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Development and application of molecular markers in fisheries, aquaculture, and industry of representative temperate and tropical sea cucumbers: a review

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Sea cucumber has emerged as a crucial economic species in aquaculture in China because of its remarkable nutritional and medicinal value. However, wild sea cucumber populations have experienced a decline due to overfishing and environmental factors, underscoring the urgent need for genetic resource conservation and biotechnology innovation within the sea cucumber aquaculture and breeding industry. The development of the sea cucumber industry is still impeded by challenges and difficulties. Nevertheless, significant progress has been made through the utilization of molecular markers, which have effectively addressed a number of fisheries and aquaculture issues. In recent years, diverse types of molecular markers including mitochondrial DNA, microsatellites, and SNP markers have been developed and extensively applied in various aspects of sea cucumber research. These markers play vital roles in genetic sex identification, germplasm resource evaluation, population structure assessment, as well as marker-assisted breeding in marine ranching and sea cucumber aquaculture and breeding industry. This review provides an overview of the fundamental principles, functions, and characteristics associated with various markers employed across various sea cucumber species while also discussing their applications within different aspects of the sea cucumber fisheries, aquaculture, and breeding industry.

KEYWORDS

sea cucumber, molecular marker, aquaculture, germplasm conservation, molecular breeding

1 Introduction

Sea cucumber is a marine invertebrate belonging to the class Holothuroidea with the phylum Echinodermata. In general, sea cucumbers appear in a cylindrical, highly contractile body with numerous papillae on the dorsal and tube feet on the ventral side. Sea cucumbers live in a wide variety of different habitats, including seaweed ecosystems and coral reefs, ranging from shallow water to depths of more than 3,000 meters (Sulardiono et al., 2022). They feed on microalgae, organic debris, and material in sediments (Madduppa et al., 2017). There are more than 1,700 species of sea cucumber in the world, of which about 50–60 are edible (Elvevoll et al., 2022). Total sea cucumber fisheries increased from 26,000 tonnes of live weight in 2007–2016 to 48,000 tonnes in 2018–2019, with a slight decline to 43,000 tonnes in 2020 (FAO). Most cultured sea cucumber species belong to the order Aspidochirotida, including two sister groups, Holothuriidae and Stichopodidae (Wen et al., 2010). In Asian countries, sea cucumber is a commercially important aquaculture species due to its high nutritional and medicinal value. The wild sea cucumber populations have declined because of overfishing and environmental factors (Peng et al., 2012).

To increase the yield of sea cucumber, the sea cucumber breeding industry is rising gradually. Since the 19th century, sea cucumber farming in most countries has experienced boom-and-bust cycles, with the exception of China, where the majority of sea cucumbers have come from the aquaculture industry since the late 1980s (Liu, 2016). Even though sea cucumber aquaculture has been developed in the past decades, a series of problems occurred that could affect the larva development and juvenile growth, mainly related to disease control, germplasm conservation, and large-scale production in sea cucumber industry (Du et al., 2012). Among these, the slow growth rate is one of the most important issues in the sea cucumber industry (Cui et al., 2021). To meet the increasing demand for sea cucumber in the market, sea cucumber aquaculture and management using molecular methods or based genomic methods are quite important.

The sea cucumber breeding industry requires the application of traditional and novel molecular methods to benefit production and ensure sustainable development. Genetic breeding has been widely used in the breeding of aquaculture species, which focuses on genetic variations among populations and individuals to improve different types of performance traits. Molecular markers are commonly used molecular methods for detecting biological diversity at the DNA level, which is a powerful informatic molecular tool in genetic breeding and population genetics. Commonly used molecular markers include mitochondrial DNA (mtDNA), restricted fragments length polymorphism (RFLP), random amplification polymorphic DNA (RAPD), microsatellite (SSR), and single nucleic polymorphism (SNP), which have been widely used for species identification, population genetics, genetic breeding, marker-assisted selection, stock enhancement, and aquaculture management (Maqsood, 2017).

At the end of the 20th century, with the emergence of restriction enzymes and polymerase chain reaction (PCR), many molecular marker technologies have been developed and widely used. RFLP marker is a highly polymorphic and co-dominant marker that can be used for species identification, which is rarely used at present due to its complex process (Wen et al., 2010). RAPD marker is a simple technique without molecular hybridization and radioautography, which can be used to evaluate the geographical origin of species and genetic diversity analysis (Yun et al., 2017). Microsatellite marker is abundant and widely distributed throughout the genome (Oliveira et al., 2006), which is a simple and fast marker that have high mutation rates and reliable results, which can identify sea cucumber species, which will benefit germplasm conservation and management (Zhan et al., 2007). Subsequently, the advent of single nucleic polymorphism (SNP) molecular marker technology made it possible to perform genetic analysis of the genome at a precise level (Oliveira and Azevedo, 2022). Microsatellite marker and SNP marker are ideal molecular markers because of their high polymorphism and co-dominant inheritance (Maqsood, 2017), which have been widely used in population structure analysis (Kanno et al., 2006), genetic linkage map construction (Yan et al., 2013), QTL mapping (Chen and Li, 2007), marker-assisted selection (MAS) (Wang et al., 2009). Mitochondrial marker is a simple, fast, reliable molecular marker that is inherited from maternal lineage, which have high mutation rates and can be used in species identification, population genetic structure analysis, and phylogenetic analysis (Sulardiono et al., 2022). The advantages and disadvantages of various types of molecular markers are summarized in Table 1. At present, molecular markers are quite useful in the sea cucumber aquaculture and breeding industry. For instance, using sex-specific tags and SNPs to identify the sex can develop and breed a single-sex population, which could improve breeding efficiency, and lay a molecular foundation for studying the sex determination mechanism of sea cucumber (Cui et al., 2021); The high-density genetic linkage map of sea cucumber was constructed based on SNPs, which laid a foundation for mapping and analyzing QTL for performance traits like growth rate (Wei et al., 2021). This review introduces and summarizes the development and application of different types of molecular markers and their based genomic methods in sea cucumbers (Figure 1, Table 2) and examines the present status and prospects in fisheries, aquaculture, and industry.

2 Development of various molecular markers in sea cucumber

2.1 Mitochondrial DNA markers

The DNA barcoding using mitochondrial COI (cytochrome C oxidase subunit I) and ITS (internal transcribed spacers) can be used for identifying species for *Stichopus* genus and analyzing genetic diversity and phylogenetic relationships (Madduppa et al.,

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TABLE 1 Characteristics, advantages, and disadvantages of various types of molecular markers.

