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NOTES ON THE GENUS DICTYOTA (DICTYOTACEAE, PHAEOPHYCEAE) IN THE PERSIAN GULF, IRAN

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In this research, the taxonomic status of the genus *Dictyota* in southern coastlines of Iran was studied. Taxonomy of the genus at species levels is complicated and difficult due to the high morphological plasticity in response to environmental conditions and overlapping of diagnostic characters. In this study, we combined morphological information with cytoplasmic barcoding data from DNA *rbc*L and *psbA*. We identified *Dictyota acutiloba* as new record and also confirmed the presence of *Dictyota ciliolata* in the Persian Gulf and Gulf of Oman. Our studies showed *D. ciliolata* species has high inter-specific morphological diversity.

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Key words: Brown algae; *Dictyota;* DNA barcoding; new record; *psbA*; *rbc*L

نکاتی در مورد جنس Dictyota (خانواده Dictyotaceae ، جلبک‌های قهوه‌ای) در سواحل ایرانی خلیج فارس

مهناز صادقی: دانشجوی دکتری بیوتکنولوژی کشاورزی، دانشگاه زابل

براتعلی فاخری: استاد گروه اصلاح نباتات و بیوتکنولوژی، دانشگاه زابل

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در این مطالعه وضعیت تاکسونومیک جنس Dictyota درسواحل جنوبی ایران مورد بررسی قرار گرفت. طبقه‌بندی این جنس در سطح گونه به‌دلیل تنوع بالای مورفولوژیک در پاسخ به شرایط محیطی و همچنین همپوشانی ویژگی‌های تشخیصی پیچیده و دشوار است. برای روشن شدن، اطلاعات مورفولوژیک را با داده‌های توالی‌های ژن‌های سیتوپلاسمی rbcL و psbA تلفیق کردیم. در این مطالعه گونه Dictyota acutiloba به عنوان یک رکورد جدید از منطقه شناسایی کرده و حضور Dictyota ciliolata را براساس مطالعات درخلیج فارس تأیید نمودیم. همچنین بررسی‌های ما نشان داد که گونه D. ciliolata دارای تنوع مورفولوژیک درون گونه‌ای بالایی می‌باشد.

INTRODUCTION

The genus *Dictyota* J. V. Lamouroux is a considerable proportion of tropical marine flora (Tronholm & al. 2010a) and grow in intertidal and shallow subtidal habitats on rocky beaches worldwide (Herren & al. 2006; Sotka and Hay 2009). Totally 97 specific and infraspecific taxa were registered for the gens in algaebase website (Guiry & Guiry 2018). The

general definition of Dictyotales is often based on morphology and anatomical characters (Altamirano-Cerecedo & al. 2007; Wang & al. 2013). Vegetative characters in the Dictyotales member are very diverse (Tronholm & al. 2008; Steen & al. 2017). Accordingly, the classifications of *Dictyota* has been reviewed several times (De Clerck & al. 2006; Tronholm & al. 2013). The morphological plasticity and inability to distinguish valuable characters, especially in the great *Dictyota* genus, have led to a long and difficult taxonomic challenges (Silberfeld & al. 2014). The same species of the genus are grouped in separate categories based on apparent differences, or vice versa, different species categories in one group according to the apparent similarity (Tronholm & al. 2010b). Some morphological features such as the presence of the dentate margin, teeth shape, and the intervals at the interdichotomies, widely used to determine the *Dictyota* members in species level boundaries while these characters may lead to wrong classifications (Gauna & al. 2013). Hwang & al., 2005 reported a change in the thalli length of the *Dictyota* on the coast of Korea as a result of environmental conditions changes in each location (Hwang & al. 2005). The reported thalli length is variable due to environmental factors, especially temperature (Pirian & al. 2016). Recent studies have shown that morphological data without approving by DNA sequence data analyses is not enough to evaluate species diversity and determine the boundaries of species (Amini & al. 2013). The emergence of molecular markers has provided a new insight into the classification and DNA-sequence based taxonomy to solve many taxonomic challenging issues (Leliaert & al. 2014). Phylogenetic analysis based on nuclear, chloroplasts and mitochondrial DNA sequences, provided detailed views on the diversity of brown algae species (Lee & al. 2011). To date, seven species of the genus *Dictyota*, have previously been reported from southern coastlines of Iran (The Persian Gulf and Gulf of Oman), which are classically identified based on morphology (Silva& al. 1996; Sohrabipour & Rabii 1999; Sohrabipour & al. 2004; Kokabi & Yousefzadi 2015). The focus of this study was to evaluate the taxonomy of *Dictyota* in this region, based on combination of morphological studies and phylogenetic analyses of DNA, using the *rbcL* and *psbA* sequences data.

