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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, *rbc*L, 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 high growth rates or efficient reproductive alternatives that promote their dispersal abilities so that they can become invasive (Liu *et al*., 2015).

The systematics of the genus *Ulva* remains challenging due to its morphological simplicity (Gao *et al*., 2016), widespread morphological plasticity (Loughnane *et al*., 2008), intraspecific variation, interspecific morphological overlaps and the 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 morpho-anatomical features with molecular features is very effective for identification of these species. Different molecular markers are utilized to differentiate *Ulva* species and to investigate their genetic variability as well as species relationships. For 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 *rbc*L 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 *rbc*L and *tufA* as suitable barcoding gene in species-level identification.

Hayden and Waaland (2004) studied species diversity and identified 12 *Ulva* species by ITS DNA and *rbc*L gene. Ismail and Mohamed (2017) investigated morphological, genetic and biochemical variation within four *Ulva* species collected from Eastern Harbor, Alexandria. In another study using *tuf*A marker, 9 genetic species groups were determined and *U. ohnoi* was identified as a new species from Tunisia (Miladi *et al*., 2018). Various molecular markers are used to identify algae, including *rbc*L 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 genetic diversity 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*, *rbc*L 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 thalli (X5), form of life (X6), proliferation (X7), thallus apex form (X8), color of thalli (X9), cell shape (X10), cell length (X11), cell width (X12), chloroplast shape (X13), chloroplast position (X14), thallus height (X15), thallus width (X16), presence or absence of calcium carbonate crystals (X17) were analyzed and recorded. These 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: Species name, code and location 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

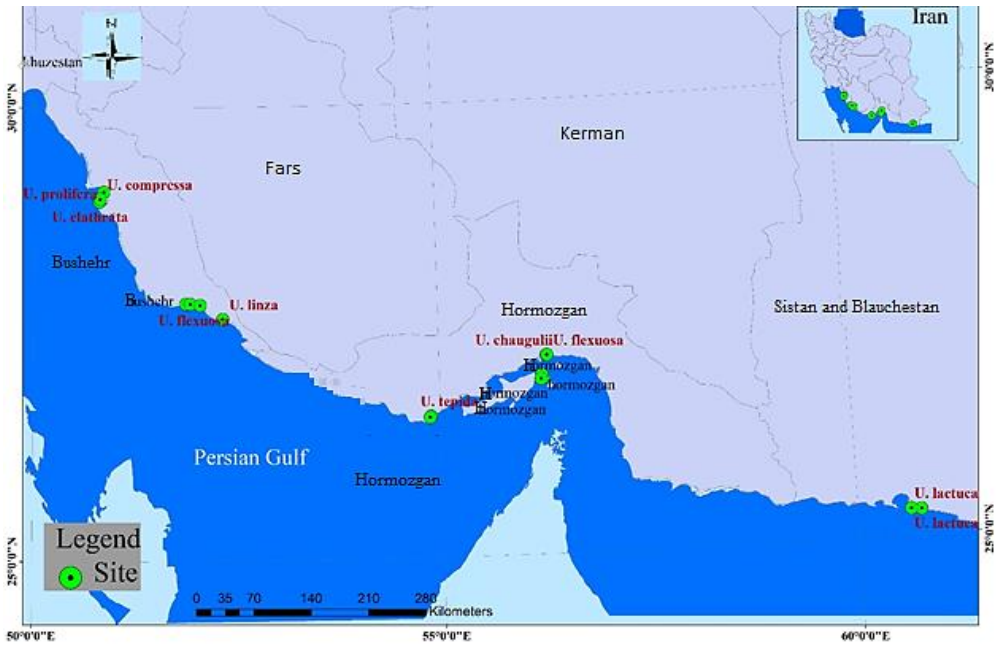

Figure 1: Study area. Sampling locations and species collected are marked on the map.

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 ng 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 GelRed™ 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 and identify taxonomically differentiating characters. CCA (Canonical 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 *rbc*L, *tufA* and ITS sequences from NCBI.

Species	Accession number for *rbc*L gene	Accession number for *tufA* gene	Accession 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 markers for genetic diversity was checked by 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 coordinates was determined by Bayesian 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 Rosenberg, 1999). For 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^2_b and $\sigma^2 w$ are phenotypic variances between and within populations respectively, C is an estimate of the proportion of total variance due to additive genetic effects among populations, and h^2 is 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, 2018). Species relationships were studied by constructing phylogenetic trees based on ISSR, SCoT and ITS data. For ISSR and SCoT, the bands obtained were treated as binary characteristics (presence=1, absence=0), and were used to estimate Nei genetic distance among the species. Different clustering methods were applied to 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	*Ulva 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**.**

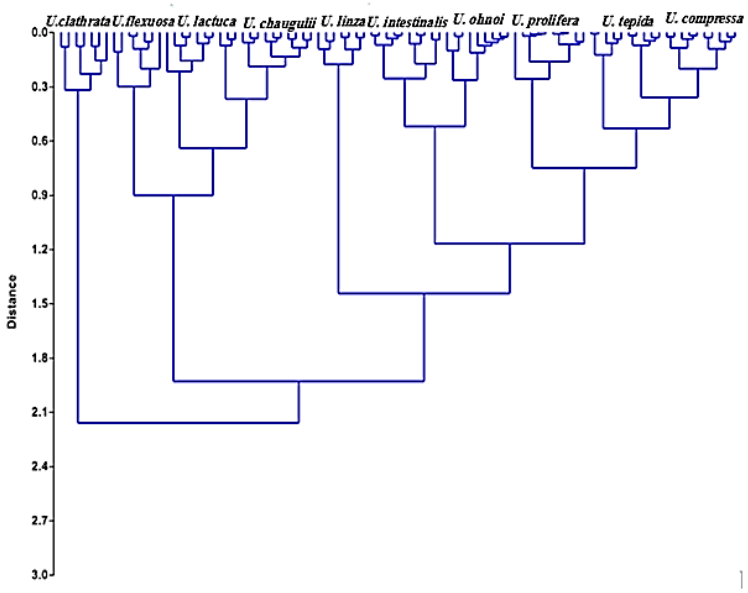

Figure 2: WARD dendrogram of *Ulva* based on morphological and anatomical features.

