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MORPHOLOGICAL DIVERSITY OF SARGASSUM SPECIES OF OMAN SEA COASTS

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Sargassum is one of the morphologically most complicated phaeophycean genera and represents the most species-rich genus of the brown algal order Fucales (Phaeophyceae). *Sargassum* is one of economically important brown algae in the Gulf of Oman. In this study, 23 characters were studied in 15 populations of 6 species of *Sargassum.* Unweight paired groups using average mean (UPGMA), ward clustering methods, principal components analysis (PCA) and principal coordinate analysis (PCoA) were performed to group the species based on morphological characters. Statistical analyses indicated that characters such as branches of receptacle, leaf apex and receptacles are the most important diagnostic characters in intera-generic variation. In general, two major clusters were formed. The species were well separated in these clusters and the UPGMA tree was able to show the relationships among the species. The species relationships in these clusters were mostly in consistent of the current classifications of this genus.

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Key words: *Sargassum*; brown algae; characters; variation; receptacles, leaf apex; UPGMA

تنوع ریخت‌شناسی گونه‌های جنس Sargassum در سواحل دریای عمان

فاطمه سرگزی: عضو هیأت علمی گروه زیست‌شناسی، دانشکده علوم زیستی، دانشگاه سیستان و بلوچستان، زاهدان، ایران

سارگاسوم یکی از پیچیده‌ترین جنس‌های جلبک‌های قهوه‌ای است و یکی از بزرگترین جنس‌ها در جلبک‌های قهوه‌ای راسته Fucales، (Phaeophyceae) را تشکیل می‌دهد. سارگاسوم یکی از جلبک‌های قهوه‌ای اقتصادی مهم در خلیج عمان است. در این مطالعه، ۲۳ صفت در ۱۵ جمعیت از ۶ گونه سارگاسوم مورد بررسی قرار گرفت. روش جفت گروه بدون وزن با میانگین متوسط (UPGMA)، روش‌های خوشه‌بندی (Ward)، تجزیه و تحلیل به مولفه‌های اصلی (PCA) و تجزیه و تحلیل به مختصات اصلی (PCoA) برای گروه‌بندی نمونه‌های گیاهان براساس خصوصیات مورفولوژیکی انجام شد. تجزیه و تحلیل آماری نشان داد که صفاتی مانند انشعابات رسپتاکل‌ها، نوک برگ و زوائد رسپتاکل از مهمترین صفات تشخیصی در تنوع درون جنسی هستند. به طور کلی دو خوشه اصلی تشکیل شده است. گونه‌ها در این خوشه‌ها به خوبی از هم جدا شده‌اند و درخت UPGMA توانست روابط بین گونه‌ها را به خوبی نشان دهد. روابط گونه‌ای در این خوشه‌ها تقریباً مطابق با طبقه‌بندی‌های جاری این جنس بود.

INTRODUCTION

Sargassum C. Agardh (Sargassaceae, Fucales), is a very large genus of brown seaweed with 360 currently accepted species (Guiry & Guiry 2007), widely distributed in the warm and temperate waters, especially in the Indian Ocean, the Gulf of Oman and the Persian Gulf, recognized in Sub-Arabian regions of this ocean. The Gulf of Oman or Sea of Oman (Gulf of Makran or Sea of Makran) borders Iran and Pakistan on the north, Oman on the south, and the United Arab Emirates on the west. This gulf is 200 miles (320 km) wide, located between Cape al-Ḥadd in Oman and Gwadar Bay on the Pakistan-Iran border. It is 350 miles (560 km) long and connects with the Persian Gulf to the northwest through the Strait of Hormuz. (Nicolini 2004). *Sargassum* is the largest and the most plant-like brown seaweed on Oman Sea shores.

