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Research Article

Dictyota pulvinata sp. nov. (Dictyotaceae) a new Indo-Atlantic brown algae

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Abstract

Dictyota, as a diverse genus of the family Dictyotaceae (brown algae) in warm temperate to tropical seas, is characterized by high morphological plasticity. The wide range of morphological variation in the members of this genus makes the species distinction based on morphological or anatomical features complicated. Persian Gulf and Gulf of Oman as a subregion of subtropical marine environments in south of Iran has various species of Dictyotaceae. This study investigated the taxonomy of *Dictyota* as one main tropical genus of the family Dictyotaceae in southern coastlines of Iran. In the current study DNA sequences of the two genes *rbc*L and *Cox*3, as well as their morphological features were analyzed to determine the species distinction of the genus in the studied areas. In *rbc*L phylogenetic tree, some of the sequences obtained from Iranian specimens of *Dictyota* formed a fully supported new clade with four sequences obtained from specimens collected from Mayotte, Bermuda, Netherland Antilles and Bahamas (Caribbean Sea). In *Cox*3 phylogenetic analyses, the sequences of the specimens from Iran were grouped with two sequences of *Dictyota* specimens collected from Egypt and Bahamas. The results showed the new clade in both *rbc*L and *Cox*3 tree represent a distinct new taxon with anastomosing points between overlaps blades which led to cushion-like (pulvinus) habit as diagnostic characteristic, so, the taxon is introduced as the new species *Dictyota pulvinata* sp. nov. The sequences of another species of Dictyotaceae in the current study were grouped with the sequences of *Canistrocarpus cervicornis* from Florida in United States of America, where the type specimen of *Canistrocarpus cervicornis* has already been reported.

Keywords: Dictyotaceae, New species, Phylogeny, cox*3*, rbcL., Persian Gulf

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Introduction

Currently 2092 species of brown algae (Phaeophyceae) have been registered in algaebase website. *Dictyota*, which was recognized for the first time by Lamouroux (1809), currently accommodates 97 species (Guiry and Guiry, 2022). To date, 79 taxa belong to seven families of brown algae have been identified from the southern coastlines of Iran, Persian Gulf, of which the Sargassaceae with 33 species and Dictyotaceae with 21 species are the most diverse genera (Sohrabipour and Rabii, 1999; Kokabi and Yousefzadi, 2015). Six species of the genus *Dictyota* has already reported from this area based on morphological features (Silva *et al*., 1996; Sohrabipour *et al*., 2004; Kokabi and Yousefzadi, 2015). Molecular studies confirmed the presence of *Dictyota acutiloba*, *Dictyota ciliolata*, *Spatoglossum crassum* and *Stoechospermum polypodioides* species of Dictyotaceae family in the Persian Gulf (Sadeghi *et al*., 2019, 2020). *Dictyota* species are common in intertidal pools and the infralittoral to relatively deep waters (Herren *et al.*, 2006; Sotka and Hay, 2009; Tronholm *et al*., 2010a). In contrast to the majority of brown algae, the genus *Dictyota* has high diversity and density in tropical waters (Lüning, 1990; Wiesemeier *et al*., 2007; Gauna *et al*., 2013). High plasticity in morphological features of *Dictyota* species, make their classification complicated at species level (De Paula *et al*., 2007; El-Shoubaky and Salem, 2014; Lozano-Orozco *et al*., 2014). Even widely used morphological traits, such as marginal teeth, are not always clear diagnostic for confident recognition of the *Dictyota* species (Hornig *et al*., 1992; De Clerck and Coppejans, 1997, 1999; De Clerck, 2003; Hwang *et al*., 2005; Tronholm *et al*., 2010b, 2013). These problems highlight the need for molecular analyses.

Progress in DNA-assisted molecular taxonomy greatly contributed to accurate classification of many organisms (Saunders and Lehmkuhl, 2005; Leliaert *et al*., 2014; Kazi *et al*., 2016). Phylogenetic analyses of Dictyotales based on nuclear, plastid and mitochondrial DNA sequences resulted in distinction of the order members at species level (De Clerck *et al*., 2001; Lee *et al*. 2011; Lozano-Orozco *et al*., 2015). Chloroplast markers (*rbc*L, *psb*A) in combination with mitochondrial markers (*cox1*, *cox3*, *nad1*) and the large subunit ribosomal DNA (*LSU* rDNA) are widely used in molecular taxonomy of Dictyotales and have gradually refined species boundaries (Ni Ni Win *et al*., 2008, 2010, 2011; Tronholm *et al*., 2010a, 2013; Vieira *et al.*, 2014, 2016), their respective ranges (Tronholm *et al*., 2012; Steen *et al*., 2015; Vieira *et al*., 2017), and identified non-native species (Verlaque *et al*., 2009; Steen *et al*., 2017).

The aim of the current study is to have a deeper sight to the previously reported species of the genus *Dictyota* (Dictyotaceae) from the Persian Gulf. Based on the previous reports six

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species of Dictyota, including *Dictyota ciliolata, D. dichotoma, D. friabilis, D. implexa, D. indica* and *D. cervicornis* have been reported. (Al-Hasan and Jones, 1989; De Clerck *et al.*, 1996, Sohrabipour and Rabii, 1999; Sohrabipour *et al.*, 2004; Kokabi and Yousefzadi, 2015). Here, we further evaluated *Dictyota* in this region using both morphology and molecular taxonomy based on the *rbc*L and *cox*3 sequences data.