MATERIALS AND METHODS

Specimen collection

Specimens of the genus *Dictyota* were collected from Hormozgan province (Hormoz and Qeshm islands, Bandar-Jask and Bandar-Lenge), Bushehr Province (Bandar-Dayyer) in northern parts of Persian Gulf (Iran) in February, March and April 2017 (table 1). The collected algae were cleaned and transferred to the laboratory for further processing and herbarium samples were prepared and deposited at the Herbarium of the Research institute of Forests & Rangelands (TARI) and the herbarium of Agricultural and Natural Resource Research and Education Centre of Hormozgan Province, Bandarabbas, Iran.

Molecular studies

Total genomic DNA was extracted using the modified CTAB method (Doyle & Doyle 1990). The DNA samples were stored at -20°C and used as templates for PCR amplification. Partial regions of *rbc*L (~ 790bp) and *psbA*(~ 1000 bp) were amplified using the primers (table 1) designed by PRIMER3 software (Untergasser & al. 2012). The 20ml reactions contained 10 ml 2x pcr master mix, 1 ml of each primer and 1 ml of template DNA (100 ng) and the final volume was adjusted up to 20 ml with distilled water. The cycle was for 5 min initial denaturation at 94°C, followed by 35 cycles of 94°C for 45s, annealing at 53°C for 45s for the *rbcL* region and 51°C for 45s for the *psbA*, extension at 72°C for 1min, and a final extension at 72°C for 5 min. The PCR products were then purified and sequenced on an automated HiSeq 2000/250 sequencer (Illumina Inc., San Diego, USA) by Macrogen (Seoul, Korea).

Table 1. Primer sequences used in this study.

Gene	**Sequence (5' > 3')**		
(*rbc*L)	Fwd	TATTCCGAATCACACCTCAGC	this study
	Rev	TTTGGCGAGCATATGTTGAA	this study
(*psbA*)	Fwd	ATGACTGCTACTTTAGAAAGACG	Olivier De Clerck*&al* (2010)
	Rev	TCATGCATWACTTCCATACCTA	Olivier De Clerck*&al*(2010)

Phylogenetic analyses

The raw DNA sequences were edited using ChromasPro ver.2.1.3. (Technelysium Pty Ltd, Queensland, Australia) and blasted against the sequences of *rbc*L and *psbA* genes of the genus *Dictyota*. The sequences were aligned using ClustalX n.2.0.8 (Larkin & al. 2007) and manually adjusted using BioEdit v.7.0.9.0 (Hall 1999). The best-fit

models were selected using the corrected Akaike information criterion for the maximum likelihood (ML) (Akaike 1973) in KAKUSAN version 3(Tanabe 2007). The sequences were analyzed for ML with 1000 bootstrap replicates using TREEFINDER version October 2008. The accession numbers and collection data of the specimens investigated in this study and the acquired accession numbers from GenBank are shown in table 2.

RESULTS

Molecular analyses

The *rbc*L phylogenetic tree showed that the sequences of specimens including JA2, QE6, LE2 and HO3 from southern coastlines of Iran grouped with *D. ciliolata* with high bootstrap support for ML analaysis (fig.1) and sequences of specimens including LE1, LE3 and DA1 from southern coastlines of Iran grouped with *D. acutiloba* with high bootstrap support for ML analysis (fig.1).

Morphological study of *Dictyota ciliolata*

The specimens identified as *D. ciliolata* using the molecular analyses (figs.1 and 2) were collected from the intertidal zones of Bandar-Lenge, Bandar-Jask, Qeshm and Hormuz Islands (table 2), revealed considerable variations in their morphology (fig. 3: A, D & G). Thalli were flattened, erect or prostrate and ribbon-like. Some of the specimens had smooth margins [HO3 (fig.3 G)] whereas others were dentate [QE6 (fig.3 A), LE2 (fig.3 D) and JA2]. The colors varied from light green to medium brown. Thalli were (3.5) 5-8 (13) cm in length and (1) 2-4 (6) mm width with marginal proliferations. The phaeophycean hairs were present in some specimens. All specimens had dichotomous branching with different branching angles of (20) 40-60 (70) degrees, showing smooth or twisted growth patterns. We mainly observed regular branching patterns (fig.3 A & G) rather than irregular ones (fig.3.D). The main axis of all thalli had almost uniform width but varied in length [short and wide: HO3 (fig.3 G) vs. slender QE6 (fig.3 A), LE2]. Inter-dichotomies sizes ranged from (1) 1.5-2.5 (3) cm in length and (1) 2-4 (6) mm in width. Apices were acute (LE2 and JA2) or truncate (QE6 and HO3). At apical segments, the dichotomous intervals are (1) 2-6 (12) mm in length and (1) 1-2 (4) mm in width. Cross sections with 50-290 µm thickness included two layers of cortex and one layer of medullary cells. Both monolayer cortex contained small and regular cells (fig.3 C, F & I), which were 15-25 µm long and 10-15 µm wide. Medulla was also monolayer and contained large cells of 50-350 µm long and 25-275 µm wide. The detailed morphological data are presented in table 3.