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**.**

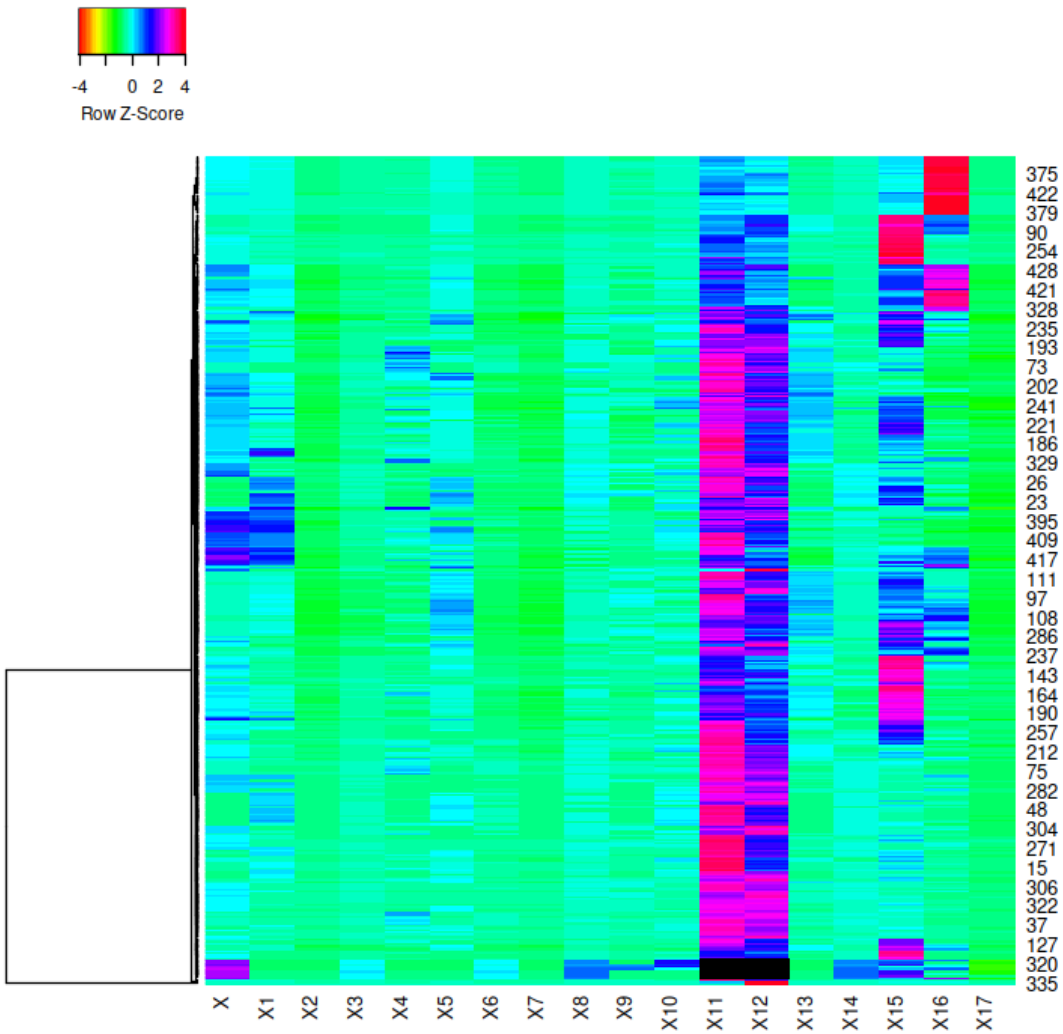

Figure 3: Heat map of *Ulva* species based on standardized morphological characters. X1- thallus form, X2- branching type, X3- number of cell layers, X4- number of pyrenoids, X5- cell arrangement in thalli, X6- form of life, X7- proliferation, X8- thallus apex form, X9- color of thalli, X10- cell shape, X11- cell length (µm), X12- cell width (µm), 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 thallus width (X16), were under influence of the species longitude distribution, while the third one, thallus height (X15), was related to latitude distribution of *Ulva* species**.**

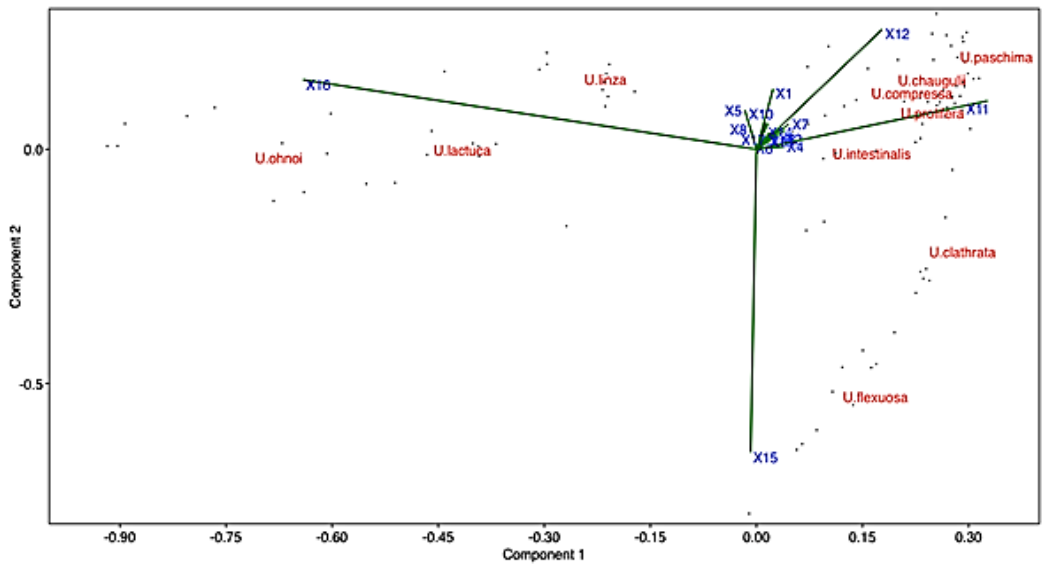

Figure 4: PCA-biplot of *Ulva* species based on morphological and anatomical data. X1- thallus form, X2- branching type, X3- number of cell layers, X4- number of pyrenoids, X5- cell arrangement in thalli, X6- form of life, X7- proliferation, X8- thallus apex form, X9- color of thalli, X10- cell shape, X11- cell length (µm), X12- cell width (µm), 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**.**