Materials and methods

Algal specimens from Iranian coasts were collected from Hormuz Island (27° 03.361' N 56° 29.965' E) and Larak Island (26° 52.891' N 56° 24.286' E) in marine waters of the Persian Gulf between January and March 2016. Specimens from Mayotte, Bermuda, Netherland Antilles and Bahamas (Caribbean Sea, Fig. 1) were kindly provide by one the authors (Professor De Clerck) and their rbcL sequences also had been obtained. After sample collection and transfer to the laboratory, the Iranian specimens were cleaned and prepared as voucher specimens and some pieces of apical parts of each specimen were completely cleaned and dried in silica gel for molecular studies. The voucher specimens were deposited at the herbarium of the Research Institute of Forests and Rangelands (TARI), Iran.

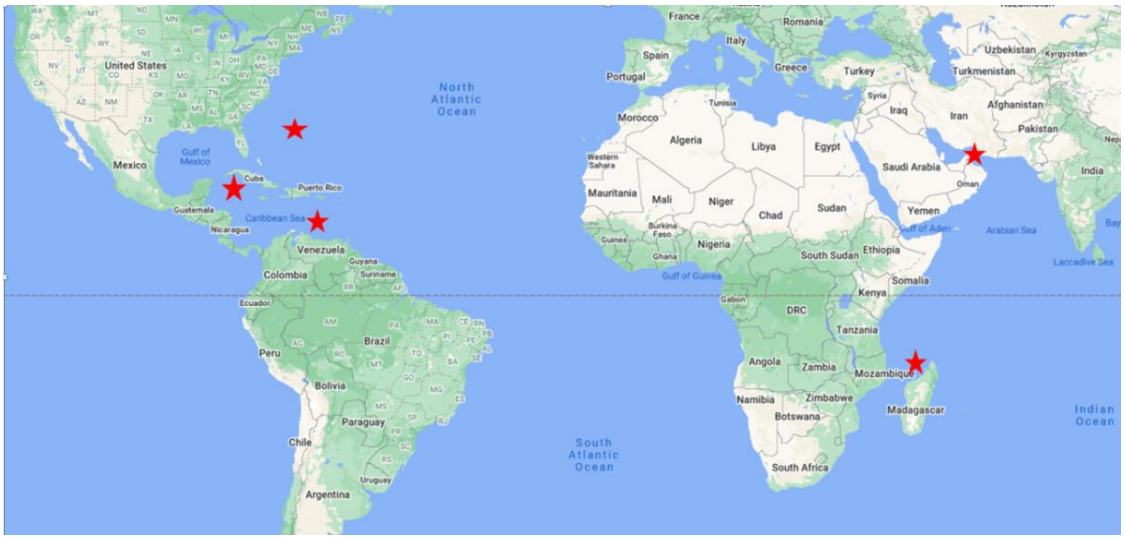


Figure 1: Red star signs on the map show the specimens collection sites.

CTAB method (Doyle and Doyle, 1990) was used for DNA extraction with moderately modification by using sarkosyl (Sodium N-Lauroylsarcosinate) to reduce the inhibition effects of polyphenolic and polysaccharide compounds. DNA samples were stored at -20°C for next PCR amplification. Partial regions of the *rbc*L (~790bp) and *cox*3 (~750bp) genes were amplified using the following designed primers:
*rbc*L_forward:
TATTCCGAATCACACCTCAGC;
*rbc*L_reverse:
TTTGGCGAGCATATGTTGAA;

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*cox*3_forward: GTAGATCCAAGCCCCTGGCC; *cox3*_reverse: ACAAAGTGCCAATACCAAGC. PRIMER3 was used to design the primers (Untergasser *et al*., 2012). The PCR reaction consisted of an initial denaturation at 94°C step for 5 min, followed by 35 cycles of 94°C for 45 sec, annealing at 55°C for 45 sec for *rbc*L or 50°C for 45 sec for *cox*3, an extension at 72°C for 1 min and a final extension at 72°C for 5 min. The PCR products were then purified and sequenced using an automated HiSeq 2000/250 sequencer (Illumina Inc., San Diego, USA) by Macrogen (Seoul, Korea).

Phylogenetic analyses

DNA sequences were edited using ChromasPro version 2.1.3. (Technelysium Pty Ltd, Queensland, Australia), then were blasted in NCBI (National Center for Biotechnology Information) and the most similar sequences were acquired from the GenBank. Totally 41 *rbc*L sequences were aligned. The aligned sequences using ClaustalX n.2.0.8. include six sequences obtained from Iranian specimens, and four unregistered sequences from Atlantic Ocean and Caribbean Sea (kindly provided by Professor De Clerck) and 17 cox3 sequences including six sequences taken in this study (Larkin *et al*., 2007). Finally, they were manually trimmed and adjusted using BioEdit v.7.0.9.0 (Hall, 1999). The best-fit models were selected using KAKUSAN version 3 (Tanabe, 2007) according to the corrected Akaike (1973) information criterion for ML and the Bayesian criterion (BIC) for BI probabilities. Maximum likelihood tree searches were performed in TREEFINDER, version October 2008 (Jobb *et al.*, 2004). Confidence was assessed using 1000 bootstrap replicates. MrBayes V.3.2.1.X86 program (Ronquist and Huelsenbeck, 2003) was used for reconstruction of the Bayesian tree with 2 chains run for 10^6 generations and sampling the data every 100 generations. Maximum parsimony (MP) analyses were carried out using PAUP version 4.0b.10. We applied a heuristic search algorithm with 1000 random sequence additions, the tree bisection and reconnection (TBR) branch swapping and bootstrap analyses using 1000 replicates. The trees were rooted with *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim, *Dilophus fastigiatus* (Sonder) J.Agardh and *Scoresbyella profunda* Womersley for *rbcL* region and with *Rugulopteryx okamurae* and *Scoresbyella profunda* for *cox*3 gene. The GenBank accession numbers of the sequences obtained from the collected and investigated specimens in this study are shown in Table 1. To determine the variation levels in *rbc*L and *cox*3 sequences, the absolute as well as corrected pairwise genetic distances were calculated in PAUP 4.0b.10.

