

Taxonomy of the Brown Algal Genus *Padina* With the Description of the New Species *Padina* sp. PG nov. (Dictyotales, Pheophyceae) from the Northern Coast of Persian Gulf

Faede Amini^{1,2*}, Hossein Riahi¹, Hossein Zolgharnain²

Received: 2018- 06- 15 Revised and accepted: 2018-09-23

Abstract

Padina is a marine brown macro algal genus, comprising of about 37 species. Representatives genus data along the Persian gulf coast is limited to a few floristic surveys. The present study introduces the *Padina* species along the Persian gulf coast, with emphasis on the molecular taxonomy. Sequences of the large subunit of RuBis-Co (rbcL) have been used in the molecular analysis of species and for phylogenetic purposes. Based on the rbcL sequences, four species are recognized along the Persian gulf coast: *Padina* sp. FA, *Padina* sp. PG, *Padina* sp. INDEGRO32 and *Dictyota ciliolata*. A new species, *Padina* sp. PG nov. is described based on morphology and molecular analyses. Twelve new sequences were generated among the samples examined.

Keywords: Macroalgea, *Padina* sp. PG nov., Molecular Systematic

Introduction

Species of the marine brown algal genus *Padina* are widely distributed through out the tropics and recognize in the field with

their “fan-like” blade. According to Algae-Base (Guiry and Guiry, 2011) 37 species are currently recognized worldwide, in which 6 species were recorded in the northern of Persian gulf coast of Iran based on morphological studies (Børgesen, 1939; Nizamuddin and Gesner, 1970; Sohrabipour and Rabiei, 1996, 1999, 2005, 2008). Several studies about systematic of macroalgae in Persian gulf along Arabian coasts have been done (Al-Hasan and Jones, 1989; Basson et al., 1992, 1989; Basson, 1979a, 1979b; Børgesen, 1939; DeClerk et al., 1997; Newton, 1955a, 1955b; Abdel-Kareem, 2009). Taxonomic studies of *Padina* species in the coast of Persian gulf, until recently were usually consisting of revisions or descriptions of the genus in this area, exclusively based on morphological characters (e.g. thallus shape, size and color) which might be variable (Trono, 1969; Ni-Ni-Win et al., 2011 a,b). Furthermore, several recent studies dealing with European and Mediterranean taxa have indicated that common morphological data without the support of DNA sequence data are an insufficient basis for estimates species diversity and knowl-

1- Faculty of Biology and Biotechnology Sciences, Shahid Beheshti University, Tehran, Iran.

2- Faculty of Marine Biology Science. Marine Science and Technology University, Khorramshahr, Iran.

*email: f.amini@kmsu.ac.ir

edge of species boundaries (De Clerck et al., 2005, 2006). However a few papers, based on taxonomic sources, were published dealing with the marine algal flora of the Persian gulf, like the molecular studied on three *Sargassum* species that were done by Noor-mohammadi et al. (2011 a, b) in which they used RAPD and ISSR markers for analyses. In addition, *Padina* could be employed as a possible environmental bioindicator in Persian gulf (Amini et al., 2013). In this study, we execute molecular phylogenetic analyses using chloroplast *rbcL* gene sequences. The chloroplast encoded large subunit of the Ru-BisCo gene (*rbcL*) has been used in molecular phylogenetic studies of brown algae and has been demonstrated to be a useful molecular marker by authors (Cho et al., 2004; Hoshina et al., 2004; De Clerck et al., 2006; Cho et al., 2007; Bittner et al., 2008; Ni-Ni-Win et al., 2008, 2010, 2011b; Phillips et al., 2008). The aim of the present study was to characterize the molecular diversity from the new sequences of *Padina* species for the

first time in Persian gulf and compare them with the *Padina* species in the other regions. Additionally, to describe the genus based on newly generated sequences from already available sequence data and describe two new species based on morphological and molecular analyses.

Materials and Methods

Padina samples (Phaeophyceae, Dictyotales, Dictyotaceae) were collected from the intertidal regions along the southern coast of Iran (the northern coast of Persian gulf) that showed in Figure 1 namely: Kish island (26° 51' N, 53°59' E), Lengeh area (26° 28' N, 54°78' E), Qeshm island include Shib Deraz (26° 42' N, 56°04' E), Messen (26° 49' N, 53°23' E), Behind Farmandari (27° 04' N, 56°59' E) from Persian gulf during May, June, August, September 2011 and March 2012 (Figure 1). The samples were obtained by hand and diving. Standard collecting and preserving proceeds follow Tsuda (1972) and molecular procedures carried out according



Fig. 1. Location of sampling for seaweeds on the intertidal Coast of Persian Gulf of Iran. 1-Kish island, 2- Lengeh area, 3-ShibDeraz (Qeshm island), 4-Messen (Qeshm island), 5-Behined Farmandari (Qeshm island).

to Siemer et al. (1998) and Ni-Ni-Win et al. (2008, 2011a). Collected samples were deposited in HSBU (Herbarium of Shahid Beheshti University). All specimens used for this study are listed in Tables 1 and 2.

Total genomic DNA was extracted from tissue samples, dried in a silica gel. DNA extraction, amplification (PCR) of the *rbcL* region and sequencing carried out using an extraction protocol detailed by Ni-Ni-Win et al. (2008 and 2011b). PCR conditions for *rbcL* were as follows: an initial denaturation step at 94°C for 3 min, followed by 94°C for 0.5 min, annealing at 58°C for 0.5 min, extension at 72°C for 2 min for 28 cycles, and final extension at 72°C for 10 min. PCR products were checked for length and yielded by electrophoresis on 1.5% agarose gels dyed with ethidium bromide. In order to minimize possible errors during PCR, three independent PCR reactions were performed for each DNA sample. Primer sequences, annealing temperatures, and bibliographic sources are provided in Table 3.

For anatomical observations, specimens were sectioned by hand and micrographed using a Dino capture Ver. 3 digital camera attached to a microscope (Olympus, Tokyo, Japan). For each specimen, morphological and anatomical characters (vegetative and reproductive) analyzed. According to Ni-Ni-Win et al. (2011a) some of the morphological characters such as shape, size, color, and thickness of the thallus are highly variable within the species and are depend on environmental conditions and age of the specimens. But other characters like the number of cell layers, presence or absence

and degree of calcification, the position and arrangement of hair lines and sporangial sori, and presence or absence of an indusium, the presence or absence of groups of rhizoid-like hairs on the thallus surface, the structure and arrangement of hair lines and reproductive sori were considered less variable within the species. The main anatomical characters used for species identification have been summarized in Table 4.

DNA sequences are deposited in DNA Data Bank of Japan (DDBJ). The 12 newly generated sequences were complemented with 31 sequences downloaded from GenBank and aligned using Thompson et al. (1994) and the alignment was refined manually. Phylogenetic analyses were carried out by maximum parsimony (MP), maximum likelihood (ML) and neighbor joining (NJ) methods, using MEGA5.1 (Tamura et al., 2011). An appropriate model of sequence evolution for maximum likelihood (ML) analysis was selected the best-fit models based on AICc values criterion with MEGA's built-in model testing suite. A ML tree was inferred using the selected GTR model using nearest neighbor interchange tree rearrangements. A neighbour-joining (NJ) distance-based tree was constructed (Saitou and Nei, 1987) using a Kimura 2-parameter method. Maximum parsimony (MP) analysis was obtained using a standard heuristic search with tree-bisection-reconnection (TBR) branch swapping options. Bootstrap resampling was carried out with 100 replicates for ML and 1000 replicates for NJ and MP (Felsenstein, 1985). *Dictyota ciliolata* and *Dictyota dicoto-*

Table1. Species used in this study, from which all new sequencing is obtained. Herbarium of Shahid Beheshti University (HSBU).