Sargassum species with more than 40 tons annual biomass production have been considered as economically important brown algae (Hosseini & Gharanjik 2003). The species of this genus are abundant in autumn and winter on the shores of the Oman Sea and are drifted to the beach by wave movements. They can easily be sampled from the shores, especially at spring tide. Several morphological studies have been carried out on *Sargassum* in the world including taxonomic revision in French Polynesia (Mattio & al. 2008), the northwest pacific (Cheang & al. 2008), Thailand (Mattio & Payri1 2010) and other warm seas. Many species of this genus have been reported from Iran in various studies (Sohrabipour & Rabii 1996, 1999, Gharanjic 2005, Abdel- Kareem 2009), but few morphological studies have been performed on them due to the difficulty of conducting such studies. Borgesen (1939) identified 26 species of brown algae in the Persian Gulf seashore of Iran. Sohrabipour & Rabii (1996 and 1999) have reported 6 *Sargassum* species in this area. Gharanjic (2005) and Noormohammadi & al. (2011) have also conducted studies on the morphology of different species of the genus *Sargassum*. Abdel- Kareem (2009) also reported sixteen *Sargassum* species in Persian Gulf in Arabic (Saudi Arabia) coasts, which 4 of them are also reported by Sohrabipour & Rabiei (1999). Kokabi & yousefzadi (2015) in checklist of Iranian benthic macroalgae, listed 24 species of *Sargassum* from Iran. Noormohammadi & al. (2011) has explained only a few characters in 3 species of this genus from Chabahar. Shams & al. (2011) studied 7 new records of *Sargassum* along the Iranian coasts. They introduced the more consistent morphological characters as the most important for each species. Some of these characters have also been used in this study.

Morphology and taxonomy of the genus *Sargassum* are very difficult due to many complicated phenotypic variations caused by polymorphisms and genetic differences among the populations of this genus. *Sargassum* species are economically important, especially in Asian countries where they are exploited by agro-food, textile, cosmetic and pharmaceutical industries. It can be utilized as a source of phycocolloids, in particular, alginate, which has many uses in food, beverage, medicine, dentistry and cosmetic industries. It is also used in vegetable soup. Young shoots also form a common ingredient of fish dishes in northern Philippines (Mon Oo & al. 2015). Moreover, the genus *Sargassum* showed antibacterial and antiviral activity (Rizvi & Shameel 2003) as well as antioxidant and antifungal activities (Costa & al. 2011).

In general, the classification system of the genus dates back to the 19th century and is based on observed differences in macro-morphological characters. Those morphological characters may display important variation within individual species and several authors have linked the taxonomic complexity of the genus to its highly polymorphic nature and phenotypic plasticity. Among the large choice of existing species and subspecies epithets (about 1000), identifying taxa accurately is a difficult task, often relying on author's interpretation of short Latin diagnoses or descriptions published in local floras. The taxonomy of this genus is very problematic and identification of *Sargassum* species is complicated on the basis of a relatively simple morphology, that is often influenced by the conditions of the habitat (Mattio & Payri1, 2010).

So far, various classifications have been offered for the genus *Sargassum*. Currently, *Sargassum* is divided into four subgenera (S. subgen. *Sargassum* J. Agardh, *Bactrophycus* J. Agardh, *Arthrophycus* J. Agardh and *Phyllotrichia (*Areschoug) J. Agardh). The subgenus *Sargassum* is now currently subdivided into eight sections: S. sect. *Sargassum* J. Agardh, *Zygocarpicae (*J. Agardh) Setchell, *Polycystae* Mattio et Payri, *Ilicifolia (*J. Agardh) Tseng et Lu, *Binderiana* Tseng et Lu, *Johnstonii* Dawson ex Norris, *Lapazaenum* Dawson ex Norris, and *Sinicola* Dawson ex Norris (Mattio & Payri 2010).

In this study, quantitative and qualitative characters in *Sargassum* species were used to identify the species and study the morphological diversity of them. I tried to select the best characters by studying the flora and various sources of the genus *Sargassum*. Some morphological characters such as branches of receptacle, leaf apex and receptacles were more effective in identification of species, but characters such as algae color, main axis appendages and midrib shape were different in different populations of a species, because being affected by habitat conditions.