Molecular marker	Description	Advantage	Disadvantage	Application
RFLP	Changes in the length of DNA fragments produced by cutting relevant DNA molecules using specific endonuclease enzymes	High polymorphism; Co-dominant marker; High reproducibility	Require target DNA sequence information for the design of amplification primers; Complicated process	Species identification; Product mislabeling correction
RAPD	Genomic DNA is amplified by PCR using a series of single random primers (usually 10 nucleotides), and the polymorphism of PCR products is detected by electrophoresis	Random primers; Simple technique without molecular hybridization and radioautography; Require low amount DNA; Low cost	Poor experimental repeatability; unreliable results; Dominant marker	Genome mapping; Geographical origin and genetic diversity analysis
AFLP	A PCR-based molecular technique that uses selective amplification of a subset of digested DNA fragments	Large amount of markers with relatively little lab effort; No prior information about genome; Genome wide coverage; Small amount of DNA needed; Low cost; High experimental repeatability; reliable results	Dominant marker; Low reproducibility	Determine genetic differences among individuals, populations, and species; Investigate population structure and phylogenetic relationships based on genetic distances; Evaluate gene flow and dispersal, outcrossing, introgression, and hybridization
SSR	non-coding repetitive DNA regions composed of small motifs of 1 to 6 nucleotides repeated in tandem	Abundant, widely distributed throughout the genome; High mutation rate; Co-Dominant marker; High experimental repeatability; reliable results	Difficult to develop; High cost	Construction of genetic maps; Analyze genetic structure; Species identification; Genetic breeding and assessment of genetic variation
SNP	DNA sequence diversity caused by single nucleotide variation at the genomic level	Co-dominant markers; Abundance; Low genotyping cost; Typically biallelic; High-throughput genotyping; Lower mutation rate; Genotyping error rates	Difficult detection; Large workload	Construction of high-dense maps; Quantitative trait loci mapping; Assessment of genetic diversity; Parentage studies; Marker-assisted breeding
Mitochondrial DNA markers	Inherited from maternal lineage and efficiently identify and classify species based on a short DNA sequence in population and evolutionary biology	High mutation rate; Maternal inheritance; Simple, fast, and reliable	Limited information compared with nuclear genes	Species identification; Examine population genetic structure; Examine phylogenetic relationships

2017; Sulardiono et al., 2022). More specifically, the DNA barcode mitochondrial COI has been used for identifying sea cucumber species in two main families, including Stichopodidae (*Stichopus herrmanni*, *Stichopus ocellatus*, *Stichopus horrens, Stichopus monotuberculatus*) and Holothuridae (*Bohadschia bivittata*, *Actinopyga lecanora*, *and Holothuria leucospilota*), which will benefit germplasm conservation and management (Madduppa et al., 2017).

Using the mitochondrial DNA marker, three local sea cucumber species in Indonesia, Crengek Gamete, Pace gamete, and Kuning gamete, were close relatives to *Stichopus monotuberculatus* (Sulardiono et al., 2022). A next-generation

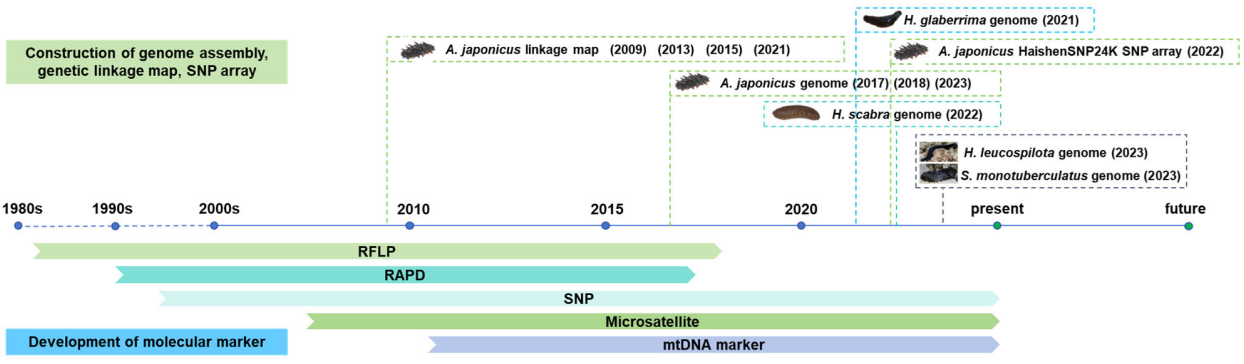

FIGURE 1
Time history of molecular markers and their based genomic methods applied in sea cucumbers.

TABLE 2 Development and application of various types of molecular markers in sea cucumbers.

Types	Sea cucumber species	Location	Method	Population number	Description	Reference
16S rRNA gene region	*Stichopus hermanni*, *Stichopus chloronotus*, *Thelenota ananas*, *Thelenota anax*, *Bohadschia argus*, *Holothuria fuscopunctata*, *Holothuria leucospilota*, *Holothuria scabra*, *Actinopyga echinites*	Sanya, China	species-specific PCR method	15 individuals per sample	a simple, fast, and reliable protocol for the accurate identification of sea cucumber species	Wen et al., 2012a
mitochondrial COI	*Holothuria atra*	Hawaiian Islands, Line Islands, Marshal Islands, Bonin Islands, and Ryukyu Islands	Sanger sequencing	385	examining population genetic structure to aid coral reef management	Skillings et al., 2011
mitochondrial COI and 16S ribosomal RNA (16S), and nuclear histone (H3)	*Holothuria edulis*	Okinawa Main Island in Ryukyu Archipelago (Motobu, Oyama, Ryugu, Sunabe, Philippine Sea and Awase)	Sanger sequencing	438	Investigating the metapopulation structure to provide valuable data to help management in vulnerable locations of Okinawa	Soliman et al., 2016
COI DNA barcoding	4 species belonged to the Stichopodidae (*Stichopus herrmanni*, *Stichopus ocellatus*, *Stichopus horrens*, *Stichopus monotuberculatus*), and 3 species to the Holothuridae (*Bohadschia bivittata*, *Actinopyga lecanora* and *Holothuria leucospilota*)	Kepulauan Seribu reefs, Indonesia	phylogenetic analysis	96	species identification	Madduppa et al., 2017
COI DNA barcoding	23 sea cucumber species	Not applicable	Sanger sequencing and next-generation DNA sequencing	23	screening of commercially processed sea cucumber products for clearer labeling	Xing et al., 2021
COI and ITS loci DNA barcoding	Stichopus	Karimunjawa Island, Central Java province, Indonesia	Not applicable	100 individuals consisting of three sea cucumber types (30 Crengek gamete, 36 Pace gamete, and 34 Kuning gamete)	genetic diversity, species identification, and examine phylogenetic relationships of the Stichopus genus	Sulardiono et al., 2022
RAPD	*Apostichopus japonicus*	Dalian and Weihai, China	RAPD-PCR	26	geographical origin and genetic diversity analysis	Yun et al., 2017
RFLP	Aspidochirotida: Stichopodidae	Sanya, Dalian, and Guangzhou, China	PCR-RFLP and FINS	70	identify six species belonging to family Stichopodidae in commercial products	Wen et al., 2010
RFLPs	*Holothuria mammata* Grube, 1840 and *H. sanctori* Delle Chiaje, 1823	The Azores in the north-east Atlantic, nine volcanic oceanic islands and several islets	PCR-RFLPs	Not applicable	quick identification of two species of sea cucumber	Madeira et al., 2018
PCR-RFLP, 16Sar/ 16Sbr primers	16 commercial sea cucumber species	Sanya and Dalian, China	PCR	6 individuals for each species	identify 16 types of sea cucumber in raw materials and processed foods	Zeng et al., 2018
EST-SSRs	*Apostichopus japonicus*	Rongcheng, Shandong Province, China	EST database	48	population structure analysis, phylogenetic and comparative genomics studies and genetic linkage map construction	Peng et al., 2009

