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Research Article

Identification and taxonomy of sea cucumbers (*Holothuria*) in Persian Gulf

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Abstract

The sea cucumbers of the genus *Holothuria* are important species in Asia. In present study in the Persian Gulf aimed to identify *Holothuria* species and investigate their relationships based on morphological and 16S rRNA molecular data. 30 specimens were collected from northern Persian Gulf coasts of Dayer (Bushehr Province) and Lengeh (Hormozgan Province). Based on ossicles morphology, three species were identified. DNA was isolated using CTAB method. Thereafter, 16S ribosomal RNA gene amplification was performed using universal primer and consequently PCR product was sequenced. Sequencing result was analyzed and identified in NCBI database using BLAST. 17 sequences of mitochondrial DNA of 16S rRNA gene (393bp length) were obtained after modification and alignment. The phylogenic tree of mentioned species showed a monophyletic group. The three identified species include *Holothuria (Thymiosycia) arenicola*, *Holothuria leucospilota* and *Holothuria parva*. *Holothuria arenicola* was found in high support in the sister clade of *H. arenicola* from China. Despite 7% genetic distance, no morphological differences were observed between *H. arenicola* with similar species.

Keywords: Morphology, Persian Gulf, 16S rRNA, Sea cucumbers, *Holothuria arenicola*, *Holothuria parva*, *Holothuria leucospilota*.

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Introduction

Sea cucumbers belong to the phylum Echinodermata, which are important food source for human, particularly in some parts of Asia (Bordbar *et al.*, 2011); they are appeared in the oceans 540 million years ago during evolution (Kerr and Kim, 2001). Holothuroids, also known as sea cucumbers, are an abundant and diverse group, which includes more than 1400 species occurring from intertidal to deepest oceanic trenches (Purcell *et al*., 2012; Gallo *et al*., 2015; Mu *et al*., 2018) and the main habitat for them are coral reefs (Bruckner *et al*., 2003). Sea cucumbers are among the main sediment bioturbators in marine ecosystems (Mansouri and Mezali, 2018). They ingest soft substratum and remove the organic matter which is necessary for their growth. This process prevents the accumulation of organic matter in the marine sediment and could help to control the effect of pathogenic agents (Uthicke, 1999). The class Holothuroidea represents 90% of deep-sea floor biomass, therefore its species are considered among the most dominant organisms in the world (Pawson and Pawson, 2008; De Leo *et al*., 2010). Moreover, commercial sea cucumbers consumption, as traditional and valuable food, has raised interests on the nutritional properties of these animals, highlighting the presence of antioxidant molecules together with a high protein content and a low fat level, also in the dried form, known as “bêche-de-mer” or trepang (Kim and Himaya, 2012; Wen *et al*., 2010; Roggatz *et al*., 2016). Other reasons of interests on sea cucumbers derive by their use as models for tissue and organ regeneration, by their peculiar adaptations and by their relevance for bioactive compounds production, such as holothurins (García-Arrarás and Dolmatov, 2010; Zhang *et al*., 2017; Eisapour *et al*., 2021). Despite the variety and wide distribution of sea cucumbers, the phylogenetic and evolutionary relationships of sea cucumbers remain largely unknown (Yang *et al*., 2020) and demands close inspection and perhaps reevaluations (Utzeri *et al*., 2020). Especially, Holothuriidae family represents 11% of the total diversity of the class Holothuroidea (Borrero-Pérez *et al*., 2009). This family contains individuals with few taxonomically informative morphological characteristics and with a high degree of intraspecific phenotypic diversity (Clouse *et al*., 2005). The species are classified into genera *Actinopyga* Bronn, 1860, *Bohadschia* Jaeger, 1833, *Holothuria* Linnaeus, 1767, *Labidodemas* Selenka, 1867, and *Pearsonothuria* Levin in Levin, Kalinen and Stonik, 1984 (Borrero-Pérez *et al*., 2009).