In this account, morphological diversity of six species of the genus *Sargassum* has been studied using 23 characters.

MATERIALS AND METHODS

Fifteen populations of 6 species of *Sargassum* were analyzed in this investigation (table 1). These populations were collected from 4 localities of Oman sea coasts in 2 seasons (autumn and winter) from 2017 to 2020. The sampling sites were Tis, Kachu, Tang and Gwadar. To minimize the effect of temporal and seasonal variations and to retain as much morphological information as possible, the specimens were collected at the same stage, once before leaf loss and development of reproductive structures and once after the development of reproductive structures (Ang 1985). Ten individuals were sampled from each population and 3 of them who were more complete and healthier samples were examined.

Table 1. *Sargassum* populations studied and their localities.

List of species	Latitude	Longitude	Location	No. of Station
S. angustifolium C. Agardh, *S. glaucescence* J. Agardh, *S. swartzii* C. Agardh, *S. tenerrimum* J. Agardh	25º 16' 57"	60º 38' 52"	Tis	1
*S. bacciferum (*Turner) C. Agardh, *S. baccularia (*Mertens) C. Agardh	25º 14' 32"	60º 50' 51"	Kachu	2
S. glaucescence, S. tenerrimum, S. angustifolium	25º 21' 52"	59º 54' 50"	Tang	3
S. glaucescence, S. swartzii, S. tenerrimum, S. bacciferum, S. baccularia, S. angustifolium	25º 10' 35"	61 º 30' 48"	Gwadar	4

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 fixed with 4% formaldehyde-seawater solution and mounted on herbarium sheets. The voucher specimens are deposited in the Herbarium of Sistan and Baluchestan University.

In total, twenty-three morphological characters (quantitative and qualitative) were used for morphometric studies (table 2). These characters including 3 measurable and twenty categorical morphological characters, as previously suggested in published papers, books and floras on *Sargassum*, were coded as binary and multistate characters.

Table 2. Morphological characters and their coding.

No.	Characters	Codes/units
1	Algae color	1: Greenish brown, 2: yellowish brown, 3: Brown
2	The height of talus	1: More than 50 cm, 2: less than 50 cm
3	Presence of stolon-like horizontal branches	1: Lower part of the main axis giving rise to stolon-like horizontal branches, 2: lower part of the main axis does not give rise to stolon-like horizontal branches
4	Holdfast shape	1: Conical shape, 2: disc shape
5	Main axis shape	1: Terete, 2: cylindrical
6	Main axis appendage	1: Glabrous, 2: with spinous appendage or covered with warts or projections
7	Primary branches type	1: Compressed and smooth, 2: flattened to compressed, 3: twisted
8	Secondary branches type	1: Slightly compress, 2: compress
9	Leaf shape	1: Elongated-lanceolate, 2: elongated-ellipsoid- lanceolate, 3: obovate-lanceolate
10	Leaf margin	1: Entire, 2: dentate
11	Leaf apex	1: Rounded, 2: obtuse, 3: acute
12	Leaf base	1: With asymmetrical bases, 2: with symmetrical bases
13	Leaf width	1: Flattened (more than 3 mm), 2: narrow (less than 3 mm)
14	Midrib shape	1: Central, 2: central, vanishing below apices
15	Number of vesicle	1: Few, 2: Numerous
16	Vesicle apex	1: Round, 2: Acute
17	Vesicle shape	1: Spherical, elliptical, 2: spherical, ovate
18	Vesicle stalk size	1: Smaller than vesicles, 2: equal to or larger than vesicles
19	Vesicle stalk type	1: Glabrous, 2: with spinous appendage
20	Vesicle stalk appendage	1: With cryptostomata, 2: without cryptostomata
21	Receptacle type	1: Monoecious, 2: dioecious
22	Branches of receptacle	1: Richly branched, 2: slightly forked, 3: simple to furcated, 4: branches are arranged in raceme to cyme
23	Receptacle appendage	1: Richly spinous, 2: slightly spinous, 3: spinous or warty