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TABLE 2 Continued

Types	Sea cucumber species	Location	Method	Population number	Description	Reference
Microsatellite	*Stichopus japonicus*	Onagawa Bay in Miyagi Prefecture, Japan	Not applicable	Not applicable	tools for genetic analyses, investigate the genetic structure of *S. japonicus* and to detect reproductive isolation among color types through mating experiments	Kanno et al., 2005
Microsatellite	*Apostichopus Japonicus*	Aomori, Hokkaido, Miyagi, Oita, Japan; Qingdao, China	Not applicable	403	population identity of sympatric green and black, difference in the population structure, and further ecological and genetic research	Kanno et al., 2006
Microsatellite	*Apostichopus japonicus*	Not applicable	screening from SSR enriched library and EST database	45	population structure and the stock enhancement in fishery, the species/hybrid identification, and the linkage map construction	Zhan et al., 2007
Microsatellite	*Stichopus japonicus*	Penglai, China	expressed sequence tags	11	examining genetic population structure, parentage analysis and mapping studies	Chen and Li, 2007
Microsatellite	*Apostichopus japonicus*	Not applicable	Not applicable	Not applicable	an efficient procedure for isolating microsatellite DNA	Hu et al., 2007
Microsatellite	*Apostichopus japonicus*	Dalian, China	combining microsatellite DNA polymorphism and its relationship with body weight	208	molecular breeding, marker-assisted selection	Wang et al., 2009
Microsatellite	*Apostichopus japonicus*, *Parastichopus parvimensis*, and Pathallus mollis warty sea cucumber (*Parastichopus parvimensis*), and black sea cucumber (*Pathallus mollis*)	Qingdao, China; United States; Peru	Not applicable	60	future genetic breeding and the assessment of genetic variation within sea cucumbers	Liao et al., 2011
Microsatellite	*Apostichopus japonicus*	Jiaonan, Shandong, China.	expressed sequence tags	30	provide sufficient polymorphism for population genetic studies and genome mapping	Jiang et al., 2011
Microsatellite	*Stichopus chloronotus*	Okinawa Archipelago, Japan	Not applicable	60	detailing the genetic structure and gene flow	Taquet et al., 2011
Microsatellite	*Apostichopus japonicus*	Rongcheng, China	enrichment-colony hybridization protocol	48	population genetic studies and molecular marker-assisted breeding	Peng et al., 2012
Microsatellite	*Apostichopus japonicus*	Noto, Shinminato, and Uozu around Toyama Bay	PCR	236 wild individuals (146 red and 90 green variants)	clarify larval dispersal among populations by describing the levels of distinctiveness and gene flow among red and green variant populations, provide useful information for genetic conservation and fisheries management	Soliman et al., 2012
Microsatellite	*Stichopus japonicus*	Jeju island, Korea	multiplex assays with eight highly polymorphic	250	reveals the genetic composition and significant genetic differentiation between wild and hatchery-produced red sea cucumber samples in Korea	An et al., 2013

(Continued)

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TABLE 2 Continued

Types	Sea cucumber species	Location	Method	Population number	Description	Reference
			microsatellite loci			
Microsatellite	*Apostichopus japonicus*	Liaoning, China	transcriptome sequencing	20	linkage mapping, comparative genome analysis and genetic breeding	Chen et al., 2013
Microsatellite	*Holothuria scabra*	Croker Island, Northern Territory, Australia	next-generation sequencing	50	population structure and mating systems, investigation of population structure, levels of gene flow and mating systems	Fitch et al., 2013
Microsatellite	*Holothuria leucospilota*	Hainan Island, China	amplified fragment length polymorphism	30	germplasm conservation, genetic diversity, population structure, and conservation strategy design	Dai et al., 2015
Microsatellite	*Holothuria scabra*	Hainan Island, China	amplified fragment length polymorphism	30	genetic diversity and its effective conservation strategy, population structure	Li et al., 2015a
Microsatellite	*Stichopus horrens*	Hainan Island, China	fast isolation by amplified fragment length	30	studying population structure and conservation strategy design, genetic diversity	Li et al., 2015b
Microsatellite	*Holothuria leucospilota*	Not applicable	amplified fragment length polymorphism	30	genetic diversity, and the design of conservation strategies, population structure studies and cultivation	Shangguan et al., 2015a
Microsatellite	*Stichopus horrens* (Selenka)	Sanya, Hainan, China	fast isolation method with amplified fragment length	35	genetic structure, population conservation, and breeding of wild *S. horrens*	Shangguan et al., 2015b
Microsatellite	*Holothuria mammata*	Atlantic and Mediterranean Sea	SSR enriched library on a Roche 454 platform	60	fisheries management including identification of stocks, assessment of genetic diversity, estimation of gene flow and monitoring the fishery effects on exploited populations	Henriques et al., 2016
Microsatellite	*Holothuria grisea*	Bitupitá beach, Barroquinha, Ceará, Brazil	Illumina paired-end reads of whole genome shotgun sequencing	30	proper conservation of the species and development of sustainable fishery and aquaculture	Pereira et al., 2018
Microsatellite	*Apostichopus japonicus* Selenka, 1867	Peter the Great Gulf, Japan	Not applicable	159	genetic variability and population structure	Yagodina et al., 2022
SNP	*Apostichopus japonicus*	Dalian, Yantai, Qingdao, and Wendeng, China	Not applicable	39	delineating population structure in the sea cucumber	Sun et al., 2010
SNP	*Stichopus monotuberculatus*	Xidao Island, Sanya, China	gene-based	80	future quantitative trait loci (QTL) analysis, and to facilitate marker-assisted selection (MAS)	Du et al., 2012
SNP	*Apostichopus japonicus*	Wendeng, China	mining EST	32	genetic diversity assessment, genome mapping, reproductive ecology analysis and SNP-based analysis in aquaculture practice	Yang et al., 2012