Morphological identification of species within the *Holothuria* genus is mainly based on the shape, size and fine details of endodermal ossicles (or sclerites) which are calcified structures that are part of the echinoderm endoskeleton (Aydın and Erkan, 2015). Most studies in Persian Gulf are based on morphological features as well, these include the identification of *Holothuria (Mertensiothuria) hilla* cucumbers from

Larak Island (Afkhami *et al*., 2012a), the identification of sea cucumbers on the northern shores of the Persian Gulf (Salari Aliabadi *et al*., 2015), and the identification of sea cucumbers on Hengam Island (Salarzadeh *et al*., 2013). Among the limited molecular studies, we can mention the study of population structure of *H. parva* on the northern coast of Persian Gulf using the mitochondrial gene 16S rRNA (Alami Naysi *et al*., 2016) and identification of the same species in Bandar-e Bostaneh - Hormozgan Province (Ehsanpour *et al*. 2016). However, doubts remain about their status as species and evaluating the taxonomic status of sea cucumber species in the northern waters of the Persian Gulf has not yet been done. Therefore, the present study aimed to identify *Holothuria* species and investigate their relationships in the Persian Gulf based on morphological and 16S rRNA molecular data.

Materials and methods

Sampling collection

A total of 30 samples were collected during three sampling periods from 2017 to 2018 from the intertidal zone and during the maximum tide of the Dayer Port, Bushehr Province (27°50′11″N, 51°53′55″E) and Lengeh Port, Hormozgan Province (26°33′29″N, 54°52′50″E) (Fig. 1). The samples were transferred to containers with 70 and 90% alcohol for morphological and molecular studies, respectively. All morphological characteristics of the samples were studied for accurate identification before DNA extraction.

Figure 1: Geographical location of the sampling stations.

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Morphological identification

For morphological studies, small pieces of abdominal and dorsal surfaces of the body wall, tubular legs and tentacles (three samples per species) were isolated and placed in 10% solution of sodium hydrochloride (Jaw Water) until the soft tissues were dissolves away leaving the ossicles. After repeated washing, the specimens were brushed on a slide and examined by light microscopy (Pawson *et al*., 2010). Diagnosis of ossicles was based on their appearance and the results of studies by other researchers (Samyn *et al*., 2006; Purcell *et al*., 2012; Prata *et al.*, 2014; Kamarudin and Rehan, 2015).

Molecular examination

Approximately 100 mg of muscles tissue was cut into small pieces. Total genomic DNA was extracted using a modified CTAB method from as described by Grewe *et al*. (1993). The quality and concentration of DNA were assessed by agarose1.0% gel electrophoresis and stored at -20°C until use. To prepare PCR solution, 1 µL (10 ng) of extracted DNA was added to 0.8 µL of each primer (10 pmol) and 12.5 µL of 2x Master Mix (containing $MgCl_2$) and the volume was distilled to 25 µL. The partial fragment of mitochondrial gene 16S rRNA was amplified using a pair of 16S rRNA Universal primer with 500-600 bp length (Table 1). Thermal cycling regimes were as follows: Initial denaturation 94°C for 5 minutes (one cycle), followed by 94°C for 30 seconds, annealing 57°C for 35 seconds, extension 72°C for 30 minutes (35 cycles) and final extension 72°C for 5 minutes (Palumbi *et al.*, 2002). The PCR products were probed on 1% agarose gel and sent to Takapozist Co., Iran for sequencing.

Table 1: Primers used for PCR amplification.

Primer	Sequence (5′ → 3′)	Reference
16sar-L (forward)	CGCCTGTTTATCAAAAACAT	Palumbi *et al.*, 2002
16sbr-H (reverse)	CCGGTCTGAACTCAGATCACGT	Palumbi *et al.*, 2002

Sequencing and molecular analysis

Sequences were modified using chromas version 2.6.5 (http://technelysium.com.au/). The sequences were then aligned using the online MAFFT software version 7 (Katoh *et al*., 2019). Then the phylogenetic tree was drawn using MEGA v7 (Kumar *et al.*, 2016) software with Maximum Likelihood (ML) method and Bootstrap 1000. Also, in order to compare the sequences of the present study with other sequences in the world and to determine the exact position of each species among other species and to better interpret the relationships among them as well as to identify more closely the sequences of this study, several sequences were extracted from the NCBI database with their access numbers visible on the plotted phylogenetic trees.