Sequence entry	Species	Origin	Voucher No.	DDBJ code for rbcl	Reference
1	<i>Padina</i> sp. PG, haplotype: 1	Qeshm Island, IR Iran	HSBU-2011300	AB793713	This study
2	<i>Padina</i> sp. FA haplotype: 2	Kish Island, IR Iran	HSBU-2011301	AB793714	This study
3	<i>Padina</i> sp. INDDR032 haplotype: 3	Kish island, IR Iran	HSBU-2011302	AB793715	This study
4	<i>Padina</i> sp. FA haplotype: 4	Kish Island, IR Iran	HSBU-2011303	AB793716	This study
5	<i>Padina</i> sp. FA haplotype: 5	Kish Island, IR Iran	HSBU-2011304	AB793717	This study
6	<i>Padina</i> sp. FA haplotype: 6	Qeshm Island, IR Iran	HSBU-2011305	AB793718	This study
7	<i>Padina</i> sp. FA haplotype: 7	Qeshm Island, IR Iran	HSBU-2011306	AB793719	This study
8	<i>Padina</i> sp. FA haplotype: 8	Kish Island, IR Iran	HSBU-2011307	AB793720	This study
9	<i>Padina</i> sp. FA haplotype: 9	Legeh Port, IR Iran	HSBU-2011308	AB793721	This study
10	<i>Padina</i> sp. FA haplotype: 57f	Kish Island, IR Iran	HSBU-2011309	AB793724	This study
11	<i>Padina</i> sp. FA haplotype: 2	Kish Island /LengehPort/ Qeshm Island, IR Iran	HSBU-2011310	AB775783	This study
12	<i>Dictyota citholata</i>	Qeshm Island, IR Iran	HSBU-2011311	AB775782	This study

Table 2. List of species from other studies investigated in this study, including collection site and GenBank accession number.

Sequence entry	Species	Origin	Voucher No.	DDBJ code for rbcl
1	<i>Padina amillarium</i>	India	INDGR032	AB096907
2	<i>Padina amillarium</i>	Diani Beach, Kenya	ODC1508	JQ364044
3	<i>P. australis</i> Hauck	Baie de Gadji, Ile des Pins, New Caledonia	IRD233	JQ364054
4	<i>P. australis</i> Hauck	Sawang, Siquijor, Philippines	ODC1459	JQ364056
5	<i>P. australis</i> Hauck	Japan	OKNNG019	AB096901
6	<i>P. australis</i> Hauck	Awase, Okinawa I., Okinawa Pref., Japan	SAP105579	AB358907
7	<i>P. australis</i> Hauck	New Caledonia	IRD241	JQ364055
8	<i>P. australis</i> Hauck	Urazoko, Okinawa I., Okinawa Pref., Japan	SAP105580	AB358906
9	<i>Padina australis</i>	Awase, Okinawa I., Okinawa Pref., Japan	SAP105579	AB358907
10	<i>P. australis</i> Hauck	Ngapali beach, Thandwel(Sandoway), Myanmar		AB489914
11	<i>P. australis</i> Hauck	Newcastle, NSW, Australia		AB489913
12	<i>Padina australis</i>	Karang Jong E, Kepulauan Seribu, Indonesia	L0609534	AB489912
13	<i>Padina australis</i>	Orano, New Caledonia	IRD167	AB512524
14	<i>Padina australis</i>	Australia		JQ364052
15	<i>Padina australis</i>	Poindimié, New Caledonia	IRD158	AB512525
16	<i>Padina australis</i>	Balabio, New Caledonia	IRD172	EU579959
17	<i>Padina boergeseni</i>	Dickwella, Sri Lanka	HEC15869	JQ364053
18	<i>Padina boergeseni</i>	Dickwella, Sri Lanka	HEC15913	JQ364057
19	<i>Padina boergeseni</i>	Cahuita, Costa Rica	LBC0930	JQ364058
20	<i>Padina boergeseni</i>	Nungwi, Zanzibar, Tanzania	TZ0520	JQ364059
22	<i>Padina boergeseni</i>	Paje, Zanzibar, Tanzania	TZ0848	JQ364061
23	<i>Padina boergeseni</i>	Makunduchi, Zanzibar, Tanzania	TZ0863	JQ364063
24	<i>Padina boergeseni</i>	Makunduchi, Zanzibar, Tanzania	TZ0872	JQ364064
25	<i>Padina tetrastromatica</i>	Malaysia		JQ364065
26	<i>Padina tetrastromatica</i>	Indonesia:KepulauanSeribu, Kelor		AB512554
27	<i>Padina tetrastromatica</i>	Thailand:Nakhon Si Thammarat, Huasai		AB512553
28	<i>Dicyota ciliolata</i>	Canary Islands	D191	AB512552
29	<i>Dicyota dichotoma</i>	Korea		GQ425109
30	<i>Dicyota dichotoma</i>	Japan: Kanagawa, Aburatsubo		AY748311
31	<i>Dicyota dichotoma</i>			AB358934

ma (Dictyotales) were considered as outgroup to root the trees.

Results

Morphological observations

***Padina* sp. PG, haplotype:** 1 nov. AB793713 (HSBU-2011300)

Habitat: Qeshm Island

The erect thalli with 2-4 cell layers, (4 cells layered at the base) is yellowish brown in color, the length between 5 to 10cm, and the width up to 4 cm, blades much divided, attached by branched rhizoidal stipe. Thalli moderately calcified on both surfaces. Sporangia rows are closely alternate with hair rows at different intervals without indusia, sometimes as isolated patches between two hair lines on the lower surface when both surfaces are viewed together. The species resembles *P. tetrastromatica* Hauck but the blades of *P. tetrastromatica* Hauck showed no calcification (Wynne et al., 1999) but this species has light calcification on two sides. In cross sections of the blades, both in mid region and in more basal portions, showed a 4-layered organization but *Padina* sp. PG has 2 cell layer and 4 layers at the base. Ecology: This species is a new species, usually grows in the lower portions of the intertidal zone on rocky substrates or shallow subtidal zones.

Etymology: The species epithet refers to Persian gulf.

***Padina* sp. FA** INDGR032, haplotype: 3AB793715 (HSBU-2011302)

Habitat: Qeshm Island, Hormozgan province

The thallus is bright brown with 2-4 cell layers,

Table 3. The name and references of the primers used in this study.

Primer name	Gene	Direction	Sequence (5'a→3')	Annealing T ₀ C	Reference
rbcl-P1	<i>rbcl</i>	Forward	GGGTAATTTGTAAGTGGATGCG	64	Ni-Ni-Win et al. (2008) Kawai et al. (2007)
rbcl-D2	<i>rbcl</i>	Reversed	CGACGAAGTCAGGAGTATCTG	61.4	Ni-Ni-Win et al. (2008) Kawai et al. (2007)
Fa(57-76)	<i>rbcl</i>	Forward	GTGGACTGTTGTTGGACTG	60.6	Present study
Ra500-519	<i>rbcl</i>	Reverse	ACATTTACGAAGAGAAAGCCC	59.7	Present study

Table 4. Main morpho anatomical characters used in the taxonomic identification of *Padina* species of Persian gulf.

