

## Before It Is Too Late: Current Genetic Diversity Status of the Exploited Sandfish *Holothuria scabra* (Echinodermata: Holothuroidea)

(Sebelum Terlambat: Status Semasa Kepelbagaian Genetik *Holothuria scabra* Gamat yang Dieksplotasi  
(Echinodermata: Holothuroidea))

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### ABSTRACT

The sandfish *Holothuria scabra* is a well exploited and among the highest-valued sea cucumber species. Owing to its economic importance and the global expansion of sea cucumber fisheries that could lead to overfishing, the aquaculture of *H. scabra* is increasing rapidly. To aid in resource management and providing sufficient molecular information to inform the selection of sea cucumber broodstock to be incorporated into aquaculture, sufficient knowledge on their genetic diversity is among the pre-requisite. Therefore, this review synthesized together the currently available information on genetic diversity of *H. scabra* in a global scale, thereby highlighting the lack of genetic baseline of *H. scabra* in some populations. The implications and importance of the availability of genetic baseline data to restocking and sea ranching, fisheries, and aquaculture of *H. scabra* are discussed. Finally, future directions, including the development of full genome, the use of other sequencing technologies, and the impact of climate change onto *H. scabra* are provided.

Keywords: Genetic diversity; *Holothuria*; sandfish; sustainable resource management

### ABSTRAK

*Holothuria scabra* adalah sejenis spesies gamat yang dieksplotasi dengan meluas dan bernilai tinggi. Aktiviti akuakultur bagi *H. scabra* telah meningkat dengan pesat disebabkan nilai pasaran dan pengembangan global perikanan gamat yang boleh menyebabkan penangkapan secara berlebihan. Bagi membantu dalam pengurusan sumber dan penyediaan maklumat berkaitan molekul gamat yang lengkap untuk pemilihan stok induk gamat yang digunakan ke dalam penternakan akuakultur, pengetahuan yang mencukupi tentang kepelbagaian genetik mereka adalah antara prasyarat yang perlu dipenuhi. Oleh yang demikian, penulisan ini merupakan rumusan yang menyediakan maklumat terkumpul mengenai kepelbagaian genetik *H. scabra* dalam skala global, sekali gus menunjukkan kekurangan garis asas genetik *H. scabra* dalam sesetengah populasi. Ia turut mengetengahkan implikasi dan kepentingan ketersediaan data asas genetik kepada penternakan semula dan penternakan laut, perikanan dan akuakultur *H. scabra*. Kesimpulannya, perbincangan berkenaan hala tuju masa hadapan, termasuk pembangunan genom keseluruhan, penggunaan teknologi penjujukan lain dan kesan perubahan iklim ke atas *H. scabra* dengan ini diberikan.

Kata kunci: Gamat; *Holothuria*; kepelbagaian genetik; pengurusan sumber mampan

## INTRODUCTION

Genetic diversity is characterised by the variations in genotype that exist between individuals, population, and species. Understanding genetic diversity that is present within and among populations provide essential insights into the basis of various population-specific phenotypes, including adaptation to environmental changes, disease susceptibility, and differing growth and reproduction traits (Waiho et al. 2022a; Weitemier et al. 2021). Various factors, including selection, genetic drift, migration, and fishing pressure, could impact the genetic diversity of a species (Duan et al. 2022), especially for species that exhibit periodic slow movement (Mercier, Battaglane & Hamel 2000; Purcell & Kirby 2006). The quantification and mapping of genetic diversity of a population allow the understanding of heterozygosity – proportion of diploid individuals with different alleles at a locus, a measure that is tightly linked with the ability of a population to adapt and evolve. Therefore, reduced genetic diversity (heterozygosity) in a population may cause reduction in population viability and increase the chances of extinction, especially when faced with extreme conditions such as climate change or heavy metal exposure (Ross et al. 2002; Thomson et al. 2021).

It is well known that anthropogenic factors such as habitat destruction and overfishing/overexploitation of a specific species may ultimately result in small, isolated populations with significantly reduced effective population size and genetic diversity (Vøllestad 2017). Therefore, understanding the genetic diversity, connectivity, and population structure of an exploited species is essential for making informed conservation and management actions to increase population connectivity and robustness (Klüttsch et al. 2019). Albeit it is evident that commercially exploited marine species exhibited lower genetic differentiation among populations (Gandra et al. 2021), the incorporation of genetics aspect into fisheries management is challenging as the traditional use of markers can be laborious whereas new sequencing techniques can be costly and require trained personnel (Petit-Marty et al. 2022).

The sandfish *Holothuria scabra* is a common sea cucumber species that can be found in sandy and muddy coastal zones along the Indo-Pacific region. They are soft-bodied echinoderms with a rough texture, greyish-gold skin color, with irregular dark brown-black stripes on the back, and a pale to almost white belly (Altamirano & Rodriguez 2022). Most of their body walls are made of

collagen tissue, which covers and protects their internal organs (Slater & Chen 2015). Sandfish are deposit-feeders that play an important role in marine ecosystems as a biocatalytic filter system (Lee et al. 2018a) by directly contributing to nutrient recycling and decomposition of detritus and organic matter (Gao et al. 2011; Zamora et al. 2018). According to the Food and Agriculture Organization database in 2023, the global aquaculture production of *H. scabra* was around 553 tonnes. *H. scabra* of all sizes are being harvested and sold in the international market, with bigger sizes correlating to higher prices. The unsustainable harvesting of *H. scabra*, especially below their length at first sexual maturity at around 15 cm (Lee et al. 2018b), will eventually result in overfishing and threatens their genetic population structure and diversity.

