Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Genetic Differentiation of Five Sea Cucumber Species from the Red Sea, Hurghada, Egypt

Diferenciação genética de cinco espécies de pepino-do-mar do Mar Vermelho, Hurghada, Egito

F. M. Abdelghany[a], A. W. A. Abdel-Warith[b], E. M. Younis[b], S. J. Davies[c], M. A. Elnakeeb[a*], M. M. El-Nawasany[a], A. A. El-Bahlol[a], M. A. Bauomi[a], A. H. Abu-Almaaty[d] and M. Y. Omer[e]

[a]Al-Azhar University, Faculty of Agriculture, Fish Production Department, Nasr City, Cairo, Egypt
[b]King Saud University, College of Science, Department of Zoology, Riyadh, Saudi Arabia
[c]National University of Ireland Galway, School of Science and Engineering, Galway, Ireland
[d]Port Said University, Faculty of Science, Zoology Department, Port Said, Egypt
[e]National Institute of Oceanography and Fisheries, Hurghada, Egypt

Abstract

This study aimed to assess the genetic differentiation and relationship among five sea cucumber species from the Red Sea in Egypt, namely *Holothuria atra*, *H. impatiens*, *H. leucospilota*, *Actinopyga crassa* and *A. mauritiana*, using Inter Simple Sequence Repeated (ISSR) and Start Codon Targeted (SCoT) markers. A collection of 100 specimens, with 20 individuals per species, was gathered for the analysis. With ten ISSR primers, 135 amplified bands were detected, including 11 distinct species-specific bands, indicating high-level polymorphism among species. Using ten SCoT primers, 151 amplicons were generated, including 30 species-specific bands, with 52% polymorphic bands indicating high-level polymorphism among species. The degree of genetic similarity (GS) among the different genotypes of species was calculated based on ISSR bands analysis, which ranged from 93% between *H. atra* and *H. impatiens* to 86% between *H. atra* and *A. crassa*. The highest genetic similarity was observed between *H. atra* and *H. impatiens* (90%), while the lowest was identified between *A. crassa* and *A. mauritiana* (75%) using SCoT bands. Notably, the ISSR and SCoT-based DNA analysis revealed similar genetic relationships between *H. atra* and *H. impatiens* compared to other sea cucumber species studied. This study provides new insights into the genetic diversity and relationship among sea cucumber species in the Red Sea, which could have implications for their conservation and management.

Keywords: genetic differentiation, relationship, sea cucumber species, ISSR, SCoT.

Resumo

Este estudo teve como objetivo avaliar a diferenciação genética e a relação entre cinco espécies de pepinos-do-mar do Mar Vermelho no Egito, quais sejam, *Holothuria atra*, *Holothuria impatiens*, *Holothuria leucospilota*, *Actinopyga crassa* e *Actinopyga mauritiana*, usando marcadores Inter Simple Sequence Repeated (ISSR) e Start Codon Targeted (SCoT). Uma coleção de 100 espécimes, com 20 indivíduos por espécie, foi reunida para análise. Com 10 primers ISSR, 135 bandas amplificadas foram detectadas, incluindo 11 bandas específicas de espécies distintas, indicando polimorfismo de alto nível entre as espécies. Usando 10 primers SCoT, 151 amplicons foram gerados, incluindo 30 bandas específicas da espécie, com 52% de bandas polimórficas indicando polimorfismo de alto nível entre as espécies. O grau de similaridade genética (GS) entre os diferentes genótipos das espécies foi calculado com base na análise das bandas ISSR, que variou de 93% entre *H. atra* e *H. impatiens* a 86% entre *H. atra* e *A. crassa*. A maior similaridade genética foi observada entre *H. atra* e *H. impatiens* (90%), enquanto a menor foi identificada entre *A. crassa* e *A. mauritiana* (75%) usando bandas SCoT. Notavelmente, a análise de DNA baseada em ISSR e SCoT revelou relações genéticas semelhantes entre *H. atra* e *H. impatiens* em comparação com outras espécies de pepino-do-mar estudadas. Este estudo fornece novas informações sobre a diversidade genética e a relação entre as espécies de pepino-do-mar no Mar Vermelho, o que pode ter implicações para sua conservação e manejo.

Palavras-chave: diferenciação genética, relação, espécies de pepino-do-mar, ISSR, SCoT.

*e-mail: Mahmoud.biotech@azhar.edu.eg; Mahmoud.biotech@gmail.com
Received: March 2, 2023 – Accepted: April 16, 2023

1. Introduction

Many marine invertebrates, particularly those that are sessile or move slowly, are rich sources of bioactive metabolites (Jiménez, 2018; Luthfi and Asadi, 2021). In Asia and the Middle East, people traditionally use marine invertebrates, such as sea cucumbers in holothurians, as food and folk medicine (Bordbar et al., 2011). The phylum Echinodermata is a group of marine animals that includes five extant groups, one of which is Holothurians, or sea cucumbers (Conand, 2006). Commercially important sea cucumbers have a global distribution, as reported by Purcell et al. (2012), and their conservation has been discussed in international meetings, such as Workshop organized by FAO entitled "Advances Aquaculture and Management Techniques of in Sea Cucumber", documented by Lovatelli et al. (2004).

Sea cucumbers possess a calcareous endoskeleton comprised of ossicles, and they have a large coelom containing an ambulacral and hydraulic mechanism for movement and feeding, which enables the integration of various physiological functions including movement, breathing, and sensory perception. The predominant form of symmetry in sea cucumbers is pentaradial, although some species exhibit secondary bilateral symmetry. The digestive system is fully functional, the neural network is decentralized, and the generative system is straightforward (Elnakeeb et al., 2020). These creatures have an elongated, pliable tubular physique that extends from the oral cavity to the anal opening, resulting in similar morphology. They inhabit the seabed and are primarily found in the three-ambulacral areas (i.e., Ambulacral basic element of the Carpenter system). To feed, they use oral appendages, and the morphology of these tentacles varies depending on the taxonomic order within the taxon group (Hyman, 1955; Booloottian, 1966).

