

Research Article

Phylogenetic relationships of *Ulva* populations in the northern Coasts of the Persian Gulf and Oman Sea based on molecular analyses

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Abstract

Genus *Ulva* includes edible seaweed species with extensive morphological overlaps, plasticity, and intra specific variation, making it taxonomically difficult to classify. *Ulva* species occur in the Persian Gulf, and Oman Sea, but there is no detailed information on their genetic structure, diversity, as well as species relationships. Aims of the present study were to: (1) identify existing species of the genus *Ulva* in selected areas of the Persian Gulf and Oman Sea, and (2) illustrate species relationships by a combination of morpho-anatomical and molecular data. Based on morphological features, 10 *Ulva* species were identified which were confirmed by their DNA homology with the reported taxa. The *Ulva* species were delineated using a combination of morphological, anatomical and molecular data (ISSR, SCoT, ITS, *rbcL*, and *tufA* region sequences). AMOVA revealed species genetic differentiation and a higher level of intra-specific diversity in *Ulva*. The species relationship is discussed based on genetic and morphological data. A kind of divergent selection on morphological characters and in response to geographical coordinates is suggested.

Key words: AMOVA, Genetic diversity, Persian Gulf, Oman Sea, *Ulva*

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Introduction

A growing number of research work on identification, genetic diversity and effective compounds of the seaweeds indicating the importance of this group of organisms as human food, feed, fertilizer, medicine, cosmetic and industry uses (Shelar *et al.*, 2012, Farasat *et al.*, 2013, Shakouri and Balouch, 2016; Azizi *et al.*, 2019).

Genus Ulva (Ulvaceae) contains 99 accepted species (Guiry and Guiry, 2022). They grow almost in all aquatic habitats ranging from freshwater through brackish to fully saline environments (Loughnane et al., 2008). Sea lettuce, a group of edible species of Ulva genus are usually found in coastal environments with high levels of eutrophication (Kang and Chung, 2017) and in large quantities in ballast waters and also attached to ship hulls (Flagella et al., 2010). Some Ulva species have growth rates or efficient high reproductive alternatives that promote their dispersal abilities so that they can become invasive (Liu et al., 2015).

The systematics of the genus Ulva challenging remains due morphological simplicity (Gao et al., widespread 2016), morphological plasticity (Loughnane et al., 2008), intraspecific variation, interspecific morphological overlaps and distromatic (two cell layers) foliose blades of some species (Hofman et al., 2010) along ecological and environmental gradients (Bermejo et al., 2019).

Many reported *Ulva* species are considered homotypic or heterotypic

synonyms (Guiry and Guiry, 2019). For example, genetic analyses indicate that *U. fasciata* Delile and *U. lobata* (Kützing) Harvey are heterotypic synonyms of *U. lactuca* (Hughey *et al.*, 2019). Another example is *U. paschima* F. Bast which is reported to be synonymous with *U. tepida* (Masakiyo and Shimada, 2014).

Morphological and anatomical characteristics of these species are very similar and these traits are highly influenced by seasonal and environmental conditions. It is shown that generational frequency and sexual and asexual reproduction cycles are affected by water salinity (Hiraoka and Higa, 2016).

It is shown that combining morphoanatomical features with molecular is effective features verv identification of these species. Different molecular markers are utilized differentiate *Ulva* species and investigate their genetic variability as species relationships. For as instance. nuclear **ITS** sequences (Internal Transcribed Spacer DNA) were utilized to investigate species diversity and seasonal changes of Ulva species in Mikawa Bay, Japan (Kawai et al., 2007). Similarly, plastid rbcL gene sequences were used to investigate relationships of *Ulva* species in China (Shen et al., 2010). Kirkendale et al. (2013), explored diversity of *Ulva* spp. in temperate zones of Australia and introduced some of endemic and new Ulva spp. by rbcL and tufA as suitable barcoding gene species-level in identification.

Hayden and Waaland (2004) studied species diversity and identified 12 Ulva species by ITS DNA and rbcL gene. Ismail and Mohamed (2017)investigated morphological, genetic and biochemical variation within four Ulva species collected from Eastern Harbor, Alexandria. In another study using tufA marker, 9 genetic species groups were determined and U. ohnoi was identified as a new species from Tunisia (Miladi 2018). Various molecular al.. markers are used to identify algae, including rbcL and tufA. Recently, petA chloroplast genes have been identified as potential genetic markers that are similar to tufA marker (Cai et al., 2021). In spite of frequent occurrence of Ulva species throughout the Persian Gulf and Oman Sea, there is no detailed study of diversity genetic and species relationships of *Ulva* species in Iran. Few molecular studies are carried out in Iran on a few species of this genus (Pirian et al., 2016), therefore, in the present study, ISSR, SCoT, and ITS markers were used for genetic diversity analysis and identifying the species. Also, tufA, rbcL and ITS data from NCBI, were obtained to evaluate species relationship. The present study was performed to achieve the following aims: (1) Identify the existing species of the genus *Ulva* in selected areas of the Persian Gulf and Oman Sea, and (2) Investigate species relationships by a combination of morpho-anatomical and molecular analyses. We also aimed to investigate potential divergent selection on phenotypes of *Ulva* species induced by Phenotypic plasticity (Pst).

Materials and methods

Sampling

About one hundred young and adult specimens from each species were randomly collected from 19 selected areas of the Persian Gulf and Oman Sea and used for morphological, anatomical and molecular investigations (Table 1, Fig. 1).

Number of cell layers (X3), number of pyrenoids (X4), cell arrangement in (X5), form of thalli life (X6).proliferation (X7), thallus apex form (X8), color of thalli (X9), cell shape (X10), cell length (X11), cell width chloroplast (X12),shape (X13),chloroplast position (X14), thallus height (X15), thallus width (X16), or absence of calcium presence carbonate crystals (X17) were analyzed recorded. These and data were standardized and used to estimate Euclidean distance (Mean=0.Variance=1).

Molecular investigation

For molecular analyses, Inter-Simple Sequence Repeats markers (ISSR), Start Codon Targeted (SCoT) and the nuclear ITS sequences and the plastid gene sequences (*rbc*L, and *tufA*) region were used. These markers were applied to assess species diversity and phylogenetic relationships (Weising *et al.*, 2005; Sheidai *et al.*, 2014).