Owing to the growing demand for sea cucumber products, overfishing has led to the depletion of various economically important sea cucumber species (Hasan 2019), including *H. scabra* (Conand 2018). To date, according to the International Union for Conservation of Nature (IUCN) red list, 16 sea cucumber species are being categorized as threatened to extinction (Conand et al. 2014; Scannella et al. 2022). Moratoriums and sea ranching programs have been initiated in various geographical locations in an attempt to alleviate the fishery harvest pressure while ensuring sustainable livelihood for coastal communities (Hair et al. 2016; Kinch et al. 2008). However, in-depth understanding of population genetic structure and genetic diversity is instrumental to ensure genetic conservation of sea cucumber populations, especially in overfished stock populations. Therefore, this review characterizes the known genetic diversity of *H. scabra*, one of the most commercially harvested sea cucumber species. The potential implications of understanding the genetic diversity of *H. scabra* at a global scale, especially in stock restoration programs and mariculture, are also discussed.

## METHODS

Systematic literature search from public database, i.e., Web of Science (WoS, <https://www.webofscience.com/>) and Scopus (<https://www.scopus.com>) were conducted to ensure all relevant literatures were included in this review. Keywords used were '*Holothuria scabra*' AND 'genetic diversity' AND 'population structure'. Articles published up to 31 March 2023 were included in the search. Only

journal articles published in English were included in the search, thereby excluding grey literature, books, editorials, and conference proceedings. The overall literature search process is depicted in Figure 1 based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow chart (Moher et al. 2015; Waiho et al. 2022b). From the initial 59 articles retrieved from WoS and Scopus databases, redundant articles between the two databases were removed, and each article was thoroughly examined for suitability. A final dataset of 11 papers that fit the description of this review were selected and filtered.

#### ECONOMIC VALUES OF SEA CUCUMBER

Although data on the fisheries aspect of *H. scabra* is lacking, as most sea cucumber producing countries classify them as holothurians/sea cucumbers without further identification up to the species level, a recent review by Conan (2018) showed that the number of Indo-Pacific countries involved in sea cucumber fisheries increased from 19 countries contributing 10,489 t/year in 1986-1996 to 30 countries contributing 16,026 t/year in 2009-2014.

Sea cucumbers, especially *H. scabra*, is traditionally marketed as a dried product and considered as a lucrative delicacy and ingredient with medicinal values for the Chinese communities. Nowadays, the development of technology has allowed sea cucumbers to be shipped in various forms in addition to the traditional dried product, such as fresh, frozen, in brine, or vacuum-packed (Conand et al. 2014). Coupled with the growth of China's market following the increase in consumer purchasing power, the demand for sea cucumber increases exponentially, especially for high value species such as *H. scabra* and *H. lessoni* (Conand 2018).

While most sea cucumber producing countries do not consume sea cucumber products, they are being harvested to produce tonics and medicines by the local communities, especially those of Asian countries (Pangestuti & Arifin 2018). The economic value of sea cucumber further escalate when they are found to contain various high value compounds with therapeutic properties such as anti-cancer (Aminin et al. 2016; Ru et al. 2022), anti-coagulant (He et al. 2020; Zheng et al. 2019), anti-microbial (Darya et al. 2020), anti-oxidant (Nugroho et al. 2022), and neuroprotective properties (Zhao et al. 2021), and induce wound healing (Zheng et al. 2022). Thus, sea cucumber fishery has a huge socio-

economic impact on the local coastal communities in the Indo-Pacific region, whereby most of them rely on the catch and sale of sea cucumbers as their household income and livelihood (Hair et al. 2019).

Among all of the commercially traded sea cucumber species, *H. scabra* remained as the most valuable species, with price increases exponentially with body size, and the average retail price reached USD369 per kg in China (Purcell, Williamson & Ngaluafé 2018). As such, overfishing of sea cucumbers, especially *H. scabra* is a global concern. Researchers and other stakeholders are also looking into ways to ensure sustainable fisheries management of *H. scabra* while protecting the livelihood of coastal households, many of which are low-income small-scale fishers (Fabinyi et al. 2022).

#### GENETIC DIVERSITY - A GLOBAL PERSPECTIVE

Genetic diversity refers to the naturally occurring variations in genetic makeup among individuals of a species. Populations with high genetic diversity are more capable of adapting to adverse and unfavourable environmental changes (Xuereb et al. 2018), and prevent inbreeding (Plough 2016). As such, understanding and the conservation of genetic diversity are the cornerstones that are crucial for the evaluation of population health and aid in management efforts of a species in their natural habitats (Petrou et al. 2021).

Sea cucumbers are known to inhabit marine coastal zones such as in the coral reefs, lagoons, and seagrass meadows of the tropical waters (Floren et al. 2021), and these nearshore marine environments are characterized by huge variation in physical parameters (temperature, salinity, and other abiotic factors) (Xuereb et al. 2018). Thus, spatial adaptation to the environmental conditions of specific geographical habitats is vital for their survival. Understanding its importance, there are growing literature that strives to quantify the spatial genetic variations of *H. scabra*. As depicted in Figure 2, although population genetic studies of *H. scabra* are gaining attention, especially in countries within the natural habitat zones of *H. scabra*, the gap of knowledge is still huge – no data is available from the north and northwest of the Indian ocean, and Malaysia.