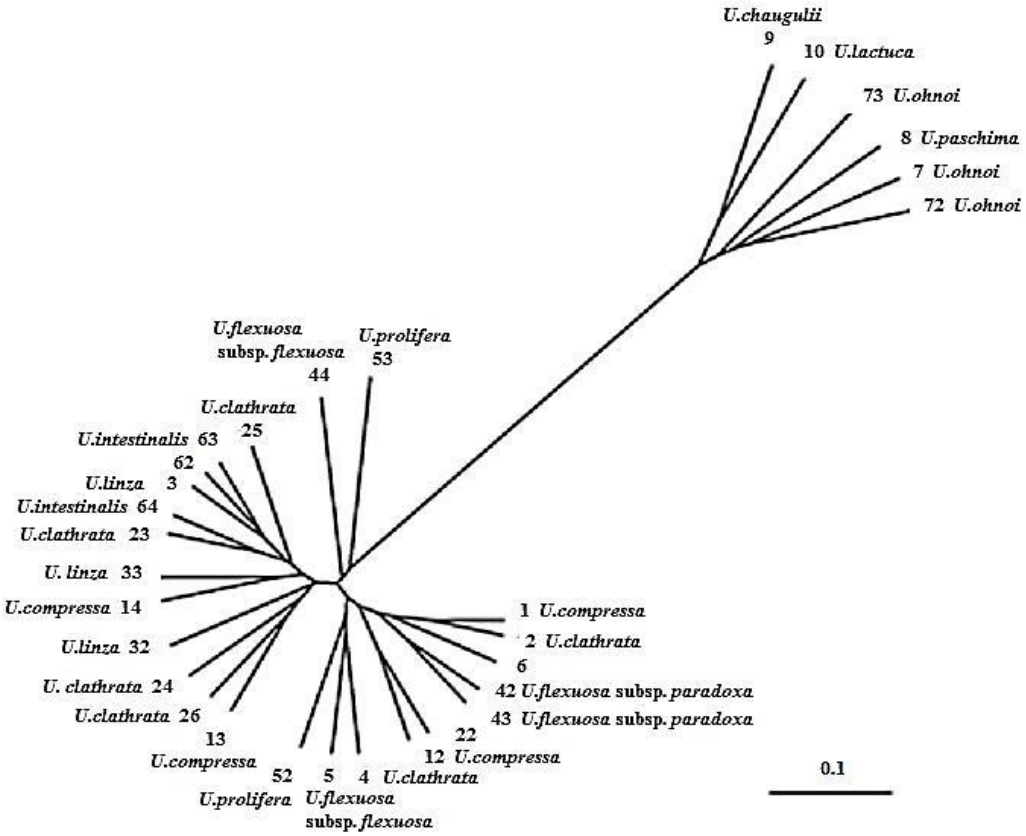

Figure 5: NJ tree of *Ulva* species based on ISSR-SCoT data, separating these taxa in two major clades.

Genetic diversity

DCA (Dentrented Correspondence Analysis) (Fig. 6), showed well scattered distribution of the loci 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***.**

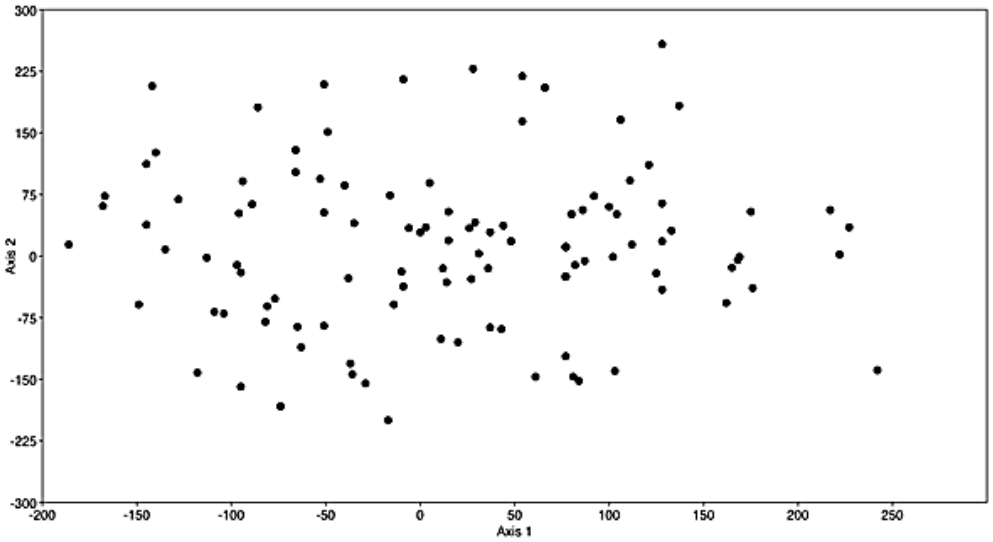

Figure 6: DCA plot of molecular markers showing well-scattered distribution.

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 to exchanging genes. Occurrence of many common shared alleles may support this hypothesis.Genetic diversity parameters determined in *Ulva* species are presented in Table 5.

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.

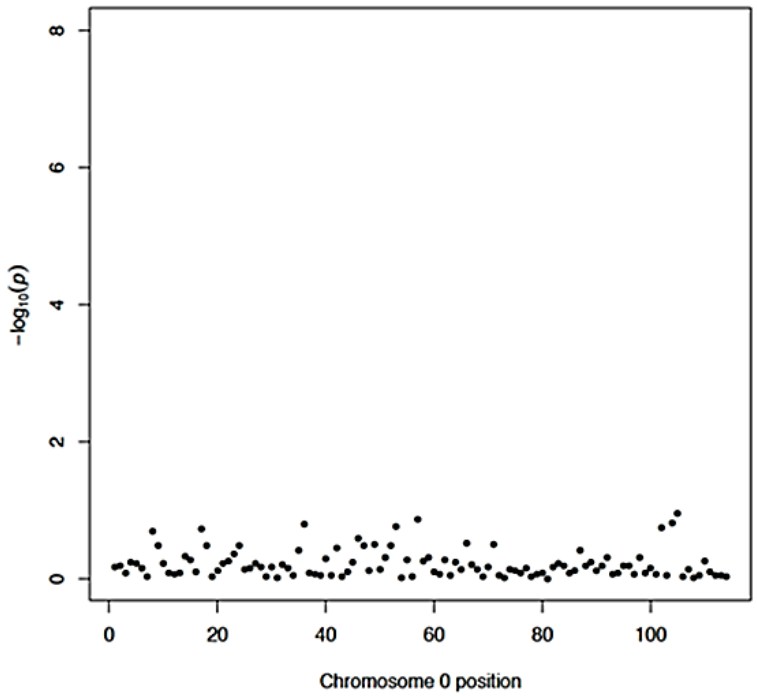

Figure 7: Manhattan plot of LFMM analysis, showing not a single locus reached the significant level of association with environmental coordinates in *Ulva* species.