Sea cucumbers are distributed widely throughout the ocean, inhabiting diverse environments ranging from the intertidal zone to the deep sea, and spanning from polar to tropical regions. Within the class Holothurian, there exist a plethora of species numbering around 1500, with the discovery of new ones being a continual process. The interest in this group has grown substantially over the years, with numerous studies dedicated to their taxonomic classification and ecological significance (Massin et al., 1999; Samyn and Berghe, 2000; Uthicke et al., 2004). The class Holothuroidea is comprised of six orders, namely Apodida, Aspidochirotida, Dactylochirotida, Dendrochirotida, Elasipodida, and Molpadida. These orders can be distinguished from each other based on various features such as the Existence or non-existence of ambulacral system. The morphology of the oral tentacles and the existence or non-existence of breathing organs, flexor muscles, and internal canals. A comprehensive illustration of the anatomy of this class can be found in Conand (1990).

The quantity and distribution of genetic diversity play a vital role in the evolution of living beings, making it a crucial antecedent to any biodiversity study (Futuyma, 1986; Fernandes et al., 2022). DNA profiling techniques such as Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR), along with other commonly used markers, have demonstrated their effectiveness in examining molecular differentiation (Costa et al., 2016). Consequently, for anonymous regions, universal primers are used for analysis (Robarts and Wolfe, 2014).

In contrast, inter-simple sequence repeat markers possess several benefits compared to different marker methods. They are uncomplicated, fast, and cheap, similar to the RAPD technique, and they have higher elongated size enables better replicability compared to primers of the RAPD technique, rendering them more reliable (Fang and Roose, 1997; Moreno et al., 1998). A potential use of ISSR patterns is to serve as prevailing methods to evaluate Species' molecular differentiation (Hartati et al., 2017; Igwe et al., 2022). Recently, there has been the development of novel and efficient marker techniques for instance the SCoT technique, a marker technique that targets the ATG (translation start codon), which is known for its replicability (Collard and Mackill, 2009). The application of SCoT markers has been effective in analyzing molecular differentiation and arrangement, identifying breeds, and mapping QTLs and DNA fingerprinting in various sea cucumber species (Tian et al., 2015; Cui et al., 2021). As a constructing marker-assisted breeding program, SCoTs have a distinct advantage over RAPDs and ISSRs. SCoT has some advantages, such as being fast and simple to implement (Mulpuri et al., 2013).

The aim of this study was to establish the phylogenetic relationships among five holothuroids species from the Red Sea in Hurghada, Egypt, using molecular genetic methods. This was the first study to use this approach in this region.

2. Materials and Methods

2.1. Sampling

In total, 100 sea cucumbers specimens were gathered, with an equal number of 20 individuals per each of the five distinct species: *Holothuria atra, H. impatiens, H. leucospilota, Actinopyga crassa* and *A. mauritiana* from Hurghada, Red Sea, Egypt. The collection site is located at (27° 17' 6.39" N, 33° 46' 28.93" E) in the front of the National Institute of Oceanography and Fisheries (NIOF). The presence of long patchy reefs characterizes this site, representing the front edge of a vast and shallow reef flat with many depressions and lagoons. It has many sea cucumber species and five sea cucumber species were collected: *Actinopyga crassa*, *Actinopyga mauritiana*, *Holothuria atra*, *Holothuria leucospilota*, *Holothuria impatiens*. Sea cucumber collecting was carried out by SCUBA-diving up to depths of 5 m. The specimens were transferred alive to the hydrobiology laboratory at (NIOF), All sea cucumber species were identified according to various technical papers and several taxonomic guides (Massin et al., 1999; Samyn, 2003; Uthicke and Purcell, 2004; Hartati et al., 2017; Cui et al., 2021). All sea cucumber individuals were measured and weighed, then muscle tissues were dissected and frozen quickly at -20°C for the following genetic analysis.

2.2. Molecular analysis

2.2.1. DNA extraction and purification

DNA from muscle tissues of five sea cucumber species was extracted according to DNeasy Kit (Qiagen) (Cat No. 69504) following the manufacturer's protocol.

2.2.2. ISSR-PCR reaction

The ISSR-PCR procedure was used to detect polymorphic bands in a group of 10 primers as shown in Table 1. In a 25 µL reaction volume, the amplification was performed using 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase, and 30 ng of the DNA template.

2.2.3. SCoT-PCR reaction

Ten primers were used SCoT-PCR to detect polymorphism as shown in Table 2. To perform the amplification, a reaction volume of 25 µL was used, which consisted of 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase, and 30 ng of the template DNA.

The amplification process was conducted using a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) that was programmed to execute 35 cycles following an initial denaturation cycle of 5 minutes at 94ºC. The amplified products were separated through electrophoresis on a 1.5% agarose gel that was prepared with 1x TBE buffer and contained ethidium bromide (0.5 µg/mL) under 95 volts.

2.3. Statistical analysis

The size range in base pairs (bp) of each band produced by ISSR and SCoT approaches was determined using the TotalLab Quant software to evaluate the genetic similarity among the studied samples. Sharp and well-defined amplified DNA fragments were given a score of 1 for band presence and 0 for band absence, while bands migrating at the same distance were deemed identical. The measure of genetic relatedness coefficient (GS) of dual genetic profiles was estimated Implementing the Dice correlation coefficient (Sneath and Sokal, 1973). This data was imported into the Multi-Variate Statistical Package (MVSP) version 19 software to create a similarity matrix and dendrogram using the unweighted pair group method with arithmetic average (UPGMA) and Jaccard's coefficient to illustrate the relationships between the studied species (Kovach, 1998). The heatmap was created using the R software package to visualize the similarity between the five sea cucumber species (*H. atra, H. impatiens, H. leucospilota, A. crassa and A. mauritiana*).