To date, four types of genetic markers, namely allozyme, partial mitochondrial gene sequences (12S rDNA gene and cytochrome oxidase subunit I gene), microsatellites, and single nucleotide polymorphisms (SNPs) have been used to investigate and characterise

the population genetic information of *H. scabra* (Table 1). Table 2 provides a brief introduction and comparison on the advantages and disadvantages of each method described in Table 1. In general, with the advancement of the next generation sequencing technologies, scientific communities have transitioned from using allozymes to partial mitochondrial genes sequences and microsatellites, and subsequently SNPs to understand the genetic diversity and population structure of *H. scabra*, both in wild and in captivity. Additionally, the choice of methods (the use of allozyme, partial mitochondrial gene sequences, microsatellites, or SNPs) is also dependent on factors such as the availability of resources and budget, intended sequencing depth, and required information that are to be mined (Table 2).

Genetic studies conducted on *H. scabra* (Table 1) highlights that haplotype diversity is, in general low, among populations within the same geographical region. This is understandable as *H. scabra* moves at a rate of approximately 40 cm per h and is active about 10 h per day. They also exhibit home-ranging behaviour, thus are often found within a limited geographical range (Lee et al. 2018b). Additionally, daily movement patterns of *H. scabra* are also affected by their body sizes, with larger sea cucumbers tend to travel greater distances (Mercier, Battaglani & Hamel 2000). The range of movement varies between species of the same genus, for example, *H. sanctori* from Spain travelled an average of  $11.12 \pm 4.24$  m per day with no clear ‘homing’ behaviour (Navarro et al. 2013).

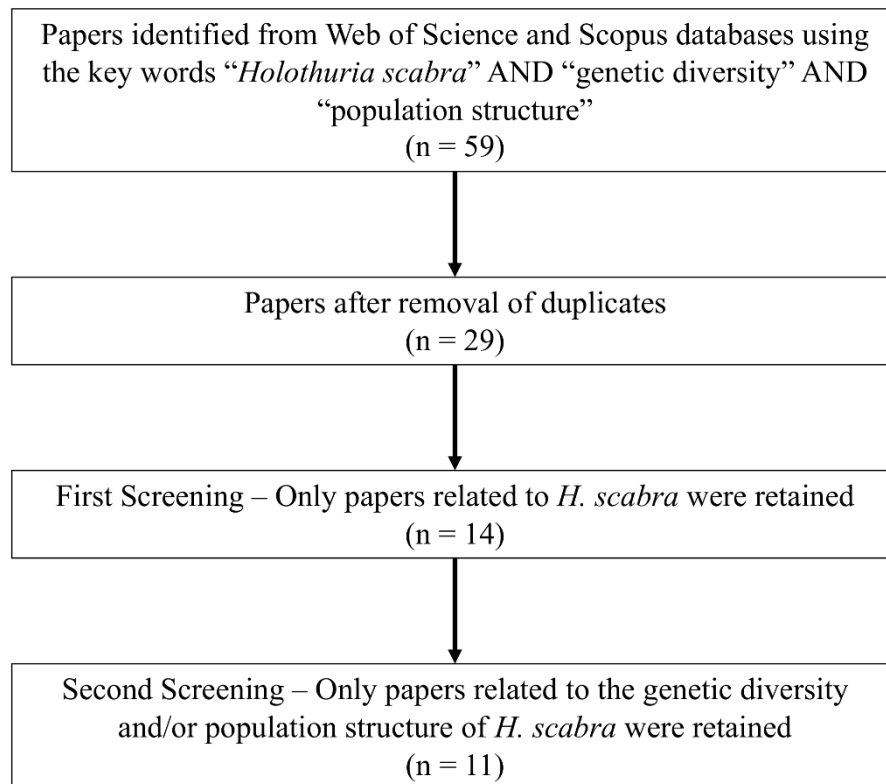


FIGURE 1. The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow chart used to retrieve and filter papers related to the current review