Table 2. Specification of morphotypes of *Dictyota* with the collection details and GenBank accession numbers for *rbc*L and *psbA* sequences.

code	Locality	Latitude and longitude	Collection date	GenBank Acc. no. (*rbcL*)	GenBank Acc. no. (*psbA*)
Dictyota ciliolata					
QE6	shibderaz, Qeshm island	26°41'48.7"N 55°57'21.8"E	Feb.2017	MF325794	MK101392
HO3	Hormuz island	27°02'37.4"N 56°29'45.1"E	Feb.2017	MF325795	MK101393
LE2	Bandar-lenge	26°32'31.6"N 54°52'30.1"E	Mar.2017	MF325796	MK101394
JA2	Bahal, Bandar-jask	25°40'54.6"N 57°51'23.7"E	Apr.2017	MF538751	MK101395
Dictyota acutiloba					
LE1	Bandar-e Lenge	26°32'31.6"N 54°52'30.1"E	Feb.2017	MG602965	MK101389
LE3	Bandar-e Lenge	26°32'31.6"N 54°52'30.1"E	Mar.2017	MG602966	MK101390
DA1	Bandar-e Dayyer	27°50'16.4"N 51°53'22.3"E	Apr.2017	MG602967	MK101391

Morphological study of *Dictyota acutiloba*

The specimens identified as *D. acutiloba* using the molecular analyses (figs.1 and 2) were collected from the intertidal zones of Bandar-Lenge and Bandar-Dayyer (table 2), (fig. 4: A & B). Thalli were flattened, ribbon-like and erect or prostrate. Thallus with smooth margins, colors varied from olive green to medium brown, no iridescence and banding. Thalli were (6) 8-12 (20) cm in length and (0.5) 0.7 (1) mm in width and show marginal proliferations, phaeophycean hairs absent, dichotomous branching with different branching angles of (30) 40-55 (75) degrees, showing smooth to sinuate growth patterns. Branching patterns was mainly regular (fig.4 A). The main axis of thalli was almost uniform in width. Inter-dichotomies sizes ranged (1.5) 1.5-3 (4.5) cm in length and (0.5) 0.7-1 (1) mm in width. Apices were acute or wrench. At apical segments, the dichotomous intervals are (2) 3-4 (5) mm in length and (0.5) 0.6-0.8 (0.9) mm in width. Dark spots present in such a way that dark vertical lines appear on the surface of the thalli (A2 & B2). Cross sections with 90-250 µm thickness included two layers of cortex and monolayer of medullary cells in the middle. The cortex contained small regular cells (fig.4 F & G), with 20-40 µm long and 15-25 µm wide. Medulla was also monolayer and contained large cells of 50-185 µm long and 25-175 µm wide. The detailed morphological data are presented in table 3.