	Table 1: Spe	cies name, code and	d location o	of studied samples
No	Location	Species	Code	Latitude, Longitude
1	Qeshm Island	Ulva tepida	Upa1	26°58'19"N, 56°15'28"E
2	Bandar-e Lengeh	Ulva tepida	Upa2	26°33'20"N, 54°53'29"E
3	Bandar-e Taheri	Ulva linza	Uli1	27°39'37"N, 52°20'59"E
4	Qeshm Island	Ulva linza	Uli2	26°58'19"N, 56°15'26"E
5	Southern Owli	Ulva prolifera	Upr1	27°50'4"N, 51°53'48"E
6	Jazireh-ye Shif	Ulva prolifera	Upr2	29°4'21"N, 50°52'25"E
7	Northern Owli	Ulva intestinalis	Ui1	27°50'18"N, 51°53'15"E
8	Bandar-e Dayyer	Ulva intestinalis	Ui2	27°49'57"N, 51°56'5"E
9	Bandar-e Jofreh	Ulva clathrata	Uc	28°58'5.6"N, 50°49'1"E
10	Bandar-e Jofreh	Ulva compressa	Uco1	28°58'20"N, 50°49'25"E
11	Qeshm Island	Ulva compressa	Uco2	26°56'3.7"N, 56°16'29"E
12	Bandar-e Kangan	Ulva flexuosa	Uf1	27°50'18"N, 52°2'48"E
13	Bandar-e Abbas	Ulva flexuosa	Uf2	27°11'10"N, 56°21'11"E
14	Qeshm Island	Ulva ohnoi	Uo1	26°58'19"N, 56°15'28"E
15	Qeshm Island	Ulva ohnoi	Uo2	26°58'30"N, 56°15'2.6"E
16	Lipar Wetland (Pink Wetland)	Ulva lactuca	Ula1	25°15'2.7"N, 60°49'56"E
17	Chabahar	Ulva lactuca	Ula2	25°21'54"N, 60°24'12"E
18	Bandar-Abbas	Ulva chaugulii	Uch1	27°11'4"N, 56°19'35"E
19	Bandar-e Lengeh	Ulva chaugulii	Upa1	26°33'40"N, 54°53'46"E

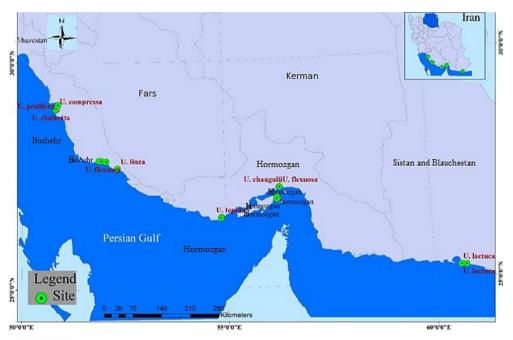


Figure 1: Study area. Sampling locations and species collected are marked on the map.

DNA extraction, PCR amplification and sequencing

Fresh leaves of specimens were dried in silica gel powder and genomic DNA was extracted by Cetyl trimethyl Ammonium Bromide-activated

charcoal protocol (CTAB). Polyvinylpyrrolidone (PVP) was used to bind polyphenolic compounds during extraction. The quality and quantity of the extracted DNA was evaluated by

running on 0.8% agarose (Sheidai *et al.*, 2012).

Eight ISSR primers were used; (CA) 7GT, (AGC) 5GG, UBC 810, (GA) 9C, UBC 807, UBC 811, (GT) 7CA and (GA) 9A commercialized by UBC (University of British Columbia) (Sheidai et al., 2012). Using a 25 µL volume containing 20 ng genomic DNA and 1 U of Taq DNA polymerase (Bioron, Germany); 50 mM KCl; 10 mM Tris-HCl buffer at pH 8; 1.5mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of each primer, polymerase chain reaction (PCR) was implemented. The PCR reactions were amplified in Techne thermal cycler (Germany) using the following procedure: 94°C for 5 min, followed by 40 cycles at 94°C for 30s, 55°C for 1 min, and 72°C for 1 min, followed by one final extension step at 72°C for 7 min.

Four primers (SCoT1, SCoT2, SCoT36, and SCoT41) based on Collard and Mackill (2009) were selected. These primer sequences are: SCoT1: CAACAATGGCTACCACCA, SCoT2: CAACAATGGCTACCACCC, SCoT36:

GCAACAATGGCTACCACC and SCoT41:

CAATGGCTACCACTGACA. A 25 µL volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany), 20 ng genomic DNA and 1 U of Taq DNA polymerase (Bioron, Germany) was used to carry out PCR reactions. The following thermal cycler parameters were used: 94°C for 5 min,

followed by 35 cycles at 98°C for 10 s, 50-55°C for 40 s, and 72°C for 2 min, followed by one final extension step at 72°C for 7 min.

ITS region DNA was amplified with 0.2 μM primer ITS1 (5' TCCGTAGGTGAACCTGCGG-3': Bioron, Germany), and primer ITS4 (5'- TCC GCT TATTGA TAT GC -3') (Chen et al., 2010). PCR reactions were performed in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany), 20 genomic DNA and 1 U of Taq DNA polymerase (Bioron, Germany). The amplification reaction was performed in a Techne thermo cycler (Germany) using the following parameters: 2 min initial denaturation step at 94°C, followed by 35 cycles of 5 min at 94°C; 1.30 min at 56°C and 2 min at 72°C. The reaction was completed by a final extension step of 7 min at 72°C.

PCR products were visualized on 2.5% agarose gels with GelRedTM Nucleic Acid Gel Staining. Fragment sizes were estimated using a 100 bp size ladder (Thermo- Fisher Scientific, Waltham, MA USA).

*rbc*L, *tufA* and ITS sequences of the species were obtained from NCBI for phylogenetic studies. The species accession numbers are shown in Table 2.

Data analysis

Morphological analyses

Morphological and anatomical data were obtained from each specimen and either measured (quantitative characters) or coded as multistage characters accordingly. The studied species were grouped by WARD (Minimum variance method) clustering method. Principal Component Analysis (PCA) was applied to investigate the species relationships identify and taxonomically differentiating **CCA** (Canonical characters. Correspondence Analysis) was used to reveal association between morphological characters and geographical coordinates. Pearson correlation coefficient was determined among quantitative characters (Podani, 2000) with PAST software (Hammer *et al.*, 2001). A heatmap was produced based on standardized morphological and anatomical characters by R package.

Table 2: Species accession numbers for rbcL, tufA and ITS sequences from NCBI.

-	Accession number	Accession number	Accession
Species	for rbcL gene	for <i>tufA</i> gene	number for ITS
			gene
Ulva paschima (=U. tepida)	KU377326.1	-	KF385506.1
$Ulva\ paschima\ (=U.\ tepida)$	KU377324.1	-	KF385505.1
$Ulva\ paschima\ (=U.\ tepida)$	KU377319.1	=	KF385504.1
Ulva linza	DQ813497.2	HQ610368.1	EU888138.1
Ulva linza	Q603601.1	KC411858.1	JN093109.1
Ulva linza	AB097620.1	MH538687.1	MN070057.1
Ulva prolifera	HQ603639.1	HQ610404.1	JQ963227.1
Ulva prolifera	JX572167.1	MH308670.1	JQ963226.1
Ulva prolifera	JQ963273.1	MH308523.1	JQ963225.1
Ulva intestinalis	HQ603541.1	HQ610323.1	KF195511.1
Ulva intestinalis	HQ603560.1	HQ610306.1	MG768946.1
Ulva intestinalis	=	EF595304.1	=
Ulva intestinalis	HQ603545.1	HQ610315.1	MF139301.1
Ulva clathrata	AY255862.1	-	HQ197901.1
Ulva clathrata	AF525940.1	-	AF153492.1
Ulva clathrata	AF525939.1	-	-
Ulva compressa	EU484397.1	HQ610429.1	KR006938.1
Ulva compressa	HQ603531.1	MF979661.1	KF195508.1
Ulva compressa	AB097615.1	JN029298.1	KR006936.1
Ulva flexuosa	HQ603532.1	HQ610296.1	MH304225.1
Ulva flexuosa	AB097619.1	JN029309.1	MN070070.1
Ulva flexuosa	KX398148.1	MF614789.1	MN070069.1
Ulva flexuosa	-	KC411863.1	-
Ulva ohnoi	AB116040.1	MF544113.1	MK910759.1
Ulva ohnoi	JN029247.1	JN029335.1	KF195516.1
Ulva ohnoi	MG704835.1	KU561325.1	KF195507.1
Ulva lactuca	EU484413.1	HQ610365.1	AF153488.1
Ulva lactuca	HQ603600.1	MH308478.1	MF041749.1
Ulva lactuca	- -	HQ610339.1	-
Ulva lactuca	HQ603598.1	JN029306.1	AY422501.1
Ulva chaugulii	MG704805.1	MG976863.1	MK426983.1
Ulva chaugulii	-	MZ582315.1	-
Ulva chaugulii	MG704804.1	MG976862.1	MK426982.1
Ulva chaugulii	-	Z582314.1	MK426981.1

