EFFECT OF ENVIRONMENTAL FACTORS ON MORPHOLOGICAL VARIATIONS OF HYPNEA SPECIES FROM OMAN SEA

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Morphological and ecological variations and inter-specific relationships of a red algal genus, *Hypnea* were investigated in 15 populations belong to 5 species from Oman Sea coasts. In this study, 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 habit are the most important diagnostic characters in intera-genus variation. In general two major clusters were formed. The first major cluster comprised *H. boergesenii*. Second major cluster contains two subclusters. The first subcluster comprised of *H. charoides*, *H. valentiae* and *H. musciformis*. The second subcluster comprised of *H. pannosa*. Results showed that variation in the cluster one was explained by average of annual salinity and morphological variation and in cluster two was related to average of annual PH, water temperature and impurities.

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Key words: *Hypnea*; morphological variations; tetrasporange; algae habit; pH; temperature; salinity

تاثیر فاکتورهای محیطی بر تنوع ریختی گونه های هیپنه آ

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تنوع ریختی و بوم شناسی و ارتباط درون گونه‌ای جلبک قرمز جنس هیپنه آ بر روی ۱۵ جمعیت متعلق به ۵ گونه از دریای عمان در این پژوهش بررسی شده است. در این پژوهش تجزیه و تحلیل خوشه‌ای، تجزیه مولفه های اصلی و تحلیل روابط بوم شناسی گونه ها انجام گرفت. تجزیه و تحلیل آماری نشان داد که صفاتی مثل وجود انشعابات خاص، موقعیت تتراسپورانژها و نحوه اتصال جلبک به زیستگاه جلبک بیشترین نقش را به عنوان صفات تشخیصی جدا کننده گونه ها در این جنس داشته اند. دو خوشه اصلی تشکیل شد که H. boergesenii در خوشه اول قرار گرفت و خوشه دوم شامل دو زیر خوشه بود که در زیر خوشه اول گونه های H. charoides ، H. valentiae و H. musciformis و در زیرخوشه دوم H. pannosa قرار گرفت. نتایج نشان داد که تنوع در زیرخوشه اول به میانگین شوری سالیانه و در زیر خوشه دوم به میانگین سالیانه اسیدیته، دمای آب و میانگین ناخالصی ها ی آن شامل مواد ارگانیک و غیر ارگانیک مرتبط است.

INTRODUCTION

Hypnea Lamouroux (1813) is a genus of red algae consists of about 53 species world-wide (Guiry *et al*. 2006) and 9 species from Iran (Gharanjik & Rohani-Ghadikolai 2009; John & Al-Thani 2014; Gharanjik 2000; Sohrabipour & Rabiei 1999, 2004, 2007). This genus is the most common red algae in the intertidal and subtidal regions of the Persian Gulf and Oman Sea coasts. This algae is important red algae commercially cultivated in various parts of the world for the production of carrageenan (Mshigeni and Chapman 1994). Agardh (1852) divided this genus into three sections based on their habits: the first known as spinuligerae including *H. charoides* Lamouroux, *H.*

valentiae **(**Turner) Montagne and *H. musciformis* (Wulfen) Lamouroux. The second section was called virgatae including *H. boergesenii* Tanaka. and *Pulvinatae* was the last section including *H. pannosa* J. Agardh (Agardh 1852).

Understanding the habitats in which these taxa survive is necessary for a variety of reasons. Habitat typification provides an opportunity to better understanding of the ecological processes influencing the distribution and survival of native populations of *Hypnea* species. Because of the direct effect of environmental factors on diversity and biomass of the algae, it is necessary to study these factors. Otherwise, their environmental variables and factors have not been previously investigated rigorously for the Oman Sea coasts.

The present study reports morphological variation and effect of environmental factors on distribution of this genus for the first time in Iran.

MATERIALS AND METHODS

Fifteen populations of five species of *Hypnea* were analyzed in this investigation (table 1).

These populations were collected from four localities of Oman sea coasts on different seasons of year 2010.

Samples from the field were transported fresh to the laboratory then washed with sterilized seawater and cleaned carefully under a dissecting microscope (Olympus, SZH model) and sorted. Materials for morphological observations were preserved in 4% formaldehyde-seawater and seawater solution.

In total, 20 morphological characters (quantitative and qualitative) were used for morphometry (table 2).

At least two randomly selected algae from each population were used for obtaining morphological data. These characters were coded as binary and multistate characters.