For morphological study, the recorded data were length and width of thallus, branching angle, marginal teeth, shape of the apices, presence of

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phaeophycean hairs and surface proliferations. In the transverse sections of blades thickness of the blades and size of cortical and medullary cells were measured using BH-2 microscope (Olympus Microscopes, Tokyo, Japan). Photographs were taken using a Nikon DXM1200 digital camera (Tokyo, Japan).

Table 1: Species of *Dictyota* and *Canistrocarpus* with collection details and GenBank accession numbers for *rbc*L and *cox*3 sequences.

Code	Locality	Latitude and longitude	Collection date	GenBank accession nr. (*rbc*L)	GenBank accession nr. (*cox*3)
***Dictyota pulvinata sp. nov*.**					
LA3	Larak island	26° 52.891' N 56° 24.286' E	Feb.2016	MG602971	MG602973
LA4	Larak island	26° 53.391' N 56° 21.321' E	Mar.2016	MG602972	MG602974
Canistrocarpus cervicornis					
HO2	Hormuz island	27° 03.361' N 56° 29.965' E	Jan.2016	MF538755	MF538758
LA1	Larak island	26° 52.891' N 56° 24.286' E	Feb.2016	MF538760	MF538764
LA2	Larak island	26° 52.891' N 56° 24.286' E	Feb.2016	MF538761	MF538765
HO4	Hormuz island	27° 04.115' N 56° 25.601' E	Feb.2016	MF538756	MF538759

Results

Molecular analyses

The *rbc*L dataset consisted of 678 bp of which 144 characters were parsimony informative. The *cox*3 alignment consisted of 557 bp of which 189 characters were parsimony informative. In the *rbc*L trees, LA3 (MG602971) and LA4 (MG602972) both from Iran formed a clade with the unregistered sequences from Bermuda (CLO12603), Mayotte (LYD10276), the Dutch Antilles (DML67430) and Bahamas (DML68137), sequences were created by De Clerck (personal communication, Fig. 2). A similar tree emerged from the *cox*3 phylogenies, where the sequences of LA3 (MG602973) and LA4 (MG602974) clustered with specimens from Egypt (SGAD1051) and the Bahamas (DML68137), with full support (Fig. 3). The new clade is described here as *Dictyota pulvinata* sp. nov.

Four sequences obtained from specimens of other species of Dictyotaceae including HO2 (MF538755), LA2 (MF538761), LA1 (MF538760) and HO4 (MF538756) were grouped with well-supported bootstrap values with the sequences of *Canistrocarpus cervicornis* (Kützing) De Paula and De Clerck from Florida in United States of America, where the type specimen of *Canistrocarpus cervicornis* has been reported. Intraspecific sequence divergence of the *rbc*L gene was 0-0.5 % and 0% in *D. pulvinata sp. nov.* and *C. cervicornis*, respectively (Table 2), while intraspecific sequence divergence of the *cox*3 gene was marginally higher 0-

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1.3% and 0.2-0.8% for *D. pulvinata sp. nov*. and *C. cervicornis*, respectively (Table 3).