Genetic analyses

Suitability of ISSR and SCoT molecular for genetic diversity was checked bv DCA (Dentrented Correspondence Analysis) (Podani. 2000) with PAST software (Hammer et al., 2001). Discriminating power of the loci versus migration was determined by GST versus Nm in pop gene program. The adaptive nature of molecular loci against geographical determined coordinates was bv Bavesian MCMC based method of LFMM (Latent factor mixed model) (Frichot et al. 2013). Genetic diversity parameters and AMOVA based on Fst was performed by GenAlex program (Peakall and Smouse, 2006). Other genetic differentiation parameters like G'st (Nei), G'st (Hed), and Dest were determined by Genodive program (Meirmans and Van Tienderen, 2004). To obtain number of genetic groups, we performed K-Means clustering by Genodive program, and Evanno test of delta K (Evanno et al., 2005), based on STRUCTURE program (Pritchard and 1999). For Rosenberg, genetic classification and grouping of the genotypes we constructed heat map by R package and STRUCTURE plot by **STRUCTURE** program. We used admixture model with 100000 permutations and 10000 burnin.

Phenotypic versus genetic differentiation

PST index was used to estimate the role of local adaptation through natural selection in *Ulva* species, compared to that of genetic differentiation. For each

population pair, pairwise PST values were calculated on each trait (and for an average PST), using the following formula:

 $P_{ST} = C\sigma^2 b / (C\sigma^2 b + 2 h^2 \sigma^2 w)$

In this formula, σ_b^2 and σ_b^2 phenotypic variances between within populations respectively, C is an estimate of the proportion of total variance due to additive genetic effects populations. and h^2 among heritability, the ratio of phenotypic variance induced by additive genetic effects (Brommer, 2011). In the present study Pst was estimated by Pstat R package 4.2 (Da Silva and Da Silva, Species relationships 2018). studied by constructing phylogenetic trees based on ISSR, SCoT and ITS data. For ISSR and SCoT, the bands treated obtained were as binary characteristics (presence=1. absence=0), and were used to estimate Nei genetic distance among the species. Different clustering methods were applied reveal Ulva species relationships based on Dice genetic distance (Hammer et al., 2001).

Results

Morphological and anatomical analyses

Our preliminary identification based on selected morphological and anatomical characters, resulted in ten distinct species. Two samples from each species ITS sequences were obtained and compared with available sequences in *Ulva* species. All identified species had at least 95% homology with the reported ITS sequence for members of

this group in NCBI (National Center for

Biotechnology Information) (Table 3).

Table 3: Ulva species identified and their ITS sequence homology to the reported species.

No	Location	Species	Code	Accession number
1	Qeshm Island	U. paschima (=U. tepida)	Upa1	KF385506.1
2	Qeshm Island	U. paschima (=U. tepida)	Upa2	KF385506.1
3	Bandar-e Taheri	Úlva linza	Uli1	EU888138.1
4	Qeshm Island	Ulva linza	Uli2	EU888138.1
5	Southern Ouli	Ulva prolifera	Upr1	JQ963227.1
6	Jazireh-ye Shif	Ulva prolifera	Upr2	JQ963227.1
7	Northern Ouli	Ulva intestinalis	Ui1	KF195511.1
8	Bandar-e Dayyer	Ulva intestinalis	Ui2	KF195511.1
9	Bandar-e Jofreh	Ulva clathrata	Uc	HQ197901.1
10	Bandar-e Jofreh	Ulva compressa	Uco1	KR006938.1
11	Qeshm Island	Ulva compressa	Uco2	KR006938.1
12	Bandar-e Kangan,	Ulva flexuosa	Uf1	MH304225.1
13	Bandar-e Abbas	Ulva flexuosa	Uf2	MH304225.1
14	Qeshm Island	Ulva ohnoi	Uo1	MK910759.1
15	Qeshm Island	Ulva ohnoi	Uo2	MK910759.1
16	Lipar lake	Ulva lactuca	Ula1	AF153488.1
17	Chabahar	Ulva lactuca	Ula2	AF153488.1
18	Bandar-Abbas	Ulva chaugulii	Uch1	MK426983.1
19	Bandar-e Lengeh	Ulva chaugulii	Uch2	MK426983.1

WARD dendrogram clustering was based on combined morphological and anatomical data is presented in Figure 2. Almost a good separation was obtained for the studied *Ulva* species, but due to overlap of some characters, some degree of intermixture was observed.

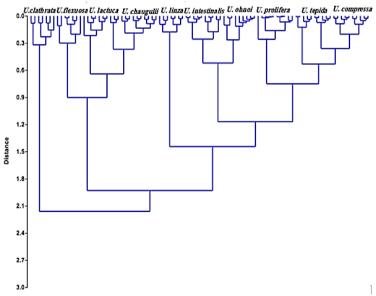


Figure 2: WARD dendrogram of Ulva based on morphological and anatomical features.

A heat map constructed based on standardized morphological and anatomical characters (Fig. 3) revealed that morphological characters, including X11, X12, X15, and X16 (length and width of cell, height and width of thallus), can differentiate *Ulva* species.

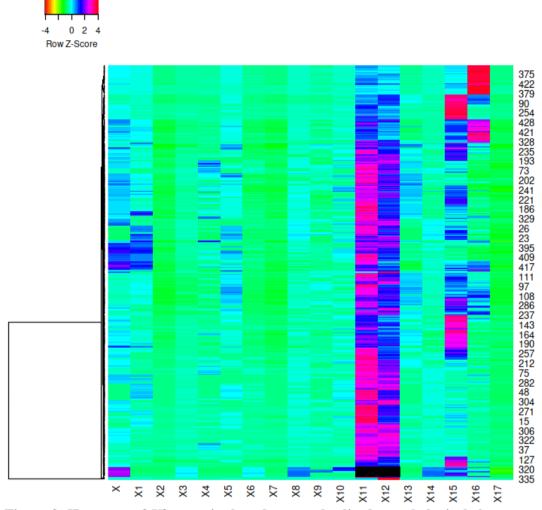


Figure 3: Heat map of *Ulva* species based on standardized morphological characters. X1-thallus form, X2- branching type, X3- number of cell layers, X4- number of pyrenoids, X5- cell arrangement in thalli, X6- form of life, X7- proliferation, X8- thallus apex form, X9- color of thalli, X10- cell shape, X11- cell length (μ m), X12- cell width (μ m), X13-Chloroplast shape, X14- Chloroplast position, X15- thallus height (cm), X16- thallus width (cm), X17- presence or absence of calcium carbonate crystals.