Environmental parameters including average of annual salinity, PH, water temperature and impurities (including organic and nonorganic materials) were measured (table 3).

The quantitative morphological characters were divided in to discrete groups and coded along with qualitative characters as binary and multistate characters. Unweighted paired groups using average mean (UPGMA) 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 *& al*. 2012).

RESULT AND DISCUSSION

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. charoides*, *H. valentiae* and *H. musciformis*. The second subcluster is comprised of *H. pannosa* (fig. 2). The results of UPGMA tree do agree with three sections that Agardh (1852) introduced in which *H. boergesinii* and *H. pannosa* are 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 were mentioned previously and these two species are relatively well separated in UPGMA tree.

PCA and PCoA plots obtained, also produced similar results. Therefore, only PCA plot is presented and discussed here. PCA biplot (figure not given) 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 70.68 % of total variation. Special branches or branchlets as the first factor (with about 35.9 % of total variance) possessed the highest positive correlation. Position of tetrasporange sorus as the second factor (with about 22.7 % of total variance) and algae habit as the third factor (with 14.53% of total variance) followed the next highest positive correlation (table 4). The present morphological analyses of *Hypnea* species is almost in agreement with different identification keys. So that diagnostic characters in these analyses are the same characters that different scientists reported previously. In the present morphometry, *H. charoides* and *H. valentiae* were very similar species so that morphological data shown high morphological similarity of these species. Because of the high morphological similarity of *H. charoides* and *H. valentiae*, genetic diversity was studied and identification of these species was confirmed.

CCA combining ordination of morphological traits on environmental variables (fig. 3) confirmed that variation in the cluster 1 (*H. boergesinii*) was explained by average of annual salinity and morphological