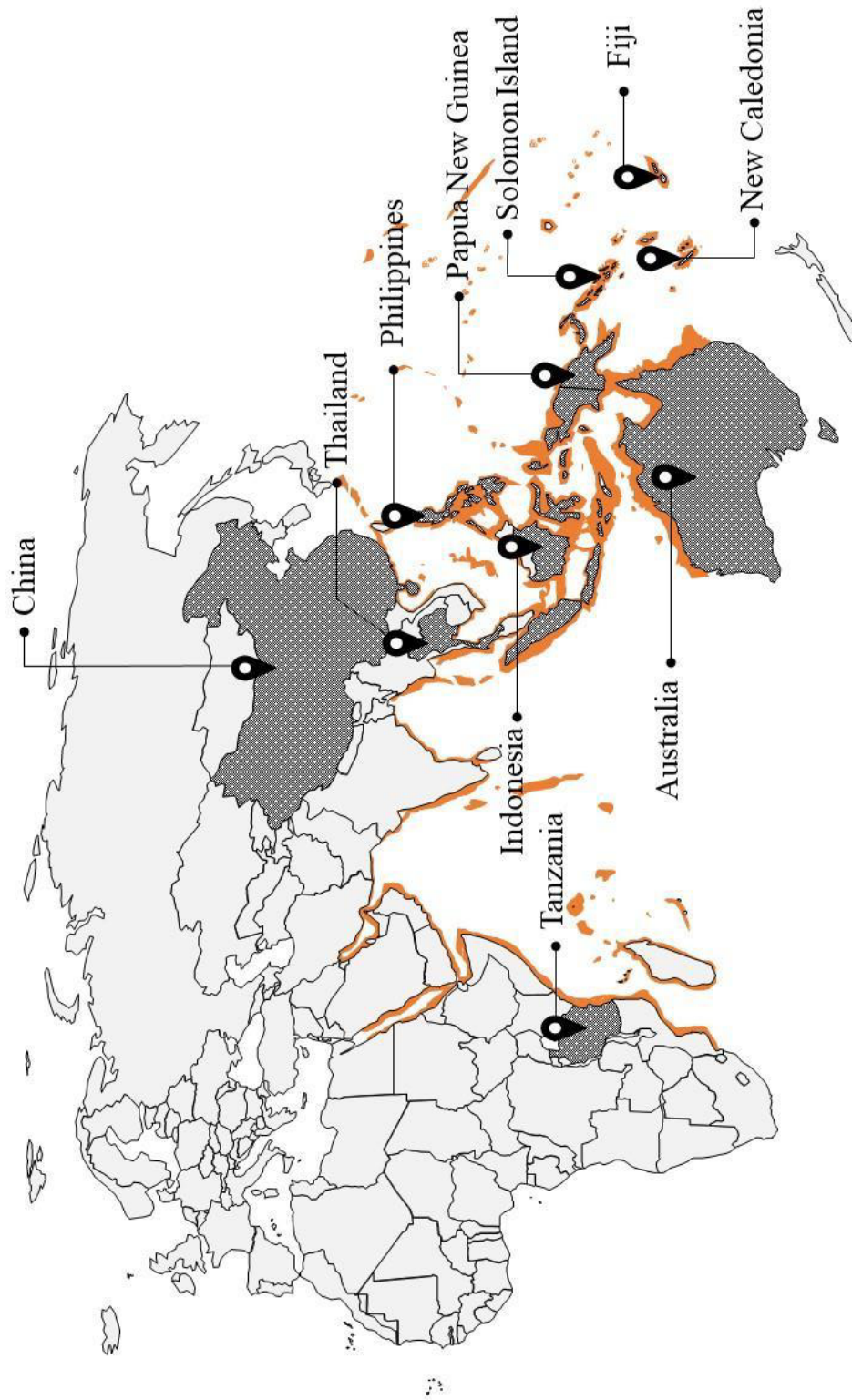


FIGURE 2. The population of *Holothuria scabra* with genetic information studies. Orange zonation indicates the global distribution of sea cucumber *H. scabra*

TABLE 1. Population genetic studies conducted on *H. scabra*

Country	Individuals sampled	Genetic markers used	Description	Highlights	Reference
China	30 adults from Sanya, Hainan	Microsatellite	35 loci tested, 6 were significant	Observed heterozygosity ( $H_o$ ): 0.0333–0.5172; expected heterozygosity ( $H_e$ ): 0.0328–0.6901	Shangguan & Li (2018)
Papua New Guinea and Australia	413 individuals: Darwin (n = 32); Cape York (n = 38); Milne Bay (n = 19); Kimbe Bay (n = 29); Belifu (n = 30); Lیماناک South (n = 37); Lیماناک North (n = 39); Sivasat (n = 45); Bangatang (n = 15); Ungakum (n = 29); Manus Island Rambutyo (n = 50); Manus Island Loi (n = 50)	Microsatellite	15 loci tested, 10 were significant	$H_o$ : 0.58 ± 0.07; $H_e$ : 0.75 ± 0.05; Inbreeding coefficient ( $F_{IS}$ ): positive - 0.25 ± 0.07 (absence of heterozygotes); Genetic differentiation test ( $F_{ST}$ ): 0.037, $P < 0.001$ (low but significant genetic substructuring)	Nowland et al. (2017)
Fiji	211 adults: Vanua Levu (n = 25); Lau Province (n = 45); Kadavu Province (n = 22); Serua Province (n = 42); Viti Levu (n = 46); Ba Province (n = 31)	Single nucleotide polymorphisms (SNPs) – Diversity Arrays Technology (DArT PL) proprietary genotyping by sequencing (DArTseq™)	6,896 SNPs were mined	Three genetically distinct populations were identified; range of $H_o$ : 0.238 ± 0.002 – 0.265 ± 0.002; range of $H_e$ : 0.266 ± 0.05 – 0.284 ± 0.002; range of $F_{IS}$ : 0.047 – 0.163; Pairwise $F_{ST}$ were low but significant, except that of Viti Levu with Serua Province	Brown et al. (2022)
Tanzania	130 individuals: Bagamoyo (n = 30); Zanzibar (n = 40); Kilwa (n = 30); Lindi (n = 30)	Partial cytochrome oxidase subunit I gene (COI)	-	Low haplotype diversity, $h_d$ (0.17–44) and nucleotide diversity, $\pi$ (0.025–0.084%); $F_{ST}$ : 0.0047, $P > 0.05$ . 13 haplotypes and 5 polymorphic sites obtained	Bugota & Rumisha (2022)
Thailand	89 individuals: Phangnga (n = 23); Satun (n = 14); Trang (n = 25); Krabi (n = 27)	Partial 12S rDNA gene; microsatellite	9 microsatellite loci tested, 6 were significant	30 haplotypes based on 12S rDNA, $\pi$ between pairs = 0.729 (Phangnga-Trang) – 0.965% (Satun-Krabi), 0.000 (Trang-Satun) – 0.067% (Phangnga-Krabi); $F_{ST}$ : 0.085, $P < 0.05$ . Based on microsatellite loci, $H_o$ : 0.58 ± 0.07; $H_e$ : 0.75 ± 0.05; $F_{IS}$ : 0.071 – 0.170; $F_{ST}$ : 0.081, $P < 0.001$	Nimwichian & Klinbunga (2020)