PCA-biplot of *Ulva* species (Fig. 4), based on morphological characters revealed that X15, differentiated the species *Ulva flexuosa*, *U. ohnoi*, *U. clathrata*, and *U. lactuca*, from other *Ulva* species which were differentiated

based on X11, X12, X15, and X16 characters, respectively.

PCA separated the studied *Ulva* species in two major groups. Three species namely, *Ulva ohnoi*, *U. lactuca*, and *U. linza* showed a higher level of morpho-

anatomical similarity and are placed close to each other. The other studied species comprised the second group, in which, *Ulva flexuosa* and *U. clathrata*, showed some difference with other species and stood with some distance from them. PCA analysis revealed that the first two PCA axes comprised about 70% of total variation, and characters X11, 12, had the highest positive correlation value (r=0.60), with the first axis, while X16, was negatively correlated (r= - 0.86), to this axis.

Similarly. X15 was negatively correlated with PCA axis 2 (r=- 0.92) (Fig. 4). CCA analysis (data not shown), showed that the first two characters including length and width of the cell (X11 and X12), along with width (X16), were under thallus influence of the species longitude distribution, while the third one, thallus height (X15), was related to latitude distribution of *Ulva* species.

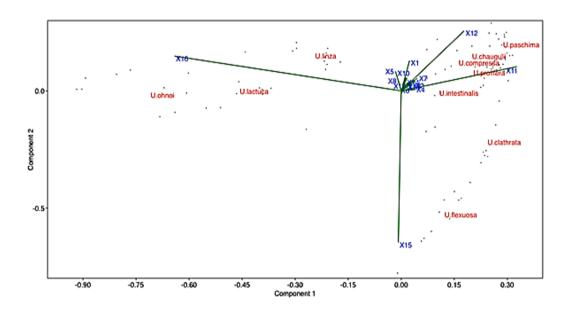


Figure 4: PCA-biplot of *Ulva* species based on morphological and anatomical data. X1- thallus form, X2- branching type, X3- number of cell layers, X4- number of pyrenoids, X5- cell arrangement in thalli, X6- form of life, X7- proliferation, X8- thallus apex form, X9- color of thalli, X10- cell shape, X11- cell length (μ m), X12- cell width (μ m), X13- Chloroplast shape, X14- Chloroplast position, X15- thallus height (cm), X16- thallus width (cm), X17- presence or absence of calcium carbonate crystals.

Genetic analyses ISSR and SCoT

Totally, we obtained 43 ISSR bands and 71 SCoT bands. All these bands showed polymorphism and not a single private band was obtained for tested *Ulva* spp. We used separate ISSR and SCoT data

as well as a combined data matrix to investigate differentiating power of these molecular markers for *Ulva* species delimitation. All attempts produced a similar result, i.e. they cannot delimit *Ulva* genus, and they are

mainly suitable to be used for genetic diversity studies.

The NJ tree of *Ulva* species based on combined ISSR-SCoT data (Fig. 5), grouped the studied samples of each species inter-mixed with others, showing that these molecular markers

may not delineate *Ulva* species. However, these markers placed the studied taxa in two major clusters that vary slightly from morpho-anatomical results.

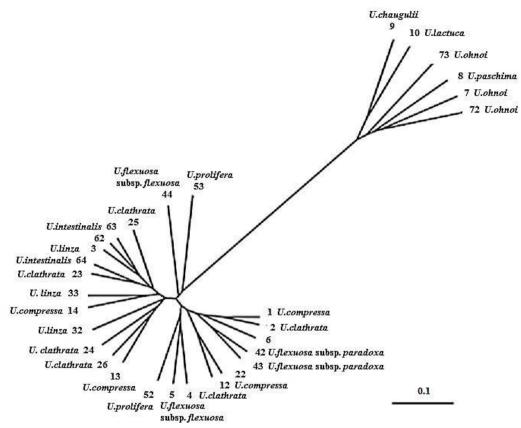


Figure 5: NJ tree of *Ulva* species based on ISSR-SCoT data, separating these taxa in two major clades.

Genetic diversity

DCA (Dentrented Correspondence Analysis) (Fig. 6), showed well distribution of the loci scattered obtained. This indicates that both ISSR and SCoT loci were distributed in the genome and were suitable molecular markers for genetic diversity investigation.

Among the 114 obtained loci, some showed a higher degree of diversity having Nei's gene diversity (h) ranging from 0.33 to 0.50, and Shannon information index (I) ranging from 0.52 to 0.69.

Discriminating (Gst) power versus migration level (Nm) analysis of the loci identified several loci from both ISSR and SCoT markers to be highly migrated (shared common) alleles among the tested *Ulva* species (Table 4). Some of the loci had high discriminating power (0.80), which means they can differentiate the studied

species. Those loci with Gst=1.00, were the private alleles occurring in a single species only. These private bands occurred 1-4 in number, in seven

examined *Ulva* species but were absent in *U. clathrata*, *U. linza*, and *U. flexuosa*.

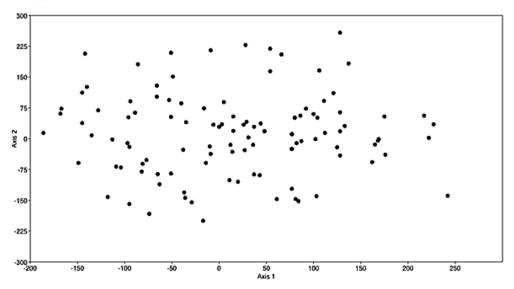


Figure 6: DCA plot of molecular markers showing well-scattered distribution.

Bayesian-based LFMM analysis for ISSR and SCoT markers did not identify any loci which are potentially associated with geographical coordinates. The result of LFMM is presented in Manhattan plot (Fig. 7). This plot shows that, none of 114 loci reached level 2 of the -log10 (p), which indicates significant association of the loci. This may be partly due to movement of these species which bring in contact with other Ulva species and colonies and thereby leading exchanging genes. Occurrence of many common shared alleles may support this hypothesis. Genetic diversity parameters determined Ulva in species are in 5. presented **Table**

We had no replicates for the three species, *Ulva paschima* (=*U. tepida*), *U. chaugulii*, and *U. lactuca*, therefore, polymorphism parameters could not be determined for them. However, among the other seven *Ulva* species, almost a moderate level of genetic polymorphism ranging from 40 (in *U. linza*), to 58% (in *U. clathrata*), was observed. This later species also had the highest value for Nei' gene diversity (0.200).

We found a high degree of genetic identity in the studied species (Table 6), ranging from 0.62 to 0.95. Their genetic distance ranged only from 0.04 to 0.46. It should be emphasized that almost the same degree of genetic distance was obtained in other sequencing-based genetic markers.

