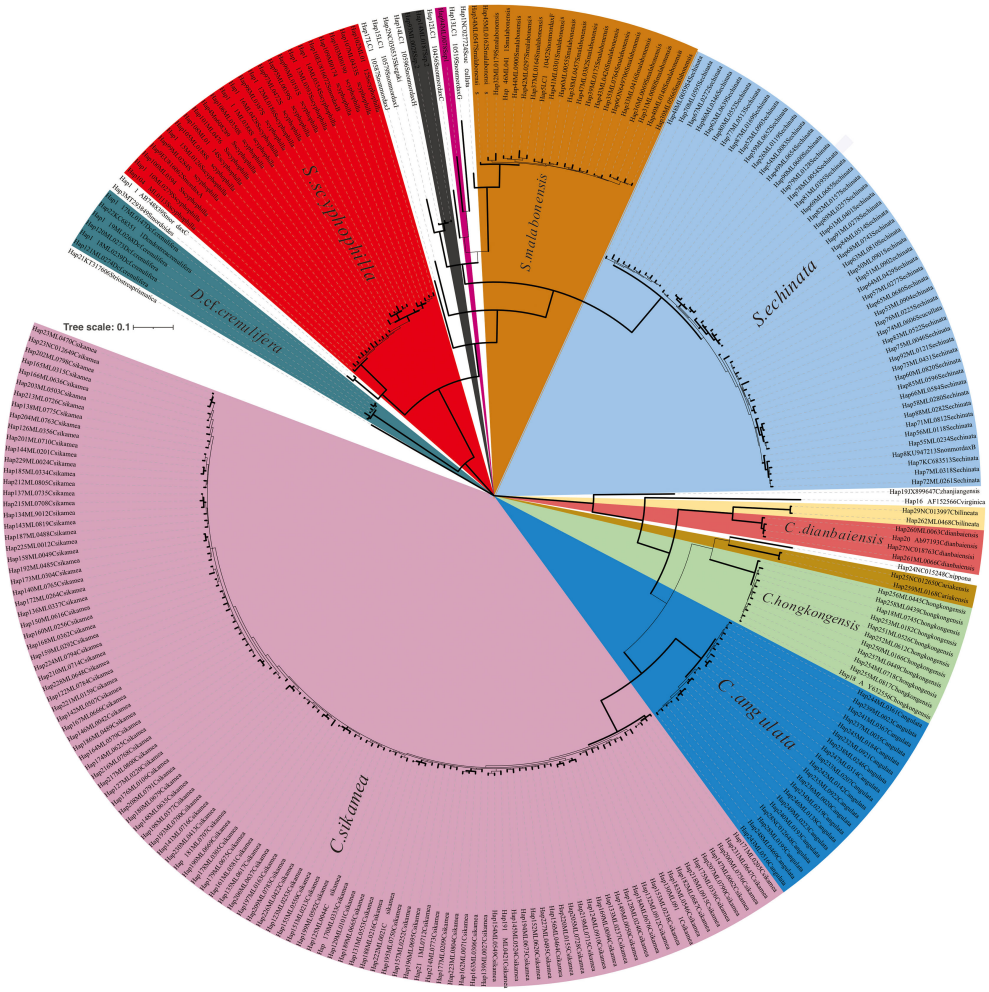

FIGURE 2
Bayesian phylogenetic tree of common oysters from oyster reefs along the Guangdong coastline, based on partial cytochrome oxidase I (COI) DNA sequences. Solid lines denote the posterior probability/bootstrap values greater than 60. Each taxon is labeled with its haplotype number, sequence number (or accession number), and tentative species name. The scale bar indicates the number of substitutions per site.

its surface, was smaller compared to that of the left valve. The growth lines were prominent and concentric, forming a densely brownish growth lamella. Hyote spines were absent on the surface of the right valve. The adductor muscle scar was distinct and horseshoe-shaped, located in the posterior ventral half of the inner right valve. The hinge line was short and obliquely oriented.

The detailed description of *S.* sp. 2 was limited owing to the scarcity of specimens. The shell shape of *S.* sp. 2 appeared flat and triangular (Figure 4I). There are no hyote spines observed on the outer surface of the right valve in our specimen. The growth lamellae and squamae were densely clustered along the shell margin, with distinct black deposition present. Radial ribs and the grooves between them were relatively faint but noticeable, especially along the shell margin of the left valve. Internally, marginal chomata were clearly present. The internal black and ochre pigment deposition was conspicuous, appearing as large spots. The adductor muscle scar was clearly visible and relatively large.

