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The ecology and morphological variation of *Hypnea* (red algae) species in Iran

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Key words: *Hypnea*, variations, tetrasporange, special branches or branchlets.

Abstract

Morphological and ecological variations and inter-specific relationships of a red algal genus, *Hypnea* were investigated based on 34 populations belong to 7 species from Persian Gulf and Oman sea coasts. cluster analyses, principal component analysis and Canonical Correspondence Analysis were done. Statistical analyses indicated that characters such as special branches or branchlets, Position of tetrasporange and algae size had the most important role as diagnostic characters in intera-genus variation. In general 2 major clusters were formed. The first major cluster comprised of *H. boergesenii*. Second major cluster contains two subclusters. First subcluster is comprised of *H. ecklonii*, *H. charoides, H. valentiae, H. musciformis* and *H. cornuta*. The second subcluster is comprised of *H. pannosa*. Results showed that all invironmental factors except the average of annual water vapor pressure had effect on the morphological distribution of *Hypnea* populations.

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Introduction

Hypnea Lamouroux (1813) is a important red alga commercially cultivated in various parts of the world for the production of carrageenan. This genus encompasses some of the most abundant intertidal seaweeds of tropical coasts of Indo-Pacific region (Geraldino *et al.*, 2010). The genus consists of about 53 species world-wide (Guiry *et al*, 2006). In Iran, there are 9 species of *Hypnea* as listed in " Atlas of the sea algae of Persian Gulf and Oman sea coasts" (Gharanjik & Rohani-Ghadikolai, 2009) that we collected 7 species of this genus from Persian Gulf and Oman sea coasts (these species listed in the Table 1).

Table 1. *Hypnea* populations studied for morphometry and their localities These populations were collected from eleven localities of Persian Gulf and Oman sea coasts (Fig.1).

species	Locality
H. ecklonii	Qeshm: Shibderaz, Bandar Lenge: Dowlat park
H. pannosa	Qeshm: Shibderaz, municipality; Bandar Lenge: Dowlat park; Kong; Tis; Remin
H. cornuta	Qeshm: Shibderaz, municipality; Bandar Lenge: Dowlat park; Kong
H. boergesenii	Qeshm: Shibderaz, Zeyton park, municipality; Tis; Remin; Kachu
H. charoides	Qeshm: Shibderaz, Zeyton park; Bandar Lenge: Dowlat park, near wharf; Tis; Beris
H. valentiae	Qeshm: Shibderaz; Kong; Tis; Remin; Kachu; Beris
H. musciformis	Qeshm: Shibderaz; Bandar Abbas: Dowlat park; Tis; Remin; Kachu; Beris

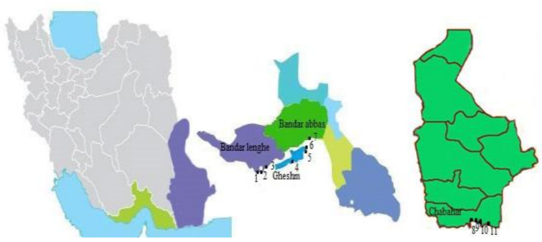

Fig. 1. Distribution map and localities of *Hypnea* populations studied. Locality codes:1- Bandar Lenge, near wharf; 2- Bandar Lenge, Dowlat park; 3- Kong; 4- Qeshm, Shibderaz; 5- Qeshm, Zeyton park; 6- Qeshm, municipality; 7- Bandar Abbas, Dowlat park; 8- Tis; 9- remin; 10- kachu 11-be.

Agard (1852) divided this genus into three sections on the basis of their habits: the first known as spinuligerae including *H. charoides* Lamouroux, *H. valentiae* (Turner) Montagne, *H. ecklonii* Suhr, *H. musciformis* (Wulfen) Lamouroux and *H. cornuta* (Kuetzing) J. Agardh. The second section was called virgatae including *H. boergesenii* Tanaka. pulvinatae was the last section including *H. pannosa* J. Agardh. These species were the most common species of red algae in Persian Gulf and Oman sea coasts that were found in suptidal zone of this area during late autumn to late spring but these species had a significant decrease in diversity and biomass in Iranian coasts in recent years.

This genus exhibits considerable morphological variation leading to taxonomic confusion. For example, there are species such as *H. charoides* and *H. valentiae* which are almost similar to each other. There are controversies among the phycologists regarding identification of these Hypnea species. Some introduced these species as complex charoides-valentiae (Yamagishi & Masuda, 1997; Lewman-omont, 1997). Phenotypic plasticity exhibited by Hypnea species for adaptation to habitat environmental changes.

Understanding the habitats in which these taxa survive is necessary for a variety of reasons. Habitat typification provides an opportunity to understand more fully the ecological processes influencing the distribution and survival of native populations of *Hypnea* species in this region. Also unfortunately *Hypnea* species is now endangered species in the Iranian coasts of Persian Gulf and Oman sea. Abundance of these species were found in these coasts in previous years but these species had a significant decrease in diversity and biomass in recent years. Otherwise, their environmental variables and factors have not been investigated rigorously for this region.