Species	Color	Length (Cm)	Width (Cm)	Cell layer (thallus)	Calcification on surface	Sporangial surface	Indusia	Phaeophyce an hairs	Stip with Rust-colored Fibrous hairs	Origin	Accession number
<i>Padina</i> sp. PG haplotype: 1	Yellowish	5-10	4-10	2-4	light / light	Upper/Lower	Absent	Upper/Lower	Present	Qeshm Island	AB793713
<i>Padina</i> sp. FA haplotype: 2	Dark brown	4-10	4-10	2	Light/ heavy	Upper	Present	Upper	Present	Kish Island	AB793714
<i>Padina</i> sp. INDGR032 haplotype: 3	Bright brown	5-9	5-9	2-4	Light/ light	Upper/Lower	Absent	Upper/Lower	Present	Kish Island	AB793715
<i>Padina</i> sp. FA haplotype: 4	Dark brown	4-6	5-9	2-6	Light/Light	Upper	Present	Upper	Absent	Kish Island	AB793716
<i>Padina</i> sp. FA haplotype: 5	Bright brown	10-15	5-8	2	Light/Heavy	Upper/Lower	Present	Upper	Absent	Qeshm Island	AB793717
<i>Padina</i> sp. FA haplotype: 6	Yellowish	4-6	6-10	2-6	Heavy/Heavy	Upper	Present	Upper/Lower	Absent	Qeshm Island	AB793718
<i>Padina</i> sp. FA haplotype: 7	Bright brown	5-15	8-15	2	Heavy/Heavy	Upper/Lower	Present	Upper/Lower	Absent	Kish Island	AB793719
<i>Padina</i> sp. FA haplotype: 8	Bright Brown	4-6	6-9	2-4-6	Heavy/Heavy	Upper	Present	Upper	Absent	Legeh Port	AB793720
<i>Padina</i> sp. FA haplotype: 9	Dark brown	5-9	4-8	2-4	Heavy/Heavy	Upper	Present	Upper/Lower	Absent	Kish Island	AB793721
<i>Padina</i> sp. FA haplotype: 57f	Darkbrown	10-17	10-13	2-4-6	Heavy/Heavy	Upper	Present	Upper	Absent	Kish Island /Lengeh Port/ Qeshm and	AB793724
<i>Padina</i> sp. FA haplotype: 2	Brightbrown	5-7	4-6	2-4-6	Heavy/Heavy	Upper/Lower	Present	Upper	Absent	Kish Island /Lengeh Port/ Qeshm Island	AB775783

the range of the wide is the same as the long, 5-9 cm. Thallus attached by a thick, discoid holdfast, stipe short with Rust-colored Fibrous hairs, lightly calcificated on both surfaces of the thallus, sporangial sori without indusium is alternating with hair lines on both surface. Ecology: This species is a new species, usually grows in the lower portions of the intertidal zone on rocky substrates.

***Padina* sp. FA**

Habitat: Qeshm Island, Hormozgan province
The sequences obtained for the rbcL of 11 *Padina* sp. FA samples collected along the coast of Persian gulf yielded 9 distinct haplotypes. There are high similarities among the haplotypes of *Padina* sp. FA and their morphological characters are very similar to those of *P. boergesenii* and *P. australis*. The following

characters are similar to more samples of haplotypes. Sporangia with indusium, relatively high calcified on lower and upper surface. They all have more than five blades.

Etymology: The species epithet refers to the name of author.

Ecology: This haplotype is a new reported for the first time in Iran, usually grows in the lower portions of the intertidal zone on rocky substrates.

***Padina* sp. FA**, haplotype: 2 AB793714 (HSBU-2011301)

Habitat: Kish Island, Intertidal

The thallus is two cells thick through out, up to 7 cm high, usually become 3 cells layered at the base. Generally blades dark brown in color, more deep develops from a stipe short with Rust-colored Fibrous hairs. Sporangia rows

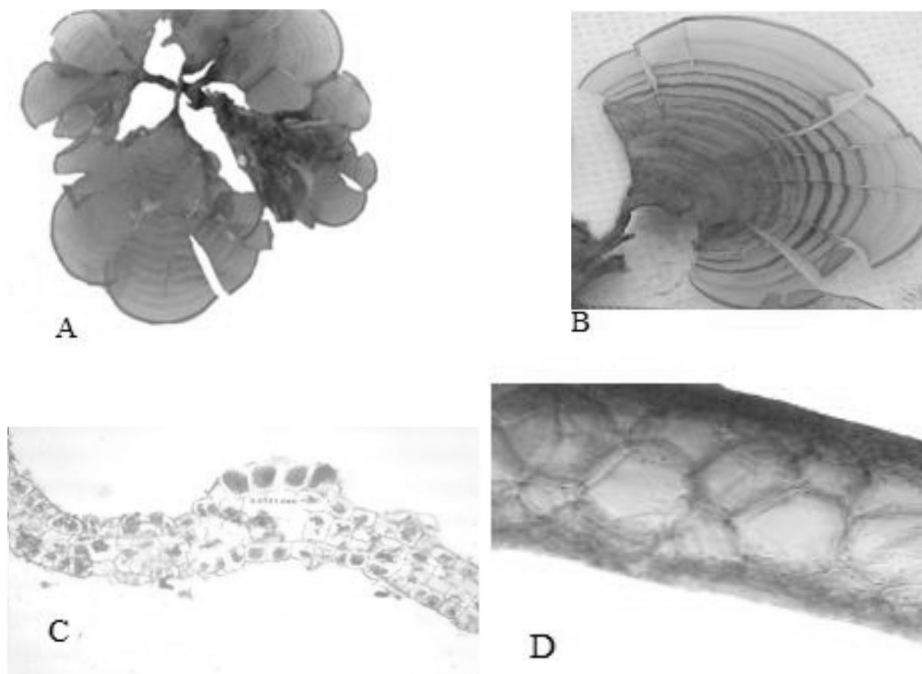


Fig. 2. A and B. Habitat, C. 2 cell layers transverse section of the middle portion of thallus, D. The view of 3 layers with hair lines (arrow) $\times 40$.

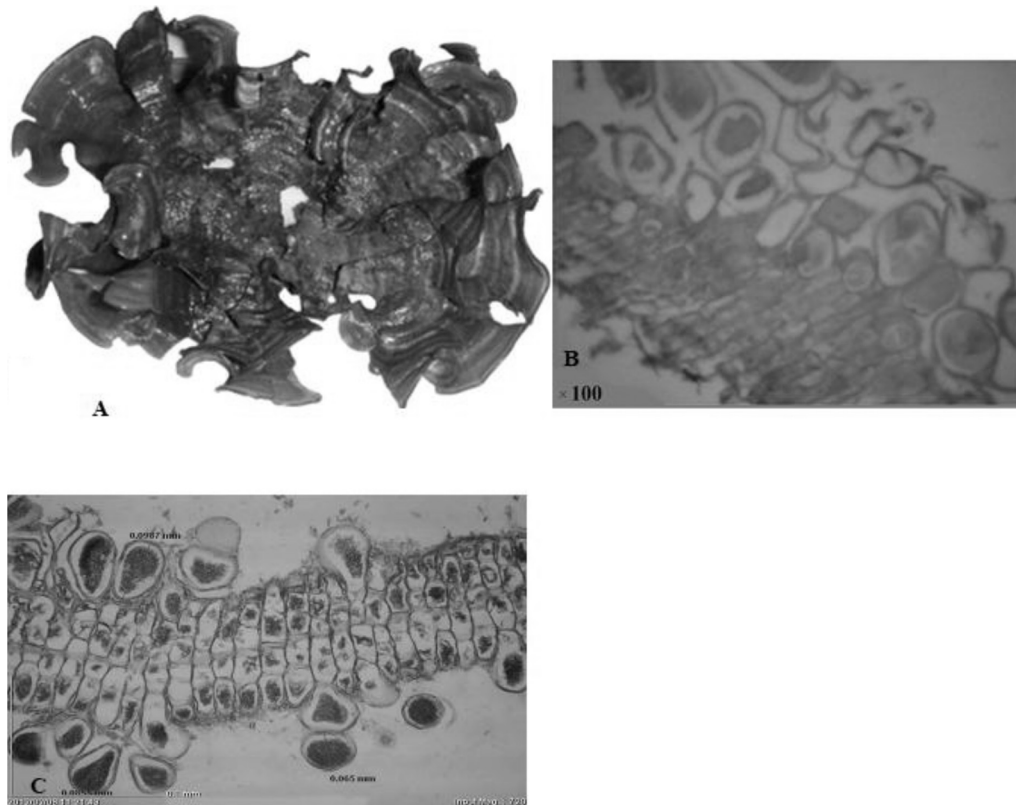


Fig. 3. A. Habitat, B. The view surface, C. Longitudinal section of the thallus×100.

were always situated on the upper surface with indusium, closely alternate with hair rows at equal intervals. Hairs in concentric lines only on the upper surface. The calcification is heavy on upper surface and light calcificated on the lower surface.