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**	**uHe**	**%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 groups, while K-Means 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.

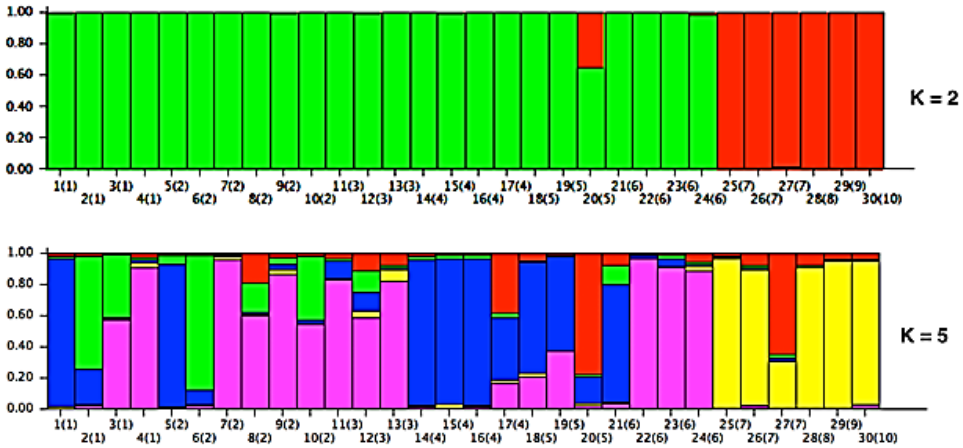

Figure 8: STRUCTURE plot of *Ulva* species based on ISSR and SCoT molecular data according to K =2 and 5. Species 1-10 are: 1. *U. compressa*, 2. *U. clathrata*, 3. *U. linza*, 4. *U. flexuosa*, 5. *U. prolifera*, 6. *U. intestinalis*, 7. *U. ohnoi*, 8. *U. paschima* (=*U. tepida*), 9. *U. chaugulii*, 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 2-parameters distance) was obtained as 0.04. A similar analysis for *rbc*L gene, produced a DNA with total length of 329 bp, with 48 polymorphic sites among *Ulva* species. These species showed genetic distance=0.05. 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 by these genes (Fig. 9). In all phylogenetic trees (ITS, *rbc*L, 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*.

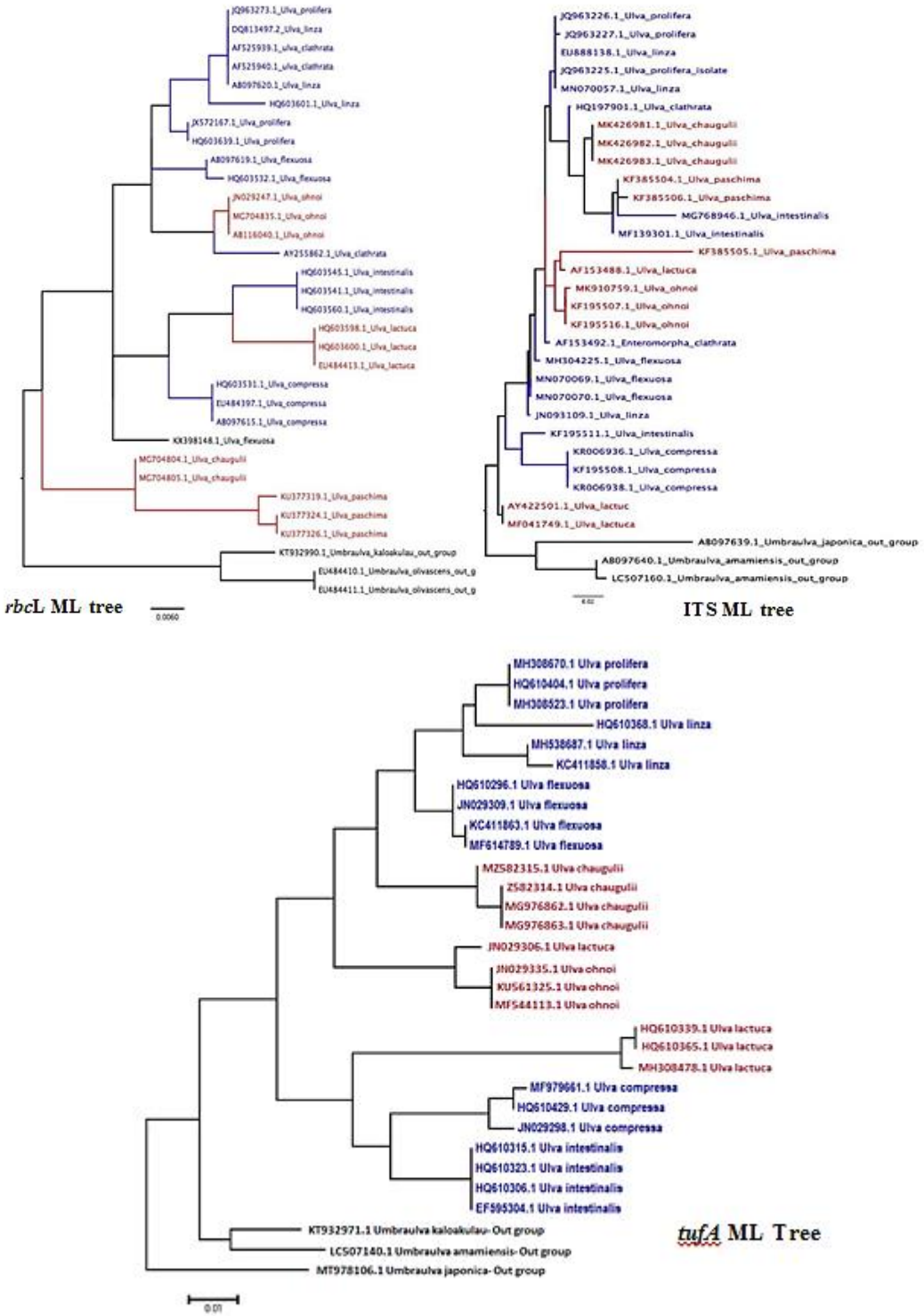

Figure 9: Maximum likelihood phylogenetic groups based on three gene sequences of ITS, *rbc*L and *tufA*.