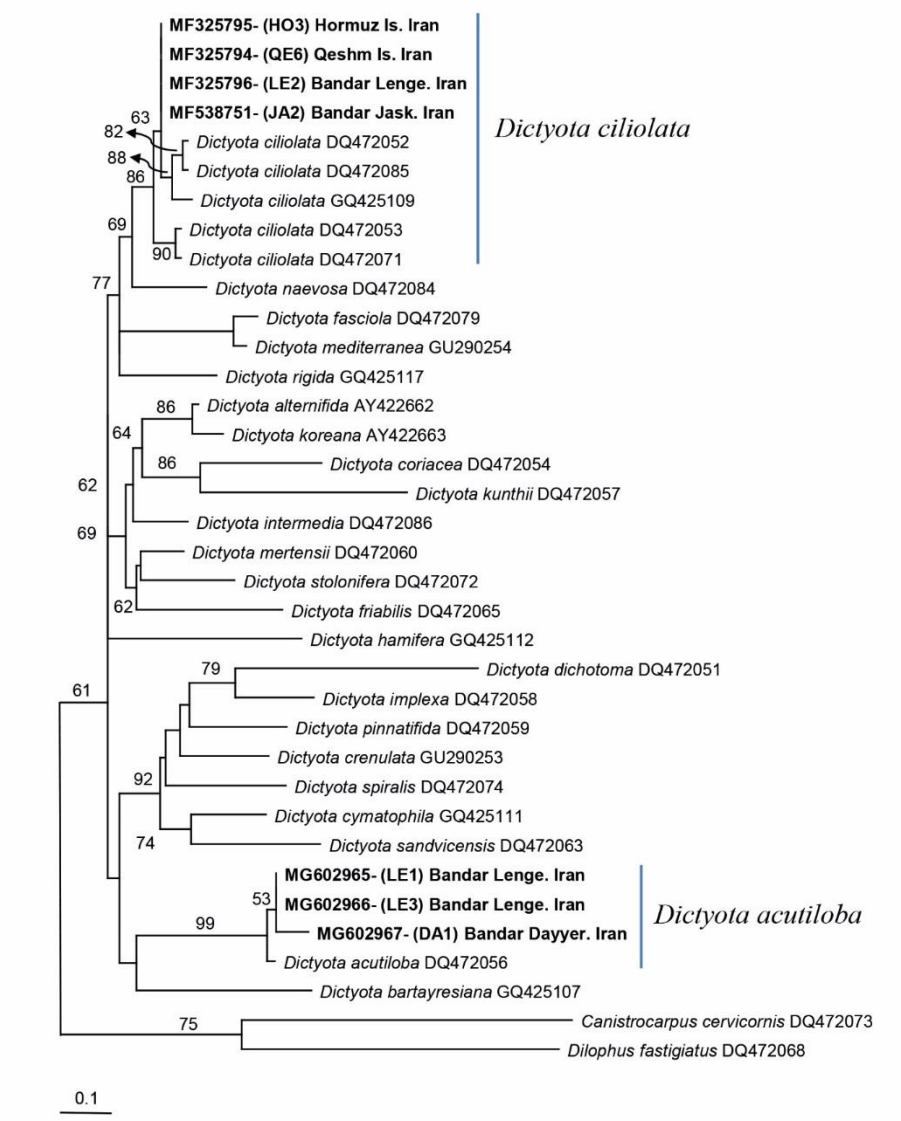

Fig. 1. Maximum likelihood (ML) tree for *rbc*L sequences of *Dictyota ciliolata* and *Dictyota acutiloba* from the southern coastlines of Iran and other regions. ML bootstrap values over 50% are shown at the nodes. Branch lengths are drawn proportional to the amount of the sequence changes.