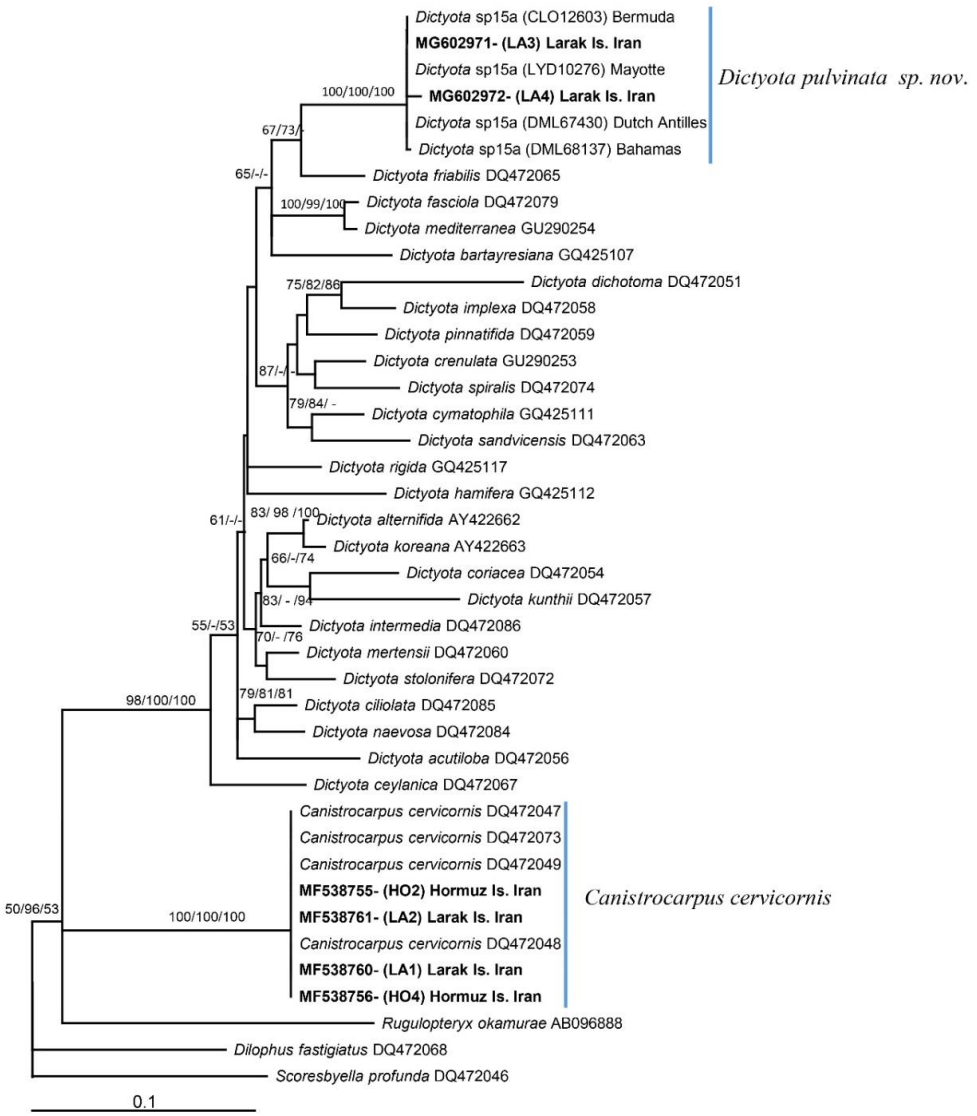


Figure 2: Maximum likelihood (ML) tree for *rbc*L sequences of *Dictyota pulvinata sp. nov.* and *Canistrocarpus cervicornis* from southern coastlines of Iran and other regions. Bootstrap support values for each node are shown for ML, MP and BI. Branch lengths are drawn proportional to the amount of sequence changes.

Taxonomy

Dictyota pulvinata Sadeghi, Sohrabipour et De Clerck, sp. nov.

Description

Thalli are flattened, greenish brown, lacking iridescence and banding, with smooth margins. Thalli are (4-) 5-6 (-7) cm long and (1-) 1.5 (-2) mm width, lacking marginal proliferations. Phaeophycean hairs are not observed. Branching is regularly dichotomous to somewhat irregular. Branching angles are (20) 30-40 (60)°. Axes are gradually narrowing from the basal segment

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toward the apical segment. Anastomosis is common among axes, which are placed on top of each other, leading to a cushion-shaped habit as the diagnostic character of the species. Upper parts of the thallus are generally narrower and thinner than basal and lower blades. Inter-dichotomies ranging from (3) 5-7 (10) mm in length and (0.5) 1 (1.5) mm in width.