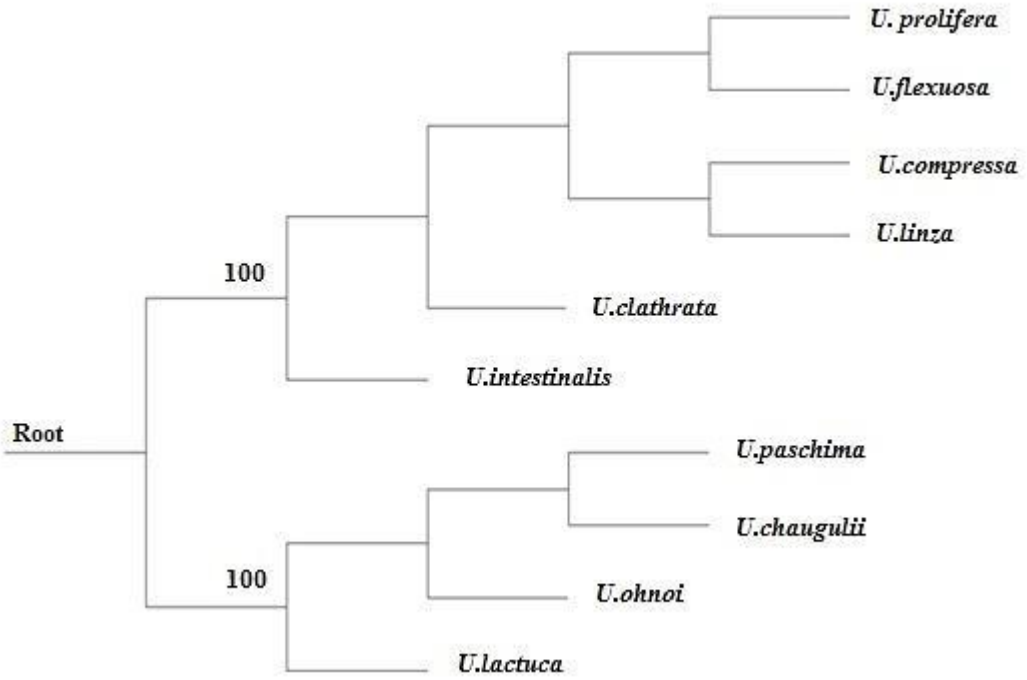

Figure 10: Consensus tree of the phylogenetic trees obtained from ISSR-SCoT, ITS, *rbc*L, and. *tufA* genetic markers. Bootstrap values are given above branches.

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 arrangement 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. clathrata***		***U. flexuosa***		***U. prolifera***		***U. intestinalis***		***U. ohnoi***	
U. compressa	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst	Pst	Fst
No of pyrenoids	0.7	0.02	0.7	0.02	--	0.02	--	0.03	0.23	0.26
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 Characters	***U. paschima* (=*U. tepida*)**		***U. chaugulii***		***U. lactuca***		***U. linza***	
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. linza***		***U. flexuosa***		***U. prolifera***		***U. intestinalis***		***U. ohnoi***	
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	0.26
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. paschima* (=*U. tepida*)**		***U. chaugulii***		***U. lactuca***		***U. compressa***	
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).

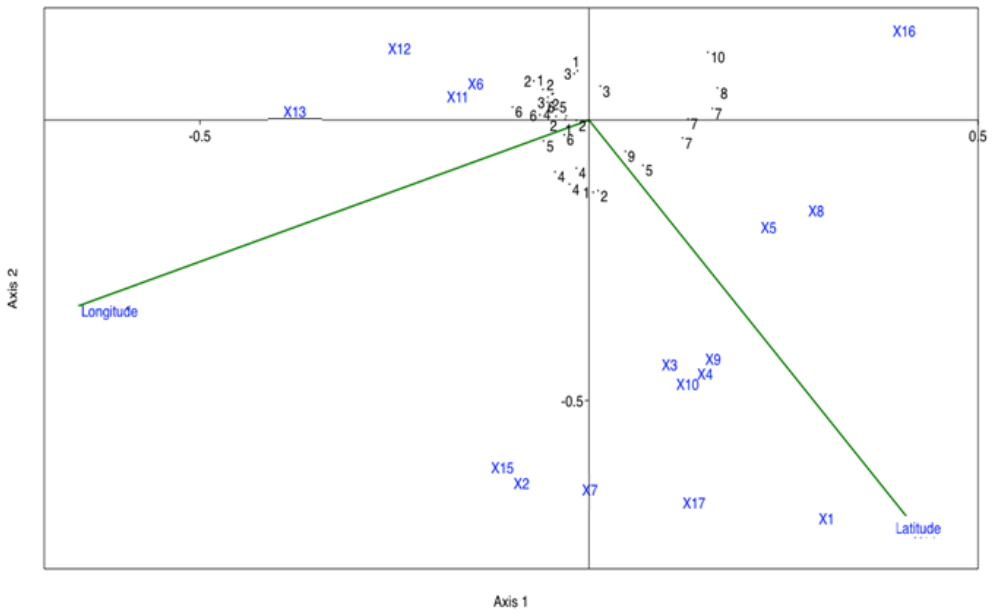

Figure 11: CCA plot of *Ulva* species geographical distribution and their morphological-anatomical features. Species numbers 1-10 are: 1. *U. compressa*, 2. *U. clathrata*, 3. *U. linza*, 4. *U. flexuosa*, 5. *U. prolifera*, 6. *U. intestinalis*, 7. *U. ohnoi*, 8. *U. paschima* (=*U. tepida*), 9. *U.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

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 previously reported 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. compress*a (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 1-2 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 physico-chemical 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 and Lopez-Bautista, 2021). For 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 Japan, using the nrITS2 region 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 and identified 4 new species for the first time based on *rbc*L 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 of 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 of intra-specific genetic 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 the 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 genetic diversity and populations' 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 intra-population genetic diversity was considered to be due to sexual reproduction which is predominant in this species. Moreover, Mantel test produced significant association between genetic distance and geographical variation of the sampling sites, which is in agreement with our study result**s.**

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 all 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 asexual 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 as index for morphological local adaptation through natural selection, but influenced by the environment (Brommer, 2011). If Pst=FST, it 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 in 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, *tufA* 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. However, detailed morphological 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.