(Continued)

TABLE 2 Continued

Types	Sea cucumber species	Location	Method	Population number	Description	Reference
SNP	*Apostichopus japonicus*	Dalian, China	SNP markers associated with defense mechanism	50	useful for the construction of genetic linkage map and comparative genome analysis	Gao et al., 2013
SNP and SSRs	*Apostichopus japonicus*	Dalian, China; Vladivostok, Russia; Rajin, North Korea	transcriptome analysis of tube foot	45	provide abundant genetics and molecular ecology resources	Zhou et al., 2016
SNPs	*Apostichopus japonicus*	Changdao, Yantai, Jiaonan, Wendeng, Penglai, China; Oita, Aomori, Japan	EST sequences	40	guides selective breeding	Dong et al., 2016
SNPs and SSR	*Apostichopus japonicus*	Yantai, China	transcriptome sequencing	150	genes discovery and functional genomic studies of the sea cucumber	Zhou et al., 2013
SNPs	*Holothuria (Metriatyla) scabra*	Six locations throughout Fiji (Namuka district, Lakeba island, Tavuki district, Serua island, Raviravi district, Nacula island)	Illumina sequencing	211	investigate genetic structure, diversity, signatures of selection, relatedness and connectivity in wild populations	Brown et al., 2022
SNPs	*Holothuria leucospilota*	Islands from Northern Vietnam, Central Vietnam, and Southern Vietnam; Darwin, Northern Australia and Mooloolaba, Eastern Australia	restricted site associated DNA sequencing	180	investigate population genetics to provide basic genetic information, design restocking management plans, genetic conservation initiatives, and sea ranching programs	Chieu et al., 2023

sequencing-based DNA mini-barcoding using COI has been developed to screen various commercial sea cucumber products and correct mislabeled for clearer labeling guidelines (Xing et al., 2021). The complete mitochondrial genome and cox1 DNA barcoding will be helpful for the sea cucumber breeding industry and studies on population genetic diversity and molecular ecology resources (Fan et al., 2012). In addition, a species-specific PCR of 16S rRNA has been used to authenticate 11 sea cucumber species from processed products, providing a rapid and useful protocol for correct labeling of commercial sea cucumber products (Wen et al., 2012a). By calculating the genetic distance of COI gene among different sea cucumber, Lu et al. proved the feasibility of COI as a DNA barcode for species identification, and the probes designed based on COI gene were used to identify four species of sea cucumber (Lv et al., 2011). Hu et al. used COI gene and 16S rRNA gene as target genes to identify sea cucumber species based on DNA barcoding technology, and the results showed that COI gene or 16S RNA gene could identify most species of sea cucumber (Hu et al., 2019). According to the specific primers for cox1 gene of *Holothuria scabra*, eDNA technology was used to investigate *Holothuria* resources in Weizhou Island, and the results showed that the eDNA monitoring method was more suitable and reliable than traditional methods (E et al., 2023).

2.2 Traditional molecular markers

A PCR-RFLP method was developed to identify six species of sea cucumbers in commercial products, which can authenticate species and detect fraudulent labeling (Wen et al., 2010). Another application of the PCR-RFLP method was to identify 16 commercial sea cucumber species from food products to correct the mislabeling (Zeng et al., 2018). Moreover, a PCR-RFLP method using restriction nuclease Sau3AI on 16S rRNA fragments has been developed to rapidly and inexpensively identify holothurian species with no need for taxonomical or genetic expertise (Madeira et al., 2018). DNA polymorphisms identified by RAPD-PCR technology were used to evaluate the geographical origin of *A. japonicus* and genetic diversity analysis (Yun et al., 2017). EST databases of sea cucumber species have been built, including *Holothuria glaberrima* during intestinal regeneration (Rojas-Cartagena et al., 2007), multiple tissues (body wall, intestine, respiratory tree) in *Apostichopus japonicus* (Yang et al., 2009), which were submitted to NCBI Genbank. EST databases have provided a rich resource for the development of molecular markers of sea cucumber species, especially microsatellite markers. SSR can be identified from EST data using screening programs, for example, SSRHUNTER (Chen and Li, 2007; Zhan et al., 2007; Peng et al., 2009; Jiang et al., 2011).

In addition, SNP markers were characterized by mining EST data of *A. japonicus* and using a homogeneous Tm- (melting temperature) genotyping method (Yang et al., 2012; Dong et al., 2016).

2.3 Microsatellite markers

The majority of microsatellite markers in sea cucumber species were from an aquaculture species in the coastal area of the Northwest Pacific, *Stichopus (Apostichopus) japonicus*. The earliest 20 microsatellite markers of sea cucumber *Apostichopus japonicus* were developed by Kanno et al. in 2004, which could be excellent molecular markers to facilitate genetic structure analysis (Kanno et al., 2005). Eleven microsatellite markers were used to identify *A. japonicus* population from green and black color variants for further genetic population structure analysis and ecological research (Kanno et al., 2006). In sea cucumber *Apostichopus japonicus*, Hu et al. developed an efficient procedure for isolating a reasonable number of microsatellites for genetic analysis (Hu et al., 2007). Forty-five informative microsatellite markers (43 genomic SSRs and 2 EST-SSRs) were developed based on screening SSR-enriched library and EST library, which may contribute to population structure analysis, linkage map construction, stock enhancement, and species identification (Zhan et al., 2007). Eleven microsatellite markers derived from expressed sequence tags were developed to investigate population structure and conduct parentage analysis and QTL mapping (Chen and Li, 2007). Microsatellite markers associated with body weight were developed for not only molecular breeding but also marker-assisted selection (MAS) (Wang et al., 2009). Jiang et al. have developed 43 EST-SSR markers for future studies in population genetics and genomics (Jiang et al., 2011). Twenty novel microsatellite markers from *Apostichopus japonicus* were used for cross-species application in *Parastichopus parvimensis* from the United States and *Pathallus mollis* from Peru, which could facilitate genetic variation assessment and genetic resource management among Holothuroidea populations (Liao et al., 2011). A larger number of ~70 microsatellite markers were developed for the implementation of marker-assisted selection and population genetic studies (Peng et al., 2012). Furthermore, 45 gene-derived microsatellite markers were identified from transcriptome sequencing, which could be useful for comparative genomics studies, genetic linkage mapping, and molecular breeding like MAS (Chen et al., 2013). A total of 8 highly polymorphic microsatellite markers were used for genetic structure studies between wild and hatchery populations and implications in breeding programs and stocking strategies (An et al., 2013). Ten microsatellite markers have revealed the genetic variation and population structure of *A. japonicus* in Peter the Great Gulf, Japan. In studying microsatellite markers, the detected high heterogeneity among populations might be due to several factors: stochastic larval settlement at a location, and selection pressures on QTL (Yagodina et al., 2022).