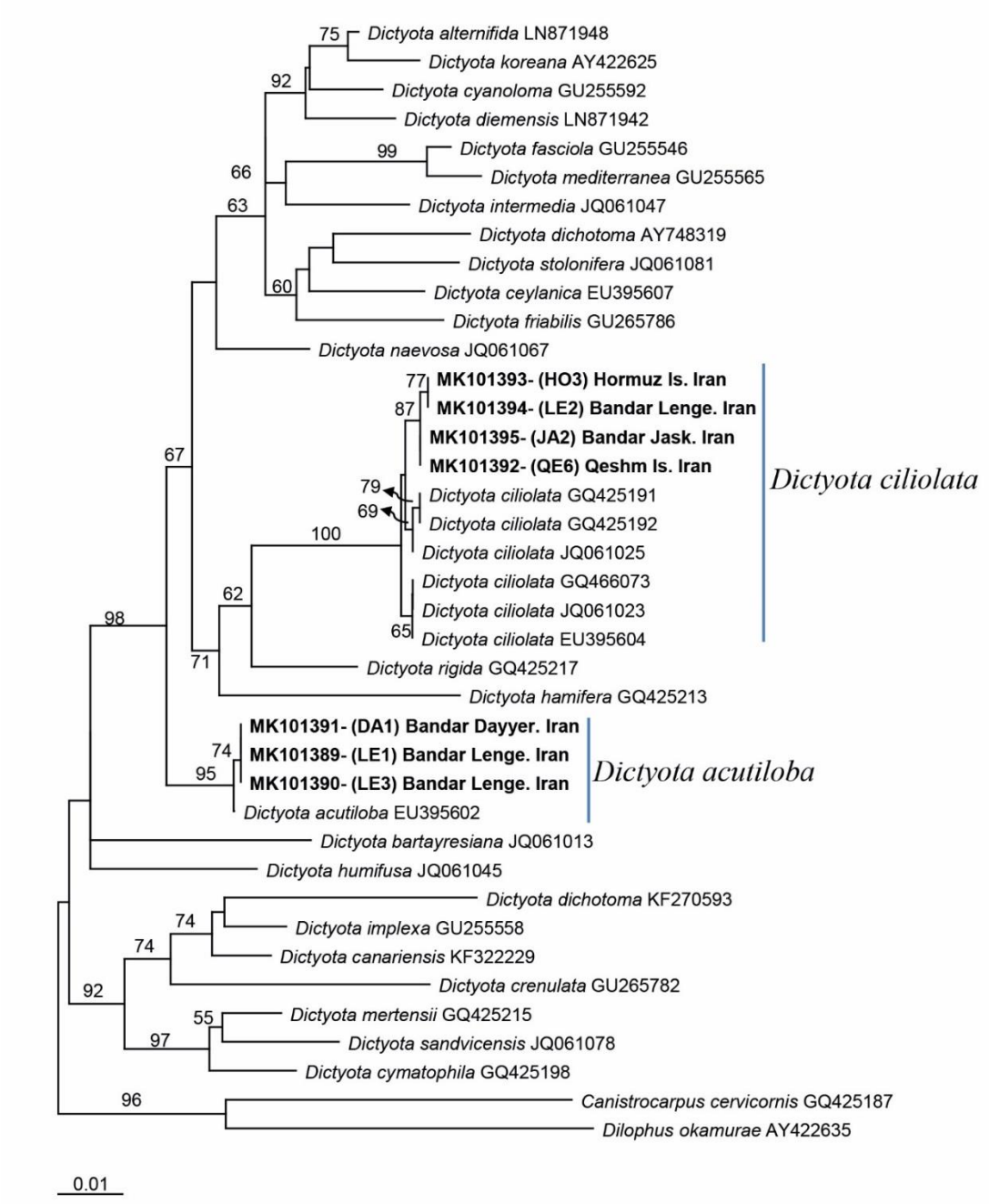

Fig. 2. Maximum likelihood (ML) tree for *psbA* sequences of *Dictyota ciliolata* and *Dictyota acutiloba* from the southern coastlines of Iran and other regions. ML bootstrap values over 50% are shown at the nodes. Branch lengths are drawn proportional to the amount of the sequence changes.