Table 4: Discriminating versus migrating loci in *Ulva* species. *Nm=estimate of gene flow from Gst or Gcs. e.g., Nm=0.5 (1 - Gst)/Gst (McDermott and McDonald, 1993).

Locus	Sample	Size Ht	Hs	Gst	Nm*
Locus4	36	0.2441	0.0488	0.8000	0.1250
Locus7	36	0.1189	0.1063	0.1058	4.2247
Locus9	36	0.2014	0.1727	0.1425	3.0099
Locus13	36	0.0862	0.0764	0.1141	3.8816
Locus15	36	0.0527	0.0459	0.1289	3.3789
Locus25	36	0.0862	0.0764	0.1141	3.8816
Locus26	36	0.0432	0.0000	1.0000	0.0000
Locus27	36	0.0264	0.0000	1.0000	0.0000
Locus28	36	0.1800	0.0000	1.0000	0.0000
Locus29	36	0.1800	0.0000	1.0000	0.0000
Locus30	36	0.1800	0.0000	1.0000	0.0000
Locus31	36	0.1800	0.0000	1.0000	0.0000
Locus33	36	0.1800	0.0000	1.0000	0.0000
Locus37	36	0.4984	0.0902	0.8190	0.1105
Locus48	36	0.3413	0.0300	0.9122	0.0481
Locus49	36	0.4340	0.0300	0.9310	0.0371
Locus50	36	0.4502	0.0488	0.8916	0.0608
Locus53	36	0.1684	0.1478	0.1223	3.5885
Locus60	36	0.0776	0.0691	0.1104	4.03309
Locus62	36	0.0432	0.0391	0.0955	4.7347
Locus70	36	0.1189	0.1063	0.1058	4.2247
Locus71	36	0.0952	0.0831	0.1265	3.4527
Locus83	36	0.0264	0.0232	0.1222	3.5912
Locus84	36	0.1800	0.0000	1.0000	0.0000
Locus85	36	0.3413	0.0300	0.9122	0.0481
Locus86	36	0.4340	0.0300	0.9310	0.0371
Locus90	36	0.4720	0.0300	0.9365	0.0339
Locus91	36	0.3561	0.0532	0.8507	0.0878
Locus92	36	0.2011	0.0232	0.8846	0.0652
Locus95	36	0.1800	0.0000	1.0000	0.0000
Locus96	36	0.1800	0.0000	1.0000	0.0000
Locus97	36	0.3413	0.0300	0.9122	0.0481
Locus99	36	0.0264	0.0232	0.1222	3.5912
Locus101	36	0.3200	0.0000	1.0000	0.0000
Locus114	36	0.2087	0.0300	0.8564	0.0838
Mean	36	0.2897	0.1216	0.5804	0.3614

Mantel test with 10000 permutation produced significant association between geographical distance and genetic distance of the studied *Ulva* species (r=0.158, P=0.0008). Therefore, genetic difference of *Ulva* species may increase as their geographical distance increases and so their contact to each other decreases. In this way less degree

of gene exchange may occur among these species.

Species genetic differentiation

AMOVA produced significant genetic difference among studied *Ulva* spp. (PhiPT=0.40, P=0.01). New genetic differentiation parameters also supported Fst result (G'st(Nei)=0.45, G'st(Hed)=0.53, Dest=0.19, P=0.001).

Pair-wise AMOVA revealed that significant genetic difference occurred among some of the species, for example two species of *U. compressa* and *U. clathrata*, differed significantly from three species of *U. paschima* (=*U. tepida*), *U. chaugulii*, and *U. lactuca*. Similarly, *U. flexuosa*, differed

genetically from almost all other studied *Ulva* species (Table 7). AMOVA revealed that about 40% of total genetic variance was due to interspecific genetic difference, while about 60% was due to intraspecific genetic variability.

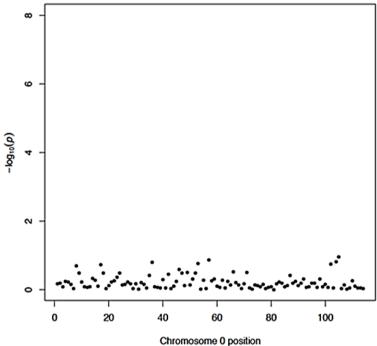


Figure 7: Manhattan plot of LFMM analysis, showing not a single locus reached the significant level of association with environmental coordinates in *Ulva* species.

Table 5: Genetic diversity parameters in *Ulva* spp. Na = mean No. of alleles, Ne = mean No. of. effective alleles, I = Shannon Information Index, He = Nei' gene diversity, uHe = unbiased Nei' gene diversity, %P = polymorphism percentage.

Ulva species	Na	Ne	I	He	uНе	%P
U. compressa	1.096	1.347	0.293	0.198	0.226	52.63%
U. clathrata	1.184	1.339	0.302	0.200	0.219	58.77%
U. linza	0.904	1.260	0.226	0.152	0.183	40.35%
U. flexuosa	1.000	1.286	0.249	0.167	0.190	45.61%
U. prolifera	1.018	1.275	0.243	0.163	0.195	43.86%
U. intestinalis	0.904	1.259	0.227	0.151	0.173	42.11%
U. ohnoi	1.009	1.317	0.273	0.184	0.221	48.25%
U. paschima (=U. tepida)	0.272	1.000	0.000	0.000	0.000	0.00%
U. chaugulii	0.219	1.000	0.000	0.000	0.000	0.00%
U. lactuca	0.263	1.000	0.000	0.000	0.000	0.00%

Table 6: Nei' genetic identity versus genetic distance in *Ulva* species. (Nei's genetic identity, above diagonal and genetic distance, below diagonal). Species 1-10 are: 1. *U. compressa*, 2. *U. clathrata*, 3. *U. linza*, 4. *U. flexuosa*, 5. *U. prolifera*, 6. *U. intestinalis*, 7. *U. ohnoi*, 8. *U. paschima* (=*U. tepida*), 9. *U. chaugulii*, and 10. *U. lactuca*.

Pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.9560	0.9229	0.9133	0.8713	0.9355	0.8538	0.7330	0.7153	0.7115
2	0.0450	****	0.9300	0.9306	0.8909	0.9584	0.8933	0.7420	0.7421	0.7389
3	0.0802	0.0726	****	0.8610	0.8834	0.9223	0.8390	0.7148	0.6777	0.7465
4	0.0901	0.716	0.1496	****	0.8990	0.9336	0.8231	0.6691	0.8886	0.6867
5	0.1378	0.1155	0.1240	0.1065	****	0.8773	0.8086	0.6259	0.6359	0.6825
6	0.0666	0.0425	0.0809	0.0687	0.1309	****	0.8472	0.7027	0.7366	0.7192
7	0.1580	0.1128	0.1755	0.1946	0.2124	0.1658	****	0.7806	0.7603	0.7307
8	0.3106	0.2984	0.3358	0.4018	0.4685	0.3528	0.2477	****	0.6316	0.7105
9	0.3350	0.2982	0.3891	0.3731	0.4527	0.3057	0.2740	0.4595	****	0.7105
10	0.3404	0.3026	0.2923	0.3759	0.3820	0.3296	0.3138	0.3417	0.3417	****