Prior to performing Maximum likelihood (ML) and Bayesian analyzes, the best nucleotide evolutionary model was selected for data based on AIC (Akaike Information Criterion) using Modeltest software version 4.1.2 (Posada and Crandall. 1998). According to this test, the HKY+G model was selected for 16S rRNA data in the present study. ML was analyzed using PAUP software version 4b10 (Swofford and Sullivan, 2009) and Bootstrap with 1000 replication was performed using RaxmlGUI program (Stamatakis, 2006; Stamatakis *et al*., 2008).

Bayesian analysis was performed using MrBayes software 3.2.3 (Ronquist *et al*., 2012). Four chains of Markov chains Monte Carlo (MCMC) consisting of one cold chain and three hot chains were run for 30 million generations at each sampling step. Finally, during the incineration phase, 25% of the collected trees (7500000 trees) were removed by the program and their residuals were plotted as a tree with values of branch support (PP). In the Bayesian method, branch support is expressed by numbers corresponding to the former probability, branch support. Chain convergence was determined by selecting the appropriate sample size (ESS>200 per sample) in Tracer version 1.5 (Baele *et al.*, 2017). The phylogenetic tree was plotted using Figtree software version 3.4.1. Also, *Cucumaria frondosa* was used as a subgroup to accurately compare relationships.

Results

Morphological data

Holothuria (Mertensiothuria) leucospilota (Brandt, 1835)

Diagnosis

Body wide and anterior part was thinner than posterior; body was smooth and had relatively thin seed coat. Ventral mouth surrounded by 20 large tentacles, anus was terminal. Trivium had large podia arranged in 4-5 rows on the radius but also dispersed in the interradial areas and were mainly distributed in ambulacral areas. The podia and papillae were randomly distributed on the bivium; bottom of the podia plate had a diameter of about 480 micrometers. The podia were distributed on multiple biviums in short and thick radial and intra-radial arrangement. The Cuvierian tubules were very thin and long. The dorsal and ventral tegument spicules were plate and button-like. The plate spicules had circular discs with 8 pores or more, crown with a large central hole. Buttons spicules were regular or irregular with 6 or 8 pores. Ventral legs had large plates and many pores. The dorsal podia had long rods (Fig. 2).

Holothuria parva Krauss, 1885

Diagnosis

Body was small, spindle-shaped was thin on both ends, had relatively hard tegument, ventral mouth had 18 short tentacles around it, anus was terminal. The short, thick trivium podia were ended with large suckers that scattered over the bivium. Cuvier's body had numerous white tubules. The tegument spicules were short, thorny and splotchy (Fig. 5**)**. Podia spicules were similar to tegument spicules with small sticky end; nearly spicules were pierced and had flat plates. The anal and oral parts were

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surrounded by stick-like spicules and perforated plates, tentacles had various thorn lengths. Some plates had 5-12 pores, circular discs, and rod spicules in dorsal and ventral surfaces. Cuvierian organs were present (Fig. 3).