***Padina* sp. FA**, haplotype: 4AB793716 (HSBU-2011303)

Habitat: Kish Island, Intertidal

The erect dark brown thalli with 2-6 cell layers, are relatively small, wider than tall at 4-6 cm long and 5-9cm wide. Calcification is light on both surfaces. Sporangia with indusium principally on the outer surface alternating with hair lines, usually in small clusters with a thin indusium. Concentric hair lines on the

upper surface of the thallus.

***Padina* sp. FA**, haplotype: 5AB793717 (HSBU-2011304)

Habitat: Qeshm Island, Intertidal

The bright brown thallus (2 cell layers) is relatively large with 10-15 length and 5-8 width, lightly calcified on the lower surface and moderately to heavily on the upper surface, sporangia principally on the outer surface and sometimes also on the inner surface, usually in small clusters with indusium, hair lines on upper

***Padina* sp. FA**, haplotype: 6AB793718 (HSBU-2011305)

Habitat: Qeshm Island, Intertidal

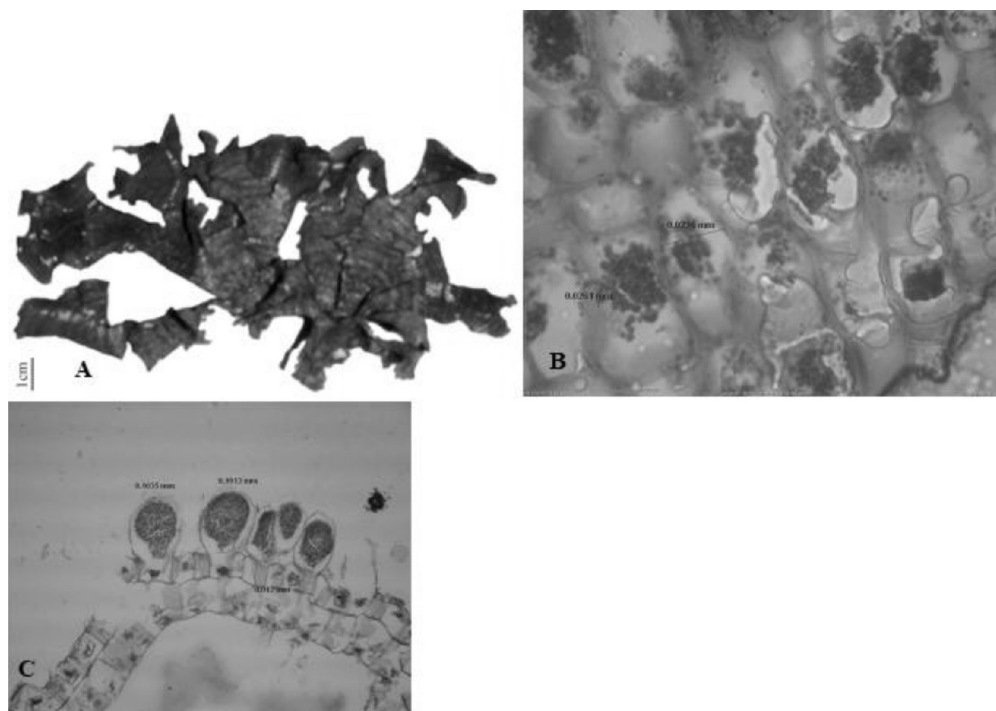


Fig. 4. A. Habitat, B. The surface view with the length of cell $\times 40$, C. Cross section showed two cell layers $\times 40$.

The yellowish thallus (2-6 cell layers) has 4-6 cm long and 6-10 cm wide. Upper and lower surfaces with heavy calcification. Sporangial sori is alternating with hair lines.

***Padina* sp. FA**, haplotype: 7 AB793719 (HSBU-2011306)

Habitat: Kish Island, Intertidal

Thalli is bright brown with 2 cell layers and 5-15 cm long and 8-15 cm wide, stipe short without rust-colored fibrous hairs, heavily calcified on both surfaces except for hair lines, indusium present, hair lines on both surfaces sporangial sori alternating with hair lines, sporangial sori on both surface.

***Padina* sp. FA**, haplotype: 8AB793720

(HSBU-2011307)

Habitat: Lengeh Port, Intertidal

Thalli (2-4-6 cell layers) with bright brown in color, 4-6 cm long and 6-9 cm wide, hair lines on upper surface and alternating with sporangial sori without indusium, heavy calcification is on upper and lower surfaces.

***Padina* sp. FA**, haplotype: 9AB793721 (HSBU-2011308)

Habitat: Kish Island, Intertidal

The length of bright brown thallus (2-4 cell layers) is 5-9 cm and the width is 4-8 cm. Calcification is heavy on Upper and lower surfaces, sporangial sori on upper surface. Thallus dark brown with 2-4 cell layers, 5-9 length, 4-8 width, high calcified on two sides spo-

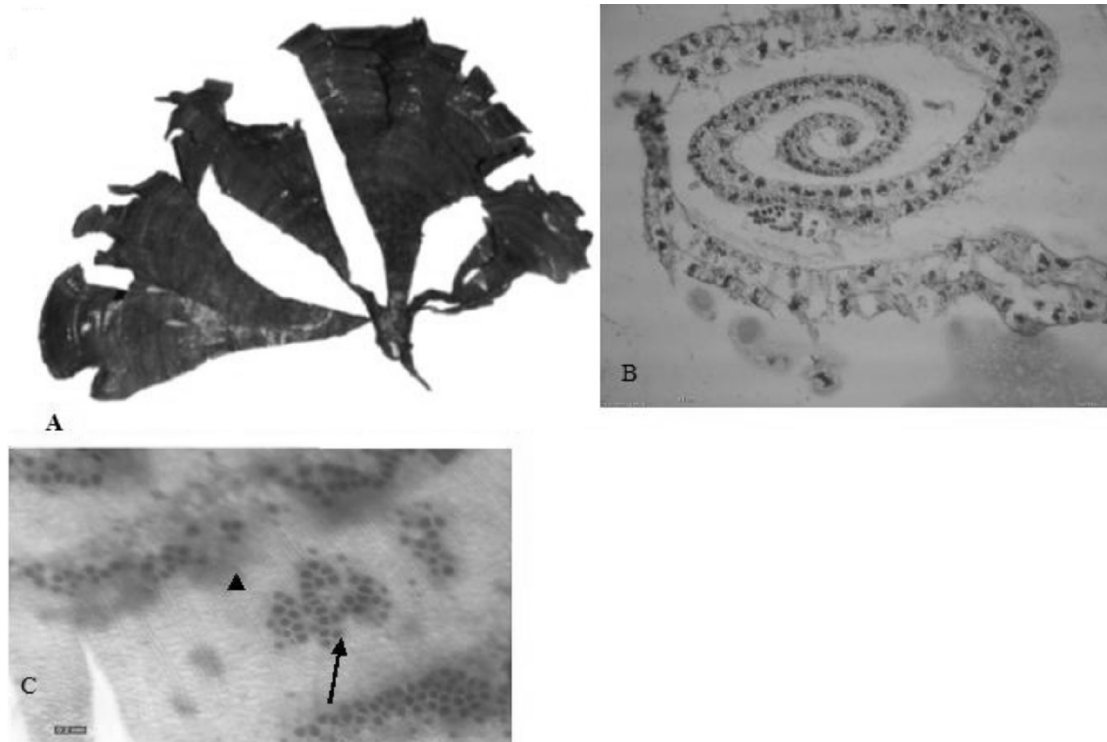


Fig. 5. A. Habitat, B. Longitudinal section $\times 40$, C. Hair lines (arrowhead) sporangium line (arrow) $\times 40$.

rangial on upper surface, reproductive organs and Phaeophyceyan hairs present in concentric zones.