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References

Azizi, R., Motallebi Moghanjoughi, A.A., Nazemi, M., Sharif Ruhani, M. and Afsharnasab, M., 2019. Isolation and identification of antibacterial steroid compounds from *Ulva fasciata* in the Persian Gulf. *Iranian Journal of Fisheries Sciences* 19(**5**), 2384 -2393. https://doi.org/10.22092/ijfs.2020.122254

Bast, F., John, A.A. and Bhushan, S., 2014. Strong endemism of bloom-forming tubular *Ulva* in Indian west coast, with description of *Ulva paschima* Sp Nov (Ulvales, Chlorophyta). *PLoS ONE*, 9(**10**), e109295. https://doi.org/10.1371/journal.pone.0109295.

Bermejo, R., Heesch, S., Monagail, M.M., O'Donnell, M., Daly, E., Wilkes, R.J. and Morrison, L., 2019. Spatial and temporal variability of biomass and composition of green tides in Ireland. *Harmful Algae*, 81, 94–105. https://doi.org/10.1016/j.hal.2018.11.015.

Blomster, J., Maggs C.A. and Stanhope, M.J., 1998. Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. *Journal of Phycology*, 34(**2**), 319–340. https://doi.org/10.1046/j.1529-8817.1998.340319.x.

Børgesen, F., 1939. Marine Algae from the Iranian Gulf. In: *Danish Scientific Investigation in Iran*, part 1. Ejnar Munksgaard, Copenhagen, Denmark.

Brommer, J.E., 2011. Whither PST? The approximation of QST by PST in evolutionary and conservation biology. *Journal of Evolutionary Biology*, 24(**6**), 1160–1168. https://10.1111/j.1420-9101.2011.02268.x.

Cai, C., Gu, K., Zhao, H., Steinhagen, S., He, P. and Wichard, T., 2021. Screening and verification of extranuclear genetic markers in green tide algae from the Yellow Sea. *PLoS ONE*, 16(**6**), e0250968. https://doi.org/10.1371/journal.pone.0250968.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. and Leon, C., 2010. Validation of the ITS_2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE*, 5(**1**), 1-8. https://doi.org/10.1371/journal.pone.0008613.

Collard, B.C.Y. 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*, 27(**1**), 86–93. https://doi.org/10.1007/s11105-008-0060-5.

Da Silva, S.B. and Da Silva, A., 2018. Pstat: An R package to assess population differentiation in phenotypic traits. *The R Journal*, 10(**1**), 447–454. https://doi.org/10.32614/rj-2018-010.

Evanno, G., Regnaut, S. and Goudet, J., 2005. Blackwell Publishing, Ltd. Detecting the number. of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14(**8**), 2611–2620. https://doi.org/10.1111/j.1365-194x.2005.02553.x.

Farasat, M., Khavari-Nejad, R.A., Nabavi, S.M., Namjooyan, F., 2013. Antioxidant Properties of two Edible Green Seaweeds From Northern Coasts of the Persian Gulf. *Jundishapur Journal of Natural Pharmaceutical Products*, 8(1), 47-52.

Favot, G., Engelen, A.H., Cunha, M.E. and Serrão, M.E.A., 2019. Identification of *Ulva* sp. grown in multitrophic aquaculture systems. *Journal of Aquaculture and Fisheries*, 3, 024. https://doi.org/10.24966/aaf-5523/100024.

Flagella, M.M., Andreakis, N., Hiraoka, M., Verlaque, M. and Buia, M.C., 2010. Identification of cryptic *Ulva* species (Chlorophyta, Ulvales) transported by ballast water. *Journal of Biological Research*, 13, 47–57.

Fleming, T., 2016. *Analysis of Freshwater and marine Ulva flexuosa in Southern California.* Master of Science Thesis in Biological Sciences, California State University, San Marcos, USA, 28 P.

Frichot, E., Schoville, S.D., Bouchard, G. and François, O., 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular Biology and Evolution*, 30(**7**), 1687–1699. https://doi.org/10.1093/molbev/mst063.

Gao, G., Zhong, Z., Zhou, X. and Xu, J., 2016. Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: A laboratory experiment. *Harmful Algae*, 59, 51–58. https://doi.org/10.1016/j.hal.2016.09.004.

Gentili, R., Solari, A., Diekmann, M., Duprè, C., Serafina Monti, G., Armiraglio, S., Assini, S. and Citterio. S., 2018. Genetic differentiation, local adaptation and phenotypic plasticity in fragmented populations of a rare forest herb. *PeerJ*, 6, e4929. https://doi.org/10.7717/peerj.4929.

Gharanjik, B.M., 2000. The marine algae of the Sistan and Baluchestan province, Iran. *Iranian Journal of Fisheries Sciences*, 2(**2**), 57–70.

Guiry, M.D. and Guiry, G.M., 2019. AlgaeBase. World-wide electronic publication. National University of Ireland Galway. http://wwwalgaebase.org.

Guiry, M.D. and Guiry, G.M., 2022. *AlgaeBase.* World-wide electronic publication, National University of

Ireland, Galway. http://www.algaebase.org.

Hammer, Ø., Harper, D.A.T. and Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(**1**), art. 4, 9pp., 178kb. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Hanyuda, T., Heesch, S., Nelson, W., Sutherland, J., Arai, S., Boo, S.M. and Kawai, H., 2016. Genetic diversity and biogeography of native and introduced populations of *Ulva pertusa* (Ulvales, Chlorophyta). *Phycological Research*, 64(**2**), 102–109. https://doi.org/10.1111/pre.12123.

Hayden, H.S. and Waaland, J.R., 2004. A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific. *Phycologia*, 43(**4**), 364–382. https://doi.org/10.2216/i0031-8884-43-4-364-1.