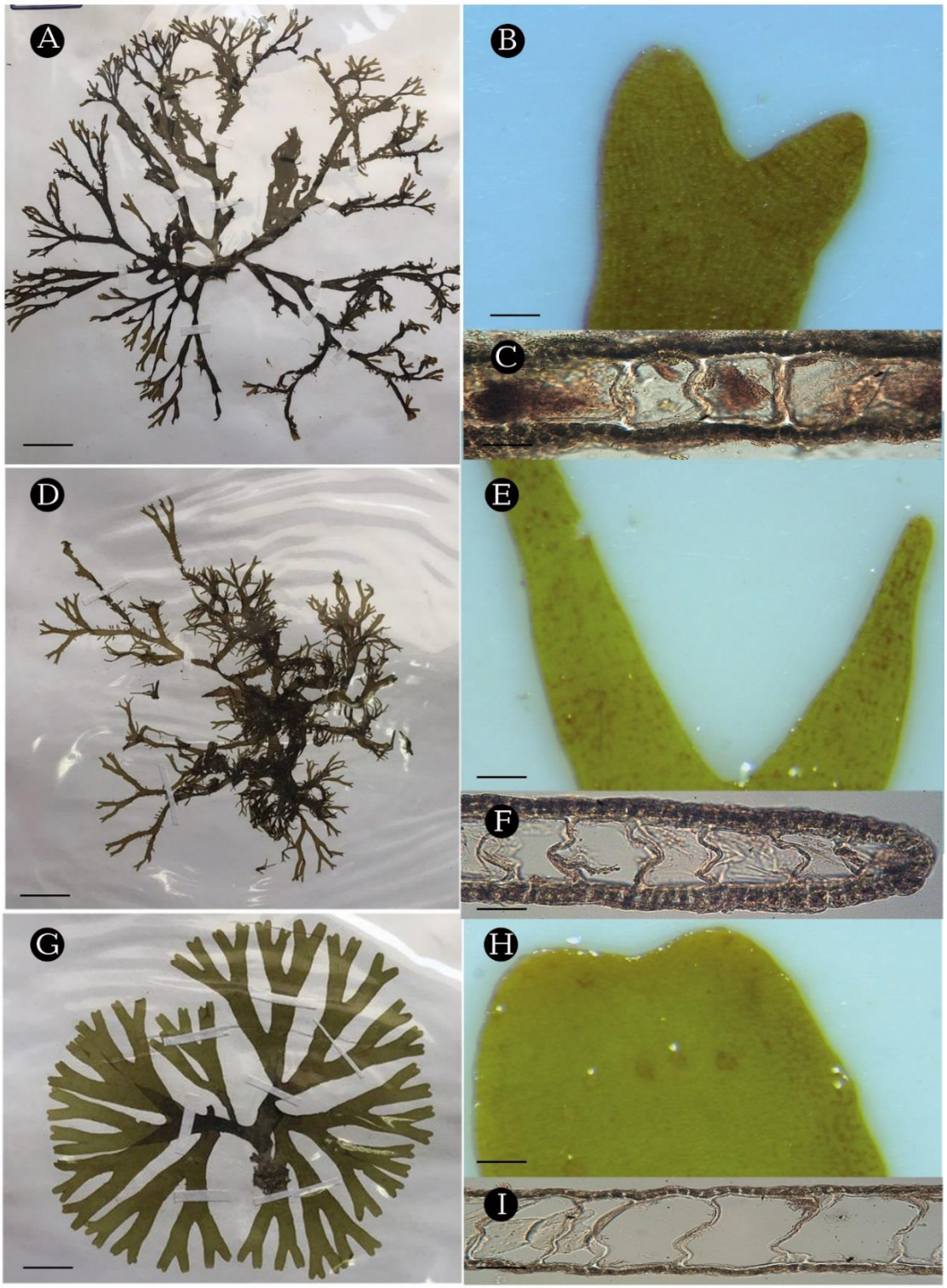

Fig. 3. Morphological types of *Dictyota ciliolata* from Persian Gulf and Gulf of Oman. A, D, G: habits of morphotypes (QE6, LE2 and HO3 respectively); Scale bars = 2 cm. B, E, H: detail of dichotomous branching; Scale bars = 1 mm. C, F, I: cross section of the middle portion of branches. Scale bars = 100 μm.

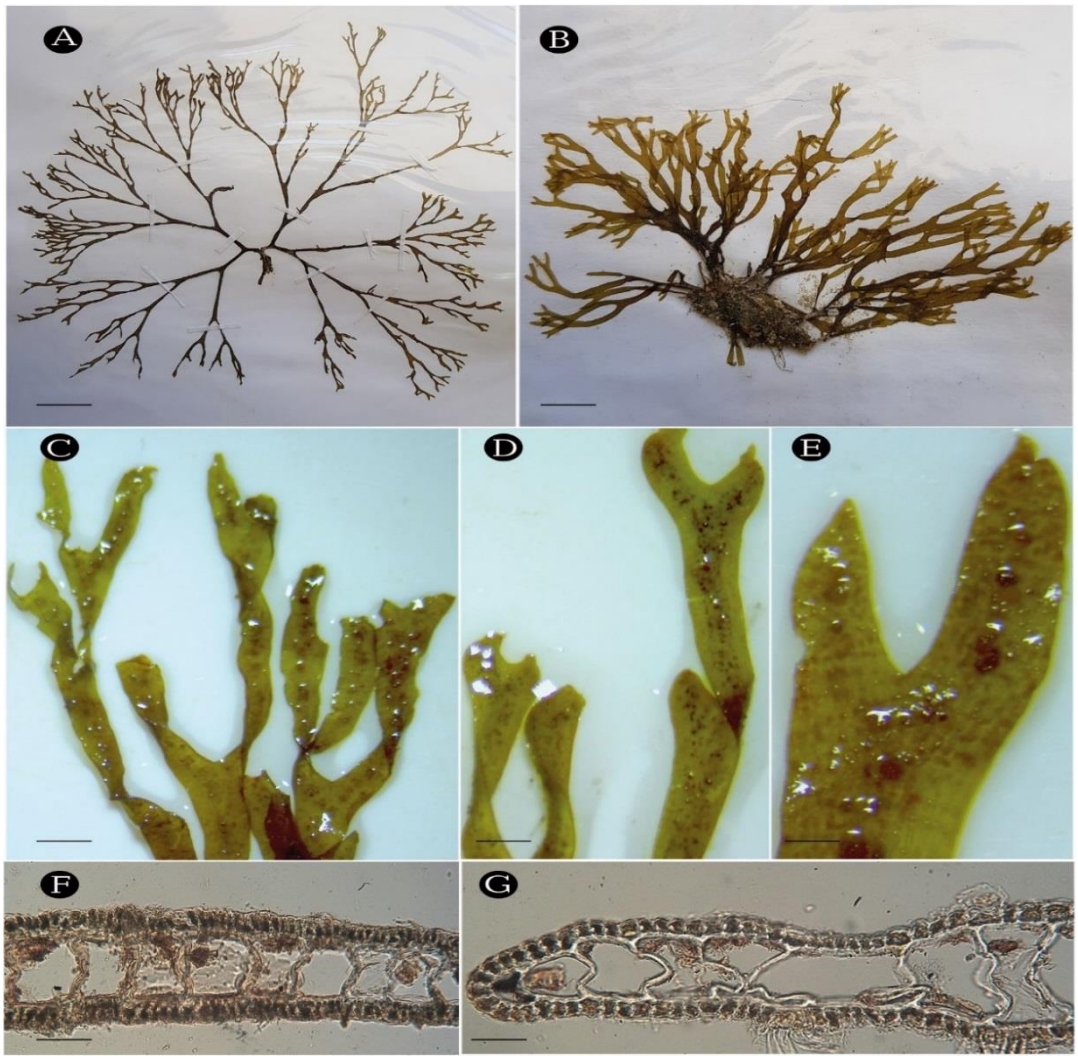

Fig. 4. Morphological types of *Dictyota acutiloba* from Persian Gulf. A, B: habits of sporophytes respectively LE1, DA1; Scale bar= 1 cm. C, D, E: detail of dichotomous branching; Scale bar respectively = 1mm; 1mm; 0.3 mm F, G: cross sections of the middle portion of a branch. Scale bars = 100µm.