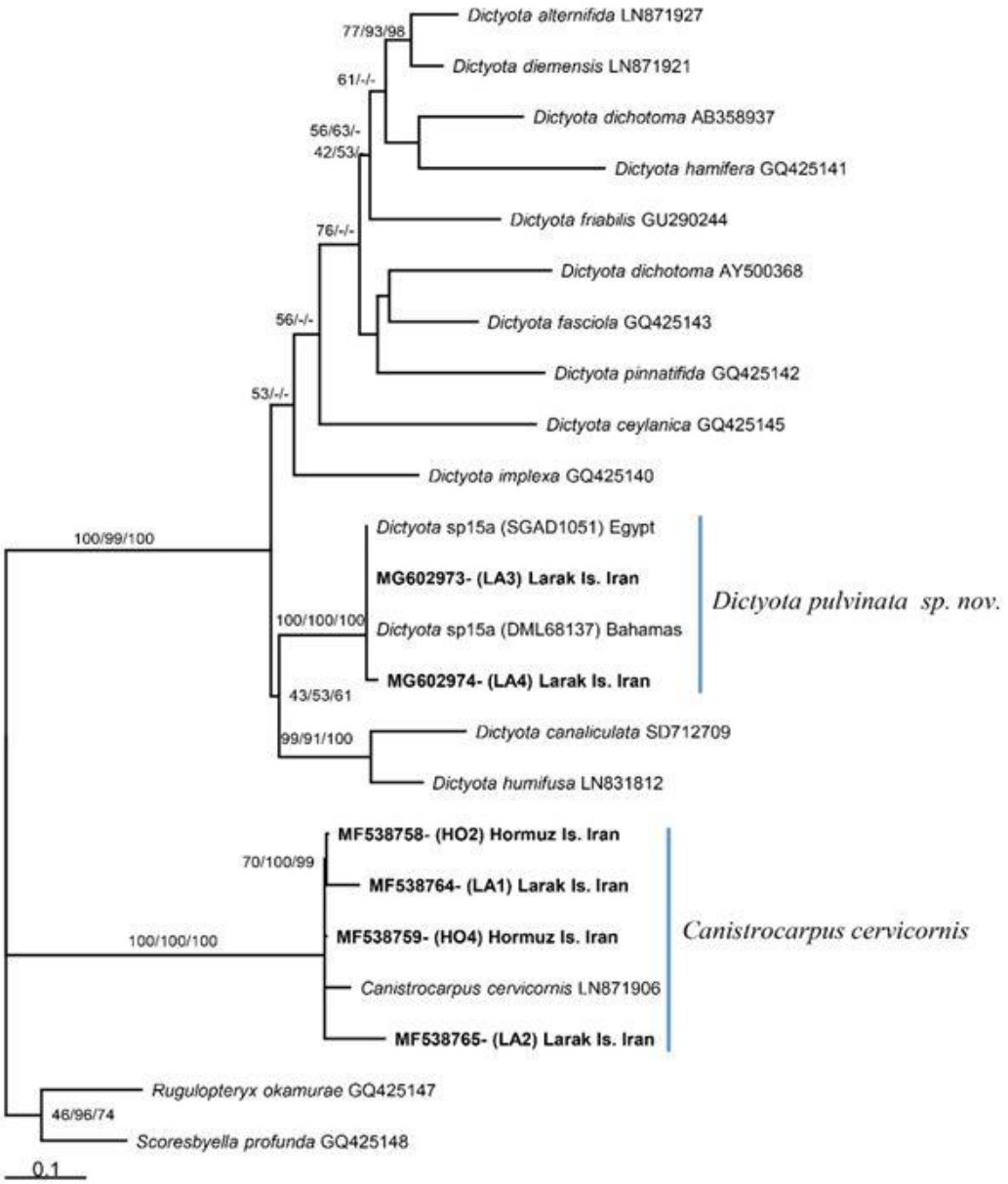


Figure 3: Maximum likelihood (ML) tree for cox3 sequences of *Dictyota pulvinata* sp. nov. and *Canistrocarpus cervicornis* from southern coastlines of Iran and other regions. Bootstrap support values for each node are shown for ML, MP and BI. Branch lengths are drawn proportional to the amount of sequence changes.

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Table 2: Divergence matrix of *rbc*L sequences showing uncorrected pairwise genetic distances between *Dictyota pulvinata sp. nov.*, *Canistrocarpus cervicornis* and other species from Iran and other geographical regions (GenBank data).

Species	1. *D. pulvinata* (IR, BR, MA, NA, BA)	2. *D. Friabilis* (HW)	3. *D. Bartayresiana* (KN)	4. *D. alternifida* (AU)	5. *D. koreana* (KO)	6. *D. Mertensii* (JM)	7. *D. Naevosa* (SA)	8. *D. Acutiloba* (HW)	9. *D. ceylanica* (FP)	10. *C. Cervicornis* (IR, PH)	11. *Dilophus fastigiatus* (AU)	12. *Scoresbyella profunda* (AU)
1.*D. pulvinata*	0.0-0.5	-	-	-	-	-	-	-	-	-	-	-
2.*D. friabilis*	4.0-4.6	0.0	-	-	-	-	-	-	-	-	-	-
3.*D. bartayresiana*	4.6-5.1	5.8	0.0	-	-	-	-	-	-	-	-	-
4.*D. alternifida*	4.4-4.9	4.6	4.9	0.0	-	-	-	-	-	-	-	-
5.*D. koreana*	4.7-5.3	4.9	5.1	0.5	0.0	-	-	-	-	-	-	-
6.*D. mertensii*	3.5-4.1	4.0	5.3	3.2	3.2	0.0	-	-	-	-	-	-
7.*D. naevosa*	5.4-6.0	5.4	5.2	4.0	4.2	4.2	0.0	-	-	-	-	-
8.*D. acutiloba*	6.0-6.5	6.3	5.9	5.3	5.6	4.6	4.9	0.0	-	-	-	-
9.*D. ceylanica*	5.1-5.6	6.1	5.6	4.7	5.1	4.4	4.7	4.9	0.0	-	-	-
10.*C. cervicornis*	9.3-9.9	9.7	10.3	9.5	9.9	9.3	9.9	9.3	7.9	0.0-0.0	-	-
11.*Dil. fastigiatus*	8.5-9.1	8.9	8.5	8.7	9.1	8.4	9.1	8.6	8.2	8.7	0.0	-
12.*Sco. profunda*	10.1-10.7	10.1	11.2	10.5	10.9	10.3	9.9	10.6	9.7	9.7	9.3	0.0

IR, Iran; BR, Bermuda; MA, Mayotte; NA, Netherlands Antilles; BA, Bahamas; HW, Hawaii; KN, Kenya; AU, Australia; KO, Korea; JM, Jamaica; SA, South Africa; FP, French Polynesia; PH, Philippines.

Holotype

10265 (Fig. 4: A1), is deposited at herbarium of the Research Institute of Forests and Rangelands, (TARI), Iran, which was collected by M. Sadeghi and J. Sohrabipour, 19 February 2016. GenBank accession numbers are for *rbc*L: MG602971 and *cox*3: MG602973.

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Table 3: Divergence matrix of *cox*3 sequences showing uncorrected pairwise genetic distances between *Dictyota pulvinata sp. nov.*, *Canistrocarpus cervicornis* and other species from Iran and other geographical regions (GenBank data).

Species	1. *D. pulvinata* (IR, EG, BA)	2. *D. Canaliculata* (IN)	3. *D. Humifusa* (MD)	4. *D. implexa* (CR)	5. *D. ceylanica* (PH)	6. *D. Friabilis* (HW)	7. *D. Hamifera* (FP)	8. *D. Dichotoma* (JP)	9. *D. diemensis* (AU)	10. *D. Alternifida* (AU)	11. *C. cervicornis* (IR, PH)	12. *Rugulopteryx okamurae* (FR)
1. *D. pulvinata*	0.0-1.3	-	-	-	-	-	-	-	-	-	-	-
2. *D. canaliculata*	15.2	0.0	-	-	-	-	-	-	-	-	-	-
3. *D. humifusa*	14.6-14.7	10.8	0.0	-	-	-	-	-	-	-	-	-
4. *D. implexa*	13.5-14.1	15..2	14.6	0.0	-	-	-	-	-	-	-	-
5. *D. ceylanica*	17.7-18.5	20.8	17.9	20.1	0.0	-	-	-	-	-	-	-
6. *D. friabilis*	14.4-14.6	19.3	15.7	18.4	18.4	0.0	-	-	-	-	-	-
7. *D. hamifera*	17.8-18.4	21.1	17.8	18.7	22.8	15.6	0.0	-	-	-	-	-
8. *D. dichotoma*	18.7-19.1	17.4	18.1	16.7	20.8	17.4	22.1	0.0	-	-	-	-
9. *D. diemensis*	17.2	20.3	17.6	15.0	18.5	15.1	17.7	16.3	0.0	-	-	-
10. *D. alternifida*	16.0	19.6	18.0	14.8	18.4	15.4	17.8	15.2	8.3	0.0-0.0	-	-
11. *C. cervicornis*	22.3-24.1	23.7-25.6	21.3-23.2	24.0-25.7	25.7-27.0	24.0-26.3	23.7-26.6	23.8-27.5	24.6-27.8	23.1-24.5	0.2-0.8	-
12. *Rug. okamurae*	22.6-24.1	25.8	23.2	20.3	26.4	23.9	24.9	20.6	22.1	23.6	21.8-25.0	0.0