***Padina* sp. FA**, haplotype: 57FAB793724 (HSBU-2011309)

Habitat: Kish Island, Lengeh Port, Qeshm Island, Intertidal

Thallus color is dark brown with 2-6 cell layers, up to 10-17cm length and 10-13cm width, hair lines on upper surface, sporangial sori alternating with hair, calcification is on upper and lower surfaces heavily.

***Padina* sp. FA**, haplotype: 2AB775783 HSBU-2011310

Habitat: Qeshm island, Intertidal

Thalli (2-6 cell layers) has 5-7 long and 4-6 wide color is bright brown, hair lines on upper surface sporangial sori alternating with hair lines sporangial sori on upper and lower surfaces, upper and lower surfaces is heavily calcified. They are yellow greenish in color. Apices are acute to round and sometimes somewhat incurved is completely erect with more than 10 cm.

Molecular phylogenetic analysis

The chloroplast-encoded *rbcL* gene has been extensively used in molecular phylogenetic studies of brown algae and has been demonstrated to be a useful molecular marker by authors (Hoshina et al. 2004; De Clerck et al., 2006; Lane et al., 2006; Cho et al., 2007; Bitner et al., 2008; Ni-Ni-Win et al., 2008 and

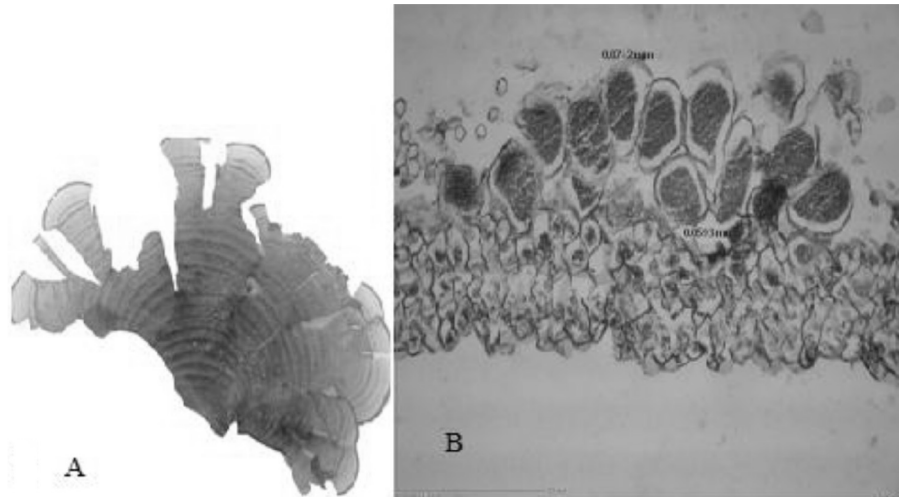


Fig. 6. *Padina* sp. FA haplotype 6: A. Habitat, B. Longitudinal section×40.

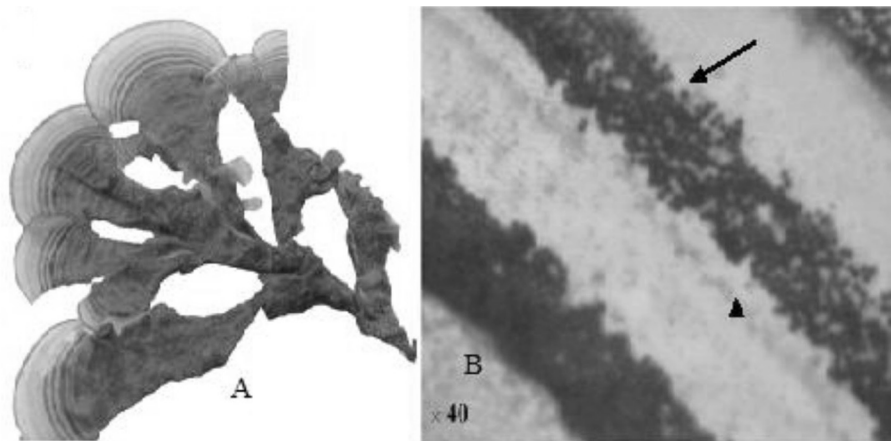


Fig. 7. *Padina* sp. FA haplotype 7: A. Habitat, B. Hair lines (arrow head) sporangium line (arrow) ×40.

2010). The *rbcL* alignment consisted of 12 sequences representing three *Padina* species and one out group taxa, including twelve new sequences and yielded 10 distinct haplotypes along the coast of Persian gulf (Table 1). Figures 13 and 14 showed the main monophyletic groups were constant in performed analyses. The first clade (Fig. 14) presented high bootstrap values (99-100% support) in all analyses (ML, NJ, MP) and included 9 haplotypes

of *Padina* sp. FA from Persian gulf which grouped with *Padina boergessenii*.

Phylogenetic trees constructed from the ML and NJ analyses showed a similar topology. *P. boergessenii* specimens and *Padina* sp. FA haplotypes were closely allied, and formed a strongly supported monophyletic group with high bootstrap confidence of 90%. *Padina boergesenii* with JQ364063 and JQ364065 accession numbers are clearly separated from the

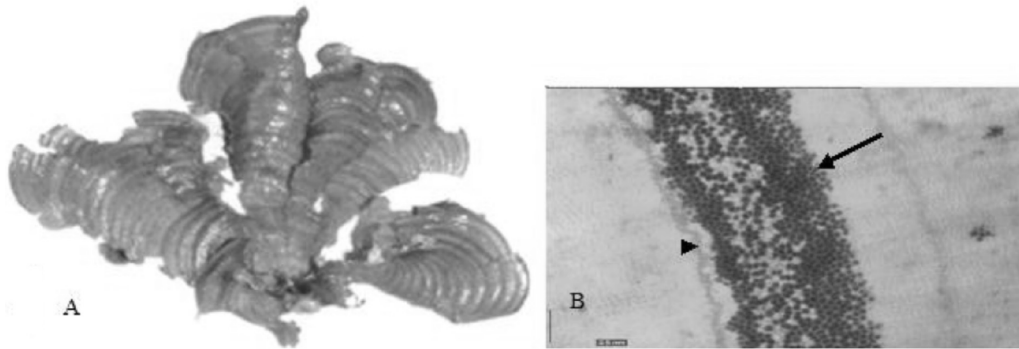


Fig. 8. *Padina* sp. FA haplotype 8: A. Habitat, B. view surface hair lines (arrow head) of tetrasporangium line (arrow) $\times 40$.

strongly monophyletic group consisting of the other *P. boergessenii* specimens and *Padina* sp. FA haplotypes. *Padina* sp. FA haplotypes are morphologically similar to *Padina australis* which have 2-6 layers but they are molecularly similar to *P. boergessenii* from Zanzibar, Tanzania which has 0-7 nucleotide differences. However, these similarities cover the partial of the nucleotides. In other words, all nucleotides of this study are almost 490 bp, but this is a comparison among 275 nucleotides. *Padina* sp. FA haplotypes are morphologically similar to *Padina australis* which have 2-6 layers but they are different from *P. Australis* with more than 10 nucleotides.. Other taxa *Padina* sp. PG and *Padina* sp. INDEGRO32 included in clade 2 in Figure 13 and in calde 3 of Figure 14. Morphological study showed that *Padina* sp. PG is closely to *P. antillarum* (*P. tetrastromatica*). As previously noted the differences between species are the calcification of blades and the number of cell layers. The phylogenetic trees confirmed *Padina* sp. PG was always nested in the diverse clade of the *P. antillarum*.