3.4 Geographic distribution pattern

In total, 33,549 oyster individuals for oyster morphological identification was sampled using a quantitative method to calculate density, biomass, and species dominance. The average density and biomass of *C. sikamea* were calculated as 1,415.38 individuals/m^2 and 4,449.42 g/m^2, making it the most predominated oyster species along the Guangdong shoreline (Figures 5, 6). This species was found across all survey sites, regardless of habitat type. Following *C. sikamea*, *S. echinata* had a mean density and biomass of 281.43 individuals/m^2 and 1102.29 g/m^2, contributing 13.11% and 13.42% to the total species richness and biomass, respectively. In this study, *C. hongkongensis* was the most common and dominant oyster species in the western district of the Pearl River Estuary, with an average density of 93.48 individuals/m^2 and a biomass of 501.68 g/m^2. *Saccostrea malabonensis*, although less abundant with a density and biomass of 40.45 individuals/m^2 and 370.26 g/m^2, respectively, was

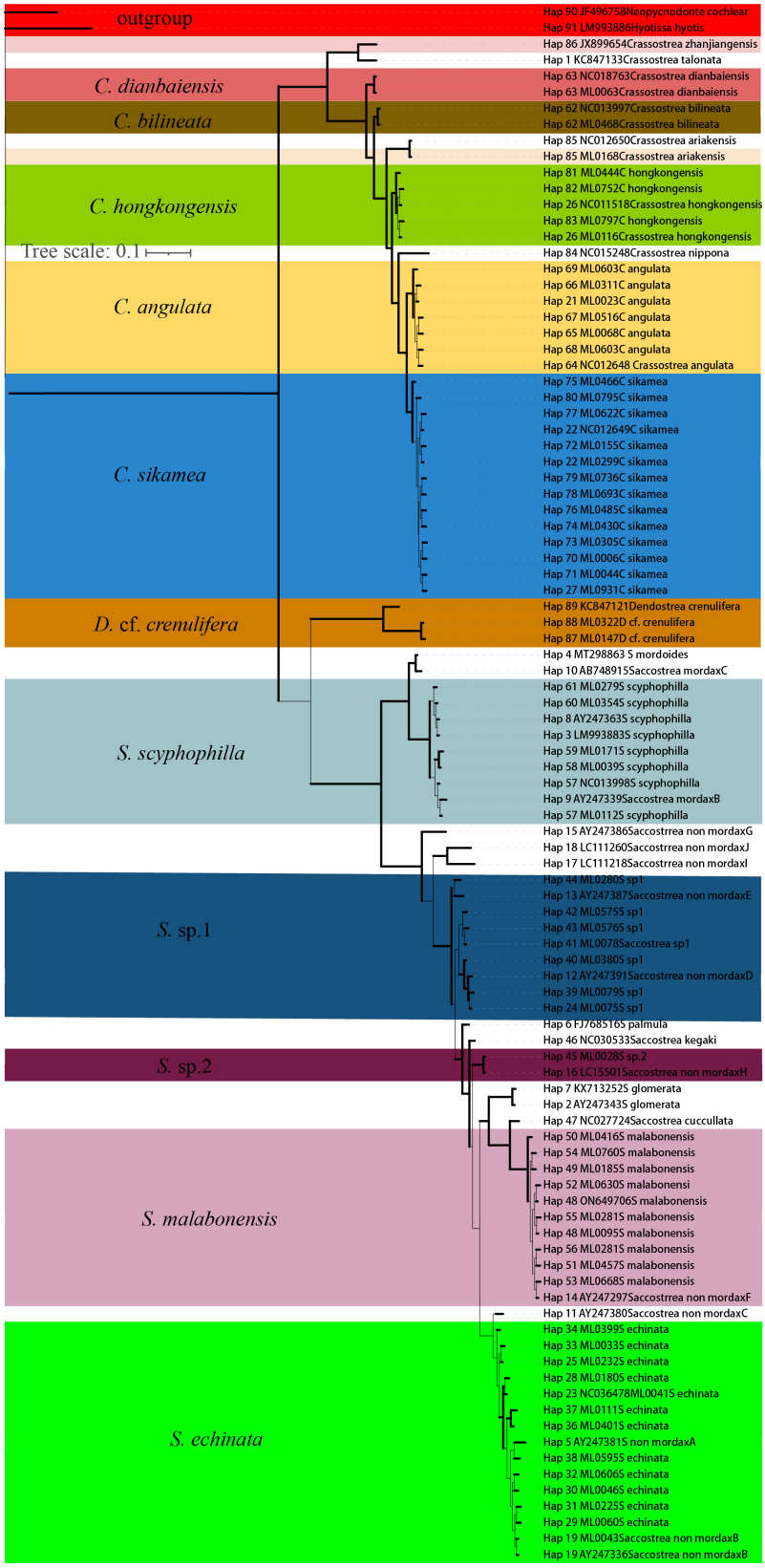

FIGURE 3
Bayesian phylogenetic tree of common oysters from oyster reefs along the Guangdong coastline, based on partial 16S rRNA sequences. Solid lines denote the posterior probability/bootstrap values greater than 60. See the Figure 2 legend for details.