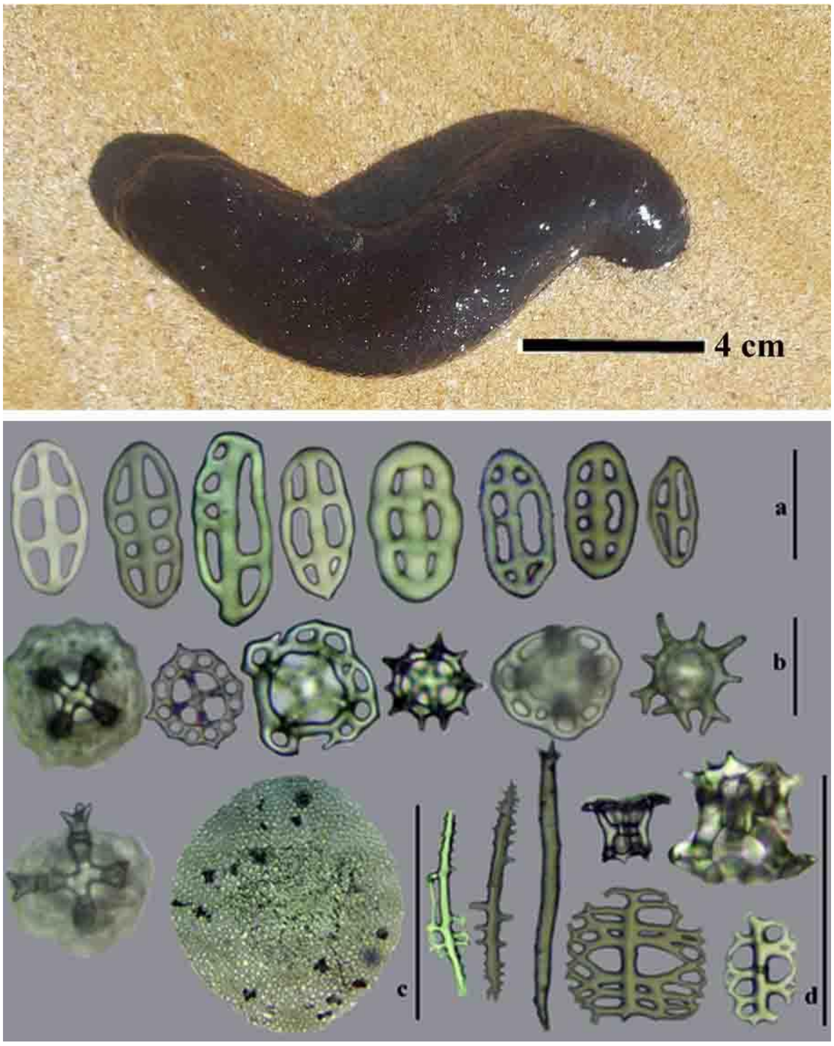

Figure 2: Photograph of external view and the spicules of *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835) with magnification of 1280×1024. a: buttons from dorsal, b: tables from tegument, c; table and plate from tentacle d: rods, tables and plate from ventral.

Holothuria (Thymiosycia) arenicola

Diagnosis

The small burrowing species (100 mm body Length and 200 mm body width) with fusiform body that narrows at both ends, tegument was relatively thin but rough. Mouth was terminal and surrounded by 20 small brown tentacles. Podia and trivium were short and wide, covering the whole surface. This species lacked Cuvier. Spicules were flat, button-like in the tegument and rod and plate in the tentacles (Fig. 4**)**. Ventral surface was white to yellowish-brown and there were two lines of dark brown spots along the body. Some specimens were very orange with white area on the anterior or posterior part and two

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irregular but continuous lines on the back.

Figure 3: Photograph of external view, tentacles and the spicules of *Holothuria parva* Krauss, 1885 with magnification of 1280×1024. A, a: rods, buttons and plate from anal, b: tables from oral; B, c: rods, button and plates from ventral, d: rods, buttons from tentacle.

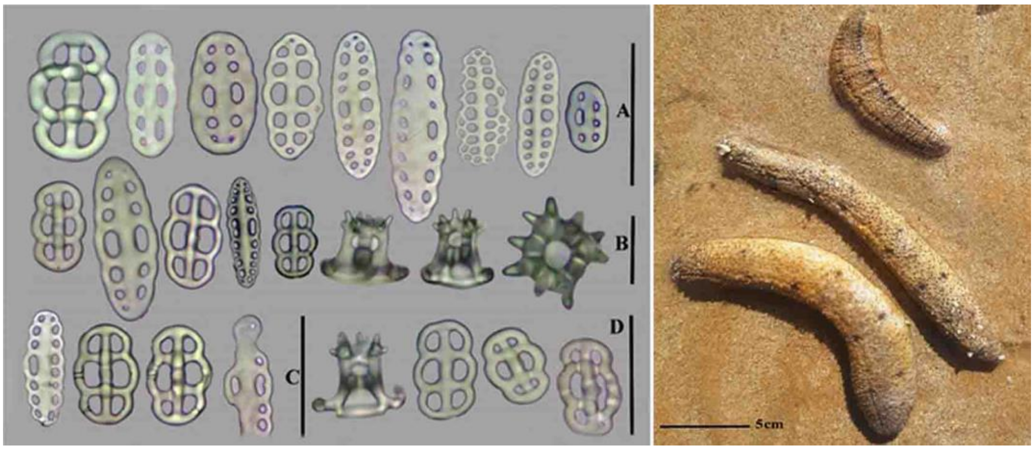

Figure 4: Photograph of external view and the spicules of *Holothuria arenicola* with magnification of 1280×1024. A: buttons from anal, B: buttons and tables from oral, C: buttons from ventral, D: buttons and table from tentacle.