IR, Iran; EG, Egypt; BA, Bahamas; IN, Indonesia; MD, Madagascar; CR, Croatia; PH; Philippines; HW, Hawaii; FP, French Polynesi;JP, Japan; AU, Australia; FR, France.

Isotypes

2969, is deposited at the herbarium of Agricultural and Natural Resource Research and Education Centre of Hormozgan Province, Bandar-Abbas, Iran, which was collected by M. Sadeghi and J. Sohrabipour, 15 March 2016. GenBank accession numbers are for *rbc*L: MG602972 and *cox*3: MG602974.

Type locality

Type locality was 26° 52.891' N 56° 24.286' E; Larak Island, Strait of Hormuz, Persian Gulf, Iran.

Etymology

Pulvinata, refers to the cushion-like habit of the species.

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Morphological features

Dictyota pulvinata grow in cushion-like forms in the intertidal and shallow subtidal zones on sandy or hard substrates and attach to substrate by patches of basal rhizoids. Thalli consisted of flattened, ribbon-like axes, greenish brown in color with smooth margins, lacking iridescence or banding. Thalli are (4-) 5-6 (-7) cm long with individual axes (1-) 1.5 (-2) mm in width (Fig. 4: A1 and A2). Apices were rounded to acute (Fig. 4: A2 and A3). Axes are (40-) 45-65 (-70) µm in thickness, and consisted of a unilayered cortex surrounding a unilayered medulla. Cortical cells were (15-) 18-20 (-25) µm in long and (12-) 15-18 (-20) µm wide. Medulla cells measured (50-) 55-75 (-100) µm in length and (15-) 25-40 (-45) µm in width (Fig. 4: A4). The detailed morphological data are presented in Table 4.

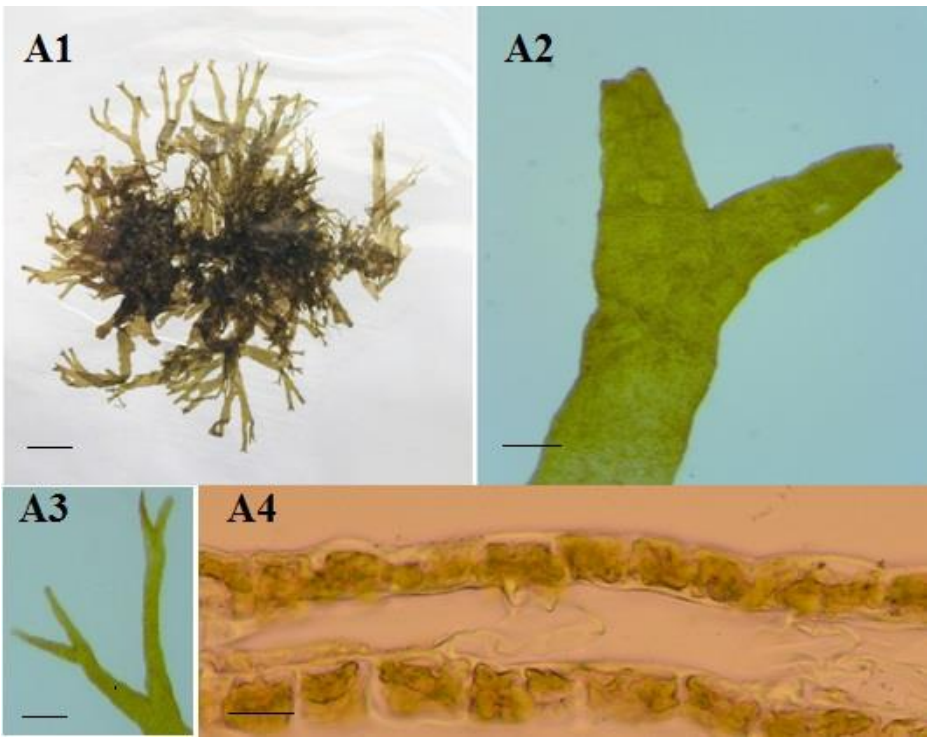


Figure 4: *Dictyota pulvinata sp. nov.*: A1. Habits of sporophytes. Scale bar=1cm; A2. Detail of dichotomous branching of the blades (rounded apical cells). Scale bar=1mm; A3. Detail of apex of the thallus (acute apical cells). Scale bar=0.3mm; A4. Transverse section at upper parts of blade. Scale bar=20µm.

Canistrocarpus cervicornis De Paula and De Clerck (2006).

The specimens identified as *C. cervicornis* via molecular analyses (Figs. 2 and 3) were collected from intertidal zones of Hormuz and Larak islands (Table 1). There was a comprehensive morphological description of the species in De Clerck (2003) as *Dictyota cervicornis* with detailed worldwide distribution.

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Table 4: Morphological characters of *Dictyota pulvinata sp. nov.* and *Canistrocarpus cervicornis* from Iran (this study).