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TABLE 4 Mean pairwise genetic divergence of the COI and 16S rRNA gene among *Saccostrea* oysters in Guangdong Province (upper diagonal: 16S RNA; lower diagonal: COI).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *S. glomerata*		0.0999	0.2687	0.2221	0.1029	0.1015	0.0933	0.1185	0.0851	0.1375	0.1789	0.1015	0.1170	0.0851	0.0851
2. *S. echinata* (*S. non-mordax A&B*)			0.3057	0.2449	0.0752	0.0566	0.0368	0.0872	0.0711	0.1337	0.1457	0.0566	0.1126	0.0295	0.0433
3. *S. mordax A&B*		0.1666		0.0576	0.2371	0.2772	0.2911	0.2341	0.2661	0.2690	0.2568	0.2772	0.2552	0.3002	0.2860
4. *S. mordax C*		0.1732	0.1067		0.1878	0.2386	0.2709	0.1986	0.2083	0.2311	0.1996	0.2386	0.2182	0.2599	0.2492
5. *S.* sp.1		0.1638	0.2104	0.2178		0.0783	0.0933	0.0252	0.0397	0.1414	0.1127	0.0783	0.1099	0.0851	0.0625
6. *S.* sp.2		0.1329	0.1841	0.2015	0.1574		0.0658	0.0981	0.0898	0.1165	0.1753	0.0000	0.1121	0.0580	0.0429
7. *S. non-mordax C*		0.1360	0.2085	0.2146	0.1674	0.1387		0.1065	0.0817	0.1165	0.1566	0.0658	0.0878	0.0069	0.0504
8. *S. non-mordax D* (*S.* sp.1)									0.0504	0.1580	0.1212	0.0981	0.1205	0.0981	0.0817
9. *S. non-mordax E*										0.1248	0.1127	0.0898	0.1039	0.0737	0.0737
10. *S. non-mordax F* (*S. malabonensis*)		0.1775	0.2075	0.1983	0.1700	0.1506	0.1581				0.1848	0.1165	0.0693	0.1082	0.1333
11. *S. non-mordax G*		0.1914	0.2098	0.2191	0.1868	0.1808	0.1691	0.2068				0.1753	0.1548	0.1475	0.1659
12. *S. non-mordax H* (*S.* sp.2)		0.1307	0.1868	0.2043	0.1547	0.0020	0.1362	0.1480	0.1835				0.1121	0.0580	0.0429
13. *S. cuccullata*		0.1691	0.1839	0.1898	0.1502	0.1502	0.1580	0.0559	0.2103	0.1477				0.0799	0.1121
14. *S. kegaki*		0.1479	0.2056	0.2213	0.1789	0.0625	0.1387	0.1684	0.1972	0.0602	0.1724	0.2003	0.1793		

widely distributed across the study area, occurring at 40 of the 53 sites. All ambiguous *Saccostrea* identified in the quantitative survey (here referred to as *S.* non-*mordax*) were present in measurable quantities in our assay. The total number of species recorded at each site ranged from 1 to 6 species by qualitative survey, while G07, G11, G15, and G38 exhibited the highest richness with 6 oyster species found. At least 7 oyster species were found at G07 by quantitative method, being the most abundant area. Our study indicated G12 was the most abundant site with a density of 6779.83 ind/m^2, while G08 was found to have the lowest abundance with a density of 626.16 ind/m^2. G32 was the greatest area of biomass with the value reaching around 16000 g/m^2. In contrast, the biomass of G46 was the lowest with only about value of 2000 g/m^2 (Supplementary Table 1).

4 Discussion

Historically, Guangdong's estuarine and coastal systems supported some of the most extensive oyster reefs in South China, providing critical nursery habitats and contributing significantly to regional water quality. However, rapid coastal development, industrial pollution, and widespread mariculture have further compounded habitat degradation in Guangdong, threatening both wild and farmed oyster populations (Cardinale et al., 2012; Jiang et al., 2024). Given that Guangdong's oyster reefs are influenced by complex salinity regimes driven by seasonal monsoons and freshwater discharges from the Pearl River, creating environmental heterogeneity that may promote high oyster species diversity and cryptic speciation. Therefore, a thorough investigation of oyster reefs in Guangdong Province is urgently needed.