Philippines	531 individuals: Cagayan (n = 50); Sorsogon (n = 33); Eastern Samar (n = 51); Zambales (n = 23); El nido, Palawan (n = 25); Coron, Palawan (n = 49); Romblon (n = 33); Concepcion, Iloilo (n = 40); Tigbauan, Iloilo (n = 15); Cebu (n = 32); Bohol (n = 29); Dumaguete (n = 38); Davao (n = 27); Sarangani (n = 44); Tawi-Tawi (n = 42)	Microsatellite	18 microsatellite loci tested, 11 were significant	$H_o$ : 0.576 – 0.742; $H_e$ : 0.737 – 0.791; $F_{ST}$ : 0.016, $P < 0.001$ ; among population variance ( $F_{CT}$ ): 1.51%.	Ravago-Gotanco & Kim (2019)
New Caledonia	400 individuals: Koumac (n = 100); Tinip (n = 100), Boyen (n = 100), Kone (n = 100). 1,226 hatchery-produced individuals	Microsatellite	16 microsatellite loci tested, 9 were significant	$H_e$ of wild populations: 0.536 – 0.581; $H_e$ of hatchery-produced populations: 0.453 – 0.569; $F_{IS}$ of wild populations: -0.001 – 0.185; $F_{IS}$ of hatchery-produced populations: -0.010 – 0.027	Riquet et al. (2022)
New Caledonia, Indonesia, and Australia	437 individuals: New Caledonia - Baie de Sainte-Marie (n = 93); Ilot Maître (n = 14); Ouano (n = 28); Ilots Kundogi (n = 31), Plateau de Béco (n = 30); Pointe Pindai (n = 28); Pouangué (n = 33); Ouaco (n = 28); Récif Thaavaam (n = 30); Bali, Indonesia (n = 87); Knocker Bay, Australia (n = 35)	Allozyme	7 polymorphic enzyme loci tested, 6 were reliably scored	$H_o$ : 0.235 – 0.294; $H_e$ : 0.221 – 0.312	Uthicke & Purcell (2004)
Australia and Solomon Islands	479 individuals: Australia - Urangan (n = 100), Tin Can Bay (n = 16), Trawl shots (n = 136), Upstart Bay (year 1998, n = 49; year 2000, n = 54), Warrior Reef (n = 41), Dungeness Reef (n = 30), Solomon Islands (A, n = 30; B, n = 23)	Allozyme	7 polymorphic enzyme loci tested, all were polymorphic except <i>MDH*</i>	$H_o$ : 0.209 – 0.385; $H_e$ : 0.237 – 0.393	Uthicke & Benzie (2001)

TABLE 2. Comparison of commonly used methods in the characterization of population genetic information in *H. scabra*

Method	Advantage	Disadvantage	Reference
Allozyme	<ul style="list-style-type: none"> <li>- Do not need prior information on a species               <ul style="list-style-type: none"> <li>- Low cost</li> </ul> </li> <li>- Show co-dominance inheritance patterns               <ul style="list-style-type: none"> <li>- Species-diagnostic characters</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Only detect coding genes</li> <li>- Low abundance and low polymorphism</li> </ul>	<p>Ayala &amp; Powell (1972); Barker et al. (1985); Appleyard &amp; Mather (2001); Allendorf (2017)</p>
Partial mitochondrial gene sequences	<ul style="list-style-type: none"> <li>- Maternal inheritance and lack of recombination, hence allow easier phylogenetic tree construction</li> <li>- Presence of conserved and less conserved regions, and higher mutation rate than nuclear DNA enable intra- and interspecific comparisons               <ul style="list-style-type: none"> <li>- Easy to use and easy to amplify</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Introgression</li> <li>- Potential co-amplification of nuclear mitochondrial pseudogenes</li> </ul>	<p>Gissi, Iannelli &amp; Pesole (2008); Song et al. (2008); Ladoukakis &amp; Zouros (2017); Makhov, Gorodilova &amp; Lukhtanov (2021)</p>
Microsatellite loci	<ul style="list-style-type: none"> <li>- Higher genetic diversity than allozyme loci</li> <li>- Able to inform genetic structure of populations and species gene flow with minimal allozyme variation               <ul style="list-style-type: none"> <li>- Very abundant and highly sensitive</li> <li>- Highly polymorphic</li> </ul> </li> <li>- Fast and low cost using next-generation sequencing</li> <li>- Demonstrate interspecific hybridisation and interspecific gene flow</li> </ul>	<ul style="list-style-type: none"> <li>- Need specific primer sequences</li> <li>- High development costs if species-specific primers are not available</li> <li>- Difficult to estimate population size (depends on number of loci, number of samples, and methods of inferences are affected by confounding factors including migration, asymmetrical migration, spatial structuring, and reproductive variance)</li> </ul>	<p>Sun et al. (1998); Degen, Streiff &amp; Ziegenhagen (1999); Butcher, Glaubitz &amp; Moran (1999); Putman &amp; Carbone, (2014); Jaskulak et al. (2022)</p>
Single nucleotide polymorphisms (SNPs)	<ul style="list-style-type: none"> <li>- Abundance and wide genomic coverage</li> <li>- Fast and high yield of genotyping               <ul style="list-style-type: none"> <li>- Low error rates</li> </ul> </li> <li>- Do not require reference genomes</li> <li>- Greater resolution in describing population structure, genetic diversity, migrant detection</li> <li>- Detect loci that are involved in selection and fitness</li> </ul>	<ul style="list-style-type: none"> <li>- Expensive</li> <li>- Require large amplicon sizes</li> <li>- High mutation rates</li> </ul>	<p>Akey et al. (2002); Hall &amp; Beissinger (2014); Allendorf (2017); Yousefi et al. (2018); Diaz et al. (2021)</p>



In general, using allozyme analysis, the genetic variability of *H. scabra*, characterized by the expected heterozygosity ( $H_e$ ), was high among most studied sites at a short geographical distance, i.e., 60-130 km distance between sites along the coast of New Caledonia (Uthicke & Purcell 2004). Additionally, the genetic variability of *H. scabra* populations at tropical zones, such as Bali, Indonesia and Knocker Bay, Australia was higher, potentially indicating the original habitat of *H. scabra* (Uthicke & Purcell 2004).