Character	*Dictyota pulvinata*	*Canistrocarpus cervicornis*
Thallus length cm	(4) 5-6 (7)	(5) 7-10 (20)
Texture	Supple	Crisp
Habit	Flattened, erect	Racemose, erect or prostrate, ribbon-like, repeatedly dichotomously branched
Margins	Smooth	Smooth
Color and Iridescence	Greenish brown	Mustard to dark brown; iridescence and banding in vivo
Branching	Dichotomous, irregular, Anastomosis, cushion-shaped	branching dichotomous, cervicorn with recurved branches
Branching angle	(20) 30-40 (60)	(20) 50-80 (110)
Phaeophycean hairs	Absent	Present
Axes width	Thallus width gradually reduces from the base toward the apical segment	Thallus width gradually reduces from the base toward the apical segment
Inter dichotomies		
Length (mm)	(3) 5-7 (10)	(5) 10-20 (30)
Average width (mm)	(0.5) 1 (1.5)	(1) 1-2 (3)
L/W	(2) 3-7 (10)	(1.6) 7-10 (20)
Apical segment		
Apical shape	Acute	Rounded, acute
Interdichotomous		
Length (mm)	(1) 2-3 (3)	(1) 2-3 (3)
Width (mm)	(0.2) 0.2-0.5 (0.9)	1-2
L/W	(1.1) 3.3-10 (15)	(1) 2-3 (4)
Cortical cells		
Cortex length (µm)	(15) 18 -20 (25)	(15) 20-30 (45)
Cortex width (µm)	(12) 15-18 (20)	(12) 15-20 (25)
Medullary cells		
Layers	Monolayer	Occasionally duplicated at sub margin
length (µm)	(50) 55-75 (100)	(60) 75-125 (200)
Width (µm)	(15) 25-40 (45)	(20) 25-100 (175)
Ml/Cl (µm)	(2.2) 2.5-3 (3.75)	(2.75) 3.4-6 (10)
Cross section thickness (µm)	(40) 45-65 (70)	(40) 50-80 (275)

Ml/Cl: ratio of the length of medullary cells to cortical cells

The species later in 2006 transferred to a new genus *Canistrocarpus* which established by De Paula and De Clerck based on molecular studies (De Clerck *et al.*, 2006). This study provided some morphological features of the species from Iranian parts of the Persian Gulf, which also abbreviated in comparison with *Dictyota pulvinata* in Table 4. This group represented morphological differences; although the specimens showed unique features. Thalli were racemose, erect or prostrate, ribbon-like, repeatedly dichotomously branched, with marginal proliferation, smooth margins, occasional spiral shape and rough touching strips and ending to the cervicorn-shape branching pattern (Fig. 5: A1 and B1). Thalli color ranged from mustard to dark brown and some specimens were iridescent and banding. The dry specimens were pale brown in apical part and slightly darker in basal sections. Thalli sizes ranged from (5) 7-

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10 (20) cm in length and (1) 1-2 (3) mm in width and had irregular branching pattern, with branching angles of (20) 50-80 (110) degrees. Twisty and spiral strips had plenty of hooked branches. The species grows on hard substrates and reef flat and attaches to the substratum by marginal and basal rhizoids. Separated thallus sometimes forms spherical turf. The width of thallus axis gradually reduces from the basal segment toward the apical part. The inter-dichotomies were (5) 10-20 (30) mm in length and (1) 1-2 (3) mm in width. Apices can be rounded or truncate and the tips have two protruding cells (Fig. 5: B2). At apical segments, dichotomous intervals are (1) 2-3 (3) mm in length and 1-2 mm in width. Cross sections with (40) 50-80 (275) µm thickness included two layers of cortex and one layer of medullary cells. Both monolayer cortex contained small and regular cells (Fig. 5: A3 and B3), which were (15) 20-30 (45) µm in length and (12) 15-20 (25) µm in width.