Remark

The burrowing behavior of this species makes it very difficult to find and can only be seen with an intra-sand flow. Therefore, they are probably much more abundant. This specimen was referred to as *H. arenicola* in previous Persian Gulf studies. Molecular studies of the species in Persian Gulf separated samples from the Eastern Pacific.

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Molecular data

The blast results of three specimens of *Holothuria (Mertensiothuria) leucospilota*, *Holothuria parva* and *Holothuria arenicola* are shown in Table 2. 17 sequences (12 sequences from Malaysia and 5 sequences from the present study) of mitochondrial DNA of 16S rRNA genes (393bp length) were obtained after modification and alignment. The frequencies of nucleotides were A: 31.6, C: 23.6, G: 21, T: 23.8, respectively; and the ratio of transient mutations to crossover mutations was estimated to be 1.3. According to the MAGA and Modeltest programs, HKY+G model was estimated to be the best evolutionary model for the 16S rRNA gene. The homogeneity rate of this model, based on the ratio of transient and crossover mutations, was as follows: Issc = 0.822, Value = 0.450 and Iss = 0.690 (Fig. 6).

Table 2: Sample information and GenBank accession numbers for specimens used in the molecular analyses.

Species	Location	16S GenBank N
Holothuria mammata	Western Mediterranean Sea	JF697311
Holothuria edulis	Malaysia, Tioman Island	FJ223854
Holothuria atra	Malaysia, Tioman Island	FJ223858
Holothuria pardalis	Malaysia, Tioman Island	FJ223861
Holothuria scabra	Malaysia, Tioman Island	FJ223862
Holothuria parva	Iran, Persian Gulf	KT159718
Holothuria coluber	Malaysia, Tioman Island	FJ223866
Holothuria leucospilota	Malaysia, Tioman Island	FJ223871
Holothuria notabilis	Malaysia, Tioman Island	FJ223872
Holothuria ocellata	Malaysia, Tioman Island	FJ223869
Holothuria arenicola	China	MG586796
Holothuria lesson	Malaysia, Kuantan, Pahang Darul Makmur	MF139544
Holothuria arenicola	Iran, Persian Gulf	Pending
Holothuria arenicola	Iran, Persian Gulf	Pending
Holothuria arenicola	Iran, Persian Gulf	Pending
Holothuria leucospilota	Iran, Persian Gulf	Pending
Holothuria parva	Iran, Persian Gulf	Pending
Cucumaria frondosa	China	KF479389

ML and BI values were -2483.957 and -2145.435, respectively. The topology of the two analyzes was very similar and the species were in the same position. BI and ML analysis showed 50% support for Bootstrap and 94% support for previous probability for most base clades and higher values in derived clades (Fig. 5). The location of clades and the ratio of all species of this genus were the same in all trees. The phylogenic tree of this genus showed a monophyletic group (Fig. 5), in which 4 lineages were described. All lineages were evaluated with high support and specific genetic intervals. *Holothuria atra* clade was placed in the sister clade of *H. pardalis*, *H. coluber*, *H. scabra*, *H. notabilis*, *H. arenicola* and *H. lesson*. The second clade consisted of *H.*

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pardalis and *H. coluber*, which was monophyletic in the sister clade of *H. leucospilota and H. notabilis* (Fig. 5). *H. leucospilota* from Persian Gulf with a genetic distance of 2% was placed next to the same species from Taiwan with high support. The other clade consisted of *H. arenicola* species, three type of which from Persian Gulf with high genetic distance (7%) and high affinity were aligned with the same species from southern China (Table 3). The next clade constituted *H. mammata, H. edulis*, and *H. parva*, in which *H. parva* corresponded to high-support with *H. parva* from Persian Gulf (Fig. 5).