The use of partial mitochondrial or nuclear genes were able to delineate individuals of *H. scabra* sampled across several geographical points within Tanzania and Thailand, into 13 and 30 haplotypes, respectively (Table 1; Bugota & Rumisha 2022; Ninwichian & Klinbunga 2020). Additionally, although all four sampling sites in Thailand (Ninwichian & Klinbunga 2020) and Tanzania (Bugota & Rumisha 2022) were separated almost at equal distance (within 3-4° latitude), the *H. scabra* populations in Tanzania showed no population differentiation among sites (based on the non-significant  $F_{ST}$ ) whereas the *H. scabra* populations for overall samples and between Phangnga and each other sampling locations in Thailand were genetically distinct. Thus, within a relatively small geographical zone, the genetic diversity of aquatic organisms, especially of a benthic dweller species such as *H. scabra*, is highly dependent on the exposed environmental and physical conditions, such as oceanographic circulation (Martinez, Willoughby & Christie 2018; Ravago-Gotanco & Kim 2019). Additionally, owing to the female inheritance characteristic, the use of mitochondrial DNA also allows the characterisation of the dominant reproduction mode employed within the specific *H. scabra* population, i.e., Ninwichian and Klinbunga (2020) suggested that *H. scabra* from Phangnga predominantly used sexual reproduction based on the large proportion of variance among individuals of the specific geographical region.

In addition to mitochondrial or nuclear gene sequences, microsatellite markers (essentially co-dominant nuclear markers) are also commonly used in population genetic studies due to its high specificity, generally transferable among related species, and relatively lower cost compared to SNPs (Table 2) (Wang et al. 2021). However, as mentioned in Table 2, microsatellite loci require specific primer sequences, and it might incur high primer development costs if species-specific primers are unavailable. Thus, six significant microsatellite loci were obtained from the tested 35 loci of

microsatellite markers of *H. scabra* by Shangguang and Li (2018). The other researchers reported 9 (Riquet et al. 2022), 10 (Nowland et al. 2017), and 11 (Ravago-Gotanco & Kim 2019) significant microsatellite loci, respectively. It is advisable that future microsatellite genotyping of *H. scabra* utilizes more than 12 microsatellite markers as less than 11 or 12 markers might result in over- or under estimation of the population genetic diversity or genetic structure (Wang et al. 2021). With the aid of microsatellite markers, regional genetic structures of *H. scabra* across the Philippine archipelago (Ravago-Gotanco & Kim 2019), Papua New Guinea and northern Australia (Nowland et al. 2017), and New Caledonia (Riquet et al. 2022) were made known (Table 1), thus, providing valuable information that can be used to inform sustainable fishery management and restocking programs of *H. scabra*.

Compared to the previous three methods, SNP genotyping is fast, has wide genome coverage, does not require reference genomes or species-specific primers, exhibit superior resolution over the other methods in describing population genetic information, and could detect selection- and fitness-related loci (Table 2). However, the major drawback of using SNPs in population genetic studies of *H. scabra* is that the sequencing is relatively expensive. Genome-wide SNPs sequencing and analysis of *H. scabra* populations from Fiji showed three shallow but genetically distinct populations, with putative local adaptation signatures observed in individuals from Lakeba Island (Brown et al. 2022). By combining the SNP data with an independent oceanographic particle dispersal model, Brown et al. (2022) further suggest the apparent compliance of Fijian *H. scabra*'s genetic structure to the isolation-by-distance model, and population fragmentation is exhibiting an eastward pattern. It is suggested that dedicated fishery management strategy, such as periodic harvest management or total harvest ban, establishment of refugia or sanctuary, prohibition of translocation, and regular monitoring efforts be conducted at geographical regions with putative local adaptation signature and distinct genetic structures. Future genome-wide SNP profiling of *H. scabra* populations from other geographically distinct locations, such as the Indian Ocean or South China Sea, will allow the characterisation of gene flow among populations across vast geographical distances, the identification of similarities and differences in genetic structure among populations, and provide robust support for the development of genetic conservation and sea

ranching programs of *H. scabra*. This is exemplified in the case of the black sea cucumber *Holothuria leucospilota*, where SNPs were subjected to restricted site associated DNA sequencing (RAD-seq) to show the genetic structure, gene flow and genetic divergence of *H. leucospilota* between Australia and Vietnam (Chieu et al. 2023).

#### APPLICATIONS OF GENETIC DIVERSITY FISHERIES

The ever-increasing fishing pressure on economically important marine species not only results in declining stock abundance and average fish size, but also reduces genetic structure and diversity, subsequently causing significant reduction in various physiological parameters, including fecundity and maturation (Pérez-Ruzafa et al. 2006). The continuous overharvesting of sea cucumbers resulted in a minimal number of viable individuals, thereby reducing the overall genetic diversity of the remaining population. The impact of fisheries is already evident in two sea cucumber species from the Mediterranean Sea, *Holothuria polii* and *Holothuria tubulosa*, whereby individuals at non-fishery area showed higher body size, weight, and genetic diversity (González-Wangüemert, Valente & Aydin 2015). Similarly, overharvesting and other coastal development resulted in significant decrease in the genetic diversity of *Holothuria atra* in commercially fished areas compared to marine protected areas (Hamamoto et al. 2021). These studies also indirectly highlight the importance of population genetic structure and diversity data of *H. scabra* as a baseline for future fishery management, monitoring, and the development of resource conservation programs.