Hiraoka, M. and Higa, M., 2016. Novel distribution pattern between coexisting sexual and obligate asexual variants of the true estuarine macroalga *Ulva prolifera*. *Ecology and Evolution*, 6(**11**), 3658–3671. https://doi.org/10.1002/ece3.2149.

Hofman, L.C., Nettleton, J.C., Neefusi, C.D. and Mathieson, A.C., 2010. Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay Estuarine System (Atlantic, USA): introduced and indigenous distromatic species. *European Journal of Phycology*, 45(**3**), 230–239. https://doi.org/1080/09670261003746201.

Hughey, J.F., Maggs, C.A., Mineur, F., Jarvis, C., Miller, K.A., Shabaka, S.H. and Gabrielson, P.W., 2019. Genetic analysis of the Linnaean *Ulva lactuca* (Ulvales, Chlorophyta) Holotype and related type specimens reveals name misapplications, unexpected origins, and new synonymies. *Journal of Phycology*, 55(**3**), 503–508. https://doi.org/10.1111/jpy.12860.

Ismail, M.M. and Mohamed, S.E., 2017. Differentiation between some *Ulva* spp by morphological, genetic and biochemical analyses. *Journal of Genetics and Breeding*, 21(**3**), 360–367. https://doi.org/10.18699/VJ17.253.

Jiang, P., Wang, J., Cui, Y., Li, Y., Lin, H. and Qin, S., 2008. Molecular phylogenetic analysis of attached Ulvaceae species and free-floating *Enteromorpha* from Qingdao coasts in 2007. *Chinese Journal of Oceanology and Limnology*, 26(**3**), 276–279. https://doi.org/10.1007/s00343-008-0276-0.

Kang, J.W. and Chung, I.K., 2017. The effects of eutrophication and acidification on the ecophysiology of *Ulva pertusa* Kjellman. *Journal of Applied Phycology*, 29(**5**), 2675–2683. https://doi.org/10.1007/s10811-017-1087-5.

Kang, J.H., Jang, J.E., Kim, J.H., Byeon, S.Y., Kim, S., Choi, S.K., Kang, Y.H., Park, S.R. and Lee, H.J., 2019. Species composition, diversity, and distribution of the genus *Ulva* along the coast of Jeju Island, Korea based on molecular phylogenetic analysis. *PLoS ONE*, 14(**7**), e0219958. https://doi.org/10.1371/journal.pone.0219958.

Kawai, H., Shimada, S., Hanyuda, T., Suzuki, T. and Gamagori, C.O., 2007. Species diversity and seasonal changes of dominant *Ulva* species (Ulvales, Ulvophyceae) in Mikawa Bay, Japan, deduced from ITS2 rDNA region sequences. *Algae*, 22(**3**), 221–228. https://doi.org/10.4490/algae.2007.22.3.221.

Kazi, M.A., Kavale, M.G. and Singh, V.V., 2016. Morphological and molecular characterization of Ulva chaugulii sp nov *U. lactuca* and *U. ohnoi* (Ulvophyceae Chlorophyta) from India. *Phycologia*, 55(**1**), 45–54. https://doi.org/10.2216/15-11.1.

Kirkendale, L., Gary, W.S. and Winberg, P., 2013. A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism. *Journal of Phycology*, 49(**1**), 69–81. https://doi.org/10.1111/jpy.12016.

Kokabi, M. and Yousefzadi, M. 2015. Checklist of the marine macroalgae of Iran. *Botanica Marina*, 58(**4**), 307–320. https://doi.org/10.1515/bot-2015-0001.

Li, Y., Huang, H.J., Li, H., Liu, J. and Yang, W., 2016. Genetic diversity of *Ulva prolifera* population in Qingdao coastal water during the green algal blooms revealed by microsatellite. *Marine Pollution Bulletin*, 111(**1–2**), 237–246. https://doi.org/10.1016/j.marpolbul.2016.07.001.

Liu, Q., Yu, R.C., Yan, T., Zhang, Q.C. and Zhou, M.J., 2015. Laboratory study on the life history of blood-forming *Ulva prolifera* in the Yellow Sea. *Estuarine Coastal Marine Science*, 163(Part A), 82–88. https://doi.org/10.1016/j.ecss.2014.08.011.

Loughnane, C.J., Mcivor, L.M., Rindi, F., Stengel, D.B. and Guiry, M.D., 2008. Morphology *rbc*L phylogeny and distribution of distromatic *Ulva* (Ulvophyceae Chlorophyta) in Ireland and southern Britain. *Phycologia*, 47(**4**), 416–429. https://doi.org/10.2216/ph07-61.1.

Mareš, J., Leskinen, E., Sitkowska, M., Skácelová, O. and Blomster, J., 2011. True identity of the European freshwater *Ulva* (Chlorophyta, Ulvophyceae) revealed by a combined molecular and morphological approach. *Journal of Phycology*, 47(**5**), 1177–1192. https://doi.org/10.1111/j.1529-8817.2011.01048.x.

Masakiyo, Y. and Shimada, S., 2014. Species diversity of the genus *Ulva* (Ulvophyceae, Chlorophyta) in Japanese waters with special reference to *Ulva tepida* Masakiyo et S. Shimada sp. nov. *Bulletin of the*

National Museum of Nature and Science, Series B. Botany, 40(**1**), 1–13.

McDermott, J.M. and McDonald, B.A., 1993. Gene flow in plant pathosystems. *Annual Reviews of Phytopathology*, 31, 353–373. https://doi.org/10.1146/annurev.py.31.090193.002033.

Messyasz, B. and Rybak, A., 2011. Abiotic factors affecting the development of *Ulva* sp (Ulvophyceae; Chlorophyta) in freshwater ecosystems. Aquatic Ecology, 45, 75–87. https://doi.org/10.1007/s10452-010-9333-9.

Meirmans, P.G. and Van Tienderen, P.H., 2004. GENOTYPE and GENODİVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, 4(**4**), 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x.

Melton III, J.T. and Lopez-Bautista, J. M., 2021. Diversity of the green macroalgal genus *Ulva* (Ulvophyceae, Chlorophyta) from the East and Gulf Coast of the United states based on molecular data. *Journal of Phycology*, 57(**2**), 551–568. https://doi.org/10.1111/jpy.13120.