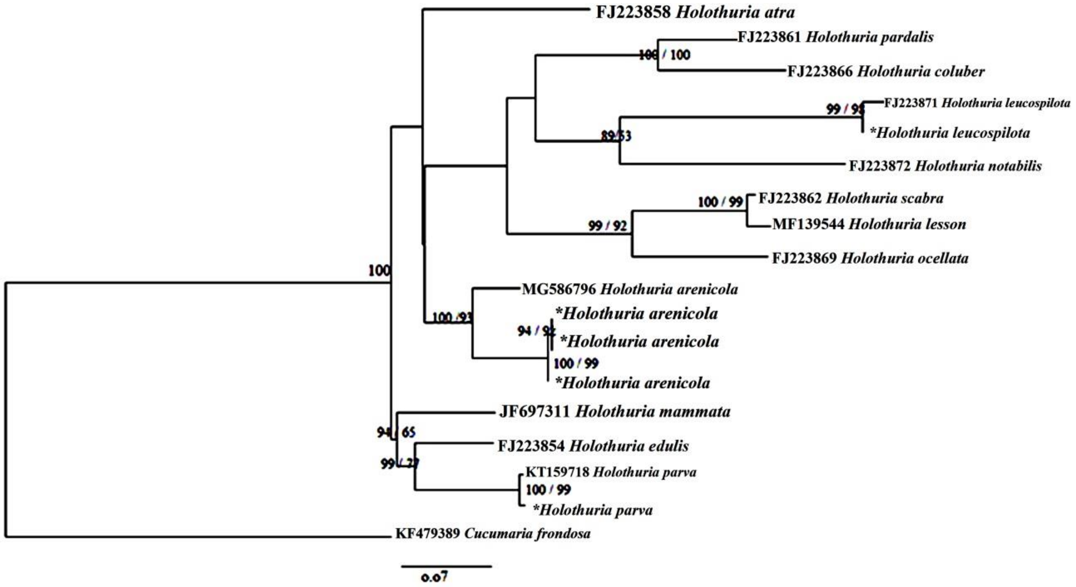

Figure 5: Maximum likelihood and Bayesian consensus tree using 16s rRNA molecular data by PAUP for *Holothuria*.

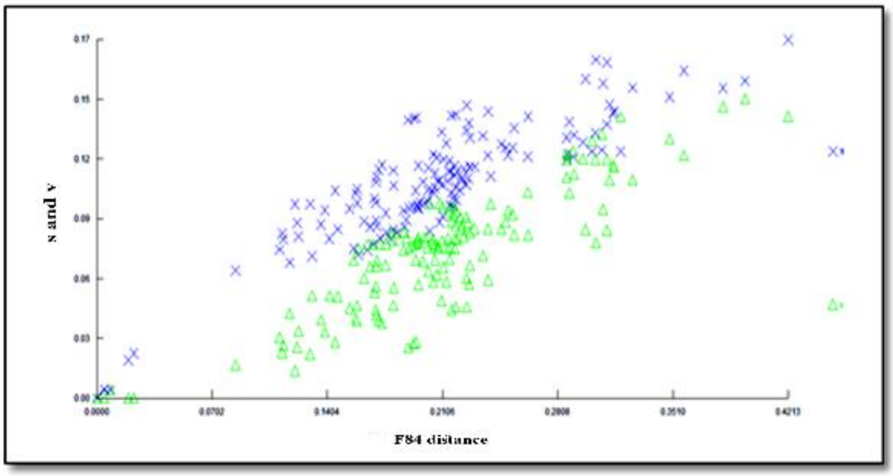

Figure 6: Saturation rate of transitions to transversion mutations.

Table 3: Table of genetic distances between species of the genus *Holothuria*.