4.1 Identification of oyster species

Currently, approximately 88 extant oyster species (Ostreidae) are recognized worldwide (Bayne et al., 2017; Li et al., 2021; Searles et al., 2021). Over 30 oyster species are found along the coast of China (Hu et al., 2019). In our analysis, population composition revealed at least 12 oyster species from three genera (*Crassostrea*, *Saccostrea*, and *Dendostrea*) and three subfamilies based on the phylogenetic systematics of Salvi, Raith, and Guo (Salvi et al., 2014; Raith et al., 2015; Guo et al., 2018) at the oyster reef of Guangdong. Given ongoing debates about the acceptance of new genera within *Crassostreinae* (e.g., *Talonostrea* Li and Qi, 1994, and *Magallana* Salvi & Mariottini, 2016), we continue to use the genus *Crassostrea* to represent cupped oysters in our study. Furthermore, because *C. iredalei* is suspected of having cryptic species and remains controversial (Guo et al., 2018), we considered it *C. bilineata* rather than *C. iredalei*. Similarly, due to challenges in phylogenetic analysis, *Dendostrea* was classified as *D.* cf. *crenulifera* in this study, as some *D. crenulifera* sequences uploaded to NCBI may be inconsistent. Further efforts are needed to clarify the relationship within *Dendostrea*. Our findings also suggest that *S. mordax* A & B lineage and *S. scyphophilla* are conspecific, as indicated by the placement of *S. scyphophilla* within the *S. mordax* A & B lineage in the phylogenetic tree, with minimal genetic divergence (Table 4). Therefore, it may be more