Table 7: Pair-wise AMOVA based on ISSR-SCoT markers in *Ulva* spp. Pop 1-10 are: 1. *U. compressa*, 2. *U. clathrata*, 3. *U. linza*, 4. *U. flexuosa*, 5. *U. prolifera*, 6. *U. intestinalis*, 7. *U. ohnoi*, 8. *U. paschima* (=*U. tepida*), 9. *U. chaugulii*, and 10. *U. lactuca*.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10
Pop1		0.024	0.024	0.100	0.101	0.038	0.266	0.570	0.60	0.601
Pop2	0.589		0.022	0.091	0.093	0.004	0.212	0.515	0.548	0.526
Pop3	0.581	0.300		0.187	0.080	0.008	0.207	0.638	0.687	0.610
Pop4	0.100	0.062	0.024		0.034	0.119	0.299	0.666	0.662	0.652
Pop5	0.104	0.037	0.092	0.338		0.139	0.214	0.684	0.695	0.650
Pop6	0.249	0.370	0.438	0.050	0.053		0.289	0.663	0.640	0.640
Pop7	0.029	0.014	0.084	0.029	0.100	0.025		0.505	0.553	0.570
Pop8	0.034	0.015	0.123	0.031	0.104	0.033	0.110		1.000	1.000
Pop9	0.027	0.011	0.100	0.026	0.102	0.031	0.087	0.094		1.000
Pop10	0.027	0.014	0.080	0.028	0.101	0.019	0.103	0.100	0.097	

Genetic grouping of Ulva species

For identification of genetic groups within tested *Ulva* species, we used both K-value from Evanno's test based on STRUCTURE analysis as well as K-Means clustering of Genodive. Both analyses produced K=2, as two major genetic while K-Means groups, clustering identified five smaller genetic groups based on Bayesian Information Criterion. Therefore, we analyzed our data for both K=2, and K=5 (Fig. 8).

The structure plot of K = 2, differentiated four species of *U. ohnoi*, *U. paschima* (=*U. tepida*), *U. chaugulii*,

and *U. lactuca* from other studied species and placed them in the first major genetic group. Similarly, the STRUCTURE plot of K=5, revealed some degree of genetic variability within the members of each genetic groups. We found many shared common allele combinations (similarly colored segments) in the studied samples.

The NJ phylogenetic tree of *Ulva* species based on the same genetic data (Fig. 5), agreed with STRUCTURE results and separated the studied species in two distinct clades. Some degree of

genetic admixture is evident in this phylogenetic tree too.

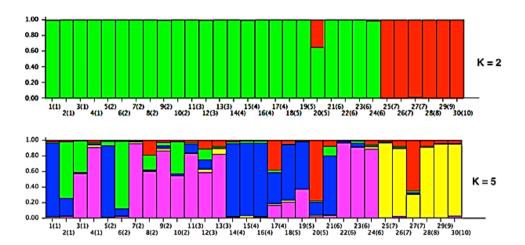


Figure 8: STRUCTURE plot of *Ulva* species based on ISSR and SCoT molecular data according to K = 2 and 5. Species 1-10 are: 1. *U. compressa*, 2. *U. clathrata*, 3. *U. linza*, 4. *U. flexuosa*, 5. *U. prolifera*, 6. *U. intestinalis*, 7. *U. ohnoi*, 8. *U. paschima* (=*U. tepida*), 9. *U. chaugulii*, and 10. *U. lactuca*.

Species relationships based on sequence data

We constructed phylogenetic trees based on available sequences in addition to ITS data sequences and compared these trees with ISSR-SCoT results. For ITS sequences, we obtained a total sequence length of 305 bp, with 82 polymorphic sites among the studied *Ulva* species.

Genetic diversity (Kimura 2parameters distance) was obtained as 0.04. A similar analysis for rbcL gene, produced a DNA with total length of 329 bp, with 48 polymorphic sites among Ulva species. These species genetic distance=0.05. showed Moreover, tufA gene analysis produced a DNA of 329 bp length, with 48 polymorphic sites among *Ulva* species. The genetic distance of 0.05 was obtained for this gene among the species.

Species relationships were revealed genes (Fig. 9). In by these phylogenetic trees (ITS, rbcL, and tufA sequences based phylogenetic trees), the out-group taxa were placed outside of in-group taxa (*Ulva* species), and showed a monophyletic picture of *Ulva* genus. Moreover, these phylogenetic trees showed some degree of genetic admixture among the studied Ulva species, which was in accordance with our ISSR-SCoT results. Similarly, all these trees, showed almost closer affinity between U. ohnoi, U. paschima (=U. tepida), U. chaugulii, and U.lactuca, which was also in agreement with ISSR-SCoT results.

Mantel test between phylogenetic trees and HGT (Horizontal Gene Transfer) of TREX program showed significant association between all obtained phylogenetic trees (p<0.01, HGT=2-5). A consensus tree of all analyses is

presented in Figure 10 which showed a closer affinity between *U. lactuca*, *U.*

chaugulii, U. paschima (=U. tepida), and U. ohnoi.

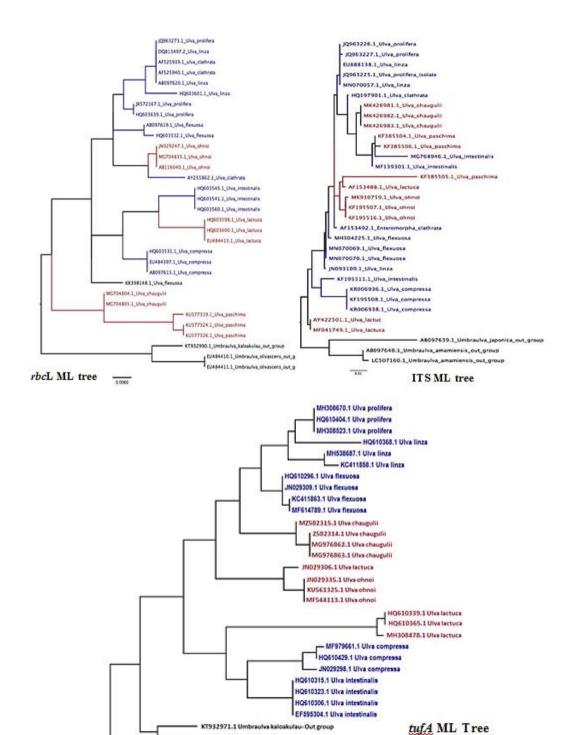


Figure 9: Maximum likelihood phylogenetic groups based on three gene sequences of ITS, rbcL and tufA.

LC507140.1 Umbraulva amamiensis- Out group MT978106.1 Umbraulva japonica- Out group

0.01

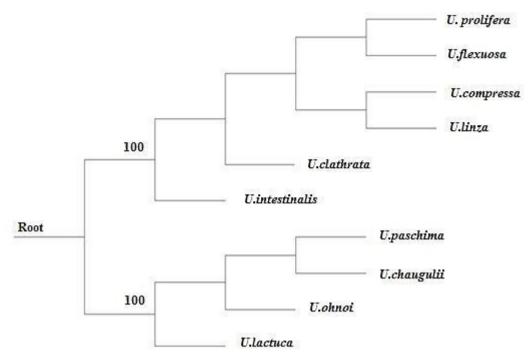


Figure 10: Consensus tree of the phylogenetic trees obtained from ISSR-SCoT, ITS, *rbc*L, and. *tufA* genetic markers. Bootstrap values are given above branches.