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*JF697311 Holothuria mammata*																	
2	*FJ223854 Holothuria edulis*	0.12																
3	*FJ223858 Holothuria atra*	0.14	0.1															
4	*FJ223861 Holothuria pardalis*	0.18	0.16	0.17														
5	*FJ223862 Holothuria scabra*	0.21	0.18	0.17	0.18													
6	*KT159718 Holothuria parva*	0.13	0.1	0.12	0.2	0.21												
7	*FJ223866 Holothuria coluber*	0.2	0.18	0.18	0.07	0.2	0.22											
8	*FJ223871 Holothuria leucospilota*	0.21	0.2	0.18	0.21	0.23	0.21	0.23										
9	*FJ223872 Holothuria notabilis*	0.19	0.18	0.18	0.18	0.17	0.21	0.18	0.19									
10	*FJ223869 Holothuria ocellata*	0.21	0.18	0.17	0.15	0.13	0.22	0.15	0.22	0.19								
11	*MG586796 Holothuria arenicola*	0.13	0.09	0.1	0.16	0.16	0.14	0.19	0.22	0.2	0.18							
12	*MF139544 Holothuria lesson*	0.21	0.18	0.18	0.18	0	0.22	0.2	0.24	0.17	0.13	0.16						
13	*Holothuria arenicola*	0.14	0.11	0.1	0.15	0.16	0.15	0.17	0.22	0.17	0.17	0.07	0.17					
14	*Holothuria arenicola*	0.14	0.11	0.1	0.15	0.16	0.15	0.17	0.22	0.17	0.17	0.07	0.17	0				
15	*Holothuria arenicola*	0.14	0.11	0.1	0.15	0.16	0.15	0.17	0.22	0.17	0.17	0.07	0.17	0	0			
16	*Holothuria leucospilota*	0.21	0.21	0.17	0.21	0.22	0.22	0.22	0.02	0.2	0.21	0.21	0.22	0.22	0.22	0.22		
17	*Holothuria parva*	0.13	0.11	0.13	0.21	0.22	0	0.22	0.22	0.19	0.22	0.13	0.22	0.15	0.15	0.15	0.22	
18	*Cucumaria frondosa*	0.27	0.27	0.26	0.29	0.27	0.28	0.3	0.26	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.28

Discussion

There is limited information about identification of holothurians in Persian Gulf. Available studies are almost limited to Heding (1940) and Price (1983). The purpose of the present study was to identify sea cucumbers in the intertidal zone of Bandar Dayer and Bandar Lengeh. Based on the morphological characteristics and molecular markers, three species of sea cucumber *H. leucospilota*, *H. parva* and *Holothuria arenicola* were identified.

H. parva and *H. leucospilota* were partly similar, but *H. leucospilota* was larger in size and darker in color. Button spicules of *H. parva* and *H. leucospilota* were almost similar in shape, but different in size. The results of this study showed that the most common type of spicule in

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sea cucumber *H. parva* was the spiny rods spicule. These results were almost similar to findings of Shakouri *et al*. (2009). Some researchers reported the number of small tentacles around the ventral mouth can reach up to 20 (Samyn *et al*., 2006; Ehsanpour *et al*., 2016); however, in the present study they were 18. There are several reports of the presence of this species in the waters of Persian Gulf (Salari Aliabadi *et al*., 2016; Ehsanpour *et al*., 2016). There is no accurate information on the global distribution of this species.

Holothuria leucospilota is an opportunistic species. It is one of the Holothurians with a wide distribution that is found in most tropical areas of the Indian Ocean, western central Pacific, Africa and Asia. Quiet and slightly deep areas on the sandy bottom or on coral rubble are their habitat (Purcell *et al*., 2012; Yang *et al.*, 2019). It is supposed that *H. leucospilota* is the dominant species in Persian Gulf too (Afkhami *et al*., 2012b). The plate spicules in *H. leucospilota* were very large and more than other types of spicules in this species. For *H. leucospilota* morphological characteristics of body, size, color and spicules were very similar to those described by Afkhami *et al.* (2012b) and Kamarudin and Rehan (2015).

Holothuria arenicola was very similar in color, tegument thickness and shape of spicules to that described by Prata *et al.* (2014). There are reports of presence of *H. arenicola* in the waters of Persian Gulf, Mediterranean Sea on Egyptian coasts (Abdel Razek *et al*., 2007), Red Sea, Comoros, the Caribbean (Purcell *et al.*, 2012) and northeast coast of Brazil (Prata *et al.*, 2014).