3. Results

3.1. Polymorphic analysis

The five sea cucumber species' genetic similarity was identified using the ISSR and SCoT-PCR molecular techniques.

The ten ISSR primers produced 135 DNA bands for all studied sea cucumber species, as shown in Table 3 and Figures 1-3. Out of these bands, 67% (90) were monomorphic, 25% (34) were polymorphic, and 8% (11) were unique. The extent of variability in the DNA markers used in this study was evaluated based on the percentage of unique and polymorphic bands. Results showed that ISSR1 exhibited the highest variability with 75%, while ISSR19 had the lowest at 7%. As shown in Table 3, the number of fragments produced per marker ranged from 8 (ISSR-2) to 18 (ISSR-10). In terms of size, the fragments amplified by the markers varied from 150 bp in the case of ISSR1 and ISSR6 primers to 1600 bp for ISSR13.

Ten markers were amplified using SCoT to a total of 151 amplicons (Table 4 and Figures 4-6) for all five sea cucumber species, with a range of 9 bands (SCoT-9) to 19 bands (SCoT-2), of which 73 were monomorphic bands (48%), 30 (20%) were unique and 48 (32%) were polymorphic. The percentage of polymorphism (which includes unique and polymorphic amplification products) ranged from 33% for SCoT-9 to 69% for SCoT-10. The size of the amplified bands varied from 100 bp in the case of SCoT-6 primer to 2000 bp in SCoT-2 (as listed in Table 4).

Table 1. Name and sequence primers ISSRs.

Name Primer	Sequence 5'-3'
ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR- 4	5'-ACACACACACACACACYG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'
ISSR-11	5'-ACACACACACACACACYA-3'
ISSR-13	5'-AGAGAGAGAGAGAGAGYT-3'
ISSR-19	5'-HVHTCCTCCTCCTCCTCC-3'

A: Adenine; T: Thymine; G: Guanine; C: Cytosine; V: (A or C or G); H: (A or C or T).

Table 2. Name and sequence primers SCoT.

Name Primer	Sequence 5'-3'
SCoT-1	ACGACATGGCGACCACGC
SCoT-2	ACCATGGCTACCACCGGC
SCoT-3	ACGACATGGCGACCCACA
SCoT-4	ACCATGGCTACCACCGCA
SCoT-5	CAATGGCTACCACTAGCG
SCoT-6	CAATGGCTACCACTACAG
SCoT-7	ACAATGGCTACCACTGAC
SCoT-8	ACAATGGCTACCACTGCC
SCoT-9	ACAATGGCTACCACCAGC
SCoT-10	ACAATGGCTACCACTACC

Table 3. Number of amplified DNA-fragments with ISSR in the five sea cucumber specimens (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa,* and 5- *A. mauritiana*).

Primer code	No. of amplified bands					Total amplified bands	Mean of band frequency	Size range (bp)	No. of monomorphic bands	No. Unique bands	No. of poly morphic bands	Poly morphism %
	(1)	(2)	(3)	(4)	(5)							
ISSR-1	9	7	4	8	9	12	0.6	150-1550	3	3	6	75
ISSR-2	4	6	4	7	7	8	0.7	1380-310	4	2	2	50
ISSR-3	15	16	12	13	14	16	0.9	1300-230	10	0	6	38
ISSR-4	11	11	10	8	9	11	0.9	1350-240	8	0	3	27
ISSR-5	11	11	11	7	11	14	0.7	170-1329	6	2	6	57
ISSR-6	10	10	10	7	7	11	0.8	150-770	7	1	3	36
ISSR-10	18	17	17	17	17	18	1.0	180-1270	17	1	0	6
ISSR-11	10	10	11	12	11	15	0.7	280-1400	8	2	5	47
ISSR-13	14	16	15	14	16	16	0.9	200-1600	14	0	2	13
ISSR-19	13	14	13	14	14	14	1.0	220-740	13	0	1	7
Total	115	118	107	107	115	135	0.6-1.0	150-1600	90	11	34	33

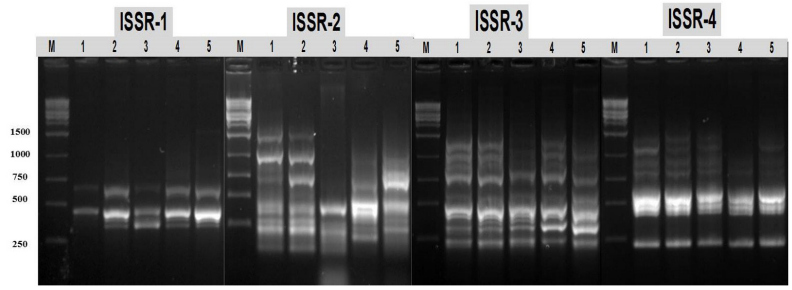

Figure 1. ISSR profile of five Sea cucumbers species using ISSR primers: (ISSR1, ISSR2, ISSR3 and ISSR4). M refers to DNA ladder marker 1 bp, (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa* and 5- *A. mauritiana*).

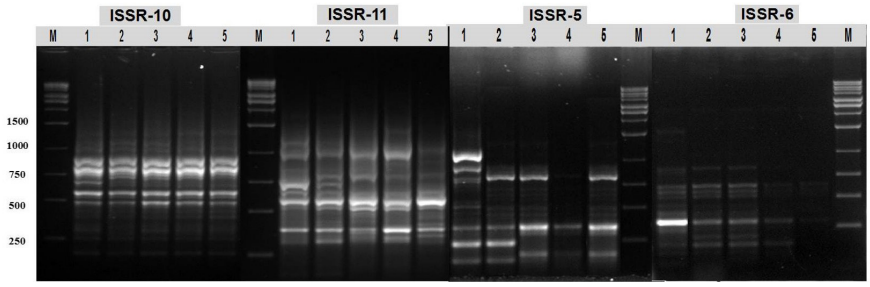

Figure 2. ISSR profile of five Sea cucumbers species using ISSR primers: (ISSR5, ISSR6, ISSR 10 and ISSR 11). M refers to DNA ladder marker 1 bp, (1- *H atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa* and 5- *A. mauritiana*).