Ten microsatellite markers were developed in a tropical sea cucumber species *Stichopus chloronotus* to investigate the genetic structure and gene flow among populations (Taquet et al., 2011). Eighteen microsatellite markers from another sea cucumber species *Holothuria scabra* were characterized, which were beneficial for genetic structure and gene flow studies for sea cucumber aquaculture and management (Fitch et al., 2013). A total of another 9 microsatellite markers of *H. scabra* were developed for conservation strategy and investigation of genetic diversity and population structure (Li et al., 2015a). Eight microsatellite markers were identified in a tropical species *Holothuria leucospilota*, which could be beneficial for genetic structure, population diversity, and germplasm conservation (Dai et al., 2015). Shangguan et al. identified 16 additional microsatellite markers from *H. leucospilota* that could be used for studies in genetic diversity and population structure and application in cultivation and conservation strategies (Shangguan et al., 2015a). Li et al. identified 9 microsatellite markers in another tropical sea cucumber *Stichopus horrens* (Selenka) that could be used as markers to study its conservation and breeding, population structure, phylogeny and evolutionary studies of Holothuroidea (Li et al., 2015b). 9 more microsatellite markers have been characterized in *S. horrens*, which could be helpful for conservation strategy and management, population structure and genetic diversity of *S. horrens* (Shangguan et al., 2015b). 9 novel microsatellite markers have been identified from *Holothuria mammata*, an exploited sea cucumber species in Mediterranean and North-Eastern Atlantic, which could be used for aquaculture and conservation management, gene flow, and genetic diversity assessment (Henriques et al., 2016). Furthermore, eight microsatellite markers in the West Atlantic sea cucumber species *Holothuria grisea* have been developed and used as markers for proper conservation and advancement in sustainable aquaculture of this species (Alves Pereira et al., 2018). Despite the popularity of SSR in diverse aspects of sea cucumber research, it is gradually replaced by genome-wide molecular markers such as SNPs.

2.4 Single nucleic polymorphism (SNP)

In *A. japonicus*, all identified 13 SNPs have two alleles and their heterozygosity could be used as molecular markers in analyzing population structure, population genetics, and QTL mapping (Sun et al., 2010). Fifteen more SNP markers were identified from ESTs and provided a complement to available molecular markers of *A. japonicus* (Yang et al., 2012). These SNPs may be implied in studies on genetic diversity, QTL mapping, and ecology analyses of *A. japonicus* (Yang et al., 2012). 26 SNP markers associated with defense mechanisms were developed by mutation scanning from unigene sequences in *A. japonicus* transcriptome, which may provide insights into genetic maps and comparative genomic studies (Gao et al., 2013). 51 gene-derived SNPs from ESTs using high-resolution melting can be used for MAS, genetic improvement, and aquaculture management (Dong et al., 2016). High throughput transcriptome analysis provided benefit to large-scale marker discovery of *A. japonicus*. 142,511 SNP markers and 6,417 microsatellite markers with high quality have been identified through transcriptome analysis, which can be used for gene discovery and functional genomic research (Zhou et al., 2014). A total of 219,860 SNP markers have been identified from tube foot

transcriptome, providing plentiful resources in genetic and molecular ecology studies (Zhou et al., 2016).

3 Construction of genome assembly, genetic linkage map, and SNP array

Since 2017, the genome assemblies of sea cucumber species have been constructed using next-generation sequencing and third-generation sequencing, including one northern sea cucumber species *A. japonicus*, and three tropical sea cucumber species, *H. scabra*, *H. leucospilota*, *H. glaberrima* and *S. monotuberculatus* (Table 3). As the most common commercial sea cucumber species, the *A. japonicus* genome assembly have been constructed in 2017 (Zhang et al., 2017) and 2018 (Li et al., 2018) and updated in 2023 (Sun et al., 2023). The construction of genome assembly has been used to understand the molecular mechanisms on morphological evolution, visceral regeneration, saponin biosynthesis, aestivation regulation, providing a referable resource for understanding evolution and biodiversity of sea cucumber. In 2022, *De novo* genome assembly of *H. scabra* has been built using nanopore sequencing, which provides excellent genetic resource for studying genetic, phylogenetic, molecular biology, and breeding studies (Luo et al., 2022). In 2023, a draft genome of *S. monotuberculatus* was assembled using Nanopore sequencing, which provides insight into critical genes in fucosylated chondroitin

TABLE 3 Construction of genome assembly and molecular markers-based genomic methods in sea cucumbers.