Hypnea species have been poorly studied ecologically and taxonomically in Iran. Available information has usually been anecdotal or observational without the necessary testing of parameters of habitat structure that predict species distributions. The present study reports the ecology and morphometry analysis among populations of this genus for the first time in Iran.

Material and methods

Algea materials

Thirty-four populations of seven species of *Hypnea* were analyzed by morphometry (Table 1) in this investigation.

Samples from the field were transported live back to the laboratory in sterilized seawater, cleaned, and sorted carefully under a dissecting microscope. Materials for morphological observations were preserved in 4% formaldehyde-seawater.

Morphometry

In total, 20 morphological characters (quantitative and qualitative) were used for morphometry (Table 2). These characters were coded as binary and multistate characters.

Table 2. Morphological characters and their coding.

no.	Character (Abbreviation for graphics)	Codes/units
1	algae color	1: purplish red, 2: greenish red, 3: dark red
2	algae size	1: larger than 50 cm,2: from 3-50 cm, 3: less than 3 cm
3	algae texture	1: cartilaginous, 2: membranous
4	Algae habits	1: caespitose, 2: intricate-caespitose, 3: cushionlike
5	main axis clearness	1: percurrent, 2: not percurrent
6	main axis shape	1: terete to subterete, 2: flattened
7	branching pattern	1: alternate-spiral, 2: alternate-distichous 3:irregular
8	abundance of lateral branches	1: profusely branched, 2: sparsely branched
9	special branches or branchlets	1: hamate branches, 2: stellate branches,3: spines branches, 5: antler branches and 4: none of them
10	direction of branching	1: wide angles- larger than 90^o, 2: narrow angles- less than 90^o
11	basal system	1: without discoid holdfast, 2: with discoid holdfast
12	small cells around axial cell	1: presence of these cells, 2: absence of these cells
13	lenticular thickenings	1: presence of these cells, 2: absence of these cells
14	size of medullary cells	1: isometric cells, 2: none isometric cells
15	width of main axes	1:larger than 1mm in diameter, 2: less than 1 mm in diameter
16	Number of cortical layers	1: 1 layer, 2: more than 1 layer
17	Width of branchlets	1:larger than 500 µm in diameter, 2: less than 500 µm in diameter
18	Position of tetrasporange sorus	1: apice of branchlets, 2: middle of branchlets 3: in angles of branchlets
19	Branchlets apex	1: acuminate apex, 2: not acuminate apex
20	Position of branchlets	1: all of branches, 2: only in midlle of branches

Ecology assay

For each of the locality, environmental parameters including average of annual air tempraturere, average of annual water vapor pressure, average of annual relative humidityity, average of annual rainfall, average of annual wind speed, and average of annual sunshine hours were measured.

Data analyses

UPGMA (Unweighted Paired Group using Average mean) and Ward clustering methods with 100 times bootstrapping as well as principal components analysis (PCA), principal coordinate analysis (PCoA) were performed to group the plants specimens based on morphological characters. The Euclidean distance and Gower distance were used for clustering methods. Cophenetic correlation was determined to check the fit of dendrograms to the original distance matrix (Podani 2000). A Canonical Correspondence Analysis (CCA) was performed on the two sets of variables, the first set containing morphometric variables weighed in the principal components (PC), and the second set composed of environmental variables. Data analyses were performed by using PAST ver. 2.17 (Hamer *et al.*, 2012).

Results

Morphometry

UPGMA and Ward clustering methods produced almost similar results and therefore UPGMA tree is only discussed. In general, two major clusters were formed in UPGMA tree with 100% bootstrap value. The first major cluster comprised of *H. boergesenii.* Second major cluster contains two subclusters. First subcluster is comprised of *H. ecklonii*, *H. charoides, H. valentiae, H. musciformis* and *H. cornuta* . The second subcluster is comprised of *H. pannosa* (Fig. 2).

PCA and PCoA plots obtained also produced similar results. Therefore, only PCA plot is presented and discussed here. PCA biplot (fig. 3) and PCA loadings were obtained to identify the most important morphological characters differentiating the studied populations. PCA analysis showed that, the first 3 components comprised about 67 % of total variation. In the first factor with about 30 % of total variance, special branches or branchlets possessed the highest positive correlation. In the second factor with about 21.5 % of total variance, Position of tetrasporange sorus possessed the highest positive correlation and in third factor with 15 % of total variance, algae size possessed the highest positive correlation (Table 3).

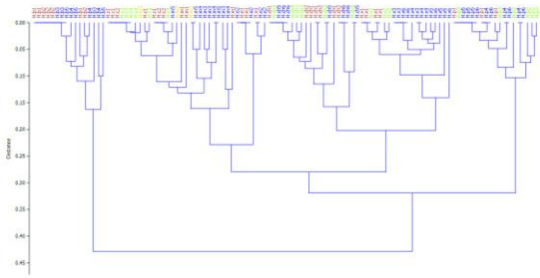

Fig. 2. UPGMA tree of morphometric data in 34 populations of *Hypnea*.