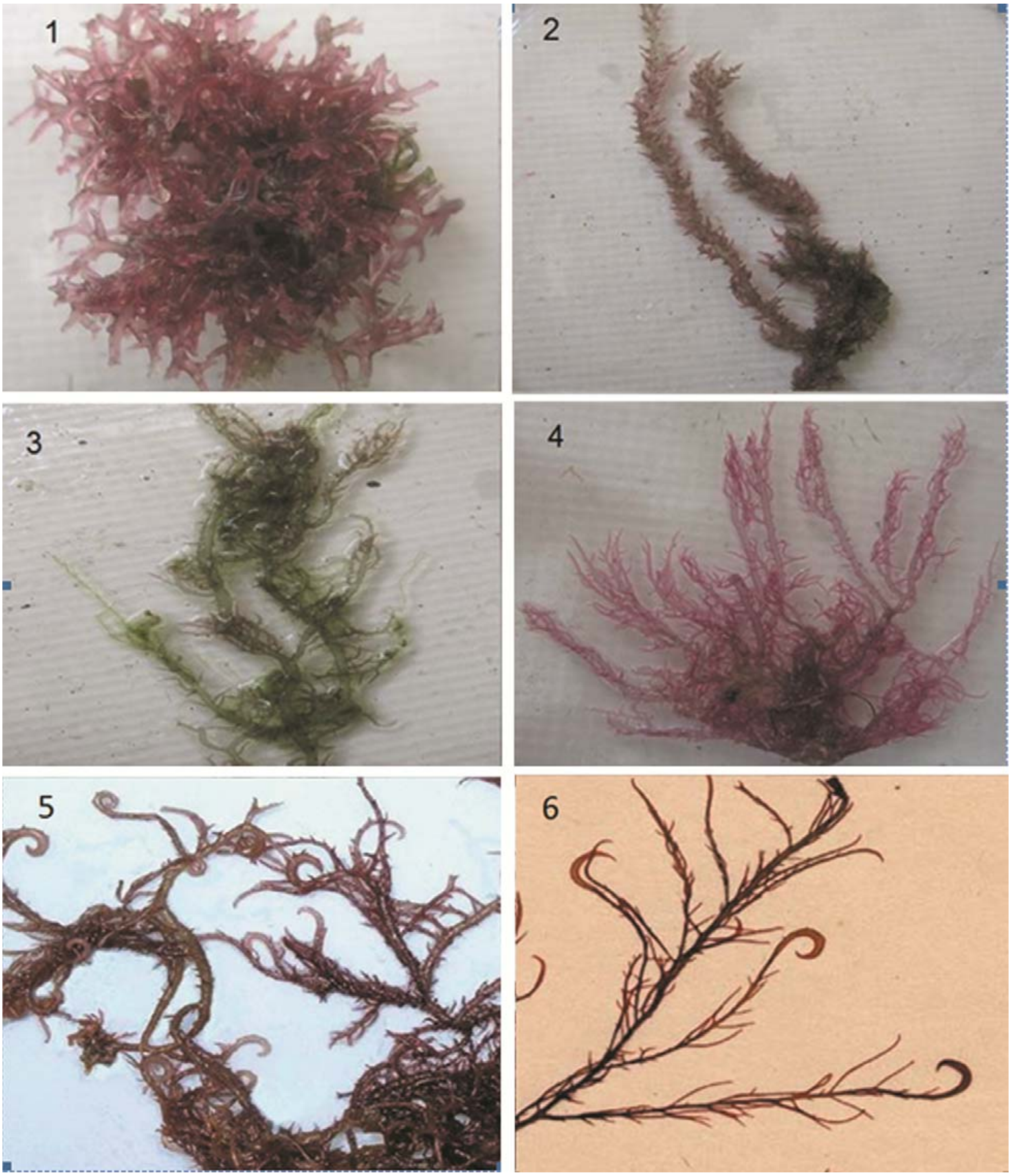

Fig. 1. Images of *Hypnea* species studied: 1, *H. pannosa*; 2, *H. boergesenii*; 3, *H. charoide;* 4, *H. valentiae*; 5 & 6, *H. musciformis*.

Table 1. Hypnea populations studied and their localities.

No. Of Station	Location	Longitude	Latitude	list of species
1	Tis	60º 38' 52"	25º 16' 57"	*H. pannosa*, *H. boergesenii*, *H. charoides*, *H. valentiae*, *H. musciformis*
2	Remin	60º 45' 6"	25º 14' 8"	*H. pannosa*, *H. boergesenii*, *H. valentiae*, *H. musciformis*
3	Kachu	60º 50' 51"	25º 14' 32"	*H. boergesenii*, *H. charoides*, *H. valentiae*, *H. musciformis*
4	Beris	61º 11' 06"	25º 07' 06"	*H. valentiae*, *H. musciformis*

Table 2. Morphological characters and their coding.

No.	Character	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 90º, 2: narrow angles- less than 90º
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

Table 3. Invironmental factors and their measurements.

Invironmental factors	Tis	Remin	Kachu	Beris
average of annual air temperature(º C)	27.07	27	27.04	27.2
average of annual salinity (gr/lit)	36.9	36.73	36.75	36.73
average of annual PH	8.10	7.90	8.70	8.02
average of annual water temperature(º C)	29.4	31.02	31.2	32.2
average of annual impurities(mgr/lit)	35456	35677	35642	35543

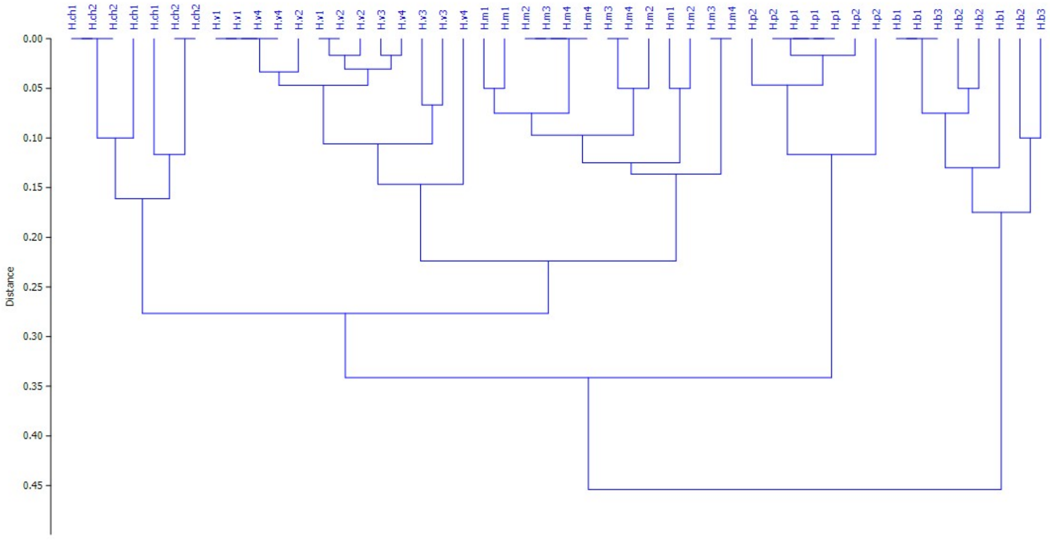

Fig. 2. UPGMA tree of morphometric data in 15 populations of *Hypnea*. (H.ch: *H. charoides*, H.v: *H. valentiae*, H.m: *H. musciformis*, H.p: *H. pannosa* and H. b: *H. boergesenii*).

variation; in cluster 2 (*H. charoides*, *H. valentiae*, *H. musciformis* and *H. pannosa*) was related to average of annual pH, water temperature and impurities.