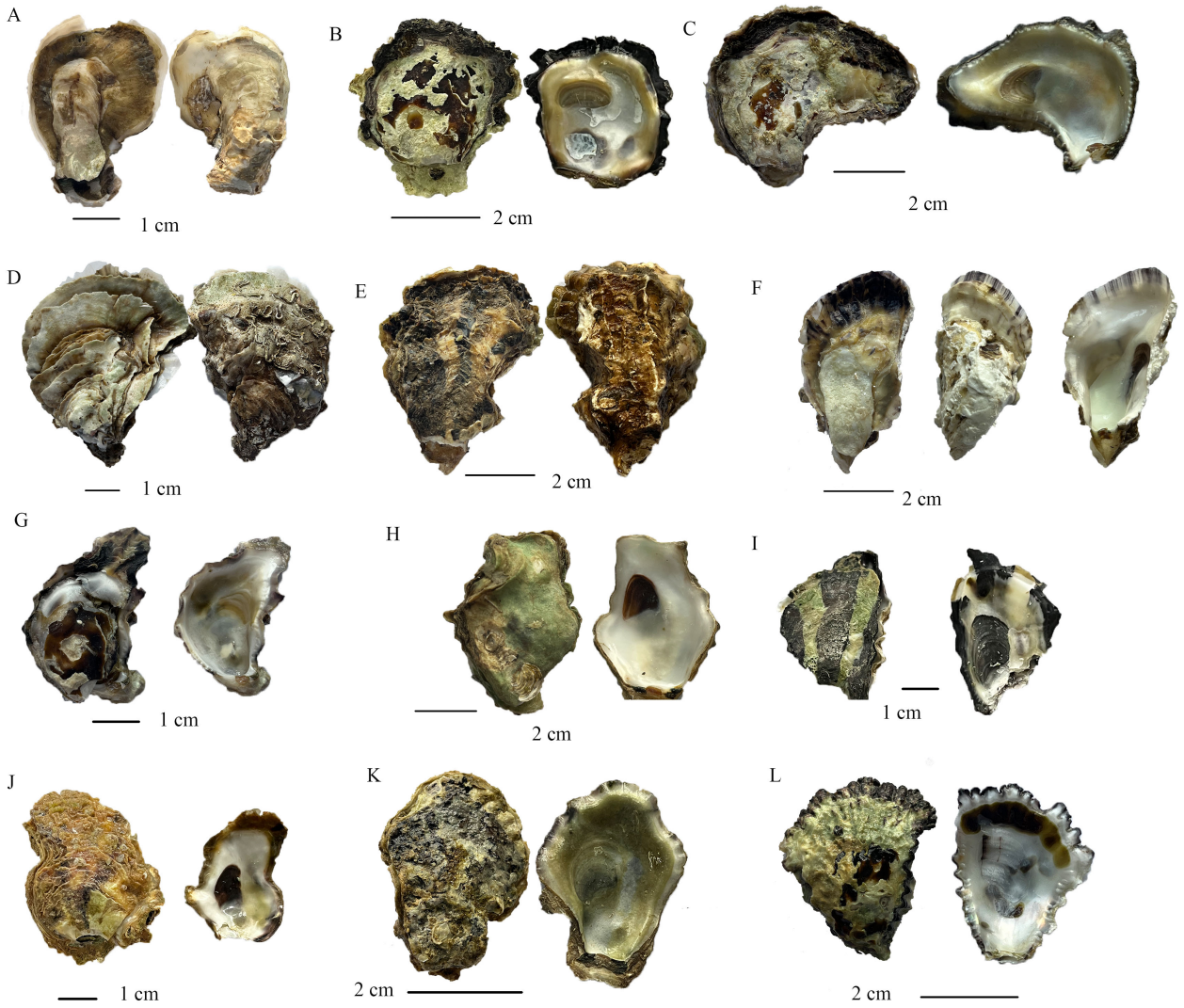

FIGURE 4
Shell morphology of oysters identified from oyster reefs in Guangdong Province, China. The identified oysters include the following: **(A)** *Crassostrea dianbaiensis*, **(B)** *Saccostrea echinata*, **(C)** *S. malabonensis*, **(D)** *C. ariakensis*, **(E)** *C. angulata*, **(F)** *C. hongkongensis*, **(G)** *S.* sp. 1 (*S.* non-*mordax* D in this study), **(H)** *C. sikamea*, **(I)** *S.* sp. 2 (*S.* non-*mordax* H in this study), **(J)** *C. bilineata*, **(K)** *Dendostrea* cf. *crenulifera*, **(L)** *S. scyphophilla*.

accurate to refer to the entire *S. mordax* A& B lineage as *S. scyphophilla* (Raith et al., 2015; McDougall et al., 2024). *S. mordax* C lineage we haven't found in our survey was referred to as *S. mordoides* (Cui et al., 2021).

4.2 Confusions of Saccostrinae

Genetic validation of rock oysters and phylogenetic divergence underpins conservation, sustainable utilization, and ecological restoration of these species. Saccostrinae remains insufficiently assessed by DNA analysis due to the subtle and unclear morphological distinction among *Saccostrea* species. Sekino and Yamashita (2016) identify 13 distinct *Saccostrea* lineages; however, most monophyletic clades have not been assigned valid species names. Currently, only a few of these lineages have been formally named. For example, *S.* non-*mordax* B is presumably recognized as *S. echinata*, while *S.* non-*mordax* F and J are assigned to *S. malabonensis* and *S. spathulata*, respectively (Cui et al., 2021; Snow et al., 2023).

S. cucullata was once considered the sole valid *Saccostrea* species in the Indo-Pacific, and this superspecies was repeatedly used in earlier records of Guangdong province to represent *Saccostrea* (Harry, 1985; Xia, 2008). However, the geographical range of *S. cucullata* is now recognized as being limited to the Atlantic, from the West Indies to the Arabian Peninsula (Huber, 2010), making this name inappropriate for oysters from the Indo-Pacific *Saccostrea* lineage. Our findings show that the rock oysters we collected did not cluster with *S. cucullata* specimens from Madagascar (Volatiana et al., 2015), with a K2P sequence divergence of 5.6%–19.1% in COI and 2.5%–9.4% in 16S rRNA, suggesting that *S. cucullata* does not occur along the Guangdong coastline. Another significant issue is the