Types	Sea cucumber species	Location	Method	Population number	Description	Reference
genome	*Apostichopus japonicus*	Laoshan, Qingdao, China	Illumina+PacBio (CLR)	NA	enables exploring key genetic mechanisms behind the unique biological characters, phylogenetic and comparative genomic analyses	Zhang et al., 2017
genome	*Apostichopus japonicus*	Shandong Province, China	Illumina+PacBio (CLR)	NA	reveals novel genomic features and molecular variations that contribute to the evolutionary adaptation, provides insights into saponin synthesis and aestivation regulation.	Li et al., 2018
genome	*Apostichopus japonicus*	Rushan, Shandong Province, China	Illumina+Pacbio (CCS)	NA	an excellent tool to better investigate the mechanisms that drive evolution and biodiversity	Sun et al., 2023
genome	*Holothuria glaberrima*	Puerto Rico, USA	Illumina	NA	a critical resource for future studies in animal regeneration	Medina-Feliciano et al., 2021
genome	*Holothuria scabra*	Guangxi Province, China	Nanopore	NA	provides a referable resource for research on genetic, phylogenetic, molecular biology, and breeding studies	Luo et al., 2022
genome	*Stichopus monotuberculatus*	Guangxi Province, China	Nanopore	NA	provides a genomic approach to investigate the structural diversity, as well as novel perspectives into evolutionary adaptation of critical genes in holothurian fucosylated chondroitin sulfates biosynthesis pathways	Zhong et al., 2023
genome	*Holothuria leucospilota*	Nanhai, China	Illumina+PacBio+BGI	NA	provides insights into molecular underpinnings of sacrificial organ expulsion in holothurian species	Chen et al., 2023
SNP array	*Apostichopus japonicus*	Liaoning and Shandong, China; Russia	a high-throughput 24 K SNP genotyping array	210	minmax concave penalty (MCP) regularization for sparse deep neural networks (DNN-MCP) high-throughput genotyping platform	Lv et al., 2022
Genetic linkage map	*Apostichopus japonicus*	Not applicable	AFLP and microsatellite markers	88	application of a marker-assisted selection breeding strategy, basis for mapping of the functional genes and quantitative trait loci, mapping QTL for growth heterosis and locating expressed genes in aquaculture.	Li et al., 2009
Genetic linkage map	*Apostichopus japonicus*	Shandong Province, China	microsatellites and SNPs	144	facilitate further sea cucumber genetic studies such as quantitative trait loci (QTL) mapping and comparative genomic analysis	Yan et al., 2013
Genetic map	*Apostichopus japonicus*	Penglai, China	genotyping-by-sequencing (GBS) methods, 2b-restriction site-associated DNA (2b-RAD) method	100	a powerful tool for research involving both fine-scale QTL mapping and marker assisted selection (MAS)	Tian et al., 2015

NA, not applicable.

sulfates biosynthetic pathway (Zhong et al., 2023). The high-resolution genome assembly of *H. leucospilota* was also built in 2023 using Illumina sequencing, Pacbio sequencing, BGI sequencing platform, which provides insights into molecular underpinnings of sacrificial organ expulsion in holothurian species (Chen et al., 2023).

Genetic linkage maps are constructed based on the gene linkage and genetic recombination rates to infer the relative positions of genes or genetic markers on chromosomes (Cui et al., 2021). Up to date, a total of four genetic linkage maps of *Apostichopus japonicus* based on molecular markers have been developed for QTL mapping and marker-assisted selection breeding since 2009 (Table 3). The first genetic linkage map was built on 484 polymorphic AFLP and microsatellite markers, which provides the application of molecular breeding and understanding the genetic basis of economic traits in sea cucumber industry (Li et al., 2009). In 2013, a medium-density genetic linkage map using co-dominant microsatellite and SNP markers was built to benefit QTL mapping, marker-assisted selection, and comparative genomic studies (Yan et al., 2013). A high-density and high-resolution genetic map of 7,839 markers based on 2b-restriction site-associated DNA (2b-RAD) was constructed in 2015, which facilitates fine-scale QTL mapping, precise marker-assisted selection, and chromosome assignment for whole genome assembly (Tian et al., 2015). A 6,144 SNP-based high-density genetic map using genotyping-by-sequencing was built, which provides an important genomic resource for marker-assisted selection with high performance traits like growth traits (Sun et al., 2021). With the development of whole genome sequencing and new molecular techniques, genome-wide molecular markers like SNPs in an SNP array chip have been used as an efficient genotyping tool for genetic research and genome breeding programs in aquaculture. In 2022, a high-throughput 24 K SNP array genotyping platform, HaishenSNP24K, was developed for genetic studies and application in selection and breeding programs with minmax concave penalty regularized deep neural networks (DNN-MCP) (Lv et al., 2022). The new SNP array has significantly improved the genomic assessment of sea cucumber populations for quantitative performance traits, including wet or dry weight, and survival time (Lv et al., 2022).

4 Application of molecular markers and based genomic methods in fisheries, aquaculture, and industry

The molecular marker technology facilitates germplasm resource evaluation, assessment of genetic diversity and population structure, sex identification for live animal, and selection of desirable traits, which has been extensively applied in various aspects of the sea cucumber aquaculture industry (Figure 2). These applications play a pivotal role in enhancing the efficiency of industrialized aquaculture and breeding programs for sea cucumbers, enabling precise breeding strategies and conservation of germplasm resources.