There are no similar studies to compare these results with previously published literatures, but some scientists explored the morphology and phylogeny of the genus based on *rbcL*, *cox1* and *psaA* sequences (Geraldino *& al.* 2006 & 2010; Rodrigues 2011). In comparison with other morphological studies, the same separating characters had been used as the key characters.

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Table 4. Contribution of the variables to components 1 (PC1), 2 (PC2) and 3 (PC3).

Variables	PC1	PC2	PC3
algae color	-0.23609	-0.066151	-0.083324
algae size	0.088942	0.23367	0.11268
algae texture	0.099247	0.17648	0.21146
Algae habits	-0.063265	0.30044	0.48123
main axis clearness	-0.14843	-0.17091	0.15067
main axis shape	0.040153	-0.034848	-0.075193
branching pattern	2.2376E-20	-2.1066E-18	2.1276E-17
abundance of lateral branches	-0.12203	-0.16503	0.0083998
special branches or branchlets	0.79358	0.25321	-0.038141
Direction of branching	0.0073496	-0.064691	0.091724
basal system	-0.091877	-0.2006	-0.26399
small cells around axial cell	-0.16251	0.12396	0.26977
lenticular thickenings	-0.16251	0.12396	0.26977
size of medullary cells	0.16441	0.08421	-0.014219
width of main axes	0.038481	-0.032391	-0.081267
Number of cortical layers	0.16251	-0.12396	-0.26977
Width of branchlets	0.038481	-0.032391	-0.081267
Position of tetrasporange sorus	-0.32861	0.75032	-0.51628
Apice of branchlets	0.087725	0.15277	0.1956
Position of branchlets	0.14421	-0.10868	-0.244

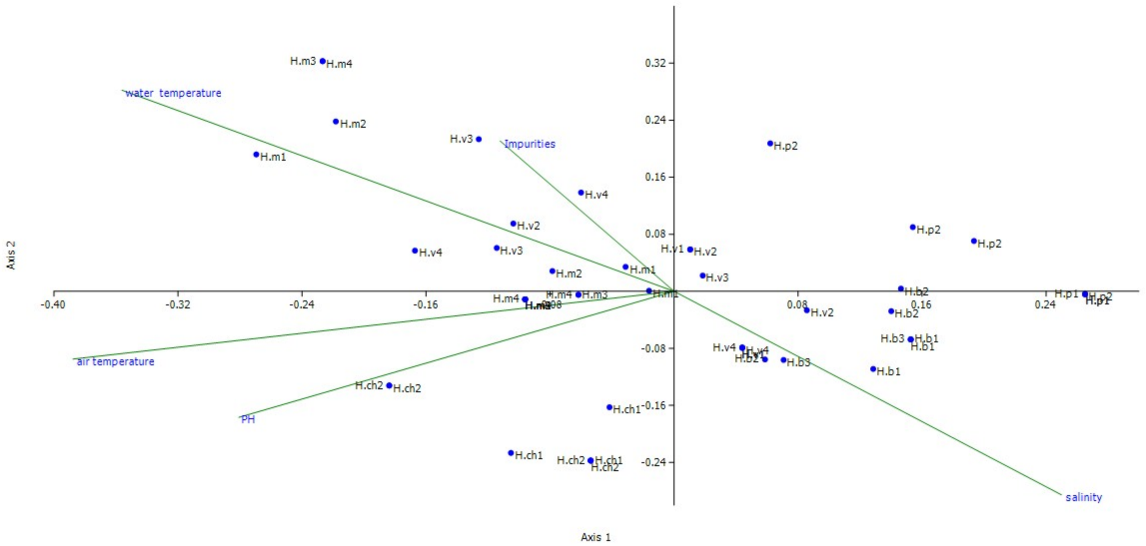

Fig. 3. CCA ordination diagram indicating the influence of environmental factors on the morphological distribution of *Hypnea* populations.