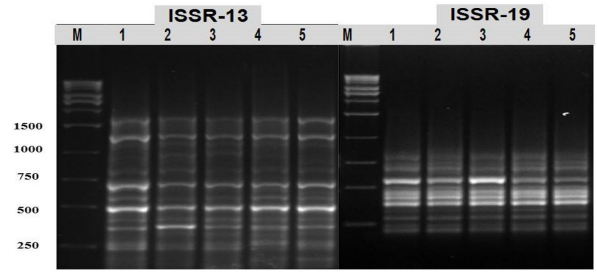

Figure 3. ISSR profile of five Sea cucumbers species using ISSR primers: (ISSR 13 and ISSR 19). M refers to DNA ladder marker 1 bp, (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa* and 5- *A. mauritiana*).

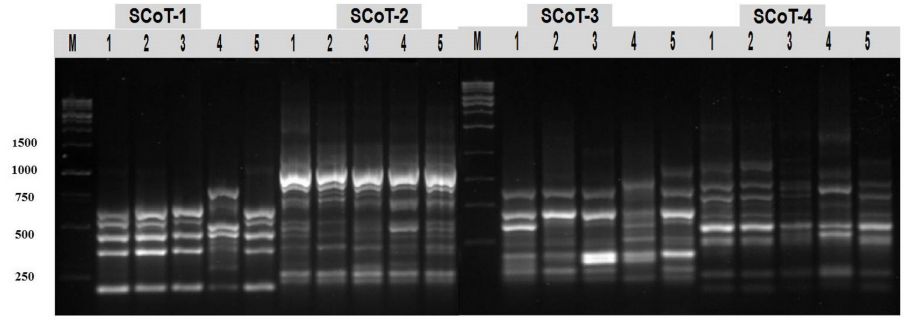

Figure 4. SCoT profile of five Sea cucumbers species using SCoT primers: (SCoT1, SCoT2, SCoT3 and ISSR 4). M refers to DNA ladder marker 1 bp, (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa* and 5- *A. mauritiana*).

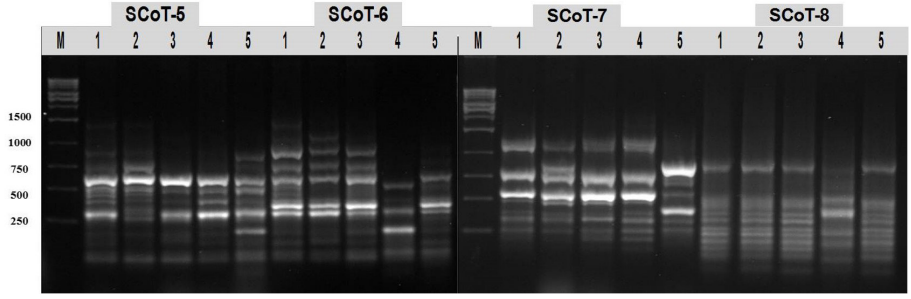

Figure 5. SCoT profile of five Sea cucumbers species using SCoT primers: (SCoT5, SCoT6, SCoT7 and ISSR 8). M refers to DNA ladder marker 1 bp, (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa* and 5- *A. mauritiana*).

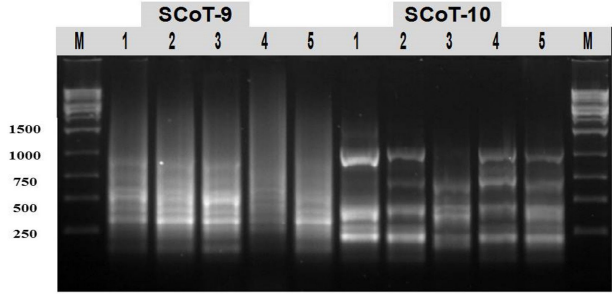

Figure 6. SCoT profile of five Sea cucumbers species using SCoT primers: (SCoT9 and ISSR10). M refers to DNA ladder marker 1 bp, (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa* and 5- *A. mauritiana*).

Table 4. Number of amplified DNA-fragments with SCoT in the five Cucumber specimens (1- *H. atra,* 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa,* and 5- *A. mauritiana*).

Primer code	No. of amplified bands					Total amplified bands	Mean of band frequency	Size range (bp)	No. of monomorphic bands	No. Unique bands	No. of poly morphic bands	Poly morphism %
	(1)	(2)	(3)	(4)	(5)							
SCoT-1	8	8	8	11	8	13	0.6	210-800	6	5	2	54
SCoT-2	17	12	15	16	15	19	0.8	180-2000	12	2	5	37
SCoT-3	10	9	10	10	11	15	0.7	150-700	6	5	4	60
SCoT-4	12	12	11	14	11	18	0.7	136-1150	8	4	6	56
SCoT-5	14	14	11	10	15	18	0.7	120-1330	9	3	6	50
SCoT-6	15	12	11	8	12	17	0.7	100-1300	7	4	6	59
SCoT-7	12	12	12	13	9	15	0.8	170-1120	7	2	6	53
SCoT-8	11	12	12	10	12	14	0.8	130-750	8	2	4	43
SCoT-9	9	8	8	7	8	9	0.9	170-930	6	0	3	33
SCoT-10	9	8	8	9	10	13	0.7	140-1370	4	3	6	69
Total	117	107	106	108	111	151	0.6-0.9	100-2000	73	30	48	52

3.1.1. Holothuria atra

Using ISSR primers on this species resulted in various band patterns, comprising a total of 115 bands that varied in size from 150 bp (ISSR-1,6) to 1600 bp (ISSR-13). The number of bands produced ranged from 4 (ISSR-2) to 18 (ISSR-10). This species displayed 117 distinct SCoT band patterns, with bands ranging from 100 bp (in the SCoT-6 primer) to 2000 bp (in the SCoT-2 primer). The number of generated bands varied from 8 (in SCoT-1) to 17 (in SCoT-2).