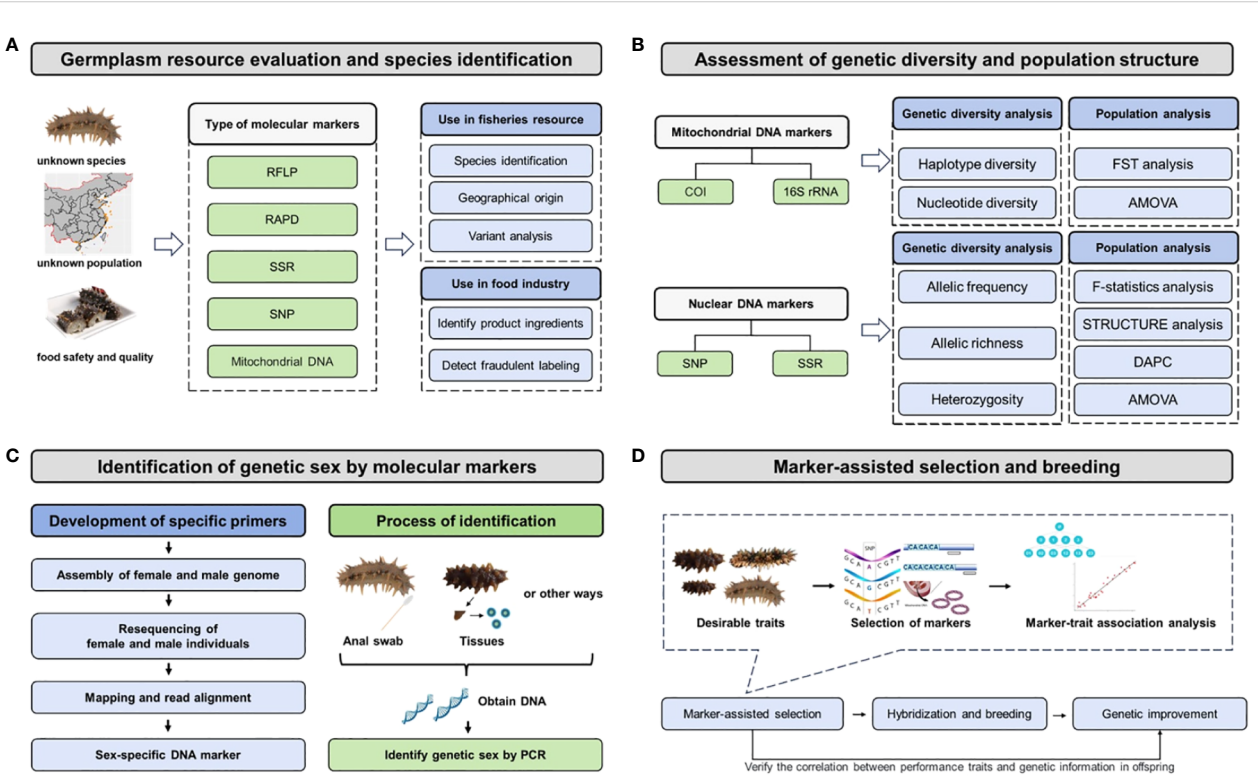

FIGURE 2
Flow chart of molecular markers or based genomic methods in sea cucumber fisheries, aquaculture, and industry. **(A)** Germplasm resource evaluation and species identification; **(B)** Assessment of genetic diversity and population structure; **(C)** Genetic sex identification in aquaculture; **(D)** Marker-assisted selection and breeding.

4.1 Germplasm resource evaluation and species identification

Germplasm resource evaluation mainly includes population resource information, ecological factors, habitat conditions, environmental variables, external disturbance factors, and strain identification. Germplasm resource identification has significant implications not only in the discovery and development of new germplasm but also in hybrid and cryptic species identification and the utilization of hybrid advantages (Ding and Chang, 2020). Specific sequence analysis plays an important role in natural hybridization and gene flow between populations. For example, skeletal characteristics could not distinguish *H. scabra*, colour morphs of *H. S.* var.*versicolor*, and intermediate phenotype hybrids in the species boundary study, as their color patterns, morphology, and small-scale distribution patterns were different (Uthicke et al., 2005). Both of population genetics and 16S mtDNA sequence analysis were used to identify new species *H. S.* var.*versicolor* and individuals with intermediate phenotypes represented F1 hybrids, demonstrating that the opportunity for introgression exists (Uthicke et al., 2005). The conventional method based on their morphology or external characteristics for species identification have gradually developed towards molecular markers with high accuracy and polymorphism, such as RFLP, RAPD, SSR, and SNP (Lv, 2012). In general, germplasm resource evaluation by molecular markers includes four important steps: sample collection, DNA extraction, PCR amplification using molecular markers, gel electrophoresis or DNA sequencing. For instance, PCR-RFLP was used to develop a method of DNA barcoding-based sea cucumber species identification, and mitochondrial COI gene was used as a DNA barcode to achieve germplasm identification of five sea cucumber species (Lv, 2012). Wen et al. successfully identified 19 commercial sea cucumber species using PCR-RFLP method (Wen et al., 2012b). With the completion of mitochondrial genome sequencing of *Apostichopus japonicus* and other sea cucumber species at the National Center for Biotechnology Information (NCBI), the basic characteristics of the mitochondrial genome of the taxonomic class Holothuroidea have been revealed, which could benefit germplasm resource evaluation using molecular methods (Shen et al., 2011). The use of all these molecular markers in fisheries resource evaluation include species identification, geographical origin, and variant analysis. In sea cucumber industry, molecular methods could identify product ingredients and detect fraudulent labeling for food safety and better quality.

4.2 Assessment of genetic diversity and population structure

Molecular marker technology has been widely applied in the assessment of population genetic structure and genetic diversity, providing guidance for the sea cucumber aquaculture industry and germplasm resource protection. Bottom-culture farming in marine ranching is an important means of germplasm resource protection, and the evaluation of genetic diversity and population structure of its natural geographical populations is crucial. In general, this includes four important steps: sample collection, DNA extraction, genotyping or sequencing, and data analysis. Numerous sea cucumber studies have utilized mitochondrial DNA markers (e.g. COI, 16S) and nuclear DNA markers (e.g. SNP, SSR) for this purpose. Mitochondrial DNA markers are used to conduct haplotype diversity (h) and nucleotide diversity (π) for genetic diversity analysis, as well as FST (fixation index) and AMOVA (Analysis of molecular variance) for population analysis. For instance, Yan (2006) used COI molecular marker methods to investigate the genetic diversity and population genetic structure of *A. japonicus* from five geographic subgroups in the Dalian Sea area and revealed that these five geographical subgroups had high genetic diversity. A mitochondrial COI gene analysis for the population genetic structure of *Holothuria atra* in the waters surrounding the Hawaiian Islands in order to aid coral reef management (Skillings et al., 2011). Additionally, nuclear DNA markers have proven to be an effective method for evaluating genetic diversity, including allelic frequency, allelic richness, and heterozygosity, as well as population analysis including F-statistics analysis (Fixation indices), STRUCTURE analysis, DAPC (discriminant analysis of principal components), AMOVA. For instance, Wang et al. conducted genetic diversity and population structure assessments of 18 geographical populations of *A. japonicus* from sea ranching in Shandong, Hebei, and Liaoning provinces using specific SNP loci, thereby enabling continuous genetic monitoring of the northern natural habitats of sea cucumbers in China (Wang et al., 2023). Whole-genome SNP data was used to identify three genetically distinct subgroups within populations of *Holothuria (Metriatyla) scabra* from Fiji, revealing a model of genetic structure isolated by distance (Brown et al., 2022). An investigation of population genetics between Australia and Vietnam using SNP markers in *H. Leucospilota* was conducted to compare gene flow and genetic similarities among populations and analyzing population genetic structure (Chieu et al., 2023). Now land et al. used population genetic analysis based on microsatellite sequences to assess the population genetic structure of *H. scabra* in Papua New Guinea and a broader region of northern Australia, in order to determine the genetic diversity within subpopulations and investigate the genetic impact of environmental changes on wild populations (Nowland et al., 2017). Eight polymorphic microsatellite loci were successfully developed in *H. leucospilota*, which provided crucial molecular markers for studying population genetic diversity and conservation strategies in sea cucumbers (Dai et al., 2015). Liao et al. utilized SSR fingerprinting technology to analyze the genetic diversity and construct fingerprinting profiles of sea cucumber populations from different regions in China, South Korea, and Russia (Liao et al., 2021). The constructed fingerprinting profiles successfully differentiated eight populations and provided technical support for the protection of sea cucumber germplasm resources and the identification of different geographical populations (Liao et al., 2021). Furthermore, genetic diversity and structure assessments of wild populations and hatchery-produced *H. scabra* in New Caledonia were achieved using nine polymorphic microsatellite markers, aiming to develop an appropriate breeding plan for restocking (Riquet et al., 2022).