#### AQUACULTURE HATCHERY PRODUCTION

In addition to stocking, the success and advancement of aquaculture and production of *H. scabra* is highly dependent on the availability of information regarding their initial population genetic structure. Knowledge on the genetic structure of individuals before being obtained and used in the hatchery as broodstock is essential to ensure high genetic diversity (Kang et al. 2011). It is a common concern that there is a lack of information on the genetic status of many aquaculture species, making resource management and genetic improvement difficult (Sonesson et al. 2023). Selective breeding to promote and select for better traits, especially with the inclusion of molecular genomic information, greatly increases the

prediction and accuracy of genetic gains (Houston et al. 2020). Specifically, knowledge on allelic diversity, inbreeding, population genetics and structure, allow for the selection of genetically distant and diverse broodstock with specific trait(s) of interest to be incorporated into an aquaculture program. For instance, although high gene flow was detected in wild *H. scabra* populations of the northwestern coast of New Caledonia (potentially exhibiting sufficient effective size of breeders to be incorporated into breeding and restocking programs), the opposite was observed in hatchery-produced *H. scabra*, with a small effective population size and detectable genetic drift and inbred individuals across only one generation (Riquet et al. 2022). The loss of genetic diversity among hatchery-reared *H. scabra* observed by Riquet et al. (2022) can be overcome by selecting wild broodstock with known population genetic information during the broodstock selection process.

Other important reasons for the understanding of population genetic structure and diversity of *H. scabra* in both wild and cultured populations is to allow for the evaluating the effect of introducing hatchery-reared individuals during stocking, and also to provide sufficient baseline data for risk assessment and management of introduced individuals, especially farm-type individuals (Sonesson et al. 2023). The intentional or unintentional release of farm-type *H. scabra*, either through restocking/sea ranching programs, or accidental escape during juvenile mariculture, could pose ecological threats to the local *H. scabra* populations. This phenomenon is already evident in other aquaculture species. For example, the introgression of genes from farmed escapees, resulted in increased growth and reduced age at sexual maturity across 105 wild Atlantic salmon populations (Bolstad et al. 2021). The population genetic information of *H. scabra* in the waters of Fiji were determined using Diversity Arrays Technology (DArT PL) proprietary genotyping by sequencing (DArTseq™) to provide essential baseline for hatchery-based restocking endeavors and three genetically distinct populations were identified (Brown et al. 2022).

#### RESTOCKING AND SEA RANCHING

Sea ranching is the introduction of hatchery-reared sea cucumber juveniles into the natural habitat, before harvesting them upon reaching marketable size; restocking focuses on building nucleus breeding population in the natural environment via the release of hatchery-reared sea cucumber juveniles (Purcell

2012). Both stocking interventions, be it sea ranching or restocking, require careful planning and considerations to ensure responsible and sustainable management efforts. In addition to ensuring hatchery-reared juveniles are free from diseases prior to stocking to avoid unnecessary disease transfer to the wild, validating the closeness of the genetic make-up within the wide genetic pool between the hatchery-reared sea cucumber juveniles and the wild stocks where the juveniles will be released is essential to preserve the original genetic diversity within the stocking habitat/geographical location (Bell et al. 2008). By doing so, the preserved genetic identity of the local stock will ensure a better survival and coping success against any stressful events (exposure to contaminants and climate change) (Mohsen et al. 2024), whereas introduction of individuals from a genetically different population will introgress the genetic identity of the local stock, and the interbred population might exhibit decrease fitness, lead to genetic erosion and loss of genetic diversity, ultimately resulting in the loss of the adapted tolerant ability to cope for stress (Klüttsch et al. 2019; Puigcerver et al. 2014).

Sea ranching and restocking of *H. scabra* is feasible and is an ongoing program in some countries (Alejandro 2019; Han, Keesing & Liu 2016; Purcell & Blockmans 2009). However, information on the use of molecular data during restocking or sea ranching is scarce. Thus far, the available molecular data on population genetics of *H. scabra* compiled in Table 1 provides a glimpse of the genetic signature of specific *H. scabra* populations. Yet, for any restocking program to be successful, the program should be designed to reduce the risk of the loss of genetic diversity and decrease the possibility of inbreeding. To do so, genetic diversity profiles of the wild cohort and broodstock, hatchery-reared individuals, and subsequent progeny should be examined (Roques et al. 2018). Genetic evaluation between wild and hatchery populations of red sea cucumber *Stichopus japonicus* via microsatellite markers indicates that high inbreeding effect, reduction in unique alleles, and loss of genetic integrity was observed in the hatchery-produced individuals (An et al. 2013). Similar reduction in genetic diversity among hatchery-produced individuals compared to the wild populations was also observed in *H. scabra* based on 6,896 selectively-neutral and 186 putatively adaptive genome-wide SNPs (Brown et al. 2022). Stocking of individuals in a high number but from relatively few parents is known to lead to bottleneck events and reduces the breeding population size and decrease in genetic diversity, in addition to

contributing towards genetic differentiation and drift effects of wild and hatchery-reared individuals (Klüttsch et al. 2019). It is hopeful that extra attention will be put forth in characterizing the population genetic structure and diversity of hatchery-reared *H. scabra* prior to the development of any restocking or sea ranching programs to ensure the genetic population health and consequently its survival within the selected geographical location.