3.1.2. Holothuria impatiens

A total of 118 different ISSR band fingerprints were produced by the primers, ranging in size from 150 bp in primer ISSR-1,6 to 1600 bp in ISSR-13. The number of generated bands ranged from 6 with ISSR-2 to 17 with ISSR-10. The application of SCoT primers on the examined species resulted in distinct band patterns consisting of 107 bands, ranging from 100 bp (SCoT-6) to 1550 bp (SCoT-2) in size. The number of generated bands varied from 8 (SCoT-1, 9, 10) to 14 (SCoT-5).

3.1.3. Holothuria leucospilota

In this species, 107 distinct banding patterns using ISSR primers were observed, with band sizes ranging from 150 base pairs (bp) for primers ISSR-1 and ISSR-6 to 1600 bp for ISSR-13. The number of amplified bands varied from 4 in (ISSR-1 and ISSR-2) to 17 in (ISSR-10). Using SCoT primers, diverse band spectra comprising 106 bands were identified in this species. These bands ranged from 100 bp (SCoT-6) to 1550 bp (SCoT-2). The number of generated bands differed from 8 in SCoT-1, 9, and 10 to 15 in SCoT-2.

3.1.4. Actinopyga crassa

This particular species exhibited distinctive band patterns using ISSR primers, with 107 bands of varying size ranging from 150 bp to 1600 bp in (ISSR-6 and ISSR-13, respectively). The number of generated fingerprints ranged from 7 in (ISSR-2) to 17 in (ISSR-10). Meanwhile, using SCoT primers, *A. crassa* yielded 108 distinct banding profile, with band sizes ranging from 100 bp in the primer (SCoT-6) to 1550 bp in (ISSR-2). The amount of resulted bands changed from 7 in (SCoT-9) to 16 in (SCoT-2).

3.1.5. Actinopyga mauritiana

The utilization of ISSR primers on this species resulted in 115 unique band patterns, varying in size from 150 bp (ISSR-1, 6) to 1600 bp (ISSR-13), in that order. The quantity of bands generated ranged from 7 (ISSR-2) to 17 (ISSR-10), for both ISSR and SCoT primers. Similarly, SCoT primers produced around 111 distinct bands in this species, with sizes ranging from 100 bp (SCoT-6) to 1550 bp (ISSR-2). The number of bands formed ranged about from 8 (SCoT-1, 9) to 15 (SCoT-2).

3.2. Clustering analysis

An analysis of ISSR bands was performed on all sea cucumber species, revealing varying genetic relationship degrees. *Actinopyga crassa* and *Actinopyga mauritiana* exhibited the lowest degree of genetic relationship, while the highest degree was observed between *Holothuria atra* and *Holothuria impatiens*. The resulting genetic similarity index ranged from 86% to 93%, as demonstrated in Table 5 and Figure 7. Additionally, the analysis of SCoT bands showed that *Actinopyga crassa* and *Actinopyga mauritiana* had the lowest genetic similarity (75%), while *Holothuria atra* and *Holothuria impatiens* had the highest (90%), as presented in Table 6 and Figure 8. The cladogram built based on genetic distance divided the dendrogram into three clades (Figures 9 and 10).

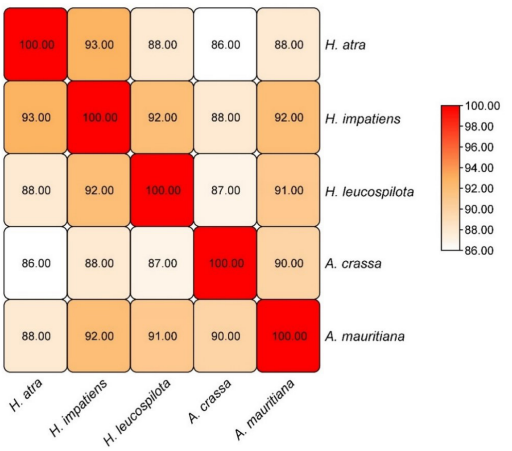

Figure 7. Heatmap visualization of the similarity between five sea cucumber species (*H. atra, H. impatiens, H. leucospilota, A. crassa and A. mauritiana*). The similarity matrix was generated by the unweighted pair group method with arithmetic average (UPGMA) and Jaccard's coefficient, based on the ISSR marker data.

Table 5. Similarity Matrix UPGMA Jaccard's Coefficient with ISSR., 1- *H. atra*, 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa*, and 5- *A. mauritiana*).

	(1)	(2)	(3)	(4)	(5)
1	100				
2	93	100			
3	88	92	100		
4	86	88	87	100	
5	88	92	91	90	100

Table 6. Similarity Matrix UPGMA Jaccard's Coefficient with SCoT., 1- *H. atra*, 2- *H. impatiens*, 3- *H. leucospilota*, 4- *A. crassa*, and 5- *A. mauritiana*).