DISCUSSION

In this study on *Dictyota* species belonging to the Dictyotaceae, we combine DNA barcoding data from two cytoplasmic genes, *rbc*L and *psbA* sequences, with morphological characteristics to reveal the clear taxonomic status of *Dictyota* genus in southern coastlines of Iran (Persian Gulf and Gulf of Oman).

The classification of Dictyotales is mainly based on the comparison of vegetative growth and reproduction Hwang & al. 2009). The species are components of warm and tropical marine flora. Nevertheless, many species are not easily defined through morphological characteristics, because of their diversity and the presence or absence of characteristics show significant changes (De Clerck & Coppejans 1999), including marginal dentation, the number of medulla layers also shows a significant difference between Dictyotales and can be varied in many species depending on the growth conditions (De Clerck and Coppejans 1999; Tronholm & al. 2013). Most tropical representatives of *Dictyota* have a similar reproductive and anatomical structure of the thallus, generally vary according to habit of thallus, size of interdichotomies, cortical and medullary cells size (De Clerck & al. 2001). Due to morphological plasticity, some of the recorded species from the previous studies of *Dictyota* may have been mistake. For example, the species *Dictyota crenulata* was misidentified as *Dictyota ciliolata*, based on the presence of marginal teeth (Tronholm & al. 2012).

Table 3. Morphological characters of *Dictyota ciliolata* and *Dictyota acutiloba*. Ml/Cl: ratio of the length of medulla cells to the cortical cells.

Characters	***D. ciliolata*** Iran	***D. acutiloba*** Iran
Thallus length cm	3.5-13	(6) 8-12 (20)
Texture	Crisp or supple	Supple
Habit	Flattened, erect or prostrate, Ribbon-like	Erect or prostrate
Margins	Smooth or dentation	Smooth
Color and Iridescence	Light green to medium brown	Olive green to medium brown
Branching	Isotomous dichotomous, often regular	Dichotomous, regular, smooth to Sinuate growth patterns
Branching angle	(20) 40-60 (70)	(30) 40-55 (75)
Phaeophycean hairs	Mostly present	Absent
Axes width	Almost uniform width	Almost uniform width
Inter dichotomies		
Length (mm)	(10) 15-25 (30)	(10) 15-20 (45)
Average width (mm)	(1) 2-4 (6)	(0.5) 0.7-1 (1)
L/W	(1.5) 2.5-3.75(5)	(10) 15-28 (45)
Apical segment		
Apical shape	Acute to truncate	Acute or wrench
Interdichotomous Length (mm)	(1) 2-6 (12)	(2) 2-4 (5)
Width (mm)	(1) 1-2 (4)	(0.4) 0.5-0.8 (0.9)
L/W	(1) 2- 3 (6)	(2.5) 3.7-8 (12.5)
Cortical cells		
Cortex length (µm)	15-25	(20) 25-35 (40)
Cortex width (µm)	10-15	(10) 15-20 (25)
Medullary cells		
Layers	Monolayer	Monolayer
length (µm)	50-350	(55) 90-130 (185)
Width (µm)	25-275	(50) 60-120 (175)
Ml/Cl (µm)	(1.5) 3.4 -7 (17.5)	(2.8) 4-4.6 (6.6)
Cross section thickness (µm)	(50) 70-150 (290)	(75) 150-190 (250)

Dictyota crenulata is very similar to *Dictyota ciliolata*, but different in the shape and frequency of marginal teeth (Tronholm & al. 2012).Also species that was reported at the first as *Dictyota bartayresiana*, in south of the Carol Sea, later shifted to a new taxa as *Canistrocarpus cervicornis* (Tronholm & al. 2013).The inability to distinguish morphologically diagnostic constants of traits that separate the different species, make specifying the boundary of the species highly variable and ambiguous (Tronholm & al. 2010a). Studies on European species have shown that morphological data without the use of DNA barcoding data is not sufficient to estimate the species diversity and boundaries (Amini & al. 2013). The DNA analysis showed is a very effective tool for investigating algal taxonomic relationships (Silberfeld & al. 2014).