This placement was highly supported with 92% bootstrap confidence (Fig. 14). *Dictyota ciliolata* and *Dictyota dicotoma* is used as out-group.

Discussion

Molecular phylogenetic analyses using *rbcl* sequences, combined with morphological observations, showed the occurrence of two undescribed *Padina* species in Persian gulf coasts. *Padina* sp. FA most closely related *P. australis* and *P. boergessenii*. The members of the *P. boergessenii* and *Padina* sp. FA haplotypes are the most strongly grouped in the trees constructed in the present study (Fig. 14). This result suggests that these species might have evolved closely to each other, but separately from the other species of the *P.boergessenii*. Given the close similarity of *Padina* sp. FA haplotype sequences to the *P.boergessenii* sequences, it seems safe to assume that these samples are conspecific, in addition, there are some differences in nucleotides between 0 to 8 positions. In order to investigate wheth-

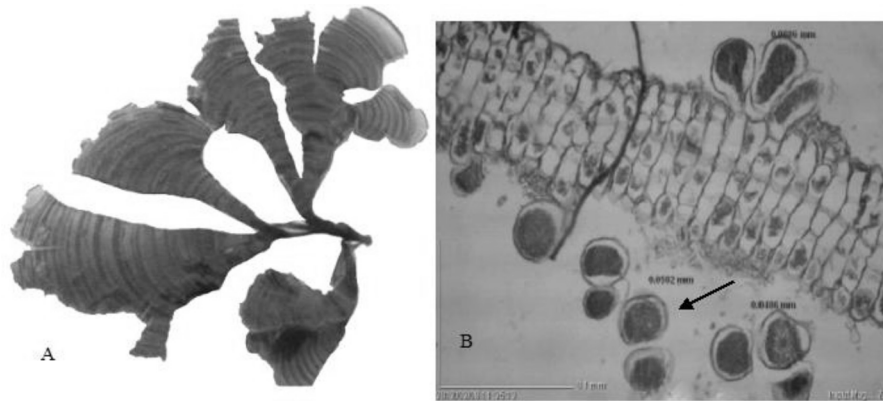


Fig. 9. *Padina* sp. FA haplotype 9A: Habitat, B. Longitudinal section Transverse section of tetrasporangial sori, showing obovate tetrasporangia (arrow) $\times 40$.

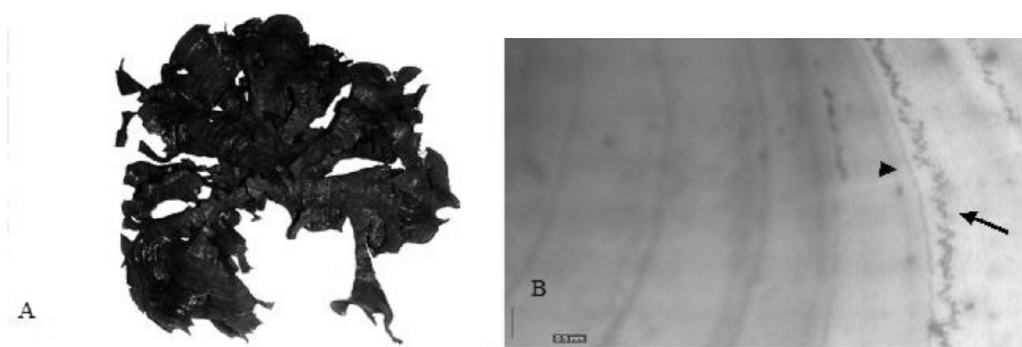


Fig. 10. *Padina* sp. FA haplotype 57FA: Habitat, B. view surface view surface hair lines (arrowhead) Tetrasporangium line (arrow) $\times 40$.

er the *Padina* sp. FA is a new species or the conspecific with the *Padina boergesenii* it is necessary to make further studies. Also more samples and using different molecular markers is needed. Until further studies clarifying their taxonomic status, we prefer to set the *Padina* sp. FA samples as a new species, since they were indistinguishable in morphology and positioned in a monophyletic clade in all analyses of *rbcL* gene and other markers.

Phylogenetic relationships among the *Padina*

sp. INDEGRO32 and *P. antillarum* species can be clear. The phylogenetic analyses of the *rbcL* data show the monophyly groupings. The monophyly of *Padina* was fully supported (De Clerck, 2006).

Padina sp. PG is very similar to *Padina antillarum* (Kützing) Piccone = *P. tetrastromatica* Hauck but the blades of *P. tetrastromatica* showed no calcification (Wynne et al., 1999). In cross sections of the blades, both in mid region and in more basal portions, *Padina* sp.

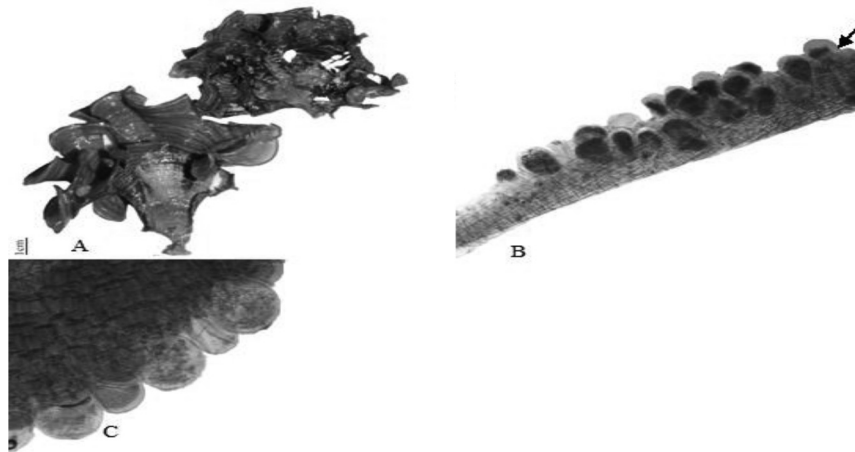


Fig. 11. *Padina* sp. FA haplotype 2: A. Habitat, B. Transverse section of tetrasporangial sori, showing obovate tetrasporangia and C. Tetrasporangium on surface *Dictyota ciliolata* AB775782.

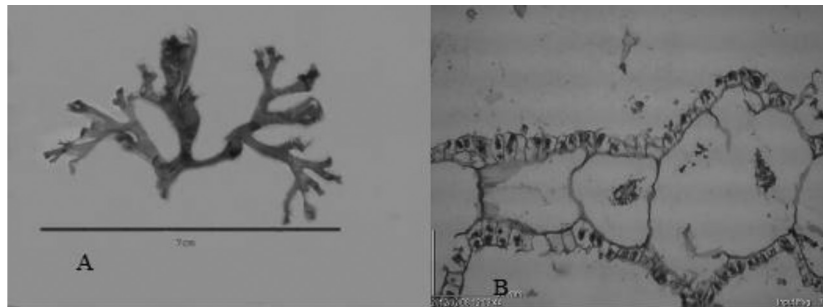


Fig. 12. *Dictyota ciliolata* AB775782: A. Habitat, B. Transverse section.