	(1)	(2)	(3)	(4)	(5)
1	100				
2	90	100			
3	86	89	100		
4	80	76	77	100	
5	88	89	86	75	100

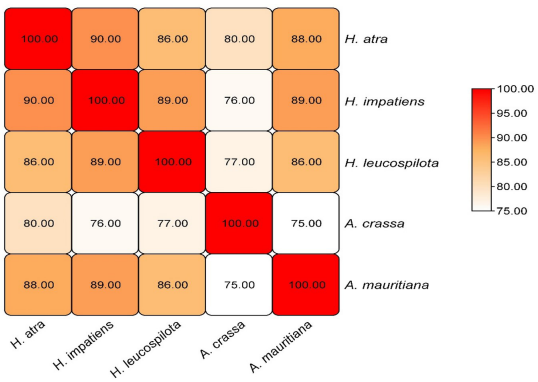

Figure 8. Heatmap visualization of the similarity between five sea cucumber species (*H. atra, H. impatiens, H. leucospilota, A. crassa and A. mauritiana*). The similarity matrix was generated by the unweighted pair group method with arithmetic average (UPGMA) and Jaccard's coefficient, based on the SCoT marker data.

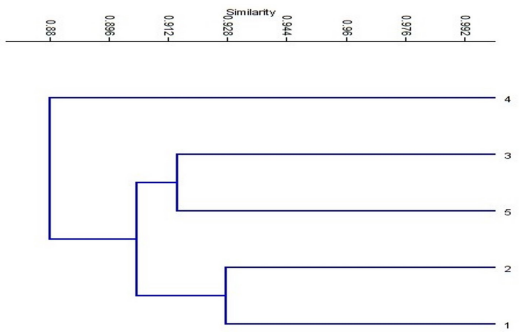

Figure 9. Dendrogram for five sea cucumber species (*H. atra, H. impatiens, H. leucospilota, A. crassa and A. mauritiana*), based on ISSR molecular markers using the UPGMA method and similarity matrices calculated according to Jaccard's coefficient.

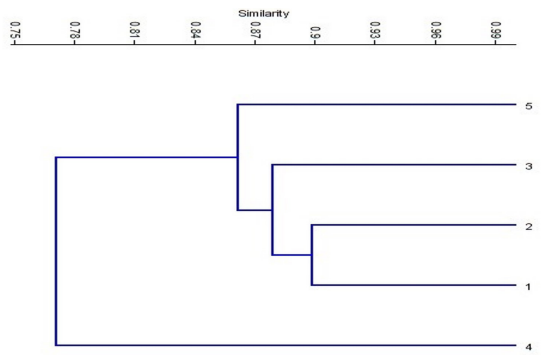

Figure 10. Dendrogram for five sea cucumber species (*H. atra, H. impatiens, H. leucospilota, A. crassa and A. mauritiana*), based on SCoT molecular markers using the UPGMA method and similarity matrices calculated according to Jaccard's coefficient.

4. Discussion

In the present study, morphological characteristics of three species from the *Holothuria* genus (*atra*, *impatiens*, and *leucospilota*) and two from the *Actinopyga* genus (*crassa* and *mauritiana*) were investigated, and DNA patterns for these species have been analyzed using molecular markers ISSR and SCoT and the resemblances and discriminate between the species was established.

Estimating molecular differentiation is vital in preserving hereditary resources (Hamrick and Godt, 1996). Implementing a genetic tracking scheme is crucial in preventing the depletion of genetic variation in farmed holothuroids. The examination of genetic differentiation is crucial in designing effective conservation programs for sea cucumber. In 2004, Uthicke and Purcell revealed significant genetic diversity through allozyme electrophoresis of 258 *Holothuria scabra* specimens from nine sites in New Caledonia. The findings referred significant genetic connectivity among populations with limited water exchange, emphasizing the importance of genetic surveillance in sea cucumber conservation programs.

ISSR analysis investigated the molecular polymorphism, and genetic variability among some species (Abu-Almaaty et al., 2017; Abu-Almaaty et al., 2020). Yao et al. (2007) employed ISSR markers to identify genetic discriminate between two stocks of sea cucumber (*Apostichopus japonicus*). Similarly, Mar'ie and Allam (2019) carried the assessment of genetic heterogeneity among several parrotfish species from the fisheries grounds of Red Sea out using the ISSR marker.

The marker targeting the start codon is anticipated to be associated with genes linked to functional characteristics, allowing for the conversion of the amplicons into molecular markers that target specific genes (as discussed by Xiong et al., 2011). The SCoT polymorphism approach, which employs a single forward and reverse primer, shares similarities with ISSR and RAPD (Bhattacharyya et al., 2013). According to Chen et al. (2009), the SCoT technique

offers several benefits, such as being cost-effective, ease of use, straightforward primer design, high polymorphism, consistent results, and broad applicability. SCoT markers have been utilized in various applications, including the identification of genetic diversity and structure, cultivar identification, and DNA fingerprinting in different species, as well as quantitative trait loci mapping (Cabo et al., 2014).

The outcomes of our study furnish insights into the genetic organization of the sea cucumber species. The findings suggest that the application of molecular techniques such as ISSR and SCoT could be advantageous tools in DNA fingerprinting, estimate genetic variability, and identifying different holothurians species sourced from different areas. Greater polymorphism is a result of differences in DNA sequences, indicating higher genetic diversity. The ISSR and SCoT markers exhibited polymorphism rates of 33% and 52%, respectively. The information obtained from this study will help to understand the genetic structure and evolution of sea cucumber species.

5. Conclusion

In conclusion, the current investigation employed ISSR and SCoT markers for evaluating the genetic distinction and molecular linkage between five distinct species of sea cucumber. The results indicated high-level polymorphism among species, with 11 distinct species-specific bands using ISSR markers and 30 species-specific bands using SCoT markers. The genetic analysis showed that *Holothuria atra* and *Holothuria impatiens* are closely related, while *Holothuria leucospilota, Actinopyga crassa* and *Actinopyga mauritiana* are genetically distinct. The findings demonstrate the potential of ISSR and SCoT markers for identifying sea cucumber species beyond traditional morphological characteristics.