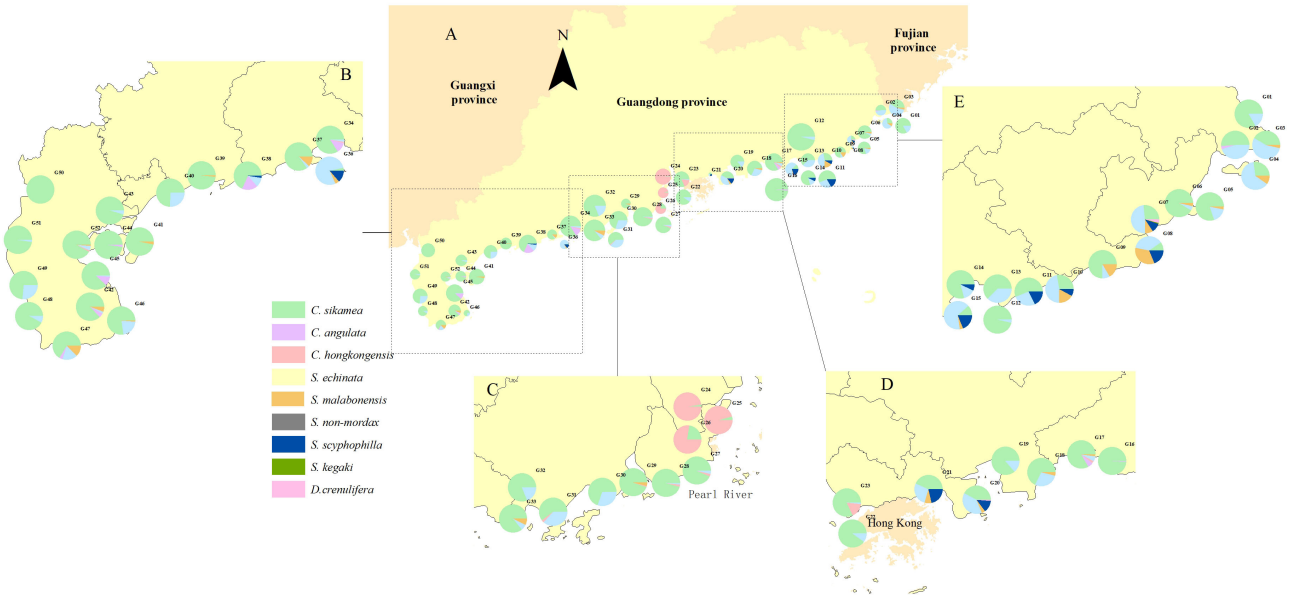

FIGURE 5
Spatial distribution of mean oyster density across 51 survey sites in Guangdong Province via quantitative survey. The circle size in the pie chart **(A)** represents the relative density of common oyster species at each site, while different colors indicate their percentage distribution within specific regions. The survey sites were divided into 4 districts: **(B)** the western part including Leizhou Peninsula, the central area **(C, D)** including Pearl River Delta, as well as the eastern part **(E)** of Guangdong Province.

presence of *S. glomerata* in the northern hemisphere. Historical data have recorded the distribution of *S. glomerata*, referred to as "tuanju oysters," along the Guangdong coastline (Phillips and Yim, 1981; Xu and Zhang, 2008; Wang, 2016; Cui et al., 2018). However, Lam and Morton, based on mitochondrial analysis, suggest that the common flattened oyster found in Hong Kong is not *S. glomerata*. They propose that native *S. glomerata* might only inhabit the temperate regions of the southern hemisphere (Lam and Morton, 2006). Our findings indicate that *S. glomerata* does not occur along the Guangdong coastline, as no taxonomic unit clustered with *S.*

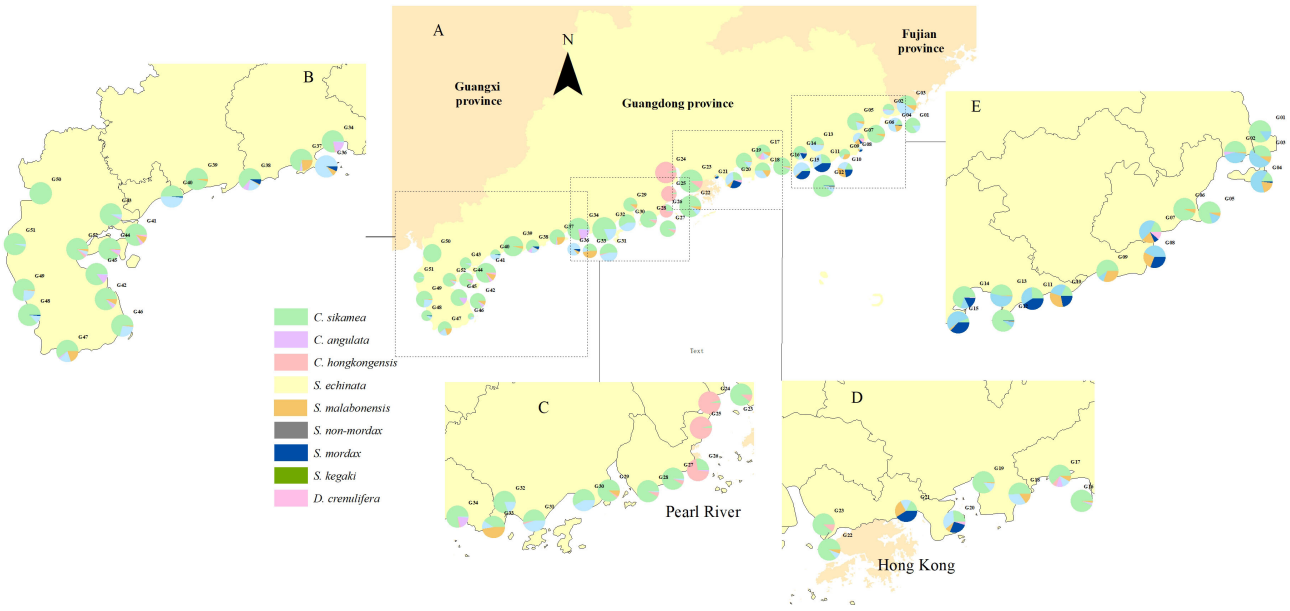

FIGURE 6
Spatial distribution of mean oyster biomass across 51 survey sites in Guangdong Province via quantitative survey. The circle size in the pie chart **(A)** represents the relative biomass of common oyster species at each site, while different colors indicate their percentage distribution within specific regions. The survey sites were divided into 5 districts: **(B)** the western part including Leizhou Peninsula, the central area **(C, D)** including Pearl River Delta, as well as the eastern part **(E)** of Guangdong Province.