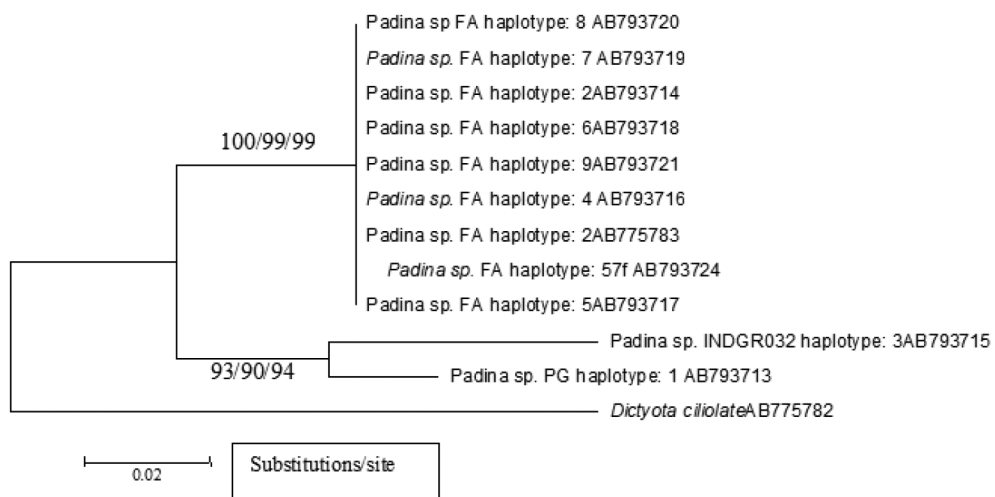


Fig. 13. Maximum likelihood tree ($-\ln L = 531.2360$) based on *rbcL* gene sequences. Numbers at each node indicate bootstrap values ($>50\%$) for maximum

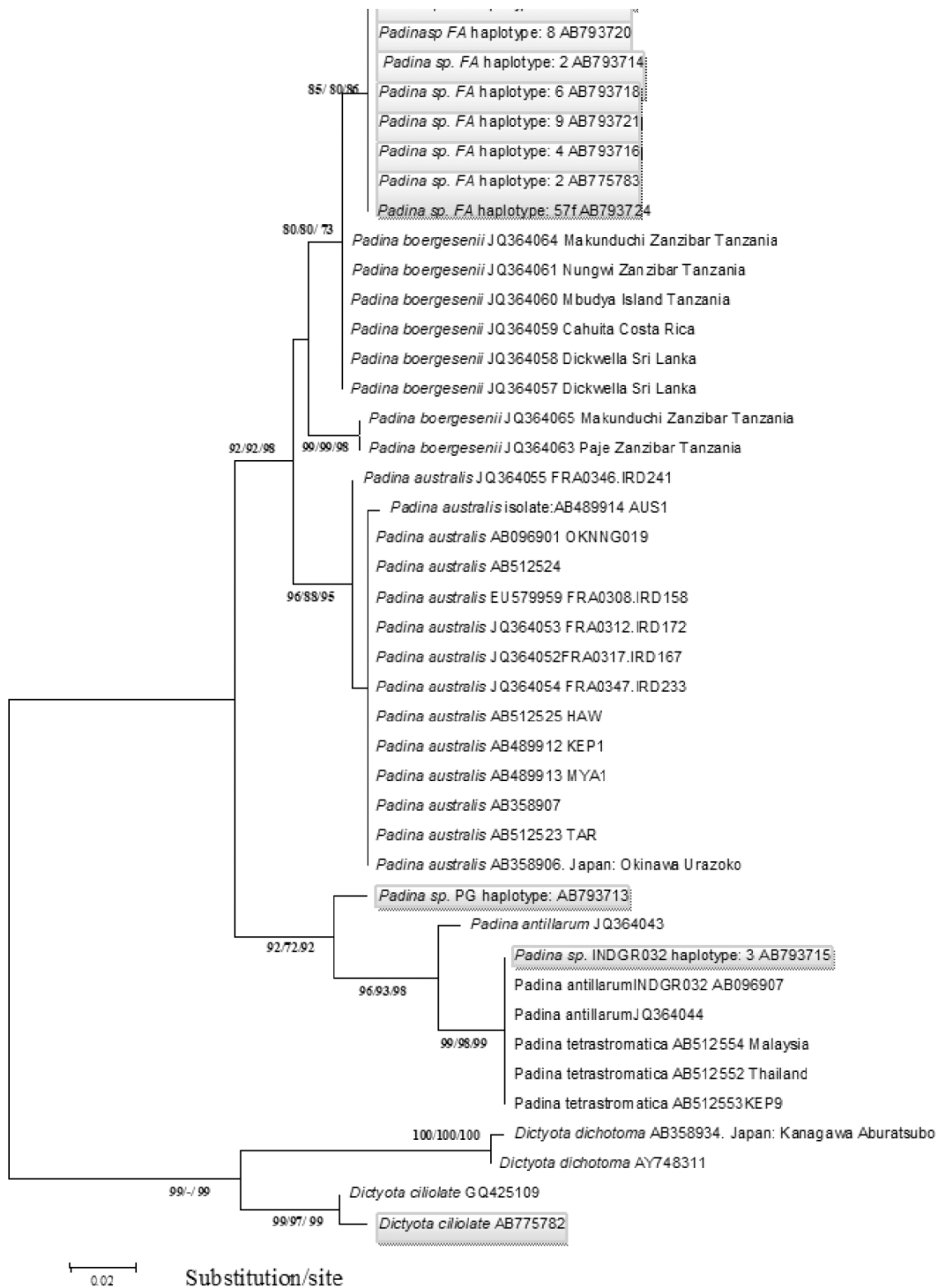


Fig. 14. Maximum likelihood tree (-ln L= 702.1252) based on *rbcL* gene sequences. Numbers at each node indicate bootstrap values with only values >70% being shown for maximum likelihood (ML) (left), maximum parsimony (MP) (middle) and neighbor joining (NJ) (right). The Persian gulf specimens whose sequenced were determined in the present work are in green boxes.

PG showed a 4 layered organization but this species has 2 cell layer and 4 layers at the base. Molecular studies confirmed this dividing. A sequence of *Padina* sp. FA is genetically identical with those of *P. australis* and *P. boergesenii*. Therefore, we consider *Padina* sp. FA to be conspecific with *P. boergesenii*. Many *Padina* species may remain to be discovered. This species is possibly endemic to Persian gulf. However, additional sampling in other regions might be able to confirm either its endemism northern or southern of Persian gulf.

Acknowledgment

Thanks are due to Professor De Clerck, Dr. Ni-Ni-Win and Dr. Vaezi for their guidance, advice and their skills.