4.3 Identification of genetic sex

Due to the lack of visible sexual dimorphism in the external appearance of most sea cucumber species, the development and application of a method based on molecular markers is useful to identify the sex of several sea cucumber species (Table 4). Generally, the development of a sex-specific marker is realized through the assembly of female and male genome sequences, resequencing of female and male individuals, mapping and read alignment, identification of sex-specific region, and design and verification of sex-specific marker. The verification of an accurate and stable sex-specific marker is usually completed by wild populations in multiple geographical regions. Tested samples can be collected by anal swab or tissue dissection, and then genomic DNA can be extracted by multiple protocols. The genetic sex of sea cucumber individual is identified by PCR amplification using sex-specific marker. The utilization of sex-specific molecular markers facilitates the determination of individual gender and enhances the efficiency of artificial breeding in large scale aquaculture. In *A. japonicus*, a sex-specific marker was developed utilizing 2b-RAD technology, which holds significant implications for genetic breeding (Wei et al., 2021). In addition, a loop-mediated isothermal amplification (LAMP) method was established based on male-specific sequences, which could rapidly identify the genetic sex of *A. japonicus* (Cong et al., 2024). Furthermore, a rapid and non-destructive method was devised for sex identification in aquaculture practices involving another tropical sea cucumber species (*Stichopus monotuberculatus*) (Wu et al., 2022). A non-invasive and rapid method based on anal swab sampling using a LAMP system (PCR and loop-mediated isothermal amplification) was employed for sex identification in tropical sea cucumber *Holothuria scabra* (E et al., 2023).

4.4 Marker-assisted selection and breeding

With the continuous development of breeding technologies, there is a gradual shift from traditional selective breeding and hybrid breeding techniques towards the integration of molecular marker-assisted breeding, whole-genome selection breeding, molecular design breeding, sex control, gene transfer, and gene editing (Ding et al., 2021). Molecular marker-assisted breeding is a technique in aquaculture breeding that involves directly selecting individuals with advantageous alleles or genotypes for specific traits using molecular markers closely associated with those traits, which applies the research findings of genomics and molecular biology to the selection and breeding of aquaculture species (Lu et al., 2019). It demonstrated that the construction and use of genetic linkage maps with a high number of molecular markers could connect desirable performance traits with molecular markers, which can benefit marker-assisted selection and breeding and accomplish genetic improvements for sea cucumber aquaculture. The accomplishments of marker-assisted selection and breeding can be evaluated through the correlation between performance traits and genetic information in offspring. In *A. japonicus*, the insertion/deletion (INDEL) marker of internal transcribed spacer 1 (ITS1) sequence divergence was successfully used to identify the genetic types of different body color varieties, and hybridization experiments showed that this marker could be used to distinguish and track the genetic composition of F1 hybrid larvae, which explored the relationship between body color variation and genetic background and its impact on larval development (Yoshida et al., 2012). The linkage map using AFLP markers serves as a foundation for constructing high-resolution genetic maps and identifying functional and quantitative trait loci (Li et al., 2009). Yan et al. constructed a common genetic map for sea cucumbers based on microsatellite and SNP markers, which was consistent with the haploid chromosome number of sea cucumbers (Yan et al., 2013). Liu et al. used a high-density genetic linkage map of sea cucumbers to preliminarily locate QTL regions associated with five traits: body length, body width, body weight, total number of spines, and survival rate (Liu et al., 2019). The "Sea Cucumber Superior No.1" was successfully bred using molecular marker-assisted breeding, which has strong resistance to *Vibrio splendidus* infection and fast growth rate (Ding and Chang, 2020).

TABLE 4 Development and application of sex-specific markers for genetic sex identification in sea cucumbers.

Types	Sea cucumber species	Location	Method	Population number	Description	Reference
sex-specific markers	*Apostichopus japonicus*	Liaoning, China.	2b-RAD	97	provide a solid basis to reveal the sex determination mechanism and the origin of sex-determining regions; improve the efficiency of genetic and sex control breeding in sea cucumbers	Wei et al., 2021
sex-specific markers	*Stichopus monotuberculatus*	Xidao Island, Sanya, China	genomic sequencing	80	an effective way to identify sex in sea cucumbers	Wu et al., 2022
PCR and LAMP system	*Holothuria scabra*	Zhanjiang, China	DNA sequencing	50	investigate the basis of sex determination in the Holothuriidae family	E et al., 2023
PCR and LAMP system	*Apostichopus japonicus*	Dalian, China	genomic sequencing	100	A rapid LAMP-based method of identifying the genetic sex without complex equipment.	Cong et al., 2024

The new germplasm was achieved through population selection, screening and verification of disease-resistant molecular markers that indicate disease-resistant functional genes (Ding and Chang, 2020). In *A. japonicus*, a new high-density genetic linkage map was constructed based on single nucleotide polymorphism (SNP) molecular markers, and quantitative trait loci (QTL) mapping analysis was performed to explore the genetic mechanism of sea cucumber growth traits, which could be helpful for future marker-assisted breeding (Cui et al., 2021).

Molecular marker technology has diverse applications in fisheries research and offers significant convenience and solutions to numerous challenges encountered in the aquaculture industry. In order to tackle the obstacles faced by the sea cucumber aquaculture and breeding industry, various molecular markers have been developed and implemented for multiple purposes, including germplasm resource evaluation, species identification, Assessment of genetic diversity and population structure, and marker-assisted selection and breeding. With innovations in genomic methods, molecular markers are expected to continue playing important roles in promoting the future development of sea cucumber fisheries, aquaculture, and industry.

Author contributions

XL: Investigation, Visualization, Data curation, Writing – original draft. XT: Investigation, Visualization, Data curation, Writing – original draft. MC: Supervision, Resources, Conceptualization, Writing – review & editing. GN: Supervision, Resources, Conceptualization, Writing – review & editing. YY: Visualization, Supervision, Resources, Conceptualization, Writing – review & editing, Writing – original draft.

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Conflict of interest

The authors declare 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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