glomerata specimens from Southeastern Australia, with a K2P sequence divergence of 9.33%–26.87% in the 16S rRNA gene (Combosch et al., 2017). Considering the flattened shell form, absence of hyote spines, plication of the right valve margin, along with its relatively large size, and attachment to rocks, the oysters previously identified as *S. glomerata* along the southern China coastline may actually be *S. malabonensis*. This aligns with our findings, where *S. malabonensis* was identified as a common species on the rocky shores of Guangdong. Recently, the *S.* non-*mordax* lineage F was reclassified as *S. malabonensis*, supported by robust phylogeny, which matches our findings (Sekino and Yamashita, 2016; Cui et al., 2021). Therefore, we speculate that the occurrence and fertility of *S. malabonensis* in southern China may have been previously underestimated.

Our findings suggest that *S. echinata* may represent *S.* non-*mordax* A and B, as its tentative haplotypes are nested within lineages A and B in the 16S rRNA gene tree. *S.* non-*mordax* A and B exhibit shell morphology with hyote spines and emerge as sympatric species in our study and related research (Lam and Morton, 2006; Snow et al., 2023), with a limited K2P distance between them. This supports the idea that they may share a common lineage.

4.3 *Saccostrea* sp. 1 and sp. 2

In our assay, two cryptic species, *Saccostrea* sp. 1 and sp. 2 were found in oyster reefs along the Guangdong Province. Molecular data indicated that *S.* sp. 1 and *S.* sp. 2 were designated to known *S.* non-*mordax* lineage D and H, respectively. The average genetic distance between *S.* sp. 1 and other Saccostrea species ranged from 15.0%–21.7% for the COI gene and 2.5%–23.7% for the 16S rRNA gene. *S.* non-*mordax* lineage D showed the closest genetic relationship with *S.* sp. 1, followed by lineage E, leading us to classify *S.* sp 1 as lineage D rather than lineage E. This is not the first record of *S.* sp. 1 in Guangdong, as previous studies have documented its occurrence in Hong Kong, Hainan, and Taiwan (Lam and Morton, 2006), and its distribution has now expanded further into another district of Guangdong. *S.* sp. 1 appears to be exclusively distributed in adjoining areas of coastal China. The spatial distribution of *Saccostrea* oysters suggests that the density and biomass of *S.* sp. 1 may have been underestimated. However, morphological identification of *S.* sp. 1 remains challenging. The most distinctive shell features of the species, such as its relatively small size and flat shape tapering towards the posterior with a pointed end, are insufficient to distinguish it from other *Saccostrea* species, highlighting the challenge of identifying species within this genus.

Saccostrea sp. 2 formed a sister clade with known *S.* non-*mordax* H and was closely related to *S. kegaki*. Regarding K2P distance, *S.* sp. 2 showed the closest relationship to *S.* non-*mordax* H, with a genetic distance of 0% for the 16S rRNA gene and 2.00% for the COI gene. The genetic distance supports the intraspecies relationship. In contrast, *S.* sp. 2 and *S. kegaki* exhibited greater genetic divergence, with a 4.3% difference in the 16S rRNA gene and 6.3% in the COI gene, reinforcing the distinction between *S. kegaki* and lineage H. Considering that lineages A, B, and H both share hytote spines, Lam suggests that these lineages align with *S. echinata* sensu Torigoe (Torigoe and Inaba, 1981). In our phylogenetic analysis, only the IQ-Tree based on the COI gene supported this hypothesis (Supplementary Figure 2). However, another tree construction analysis indicated that lineage H did not cluster with lineages A or B but instead formed an independent lineage with *S. kegaki* as the basal clade (Figures 2, 3).