Acknowledgements

This work was supported by the Researchers Supporting Project (RSP2023R36), King Saud University, Riyadh, Saudi Arabia.

References

ABU-ALMAATY, A.H., BAHGAT, I.M. and AL-TAHR, Z.M., 2020. Using SDS-page and ISSR as biochemical markers for assessment the genetic similarity and protein analysis of some Cyprinid fish species. *Genetika*, vol. 52, no. 1, pp. 161-175. http://dx.doi.org/10.2298/GENSR2001161A.

ABU-ALMAATY, A.H., HASSAN, M.K., BAHGAT, I.M. and SULEIMAN, M.E.E., 2017. Inter Simple Sequence Repeat (ISSR) and Cytogenetic Analysis of Three Fish Species of Family Osphronemidae. *Egyptian Journal of Aquatic Biology & Fisheries*, vol. 21, no. 2, pp. 1-15. http://dx.doi.org/10.21608/ejabf.2017.2436.

BHATTACHARYYA, P., KUMARIA, S., KUMAR, S. and TANDON, P., 2013. Start Codon Targeted (SCoT) marker reveals genetic diversity of *Dendrobium nobil Lindl*., an endangered medicinal orchid species. *Gene*, vol. 529, no. 1, pp. 21-26. http://dx.doi.org/10.1016/j.gene.2013.07.096. PMid:23939470.

BOOLOOTIAN, R.A., 1966. *Physiology of echinoderms*. New York: Wiley, 822 p. https://doi/10.1016/C2013-0-02511-X.

BORDBAR, S., ANWAR, F. and SAARI, N., 2011. High-value components and bioactives from sea cucumbers for functional foods, a review. *Marine Drugs*, vol. 9, no. 10, pp. 1761-1805. http://dx.doi.org/10.3390/md9101761. PMid:22072996.

CABO, S., FERREIRA, L., CARVALHO, A., MARTINS-LOPES, P., MARTIN, A. and LIMA-BRITO, J.E., 2014. Potential of Start Codon Targeted (SCoT) markers for DNA fingerprinting of newly synthesized tritordeums and their respective parents. *Journal of Applied Genetics*, vol. 55, no. 3, pp. 307-312. http://dx.doi.org/10.1007/s13353-014-0211-3. PMid:24733248.

CHEN, H., HE, X., LUO, C., GAO, M. and ZHU, J., 2009. *The optimization of SCoT-PCR system of Longan (Dimocarpus longan). Genomics and Applied Biology*, no. 28, pp. 970-974.

COLLARD, B.C. and MACKILL, D.J., 2009. Start Codon Targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, vol. 27, no. 1, pp. 86-93. http://dx.doi.org/10.1007/s11105-008-0060-5.

CONAND, C., 1990. *The fishery resources of Pacific island countries*. Rome: Part 2: Holothurians. Fisheries Technical Paper/FAO, vol. 272, no. 2, 143 p.

CONAND, C., 2006. *Sea Cucumber Biology, Taxonomy, Distribution and Conservation Status*. Silver Spring: NOAA Technical Memorandum NMFS-OPR-34, 244 p.

COSTA, R., PEREIRA, G., GARRIDO, I., TAVARES-DE-SOUSA, M.M. and ESPINOSA, F., 2016. Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify Orchardgrass (*Dactylis glomerata L.*) germplasm variations. *PLoS One*, vol. 11, no. 4, pp. e0152972. http://dx.doi.org/10.1371/journal.pone.0152972. PMid:27070939.

CUI, W., HUO, D., LIU, S., XING, L., SU, F., YANG, H. and SUN, L., 2021. Construction of a high-density genetic linkage map for the mapping of QTL associated with growth-related traits in sea cucumber (*Apostichopus japonicus*). *Biology*, vol. 11, no. 1, pp. 50. http://dx.doi.org/10.3390/biology11010050. PMid:35053048.

ELNAKEEB, M., VASILYEVA, L.M., SUDAKOVA, N.V., ANOKHINA, A.Z., and GEWIDA, G.A., 2020. Influences of feeding habitat and age composition on the growth patterns, length-frequency and gut contents in maternal paddlefish *Polyodon spathula* (Walbaum, 1792). *Egyptian Journal of Aquatic Biology and Fisheries*, vol. 24, no. 5, pp. 361-374. http://dx.doi.org/10.21608/ejabf.2020.106609.

FANG, D.Q. and ROOSE, M.L., 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, vol. 95, no. 3, pp. 408-417. http://dx.doi.org/10.1007/s001220050577.

FERNANDES, D.S., OKUMA, D., PANTOJA-GOMEZ, L.M., CUENCA, A. and CORRÊA, A.S., 2022. *Bemisia tabaci MEAM1* still remains the dominant species in open field crops in Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, no. 1, pp. e256949. http://dx.doi.org/10.1590/1519-6984.3828. PMid:35239823.

FUTUYMA, D.J., 1986. *Evolutionary Biology*. Sunderland: Sinauer Associates.

HAMRICK, J.L. and GODT, M.J.W., 1996. Conservation genetics of endemic plant species. In: J.C. AVISE and J.L. HAMRICK, eds., *Conservation genetics: case histories from nature*. New York: Chapman and Hall, pp. 281-304. http://dx.doi.org/10.1007/978-1-4757-2504-9_9.

HARTATI, R., WIDIANINGSIH, W., TRIANTO, A., ZAINURI, M. and AMBARIYANTO, A., 2017. The abundance of prospective natural food for sea cucumber *Holothuria atra* at Karimunjawa Island

waters, Jepara, Indonesia. *Biodiversitas*, vol. 18, no. 3, pp. 947-953. http://dx.doi.org/10.13057/biodiv/d180311.