To carry out the molecular analysis on the of the collected specimens from the southern coastlines of Iran we chose two cytoplasmic genes, i.e. chloroplast *rbc*L and *psbA*, because the cytoplasmic genes seem to provide clearer information in evolution (Bittner & al. 2008). *Our phylogentic analyses revealed* 0-1.6 % intraspecific divergence in *rbc*L sequences for *D. ciliolata* and showed a divergence of 2.5-3 % from the closest sister clade including *D. naevosa* and 7-9.6% divergence from the out group, *Canistrocarpus cervicornis*. In contrast, the intraspecific distances of *psbA* sequences was 0-0.6% and the nearest sister species, *D. rigida*, showed a divergence of 3.4-3.7% and divergence from the out group, *Dilophus okamurae* was 7.8-9.1%. On the other hand, in *D. acutiloba* the intraspecific divergence based on *rbc*L sequences was found to be 0-1.5 % and the closest species, *D. bartayresiana*, showed 5.7-6.8 % divergence from *D.*

acutiloba and 9.1-10.3% from the out group, *Canistrocarpus cervicornis*. However, the intraspecific distance of *psbA* sequences was found to be 0-0.1% and the closest species, *D. bartayresiana*, showed 4.8-4.9% and 7.67.8-25% divergence from the out group, *Dilophus okamurae*. Accordingly, we confirm the presence of *Dictyota ciliolata* and introduce a new record from *Dictyota acutiloba* in the southern coastlines of Iran.

In a revision of the *Dictyota* genus, using the phylogenetic analysis of *rbc*L and *26SrRNA*, *Pachydictyon*, *Glossophorella*, *Glossophora* and *Dilophus*, instead of five distinct genuse, were considered in the large *genus Dictyota Lamouroux*, which included the species with monolayer cortex and monolayer/multilayer medulla(De Clerck & al. 2006). Also, some species were separated from this taxon and two new genera of *Canistrocarpus De Paula & De clerck* and *Rugulopteryx De clerck & Coppejans* were formed. The species previously known as *Dictyota cervicornis* was changed to *Canistrocarpus cervicornis*(Hwang & al. 2009). Also, phylogenic studies showed that the species previously reported as *D. ciliolata* from the Mediterranean Sea which was introduced as new species named *D. cyanoloma*(Tronholm & al. 2010b).

Molecular data showed that some of the specimens collected in this study belong to *Dictyota ciliolate,* thalli of the species is completely erect, 8-15 cm long, texture somewhat crisp, color in situ brown, generally retain their color in dry specimens. The margins are normally dentate, but specimens with a reduced number of teeth or specimens without any dentation have also been observed. Interdichotomies width is mainly same size in whole thalli. Apices generally rounded, sometimes truncate, apical cell protruding (De Clerck 2003). The common characteristics of *D. ciliolata* collected in this study are a medium sized to large specimens, up to 15 cm long, dichotomously branched, wide of thalli in various morphotypes is narrow to slightly wide, main axis of thalli of a specimen has almost uniform width. The margins in almost morphotypes are dentate and sometime are smooth. Branching isotomous dichotomous and sometimes anisotomous dichotomous. branching angles of 20-70°. With marginal proliferations and without surface proliferations. The apices are rounded, acute or truncate. Cortex and medulla is monolayered.

According to barcoding data, other some collected specimens from the current studied area are *Dictyota acutiloba*, originally described from the Hawaiian Archipelago, is generally accepted that is a Pacific species (De Clerk & al. 2006; Lozano-Orozco & al. 2015). *D. acutiloba* thalli is erect, 4.5-16.5 cm long, medium brown in color, growth form often twisted, difficult to separate and spread out of branches when is out of water, mostly with dichotomous branching, margins smooth. Single medullary cell layer and single cortical layer. Apices extending to acute tips (Kraft 2009). Thalli of *D. acutiloba* is up to 20 cm in length, medium brown color, sinusoidal form, smooth margins, blades become narrow from the base to the tip, apices acute to round, distance between Inter dichotomies of thalli often unequal, base and middle part of the thalli are equal and longer, with no surface proliferations (fig 4 &table 3). Variable environmental conditions affect the occurrence of brown algae. Population of *Dictyota* mostly appear in the cold season. In fact, visible part of the specimens of the species disappear completely in summer (Tronholm & al. 2008). Similarly, the maximum abundance of *D. acutiloba* and *D. ciliolata* from the Persian Gulf was observed in the intertidal to shallow subtidal zones on hard and rocky substrate from January to march while nothing during the summer.

In this study, we investigated morphological characterizations combined with the molecular analysis of the *Dictyota* algae species in the Persian Gulf and Gulf of Oman, Iran. We reported new record of *D. acutiloba* and confirmed the presence of *D. ciliolata*. Future studies may lead to identification of new taxa and increasing the total number of speies in the area.

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