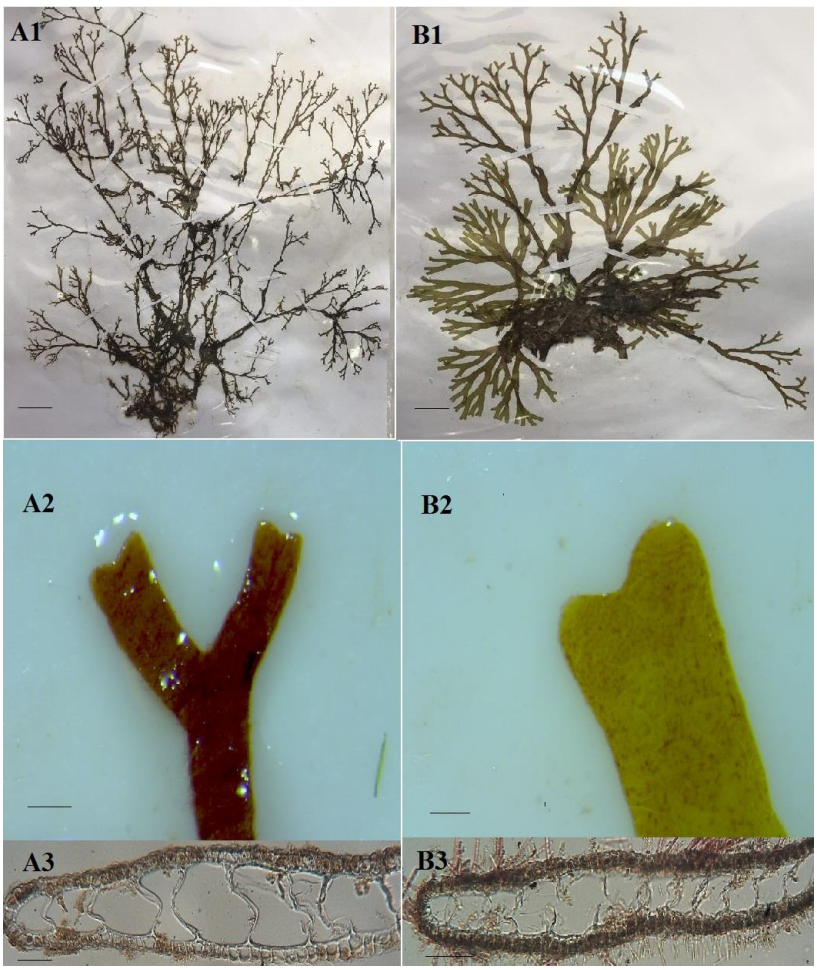


Figure 5: ***Canistrocarpus cervicornis*****; A1. Habits of species collected sample encoded as HO1. Scale bar = 1 cm; B1. Habits of species collected sample encoded as HO2. Scale bar = 1 cm; A2. Detail of dichotomous branching pattern and apex of HO1 morphotype. Scale bar = 1 mm; B2. Detail of dichotomous branching pattern and apex of HO2 morphotype. Scale bar =1 mm; A3. Transverse section at the middle parts of blade in dried specimen HO1 morphotype. Scale bar = 100µm; B3. Transverse section at the middle parts of blade in dried specimen HO2 morphotype. Scale bar = 100µm.**

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Medulla was also monolayer and contained large cells of (60) 75-125 (200) µm long and (20) 25-100 (175) µm wide with infrequent duplication in sub margin. The detailed morphological data are presented in Table 4.

Discussion

In this study we combined the morphological characteristics of the *Dictyota* and *Canistrocarpus* species, which belong to the family Dictyotaceae (brown algae) with the DNA sequences data obtained from the sequences of two cytoplasmic genes, *rbc*L and *cox*3, seeking a deeper insight into the diverse flora of Dictyotales in Persian Gulf, south of Iran.

In *Dictyota* genus, species distinction is based on a combination of qualitative characteristics including growth form, apical shape, presence or absence of dentate margin, branching pattern, reproductive structures, and quantitative characteristics such as size of inter-dichotomies, branching angle, size of cortical and medullary cells, size of the reproductive structure (Tronholm *et al*., 2008), which may easily vary under different spatial and temporal conditions. In Europe, numerous morphotypes of *Dictyota* are described as *D. dichotoma*, which shows this species is highly polymorphic (De Clerek, 2003; Tronholm *et al*., 2008, 2013; Darakrai, 2012). In *D. pulvinata sp. nov.*, we observed a 0-0.5% intraspecific divergence of *rbc*L sequences and a divergence of 4-4.6% from the closest sister clade *D. friabilis* (Table 2). In contrast, the intraspecific distances of *cox*3 sequences was 0-1.3% and the nearest sister species *D. canaliculata*, showed a divergence of 15.2% (Table 3). Actually, Cox3 sequences are not provided for all the species that their rbcL sequence are accessible in GenBank and this issue causes high divergence between the sister groups in *Cox*3 sequences divergence. About *C. cervicornis* the intraspecific divergence based on *rbc*L sequences was 0% and the closest species, *D. ceylanica*, showed 7.9% divergence (Table 2). However, the intraspecific distance of *cox*3 sequences was found to be 0.2-6.8% and 21.8-25% divergence from the outgroup *Rugulopteryx okamurae* (Table 3). *Cox*1 shows a greater divergence than the *rbc*L gene (Sohrabipour *et al.*, 2013), which is also valid for *cox*3. Molecular data showed that some of the specimens collected from Persian Gulf (this study), and specimens collected from Atlantic Ocean, Indian Ocean and Red Sea (Sequences created by De Clerck, Belgium) were grouped together in the same group for both *rbc*L and *cox*3 genes, and here this taxon is introduced as new species *Dictyota pulvinata sp. nov*. (Figs. 2 and 3).

D. pulvinata sp. nov can be distinguished from other species of *Dictyota*, mainly based on the smaller size and shape of the thalli, anastomosis connection cushion-shaped habit, delicate blade and small thickness of the cross sections.

Spatiotemporal changes affect the occurrence of brown algae. *Dictyota*

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populations reach the peak in coldest season of the year. In fact the vegetative parts of the species completely disappear in summer during September -October (Tronholm *et al*., 2008). *C. cervicornis* reaches the maximum reproduction and biomass during the cold season in the Red Sea, whereas no macro thalli are available during the warm season (Ateweberhan *et al*., 2005; Gauna *et al*., 2013). Similarly, maximum abundance of *Dictyota* and *Canistrocarpus* species from Persian Gulf was observed in intertidal to shallow subtidal zones on hard and rocky substrates from January to April, but nothing during the summer.

Morphological characterizations combined with molecular analyses disclose further species in Dictyotales and provide a more comprehensive and precise image of the algae. In this study, we investigated the Dictyotales flora in Persian Gulf, Iran and reported one new species, *D. pulvinata sp. nov.*, which was similar to the sequences of the specimens of Dictyota genus from Atlantic Ocean and Caribbean Sea, which showed that the introduced species is a wide spread species in Indo-Atlantic region. This study also confirmed the presence of *C. cervicornis* in this area, which was previously identified as *D. cervicornis*. The current study concluded that still there is a need to consider the morphological and phenological features in marine flora especially in *Dictyota* genus, the robust DNA barcoding data seem necessary for precise identification of the genus populations.

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