HYMAN, L.H., 1955. The Invertebrates: Echinodermata. In: M.G. HILL, ed. *The Coelomate bilateria*, vol. IV, pp. 1-763.

IGWE, D.O., IHEARAHU, O.C., OSANO, A.A., ACQUAAH, G. and UDE, G.N., 2022. Assessment of genetic diversity of Musa species accessions with variable genomes using ISSR and SCoT markers. *Genetic Resources and Crop Evolution*, vol. 69, no. 1, pp. 49-70. http://dx.doi.org/10.1007/s10722-021-01202-8.

JIMÉNEZ, C., 2018. Marine natural products in medicinal chemistry. *ACS Medicinal Chemistry Letters*, vol. 9, no. 10, pp. 959-961. http://dx.doi.org/10.1021/acsmedchemlett.8b00368. PMid:30344898.

KOVACH, W.L., 1998. *MVSP-A Multivariate Statistical Package for Windows, Version 3.0*. Pentraeth: Kovach Computing Services.

LOVATELLI, A., CONAND, C., PURCELL, S., UTHICKE, S., HAMEL, J.-F. and MERCIER, A., 2004. Advances in sea cucumber aquaculture and management. Rome: FAO Fisheries Technical Paper No. 463/FAO, 425 p.

LUTHFI, O.M. and ASADI, M.A., 2021. Recruitment of crustose coralline algae on tile material for monitoring coral larvae settlement's consolidators at Nature Reserve Pulau Sempu, East Java, Indonesia. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83. http://dx.doi.org/10.1590/1519-6984.245922.

MAR'IE, Z. and ALLAM, M., 2019. Usage of Inter Simple Sequence Repeat (ISSR) marker in assessing the genetic variation of six parrotfish species from the Egyptian Red Sea. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, vol. 11, no. 3, pp. 109-116. http://dx.doi.org/10.21608/eajbsc.2019.63943.

MASSIN, C., RASOLOFONIRINA, R., CONAND, C. and SAMYN, Y., 1999. A new species of Bohadschia (Echinodermata, Holothuroidea) from the Western Indian Ocean with a redescription of Bohadschia subrubra. *Bulletin of the Institute of Royal Sciences Belgique*, vol. 69, pp. 151-160.

MORENO, S., MARTIN, J.P. and ORTIZ, J.M., 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica*, vol. 101, no. 1, pp. 117-125. http://dx.doi.org/10.1023/A:1018379805873.

MULPURI, S., MUDDANURU, T. and FRANCIS, G., 2013. Start codon targeted (SCoT) polymorphism in toxic and non-toxic accessions of *Jatropha curcas L.* and development of a codominant SCAR marker. *Plant Science*, vol. 207, pp. 117-127. http://dx.doi.org/10.1016/j.plantsci.2013.02.013. PMid:23602106.

PURCELL, S.W., SAMYN, Y. and CONAND, C., 2012. Commercially Important Sea Cucumbers of the World. In: FAO, org. *Species catalogue for fishery purposes*. Rome: FAO, no. 6.

ROBARTS, D.W.H. and WOLFE, A.D., 2014. Sequence-related amplified polymorphism (SRAP) markers: a potential resource for studies in plant molecular biology. *Applications in Plant Sciences*, vol. 2, no. 7, pp. 1400017. http://dx.doi.org/10.3732/apps.1400017. PMid:25202637.

SAMYN, Y. and BERGHE, V.E., 2000. Annotated checklist of the echinoderms from the Kiunga Marine National Reserve, Kenya. Part 1: echinoidea and Holothuroidea. *Journal of East African Natural History*, vol. 89, no. 1, pp. 1-36. http://dx.doi.org/10.2982/0012-8317(2000)89[1:ACOTEF]2.0.CO;2.

SAMYN, Y., 2003. Shallow-water Holothuroidea (Echinodermata) from Kenya and Pemba Island, Tanzania. *Std Afrotropical Zoology*, vol. 292, pp. 158.

SNEATH, P.H.A. and SOKAL, R.R., 1973. Numerical taxonomy; the principles and practice of numerical classification. New York: Stony Brook.

TIAN, M., LI, Y., JING, J., MU, C., DU, H., DOU, J., MAO, J., LI, X., JIAO, W., WANG, Y., HU, X., WANG, S., WANG, R. and BAO, Z., 2015. Construction of a high-density genetic map and quantitative trait locus mapping in the sea cucumber *Apostichopus japonicus. Scientific Reports*, vol. 5, no. 1, pp. 14852. http://dx.doi.org/10.1038/srep14852. PMid:26439740.

UTHICKE, S., O'HARA, T. and BYRNE, M., 2004. Species composition and molecular phylogeny of the Indo-Pacific teatfish (Echinodermata: Holothuroidea) bêche-de-mer Fishery. *Marine and Freshwater Research*, vol. 55, no. 8, pp. 837-848. http://dx.doi.org/10.1071/MF04226.

UTHICKE, S. and PURCELL, S., 2004. Preservation of genetic diversity in restocking of the sea cucumber *Holothuria scabra* investigated by allozyme electrophoresis. *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 61, no. 4, pp. 519-528. http://dx.doi.org/10.1139/f04-013.

XIONG, F., ZHONG, R., HAN, Z., JIANG, J., HE, L., ZHUANG, W. and TANG, R., 2011. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea L.*) genotypes. *Molecular Biology Reports*, vol. 38, no. 5, pp. 3487-3494. http://dx.doi.org/10.1007/s11033-010-0459-6. PMid:21104441.

YAO, B., HU, X., BAO, Z., LU, W. and HU, J., 2007. Genetic variation in two sea cucumber (*Apostichopus japonicus*) stocks revealed by ISSR markers. *Chinese Journal of Oceanology and Limnology*, vol. 25, no. 1, pp. 91-96. http://dx.doi.org/10.1007/s00343-007-0091-z.