Phenotypic plasticity versus genetic differentiation (Pst versus Fst)

Some of the qualitative characters are significantly correlated (p<0.01, Table 8). Almost for all the studied

quantitative characters, Pst values were much higher than Fst values obtained among the same *Ulva* species by molecular data (both ISSR and SCoT markers).

Table 8: Pearson coefficient of correlation among quantitative morphological characters in *Ulva* species studied. (r. value below diagonal, P- value above diagonal).

	Branching type	No. of cell layers	No. of pyrenoids	Cell arrangeme nt in thalli	Cell length	Cell width	Thallus height	Thallus width
Branching type	-	0.089322	0.34562	0.00074411	0.016397	0.096928	0.12612	0.069892
No. of cell layers	0.27217	-	0.11975	0.032889	0.1358	0.43131	0.39763	7.7095E- 08
No. of pyrenoids	0.15309	0.25	-	0.0053167	9.8801E-06	0.14217	4.8635E-05	0.021655
Cell arrangement in thalli	-0.51144	-0.33804	-0.4325	-	0.013765	0.278	0.61899	0.0026463
Cell length	0.37729	0.24	0.63704	-0.38648	-	1.9722E-07	0.022077	0.076048
Cell width	0.26614	-0.12797	0.23627	-0.17576	0.71674		0.51941	0.30543
Thallus height	-0.24591	0.13746	0.5965	0.081069	0.36107	0.10491	-	0.65
Thallus width	-0.28961	-0.73259	-0.36215	0.46268	-0.28371	0.16618	-0.073993	-

The representative Pst versus Fst values of only two species *U. compressa* and

U. clathrata are provided in Tables 9 and 10.

Table 9: Representative Pst versus Fst values in *Ulva compressa*.

Species Characters	U. clati	hrata	U. flexu	iosa	U. pro	lifera	U. intest	inalis	U. oh	noi
U. compressa	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fs
No of pyrenoids	0.7	0.02	0.7	0.02		0.02		0.03	0.23	0.2 6
Cell arrangement in thalli	1		1		1		1		1	
Cell length	0.93		0.05		0.63		0.56		0.72	
Cell width	0.85		0.46		0.19		0.04		0.8	
Thallus height	0.24		0.87		0.1		0.32		0.51	
Thallus width	0.1		0.1		0.11		0.71		0.46	
Species	U. paschii	ma (=U.	U. chaus	aulii	U. lac	rtuca	U. lin	ıza		

Species Characters	U. paschi tepio		U. chai	ıgulii	U. lac	etuca	U. lin	ıza
U. compressa	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst
No of pyrenoid	1	0.26	0.37	0.26	0.37	0.26	1	0.02
Cell arrangement in thalli	1		1		1		1	
Cell length	0.42		0.75		0.08		0.45	
Cell width	0.35		0.88		0.02		0.32	
Thallus height	0.68		0.43		0.79		0.21	
Thallus width	0.43		0.1		0.7		0.1	

Table 10: Representative Pst versus Fst values in *Ulva clathrata*.

Species Characters	U. la	inza	U. flex	uosa	U. pro	lifera	U. intes	tinalis	U. oh	noi
U. clathrata	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst
No of pyrenoids	0.7	0.02	0.27	0.18	0.7	0.02	0.7	0.03	0.6	0.2 6
Cell. arrangement in thalli	1		1				1		1	
Cell length	0.98		0.05		0.99		0.97		0.96	
Cell width	0.74		0.83		0.91		0.75		0.94	
Thallus height	0.19		0.83		0.2		0.16		0.02	
Thallus width	0.1		0.48		0.1		0.71		0.43	

Species Characters	U. pasch tepi	ima (=U. ida)	U. chau	ıgulii	U. lact	tuca	U. comp	pressa
U. clathrata	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst
No of pyrenoids	0.6	0.26	0.51	0.26	0.51	0.26	0.7	0.02
Cell arrangement in thalli			1.00		1.00		1	
Cell length	0.89		0.96		0.84		0.93	
Cell width	0.74		0.96		0.57		0.85	
Thallus height	0.37		0.32		0.002		0.24	
Thallus width	0.91		0.02		0.71		0.1	

A higher value of Pst versus Fst indicates that, some sort of divergent selection has been imposed on *Ulva* species by environmental parameters, which has resulted in morphological divergence of these taxa along with genetic differentiation. These results also indicate that genetic drift and small size of *Ulva* populations for each

species is not affecting morphological differentiation. The CCA of morphological data based on *Ulva* species geographical distribution coordinates (longitude versus latitude), identified morphological characters which diverged under influence of these geographical parameters (Fig. 11).

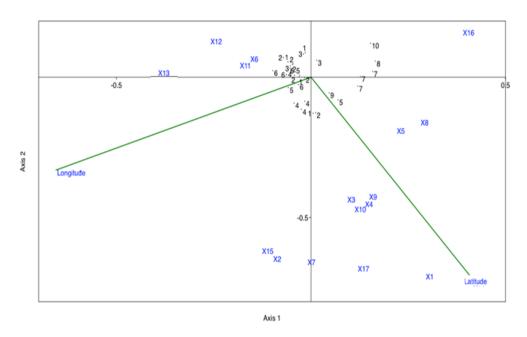


Figure 11: CCA plot of *Ulva* species geographical distribution and their morphological-anatomical features. Species numbers 1-10 are: 1. *U. compressa*, 2. *U. clathrata*, 3. *U. linza*, 4. *U. flexuosa*, 5. *U. prolifera*, 6. *U. intestinalis*, 7. *U. ohnoi*, 8. *U. paschima* (=*U. tepida*), 9. *U. chaugulii* and 10. *U. lactuca*.

Discussion

Species identification and delineation Several studies are conducted to identify the Persian Gulf algae species. For example, Sohrabipour and Rabii (1999) published the first checklist of marine algae and reported 8 species of Ulva from Hormozgan province. Gharanjik (2000)introduced two species of Ulva from Sistan and Baluchestan Province. Sohrabipour et al. (2004) reported 5 species of Ulva from the port of Bandar Lengeh.

Kokabi and Yousefzadi (2015) reported 10 *Ulva* spp. from Iran. None of these studies focused on anatomical or morphological features. The first report of molecular studies on the genus *Ulva* in southern waters of Iran was related to the research of Pirian *et al.* (2016), in which 3 species including *Ulva chaugulii*, *Ulva paschima* and *Ulva ohnoi* as newly recorded for the Persian Gulf waters.

We used a combination of morphological and anatomical features

to identify and delineate *Ulva* species which was also confirmed by ITS sequencing. Both morphological and anatomical features used in the present study correspond to the characteristics reported previously for species description, and in a few features, such as length and width of thallus or length and width of cells, some differences were seen. In U. linza, U. prolifera, U. intestinalis, U. lactuca, U. ohnoi, U. paschima (=U. tepida) and U. chaugulii most of studied features matched with previous references (Bast et al., 2014; Kazi et al., 2016). U. clathrata was relatively more variable compared to previous reports on the same taxon (Børgesen, 1939; Tseng, 1984; Milchakova, 2011). The same holds true for the color of thallus in U. compressa (Blomster et al., 1998).