Table 3. Contribution of the variables to components 1 (PC1), 2 (PC2) and 3 (PC3**).**

Variables	PC1	PC2	PC3
algae color	-0.17043	-0.17204	0.14587
algae size	0.15604	0.23142	0.55196
algae texture	0.010019	0.24711	0.22595
Algae habits	-0.066671	0.31442	0.38199
main axis clearness	-0.067	-0.063128	-0.18156
main axis shape	0.041833	-0.017977	-0.040058
branching pattern	-0.025345	-0.049376	0.058119
abundance of lateral branches	-0.06045	-0.080154	-0.16387
special branches or branchlets	0.6209	0.47893	-0.25087
Direction of branching	-0.022518	0.018731	-0.22036
basal system	0.011983	-0.26504	0.14946
small cells around axial cell	-0.14796	0.080592	0.14467
lenticular thickenings	-0.15888	0.058007	0.17791
size of medullary cells	0.14945	0.19316	-0.24066
width of main axes	0.034732	-0.061798	-0.039512
Number of cortical layers	0.16686	-0.067311	-0.15604
Width of branchlets	0.019893	-0.06045	-0.03752
Position of tetrasporange sorus	-0.63914	0.58877	-0.30083
Apice of branchlets	0.079485	0.18535	0.17678
Position of branchlets	0.14724	0.059991	-0.13745

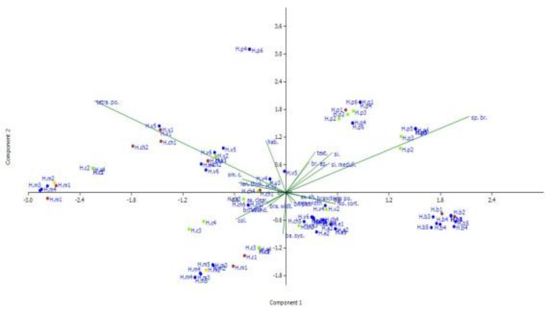

Fig. 3. PCA biplot of the populations of *Hypnea*.

PCA biplot showed that morphological characters like number of cortical layers and Position of branchlets separated *H. boergesinii* from *H. valentiae.* Similarity characters such as size, texture, size of medullary cells, special branches or branchlets, branchlet apex and habit separated *H. pannosa* from the other species. Also direction of branching, aboundance of lateral branches and main axis clearness separated *H. charoides* and *H. valentiae* from each others (Fig. 3).

Ecology assay

CCA combining ordination of morphological traits on environmental variables (Fig. 4) confirmed that that variation in the Cluster 1 (*H. boergesinii*) was explained by average of annual relative humidity. Morphological variation in first subcluster of cluster 2 (species of *H. charoides*, *H. valentiae*, *H. cornuta*, *H. musciformis* and *H. ecklonii*) was related to average of annual air tempraturere, average of annual rainfall, average of annual wind speed and average of annual sunshine hours. average of annual rainfall is the main factor which explained morphological variation in second subcluster of cluster 2 (*H. pannosa*). And average of annual water vapor pressure had a little effect on the morphological distribution of *Hypnea* populations.

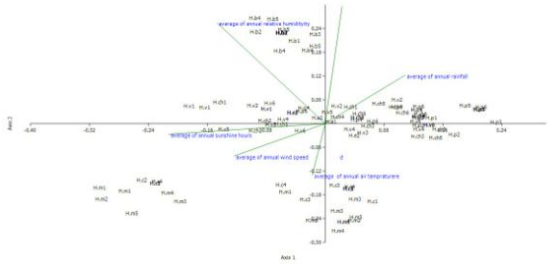

Fig. 4. CCA ordination diagram indicating the influence of environmental factors on the morphological distribution of *Hypnea* populations.

Discussion

There are no studies for comparison on morphological and ecological variations in this genus. But in comparison with other morphological studies, the same separating characters were obtained and the results are consistent with the different identification keys. Such as, direction of branching, aboundance of lateral branches and main axis clearness were the separating characters of *H. charoides* and *H. valentiae* (Womersley, 1994). Also characters such as size, texture, size of medullary cells, special branches or branchlets and habit were separating characters of *H. pannosa* (Yamagishi & Masuda, 1997; Lewmanomont, 1997).

We had a very good selection of morphological characters. We tried to select characters that are more diverse among species and less affected by habitat conditions.

Some population such as populations of *H. cornuta*, *H. charoides*, and *H. musciformis* showed high morphological variations as even individuals of these populations were placed in different place of subcluster. In population of these species of each erea (Gheshm, Bandar Lenge, Bandar Abbas and Chabahar) were seen morphological variations but these variatins is not enough to produce ecotypes.

The results of UPGMA tree are in consistent with three sections that Agard (1852) introduced. So that *H. boergesinii* and *H. pannosa* separated from other species in clusters. In addition, despite the high morphological similarities of *H. charoides* and *H. valentiae*, we were able to recognize these species with three characters that mentioned previously and these two species relatively well separated in UPGMA tree.

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