#### TRANSLOCATION RECOMMENDATION

Fisheries and management of sea cucumbers, particularly *H. scabra* vary among countries. For example, some countries such as Mozambique has no management plan, some such as Zanzibar prohibit sea cucumber fisheries and promote sea cucumber farming (for full review of the fisheries and management of sea cucumbers in the Indian Ocean, see Conand et al. 2022), while some such as Fiji bans the export of all sea cucumber species (Brown et al. 2022). Regardless of the presence or absence of management plan for *H. scabra*, local communities are aware of the intensified sea cucumber harvesting and the decline in wild populations (Hair et al. 2020). Owing to its high market value, it is anticipated that governmental authorities and other relevant stakeholders will soon endeavor to implement comprehensive fishery management and conservation strategies for *H. scabra* based on their population genetic profiles to safeguard the remaining natural resources. Countries such as Fiji (Brown et al. 2022), New Caledonia (Riquet et al. 2022), Papua New Guinea and northern Australia (Nowland et al. 2017), the Philippines (Ravago-Gotanco & Kim 2019), Tanzania (Bugota & Rumisha 2022), and Thailand (Ninwichian & Klinbunga 2020) are already equipped with the baseline population genetic profiles of their native *H. scabra* populations that are useful to inform sustainable fishery management, facilitate translocation, and guide the development of marine policy related to the translocation and conservation of *H. scabra*.

The restoration and recovery of depleted or almost-depleted populations rely heavily on the restocking process whereas the primary method of sea cucumber mariculture is sea ranching. Both restocking and sea ranching involve the collection of broodstock, the production of juveniles in the hatchery, and translocation of *H. scabra* into the designated area. Therefore, it is imperative that the translocation process be conducted carefully to avoid unwanted detrimental impacts towards the genetic diversity and population structure of the local *H. scabra* populations.

With the current understanding of the genetic diversity and population structure of *H. scabra*, it is extremely important for any hatchery-based translocation strategy to examine the genetic diversity of the broodstock to ensure adequate genetic diversity will be present among offspring. In addition, hatchery-reared individuals should only be restocked into the original broodstock population to avoid potential deletion effect due to the introduction of individuals with reduced genetic diversity into the natural population, or potential translocation of individuals into a location beyond their natural dispersal limits (Brown et al. 2022). Continuous restocking of the natural population with hatchery produced cohorts of similar genetic diversity profiles is known to reduce heterozygosity, inflate inbreeding coefficient, and reduce effective population size in the wild population that is being translocated (Brown et al. 2022; Ravago-Gotanco & Kim 2019).

It is also important to note that hatchery practices can also lead to the loss of genetic diversity in *H. scabra* (Brown et al. 2024). When cultured in the hatchery, the surviving families to juveniles were reportedly low (13-16), indicative of low effective population sizes among offspring. Therefore, detailed pedigree tracking and controlled batch spawning should be conducted to ensure genetic variability among offspring (Brown et al. 2024).

Ensuring adequate genetic diversity within a population through translocation will not only benefit the wild population in the long run, but also fishers that rely on the harvest of *H. scabra*, as populations with high genetic diversity are more resilient towards fluctuation or changes in environmental factors and disease resistance.

#### CONCLUSION

Understanding of the population genetic makeup of *H. scabra* is instrumental to the sustainable harvest, resource management, conservation, and aquaculture of this important natural resource. The systematic review analysis conducted in this review retrieved a final dataset of 11 papers and highlighted the urgency of *H. scabra* population genetic research in many of its naturally occurring habitats. Nonetheless, some countries have already started to incorporate genetic information as a preliminary criterion for broodstock selection for restocking or sea ranching purposes. Among the main problems identified in hatchery-reared individuals, especially when they are cultivated without considering their population genetic background, lead to the production of hatchery population with low genetic

diversity and has detrimental effects of genetic drift and inbreeding. The collective data made available in this review provide a baseline for future restocking or sea ranching programs of *H. scabra*.

To date, the whole genome (Luo et al. 2022) and full mitogenome sequences (Utzeri et al. 2020) of *H. scabra* are available, thus enabling more precise and defined inference of the population structure, genomic diversity, and intraspecific demographic dynamics (Iannucci et al. 2021), consequently aiding in the sustainable management of *H. scabra*.

Conservation and management of *H. scabra* can be further enhanced via the incorporation of environmental association analysis, thereby uncovering the spatial scale of local adaptation and the potential environmental factors that might contribute to the adaptive diversity of *H. scabra* at specific geographical location. By linking environmental factors with candidate markers obtained from RAD sequencing, Xuereb et al. (2018) successfully identified specific candidate SNPs that were significantly associated with environmental factors, allowing better understanding of the putative adaptive variation in the giant California sea cucumber *Parastichopus californicus*.

Considering the imminent threat of climate change, it is also essential to evaluate the changes in distribution, and thus the impact on genetic structure of *H. scabra* as environmental factors such as temperature and pH changes due to ocean warming and ocean acidification. The decrease in pH has already been linked with changes in larval dispersal phase of the royal sea cucumber *Parastichopus regalis* in the central Mediterranean Sea (Scannella et al. 2022). The impact of climate change on the population dynamics and genetic structure of *H. scabra* will be made clear with the availability of sufficient baseline genetic data for comparison purposes, of which we still lack.

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