4.4 Transformation of oyster composition

A putative quantitative survey by morphological analysis were conducted to study oyster composition of Guangdong province. Previous studies of China's southern coastline (Zhou and Jr., 2003; Lu et al., 2024) and the 2007 national coastal survey (Wu et al., 2025) in Guangdong Province reported substantial populations of *C. ariakensis* and *C. angulata* (Wang et al., 2010; Xia et al., 2014). In contrast, our study revealed that *C. sikamea* and *S. echinata* were the most predominant and endemic species at coastal of Guangdong Province, while historically dominant species like *C. ariakensis* and *C. angulata* were comparatively rare. Similarly, *C. sikamea* was also identified as the dominant oyster species along the Zhejiang coast to the north of Guangdong (Liu et al., 2021). These discrepancies may result from earlier studies relying on morphological traits or limited spatial sampling. Alternatively, climate change, widespread mariculture, and habitat alteration threatening both wild and farmed oyster populations over the past few decades could explain shifts in oyster species composition in this region. On the other hand, *C. sikamea* owning to its euryhaline and eurythermal characteristic possessed a specific capacity for adaption to coastline of China. A sufficient genetic variability and strength of gene flow also promote its performance of regional acclimation (Liu et al., 2021). Although historically confined to southern China, *C. sikamea* has recently expanded beyond its traditional range, now aggressively invading artificial shorelines in northern China (Hu and Dong, 2022). Its remarkable regional adaptation and acclimation may play a key role in its expansion. As such, our study provides an updated baseline for detecting future changes of this species and its distribution along the Guangdong coastline. From this view, the current situation underscores the need for proactive measures to safeguard and preserve oyster diversity. Additionally, *C. talonata* was found in mangrove of Beibu gulf and coastline in Hainan Island by previous surveys (Li and Qi, 1994; Lu et al., 2024), indicating that it was a common oyster in South China Sea. There are no specimens of *C. talonata* were detected in our survey though. The result indicated *C. talonata* was unusual species at oyster reefs in Guangdong province, which was identical to Xia's result (Xia, 2008). The contrast might be due to its sparse distribution and its preference to subtidal area where it adhered to the surface of mesh cages or shells (Wang, 2016).

4.5 Habitat management and protection

Notably, wild-type *C. hongkongensis* were found between Yangjiang and Zhuhai (G24 - G37) mostly situated at Pearl River Delta in our study. However, this area suffered from habitat losses, tourist pressure, and contamination of terrestrial runoff as a result of rapid urban expansion. In consideration of the restricted area of this species and its essential position for traditional aquaculture in Guangdong Province, more efforts are needed to monitor its population dynamic process and genetic resources. Similarly, there was an ancient and naturally occurring oyster reef first documented at site G47 of Xuwen. However, the low diversity and richness of this site might suggest the poor condition recently. Establishing protected zones of this area for strengthening management may be a viable solution. In addition, our study outlined several critical regions that implied high levels of oyster diversity and abundance. At least 7 oyster species were found at site G07, which might suggest an oyster hotspot of species diversity for this area. Other sites of G38, G12 and G32 were another noteworthy area with high species richness, abundance and biomass. Overall, this study revealed some potential places for site selection of oyster habitat management, protection and restoration efforts. More importantly, it provided insights into where or which oyster species was presumably under population outbreak or population bottleneck.

5 Conclusion

In conclusion, this research enhances our understanding of oyster assemblages and the spatial distribution of oyster reefs in Guangdong Province. We collected over 30,000 oyster specimens, which were classified into 12 species across three genera within the Ostreidae family. The findings validate the monophyly of *Crassostrea*, *Saccostrea*, and *Dendostrea* and identify two cryptic species within *Saccostrea*. By combining molecular and morphological data, this study updates the species inventory of oysters along the Guangdong coastline and clarifies the existing *Saccostrea* identities and their systematic relationships. In the analysis of dominant species, *C. sikamea* and *S. echinata* were the most abundant across 51 survey sites. *C. sikamea* was more abundant than that of previous studies, while fewer *C. ariakensis* and *C. angulata* specimens were found. We also suggest several potential places including G07, G12, G32 and G38 for oyster management and protection. To conserve the environment and restore ecosystem service, implementing more effective management measures is crucial to alleviating local pressures. The findings presented here can serve as a foundation for developing corresponding management strategies.

Data availability statement

Sequences of *S.* sp.1 haplotype are deposited in NCBI with accession numbers PQ452111 and PQ459553-PQ459559 for COI gene and 16S rRNA gene respectively. Sequences of *S.* sp.2 haplotype were linked to accession number PQ455391-PQ455392 for COI gene and PQ459552 for 16S rRNA gene.

Ethics statement

The animal studies were approved by South China Sea Bureau, Ministry of Natural Resources. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

ZZ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HQ: Investigation, Writing – original draft, Writing – review & editing. YC: Investigation, Writing – original draft, Writing – review & editing. J-wQ: Formal analysis, Writing – original draft, Writing – review & editing. YZ: Supervision, Validation, Writing – original draft, Writing – review & editing. JZ: Formal analysis, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

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