Morphological approach is simpler and easier to apply compared with genetic approach (Hebert and Gregory, 2005). However, because the ossicles have intraspecific differences in shape, typology, and position in different parts of the body, morphological analysis can lead to misidentification of *Holothuria* species (Kim *et al*., 2013). On the other hand, no detailed characterization is available for ossicles of most species such as *H. arenicola*. Molecular information is therefore necessary in order to complete diversity information of sea cucumbers (Aydın and Erkan, 2015; Utzeri *et al.*, 2020). Due to high accuracy, slow evolutionary rate and accurate determination of relationships, the present study selected 16S rRNA gene as a suitable marker for the Holothuridea family (Clarridge, 2004; Uthicke *et al.*, 2005; Borrero-Pérez *et al.*, 2009). The results of the present study indicated that individuals of this genus form a monophyletic group. Ehsanpour *et al*. (2016) introduced Holothurians as a monophyletic group based on molecular and morphological data. In the present study the range of interspecies distance was 7 to 22% and intraspecific was 1 to 2%. Kamarudin *et al*. (2015) estimated this range to be 19 to 22 percent for the genus *Holothuria*. In the present study, two individuals of *H. leucospilota*, one from Persian Gulf and the other from East Malaysia-Tioman Island, with a 2% genetic distance and high support, were clustered. This 2% difference is probably due to the specific physical and environmental conditions of Persian Gulf as well as geographical distance and larval stage of these species (El-Naggar *et al*., 2008). Sea cucumbers like other marine invertebrates are dispersed during their larval stage. There are different mechanisms for long plankton migration. Ocean currents are probably the main factor in this case. *H. leucospilota* is within the order Holothuriida. This species, like other members of this order, has planktotrophic larvae. Planktotrophic larvae feed while they are in the water column and can be pelagic over a long period of time and so disperse over long distances (Strathmann *et al*., 2002). Considering the connection of the waters of Persian Gulf with Indian Ocean and Indo-Pacific region, through the Sea of Oman, ocean currents may have played a role in the dispersion of this species. Therefore, the genetic distance between the species of Persian Gulf and that in the waters of Malaysia can be explained.

Ghobadian *et al.* (2019) reported the shortest genetic distance between this species and Malaysian species. They stated that the wide distribution of this species could be due to presence of planktotrophic larvae and passive ocean currents. Also, *H. notabilis* and *H. leucospilota* clades were placed in the sister clade of *H. pardalis* and *H. coluber*, respectively. *H. leucospilota* had a genetic distance of 22% with all species except *Holothuria atra* which had the lowest distance to it (17%). Kamarudin *et al*. (2015) found that the genetic relationship between *H. leucospilota* and *H. coluber* was more than *H. atra*, but the genetic distance between *H. coluber* and *H. atra* was more. These results were in agreement with the findings of the present study.

Another identified species from Persian Gulf was *Holothuria arenicola* which was found in high support in the sister clade and 7% genetic distance with *H. arenicola* from China (Unpublished). Based on the results of the present study and the study of Ward *et al.* (2008) that proposed the interspecific divergence range between 8%, *H. arenicola* from Persian Gulf showed the beginning of separation from samples from the China. Morphologically, *H. arenicola* in Persian Gulf was still similar to those described from other regions, but had begun to change genetically. Alami Naysi *et al*. (2016) pointed out that due to the semi-closed nature of Persian Gulf and its location in subtropical latitudes and restrictive environmental conditions, there is a possibility of native species formation. Most studies on *H. arenicola*, mainly described stages of asexual reproduction, extraction of compounds or morphological identification. According to the results of the present study, this species needs more studies.

H. parva was compared to a similar sample from Persian Gulf (Salari Aliabadi, 2016) were clustered without genetic distance (0%) and high support, which supports and confirms the molecular identification of this species.

Due to the presence of common haplotypes of *H. parva* in northern coasts of Persian Gulf, Alami Naysi *et al*. (2016) reported a common ancestor for this species due to the limited Persian Gulf region and high gene flow. *H. parva* was monophyletic in the sister clade of *H. edulis*. Ehsanpour *et al*. (2016) examined the population structure of *H. parva* in Persian Gulf and placed it in the sister clade of *H. edulis*, which is consistent with the results of the present study. Limited studies have been performed on this species. Due to distribution of *H. parva* in different regions of Persian Gulf, this species deserves further studies.

This study investigated, for the first time, the phylogenetic of three dominant species of *Holothuria* on northern coasts of Persian Gulf. Based on morphological evidence and the scientific literature we have identified three species and, based on molecular data, we refer to these species as *Holothuria parva, H. leucospilota*, and *H. arenicola*. Holothuriidae is an ancient group with low diversity in the study area; however a more extensive description of ossicle variability is still needed to obtain a better description of these species. It appeared clear, however, that ossicle data should be coupled with molecular data to confirm species identification as we did in our study. Nevertheless, our results showed the importance of a molecular approach, as compared with a morphological approach, in addressing the ossicle taxonomic problem because molecular evidence provides a reference to confirm the range of morphological variability in each species.

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