In case of *U. flexuosa*, collected specimens from two sampling sites (Uf1 and Uf2) differed in branching form as well as the pyrenoid number. It was suggested that U. flexuosa had two subsp. in this region, namely, 1- U. flexuosa subsp. flexuosa, which was almost unbranched and has pyrenoids, and, 2- U. flexuosa subsp. paradoxa with filamentous branches with often 1 to 3 or sometimes more pyrenoids (Sahoo et al., 2003; Mareš et al., 2011; Fleming, 2016). It seems that morphological difference in the studied samples were related to the presence of two subspecies in the Persian Gulf waters. In some populations, differences were observed in shape of thallus, for example: intestinal and twisted thalli, tubular and flat thalli with

obvious knots and filamentous thalli. These differences in shape of thallus or even in color of thallus can be a reflection of difference in physicochemical parameters of seawater (Messyasz and Rybak, 2011; Rybak, 2018). This assumption was then supported by NJ tree of ISSR-SCoT markers (Fig. 4), as these samples were also differentiated by molecular data.

Several studies have shown that a multiple approach using molecular data combined with morphological and anatomical features can resolve taxonomic complexities of the genus Ulva (Sherwood et al., 2000; Shimada et al., 2003; Jiang et al., 2008; Melton 2021). Lopez-Bautista, and example, Favot et al. (2019) based on ITS marker and morphological analysis identified six species of *Ulva*, out of which Ulva flexuosa was a new record for South Portugal. Similarly, Hofman et al. (2010), using morphological features and ITS sequencing reported four distromatic form of Ulva species including U. lactuca, U. rigida, U. compressa and U. australis in the Great Bay Estuarine System (GBES) region of New Hampshire, USA. Previously, only U. lactuca was reported as distromatic species for this area. In using the nrITS2 region Japan, sequencing, Masakiyo and Shimada (2014) identified 164 specimens of the genera Ulva and Umbraulva, and introduced 29 distinct clades composed of a number of known species as well as a number of species with novel sequences. Melton and Lopez-Bautista (2021) studied *Ulva* species from East and Gulf Coast of the United states and identified U. compressa, U. lactuca, U. rigida, and U. torta as the species with a low intraspecific diversity and *Ulva* tepida was introduced as a native species of these regions due to its high intraspecific diversity compared to other species reported in the world. They confirmed nine of 24 taxa previously reported in Gulf of Mexico and Atlantic coast of USA identified 4 new species for the first time based on rbcL and tufA and nuclear (ITS1-5.8S-ITS2) molecular markers. Hanyuda et al. (2016) studied native populations of Ulva australis and based on genetic markers chloroplast, mitochondria and nuclear non-coding region sequences concluded that this species is a temperate species and not distributed predominantly in tropical regions.

Genetic diversity

The present study revealed a high degree intra-specific genetic of variability in the studied *Ulva* species, and AMOVA revealed a higher degree of within species/population variance. This is in agreement with other studies carried out by different molecular markers in Ulva species and populations. For example, Zhao et al., (2011), investigated genetic variation of Ulva prolifera as a causative species of green tides in Yellow Sea, China by inter-simple sequence repeats (ISSR). They reported a lower level of genetic diversity in the floating samples (H=0.1663, I=0.2608), than that of the attached U. prolifera samples

(H=0.2105,I = 0.3346). **AMOVA** produced significant genetic difference and limited gene flow among attached U. prolifera populations. Similarly, Zhao et al. (2010), analyzed genetic variation within and among Ulva australis populations by using ISSR markers. They reported Nei's gene diversity (H) ranging from 0.0729 to 0.1496, and Shannon's information index (I) from 0.1072 to 0.2196. Moreover. **AMOVA** showed greatest variance within populations (68.57%), but much less variance among populations (22.63%)and among areas (8.79%).

Zhang et al. (2014), used EST-SSR markers (microsatellite markers based expressed sequence tag), to investigate diversity and populations' genetic relationship in *Ulva prolifera*. The samples were different in genetic content and were differentiated in **UPGMA** dendrogram. Similarly, Steinhagen et al. (2019), reported as high as 68.57% of total variance in U. australis populations. This High intradiversity population genetic considered to be due to sexual reproduction which is predominant in this species. Moreover, Mantel test produced significant association genetic distance between and geographical variation of the sampling sites, which is in agreement with our study results.

Genetic distance and phylogenetic relationship

Zhao et al. (2011), utilized both ISSR and ITS genetic markers to study

genetic diversity of Ulva prolifera, and reported that the samples differed only in one nucleotide of ITS sequences and therefore, ISSR markers are suitable for genetic diversity studies. In the present study we obtained almost very similar genetic difference of about 0.04-0.05 in molecular markers utilized. However, Li et al. (2016) reported a low level of genetic diversity in U. prolifera populations by microsatellite markers (SSRs). They proposed that the limited origin area of the free-floating biomass and as $ext{sex}$ are reproduction of U. prolifera might be responsible for lower diversity of free floating *U. prolifera*.

Pst versus Fst

Genetic differentiation measured by quantitative traits (Qst or Pst) were compared to genetic differentiation measured by neutral traits (Fst) in the tested *Ulva* species. We obtained a higher value for Pst versus Fst, almost in all *Ulva* species studied and for all quantitative characters. The Pst is taken index for morphological local adaptation through natural selection, but influenced by the environment 2011). If Pst=FST, it (Brommer, indicates that divergence is due to genetic drift; and if Pst > Fst, indicates the role of directional selection (i.e., when one extreme phenotype is favored over the others) among populations; and finally, if Pst< Fst, it indicates that the same phenotypes are favored different populations due to stabilizing selection (Gentili et al., 2018).

In the study of Kang *et al.* (2019), 215 specimens that were

morphologically identified as Ulva.were phylogenetically studied using two chloroplast markers cpDNA tufA and nuclear nuDNA ITS. They reported 193 specimens as 9 species of *Ulva*, 14 specimens as Blidingia spp., and 8 specimens as unidentified. The results of this study showed that the largest genetic distance between species for tufA and ITS were 11% and 9.8%, respectively. They showed that in combining the two markers, marker is a more efficient marker in species delimitation than the ITS region. Because ITS phylogeny had not been able to provide high resolution in several species groups such as U. ohnoi with other closely related species. detailed morphological However, assessments along with molecular analysis using chloroplasts and nuclear markers, especially tufA, can be applied as an efficient and reliable approach for accurate delineation and identification of *Ulva* species.

Based on multiple approach data, we identified 10 *Ulva* species in the coastal areas of the Persian Gulf and Oman Sea. A low to moderate level of genetic diversity occurred in these species and AMOVA revealed that these species differ genetically from each other and exhibit a higher degree of intraspecific genetic variability. The phylogenetic trees obtained from different molecular markers produced similar species grouping and relationship.

We may, therefore, suggest that, due to global warming, anthropogenic effects (such as eutrophication, some local environmental/or tidal conditions), some adaptive changes have occurred in *Ulva* genus in the Persian Gulf and Oman Sea, which needs more detailed studies in the future.

Acknowledgment

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