Miladi, R., Manghisi, A., Minicante, S.A., Genovese, G., Abdelkafi, S. and Morabito, M., 2018. A DNA barcoding survey of *Ulva* (Chlorophyta), Tunisia and Italy reveals the presence of the overlooked alien *U. ohnoi*. *Cryptogamie Algologie*, 39(**1**), 85–107. https://doi.org/10.7872/crya/v39.iss1.2018.85.

Milchakova, N., 2011. *Marine Plants of the Black Sea, an illustrated field Guide*. DigiPrint Press, Sevastopol, Russia,144 P. https://doi.org/10.21072/978-966-02-5801-3.

Peakall, R. and Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(**1**), 288–295. https:/doi.org/10.1111/j.1471-8286.2005.01155.x.

Pirian, K., Piri, K., Sohrabipour, J., Tamadoni Jahromi, S. and Blomster, J., 2016. Molecular and morphological characterisation of *Ulva chaugulii U. paschima* and *U. ohnoi* (Ulvophyceae) from the Persian Gulf Iran. *Botanica Marina*, 59(**2–3**), 147–158. https://doi.org/10.1515/bot-2016-0009.

Podani, J., 2000. *Introduction to the exploration of Multivariate biological data*. Backhury Publishers, Leiden, Netherlands,407 P.

Pritchard, J.K. and Rosenberg, N.A., 1999. Use of unlinked genetic markers to detect population stratification in association studies. *The American Journal of Human Genetics*, 65(**1**), 220–228. https://doi.org/10.1086/302449.

Rybak, A.S., 2018. Species of *Ulva* (Ulvophyceae, Chlorophyta) as indicators of salinity. *Ecological*

Indicators, 85, 253–261. https://doi.org/10.1016/j.ecolind.2017.10.061.

Sahoo, D., Sahu, N., and Sahoo, D., 2003. A critical survey of seaweeds diversity of Chilika Lake, India. *Algae*, 18(**1**), 1–12. https://doi.org/10.4490/ALGAE.2003.18.1.001.

Shakouri, A. and Balouch, G.M., 2016. The effects of nitrate and phosphate on growth of algae, *Ulva rigida*. *Iranian Journal of Fisheries Sciences*, 19 (**1**), 59 -66. doi.org/10.22092/ijfs.2018.117843

Sheidai, M., Seif, E., Nouroozi, M. and Noormohammadi, Z., 2012. Cytogenetic and molecular diversity of *Cirsium arvense* (Asteraceae) populations in Iran. *Journal of Japanese Botany*, 87(**3**), 193–205.

Sheidai, M., Ziaee, S., Farahani, F., Talebi, S. M., Noormohammadi, Z. and Hasheminejad Ahangarani Farahani, Y., 2014. Infra-specific genetic and morphological diversityin *Linum album* (Linaceae). *Biologia*, 69, 32-39. https://doi.org/10.2478/s11756-013-0281-4.

Shelar, P.S., Reddy, V.K., Shelar, G.S., Kavita, M., Kumar, G.P. and Reddy, V.S., 2012. Medicinal value of seaweeds and its application- a review. *Continental Journal of Pharmacology and toxicology Research*, 5(**2**), 1–22.

Shen, S., Li, Y., Wu, X. and Ding, L., 2010. Sequences and phylogeny analysis of *rbc*L gene in marine Chlorophyta. *Journal of Ocean University of China*, 9, 145–150. https://doi.org/10.1007/s11802-010-0145-2.

Sherwood, A.R., Garbary, D.J. and Sheath, R.G., 2000. Assessing the phylogenetic position of the Prasiolales (Chlorophyta) using *rbc*L and 18S rRNA gene sequence data. *Phycologia*, 39(**2**), 139–146. https://doi.org/10.2216/i0031-8884-39-2-139.1.

Shimada, S., Hiraoka, M., Nabata, S., Lima, M. and Masuda, M., 2003. Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae) with special reference to the free-floating *Ulva*. *Psychological Research*, 51(**2**), 99–108. https://doi.org/10.1046/j.1440-1835.2003.00296.x.

Sohrabipour, J. and Rabii, R., 1999. A list of marine algae of seashores of Persian Gulf and Oman Sea in the Hormozgan Province. *Iranina Journal of Botany*, 8(1), 131–162.

Sohrabipour, J., Nejadsatari, T., Assadi, M. and Rebei, R., 2004. The marine algae of the southern coast of Iran, Persian Gulf, Lengeh area. *Iranian Journal of Botany*, 10(**2**), 83–93.

Steinhagen, S., Karez, R. and Weinberger, F., 2019. Cryptic alien and lost species: molecular diversity of *Ulva sensulato* along the German coasts of the North and Baltic Seas. *European Journal of Phycology*, 54(**3**), 466–483. https://doi.org/10.1080/09670262.2019.1597925.

Tseng, C.K., 1984. *Common Seaweeds of China*. Science Press, Beijing, China, 316p. https://doi.org/10.

Weising, K., Nybom, H., Wolff, K. and Kahl, G., 2005. *DNA fingerprinting in plants; principles, methods, and applications*, 2nd edition. CRC Press, Boca Rayton USA. https://doi.org/**10**

Zhao, J., Jiang, P., Li, N., Wang, J.F., Liu, Z.Y. and Qin, S., 2010. Analysis of genetic variation within and among *Ulva pertusa* (Ulvaceae, Chlorophyta) populations using ISSR markers. *Chinese Science Bulletin*, 55(**8**), 705−711. https://doi.org/10.1007/s11434-009-0715-0.

Zhao, J., Jiang, P., Liu, Zh., Wang, J., Cui, Y. and Qin, S., 2011. Genetic variation of *Ulva* (*Enteromorpha*) *prolifera* (Ulvales, Chlorophyta)—the causative species of the green tides in the Yellow Sea, China. *Journal of Applied Phycology*, 23(**2**), 227–233. https://doi.org/10.1007/s10811-010-9563-1.

Zhang, L., Wang, G., Liu, C.C.S. and Liu, T., 2014. Development and utility of EST-SSR markers in *Ulva prolifera* of the South Yellow Sea. *Acta Oceanologica Sinica*, 33(**10**), 105－113. https://doi.org/10.1007/s13131-014-0545-z.