References:

- Abdel-Kareem MSM. (2009). New algal records from the Persian gulf Coast of Saudi Arabia. *Botany Research International*. 2 (4): 268-276
- Al-Hasan RH and Jones WE. (1989). Marine algal flora and sea grass of the coast of Kuwait. *Journal University of Kuwait (Science)*. 16: 289-339.
- Amini F, Riahi H, Zolgharnain H. (2013). Concentrations Metal in *Padina* Species and Associated Sediment from Nayband Bay and Bostaneh Port, Northern Coast of the Persian gulf, Iran. *Journal of the Persian Gulf Marine Science*. 4 (11): 17-24.
- Basson PW, Mohamed S, Arora DK. (1989). A survey of the benthic marine algae of Bahrain. *Botanica Marina*. 32: 27-40.
- Basson PW. (1992). Checklist of marine algae of the Persian Gulf. *Journal University of Kuwait (Science)*. 19: 217-230.
- Basson PW. (1979a). Marine algae of the Persian Gulf coast of Saudi Arabia (first half). *Botanica Marina*. 22: 47-64.
- Basson PW. (1979b). Marine algae of the Persian Gulf coast of Saudi Arabia (second half). *Botanica Marina*. 22: 65-82.
- Bittner L, Payri CE, Couloux A, Cruaud C, de-Reviers B, Rousseau F. (2008). Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*. 49 (1): 211-26.
- Børgesen F. (1939). Marine algae from the Iranian Gulf. In: *Danish Scientific Investigations in Iran* (K. Jessen and R. Sparck, eds.), Einar Munksgaard. 1: 47-141.
- Cho GY, Kogame K, Kawai H, Boo SM. (2007). Genetic diversity of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) from the Pacific and Europe based on RuBisCO large subunit and spacer, and ITS nr DNA sequences. *Phycologia*. 46: 657-65.
- Cho GY, Lee Sh, Boo SM. (2004). A new brown algal order, Ishigeales (Phaeophyceae), Established On the Basis Of Plastid Protein-Coding *rbcL*, *psaA*, and *psbA* region comparisons. *Journal of Phycology*. 40: 921-936.
- De Clerck O, Gavio B, Fredericq S, Bárbara I, Coppejans E. (2005). Systematics of *Grateloupia filicina* (Halymeniaceae, Rhodophyta), based on *rbcL* sequence analyses and morphological evidence, including the rein-

- statement of *G. minima* and the description of *G. capensis* sp. nov. *Journal of Phycology*. 41: 391-410.
- De Clerck O, Leliaert F, Verbruggen H. (2006). Arevised classification of the Dictyoteae (Dictyotales, Phaeophyceae) based on rbcL and 26S ribosomal DNA sequence analyses. *Journal of Phycology*. 42: 1271-88.
- De Clerck O and Coppejans E. (1997). Notes on the *Dictyota vieillardii* and *D. adnata* (Dictyotaceae, Phaeophyta). *Taxon*. 46: 33–36.
- Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. 39: 783-791.
- Guiry MD and Guiry GM. (2013). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway.
- Hoshina R, Hasegawa K, Tanaka J, Hara Y (2004). Molecular phylogeny of the Dictyotaceae (Phaeophyceae) with emphasis on their morphology and its taxonomic implication. *Japan Journal of Phycology*. 52:189–194.
- kawai H, Hanyuda T, Draisma SGA, Muller DG. (2007). Molecular Phylogeny Of *Discosporangium measarthrocarpum* (Phaeophyceae) With a Reinstatement of the order Discosporangiales. *Journal of Phycology*. 43:186-194.
- Lee J and Bae KS. (2002). Phylogenetic relationships among several genera of Dictyotaceae (Dictyotales, Phaeophyceae) based on 18S rRNA and partial rbcL gene sequences. *Marine Biology*. 140:1107
- Nei M and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press. 333 P.
- Newton L. (1955a). The marine algae of Kuwait. In: Dickson V. (Ed.). *The wild flowers of Kuwait and Bahrain*, Allen and Unwin. 100 p.
- Newton L. (1955b). The marine algae of Bahrain. In: Dickson V. (Ed.). *The wild flowers of Kuwait and Bahrain*. Allen and Unwin. 141 p.
- Ni-Ni-Win W, Hanyuda T, Arai S, Uchimura M, Abbott I. A, Kawai H. (2010). Four new species of *Padina* (Dictyotales, Phaeophyceae) from the Western Pacific Ocean, and reinstatement of *Padina japonica*. *Phycologia*. 49: 136-53.
- Ni-Ni-Win W, Hanyuda T, Arai S, Uchimura M, Abbott IA, Kawai H. (2008). Three new records of *Padina* in Japan based on morphological and molecular markers. *Phycological Research*. 56: 288–300.
- Ni-Ni-Win W, Hanyuda T, Arai S, Uchimura M, Prathep A, Draisma SGA, Phang SM, Abbot IA, Millar AJK, Kawai H. (2011a). A taxonomic study of the genus *Padina* (Dictyotales, Phaeophyceae) including the description of four new species from Japan, Hawaii, and the Andaman Sea. *Journal of Phycology*. 47: 1-17.
- Ni-Ni-Win W, Hanyuda T, Draisma SGA, Furnari G, Meinesz A, Kawai H. (2011b). *Padina ditristromatica* sp. nov. and *Padina pavonicoides* sp. nov. (Dictyotales, Phaeophyceae), two new species from the Mediterranean Sea based on morphological and molecular markers. *European Journal of Phycology*. 46 (4): 327-341.
- Nizamuddin M and Gessner F. (1970). The marine algae of the northern part of the Arabian Sea and of the Persian gulf. "Meteor" *Forsch-Ergebnisse, Reihe D. Biologie*. 6:

- 1-42.
- Noormohammadi Z, Ghasemzadeh Baraki S, Sheidai M. (2011a). Preliminary report on molecular diversity of Sargassum species in Oman Sea by using ISSR and RAPD markers. *Acta Biologica Szegediensis*. 55 (1): 19-26.
- Noormohammadi Z, Ghasemzadeh Baraki S, Sheidai M, Rafiee F, Mohammad Gharanjik B. (2011b). Morphological diversity of Sargassum species of Iran. *Gene Conserve*. 10 (39): 1-22.
- Saitou N and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. 4 (4): 406-425.
- Siemer BL, Stam WT, Olsen JL, Pedersen PM. (1998). Phylogenetics relationships of the brown algal orders Ectocarpales, Chordariales, Dictyotales and Tilopteridales (Phaeophyceae) Based on RuBisCo Large Subunit and Spacer Sequences. *Journal of Phycology*. 34 (6): 1038-1048.
- Silberfeld T, Bittner L, Fernandez-Garcia C, Cruaud C, deReviere B, Rousseau F, Payri CE, DeClerck O. (2013). Species diversity, Phylogeny and large scale biogeographical patterns of the genus *Padina* (Phaeophyceae, Dictyotales). *Journal of Phycology*. 49 (1): 130-142.
- Sohrabipour J and Rabii R. (2008). Rhodophyta of Oman Gulf (South East of Iran). *Iranian Journal of Botany*. 14 (1): 70-74.
- Sohrabipour J and Rabii R. (1996). New Records of Algae for Persian Gulf and flora of Iran. *Iranian Journal of Botany*. 7 (1): 95-115.
- Sohrabipour J and Rabii R. (1999). A list of marine algae of sea shores of the Persian Gulf and Oman Sea in the Hormozgan province. *Iranian Journal of Botany*. 8 (1): 131-162.
- Sohrabipour J and Rabii R. (2007). The checklist of green algae of the Iranian coastal lines of the Persian Gulf and Gulf of Oman. *Iranian Journal of Botany*. 13 (2): 146-149.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*. 28: 2731-2739.
- Thompson JD, Higgins DG, Gibson TJ. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*. 22: 4673-4680.
- Tronholm A, Steen F, Tyberghein L, Leliaert F, Verbruggen H, Ribera Siguan MA, De Clerck O. (2010). Species delimitation, taxonomy and biogeography of *Dictyota* in Europe (Dictyotales, Phaeophyceae). *Journal of Phycology*. 46: 1301-1321.
- Trono CJ. (1969). The marine benthic algae of the Caroline Islands. II. Phaeophyta and Rhodophyta. *Micronesica*. 5: 25-119.
- Tsuda RT. (1972). Marine benthic algae of Guam Phaeophyta. *Micronesia*. 8: 87-115.
- Wynne MJ. (1998). A study of *Padina antillarum* (Kützinger) Piccone and a comparison with *P. tetrastratica* Hauck (Dictyotales, Phaeophyta). *Cryptogamie Algologie*. 4: 271-89.