Different clustering methods including unweight paired groups using average mean (UPGMA), Neighbor Joining (NJ) and ward clustering methods as well as principal components analysis (PCA) and principal coordinate analysis (PCoA) were performed to group the plants specimens based on morphological characters. Co-phenetic correlation and bootstrapping (100 replicates) were performed to check the fit of dendrograms to the original distance matrix. The Euclidean distance and Gower distance were used for clustering methods. After performing the analyzes, the best trees were selected (Podani 2000).

RESULTS

UPGMA, Neighbor Joining and ward clustering methods produced almost similar results but in UPGMA with Gower distance, the species relationships were more in consistent to the existing classifications of this genus (Podani 2000). Therefore, UPGMA's tree is only discussed (fig. 1). In general, two major clusters were formed in UPGMA's tree with 100% bootstrap value. The first major cluster comprised of *S. baccularia* and *S. swartzii*. *Sargassum baccularia* and *S. swartzii* are each placed in separate subclusters. *Sargassum baccularia* belongs to Sect. *Polycysta*, but, *S. swartzii* is recognized in Sect. *Bindariana*. Therefore, in the first cluster, the species of these two sections are well separated.

The second major cluster contains two major subclusters. First subcluster is comprised of *S. bacciferum* and *S. angustifolium*. The populations and individuals of these two species are also well separated in different subclusters. *Sargassum bacciferum* belongs to Sect. *Sargassum* and *S. angustifolium* belongs to Sect. *Zygocarpiae*. The second major subcluster is comprised of *S. tennerium* and *S. glaucescens*, belonging to Sect. *Zygocarpiae*. The grouping of species in the second cluster shows that the specimens of *S. angustifolium* may be separate from Sect. *Zygocarpiae*, because it made a distinct group in the tree, regardless to its current classification, which recognizes *S. angustifolium*, *S. tennerium* and *S. glaucescens* in Sect. *Zygocarpiae*.

According to the morphological key described by Agardh (1848, 1889), Womersley (1954), Yoshida (1983, 1989), Abbott & al. (1992, 2004), Tseng & Lu (1988, 1992a, b, 1995a, b, c, 1997a, b, 1999, 2002a, b, c, d), Stiger & al. (2003), Mattio & Payri (2009, 2010), Mattio & al. (2009), Norris (2010), a variety of subgenera and sections are explained for the genus *Sargassum*. The species studied in this paper are classified as subgenus *Sargassum.* In this subgenus, these 6 species were classified in 4 sections. Three species, including *S. angustifolium*, *S. tennerium* and *S. glaucescens* are placed in the same section, but as the result of this study shows, *S. angustifolium* is independent and may be transferred to a separate section. The characters selected in this study separated the species in accordance with classic classification of the genus, but with minor difference.

PCA and PCoA plots obtained, also produced similar results. Therefore, only PCA plot is presented and discussed here (fig. 2). PCA biplot and PCA loadings were obtained to identify the most important morphological characters differentiating the studied populations.

PCA analysis identified 3 factors which explain 79.49 % of the total variance. The first factor comprised about 37. 28 % of total variance, while second and third factors comprise about 25% and 17.2% of total variances respectively (table 3). In the first factor, branches form of receptacle (with about 37.28% of total variance) possessed the highest positive correlation. Leaf apex as the second factor (with about 25% of total variance) and receptacles as the third factor (with 17.2% of total variance) had the next highest positive correlation. These results show that these characters may be used to identify and discriminate *Sargassum* species. According to the results of these analyzes, although some characters were more valuable in identifying species, but all of these characters are useful in identification of species and can be used.

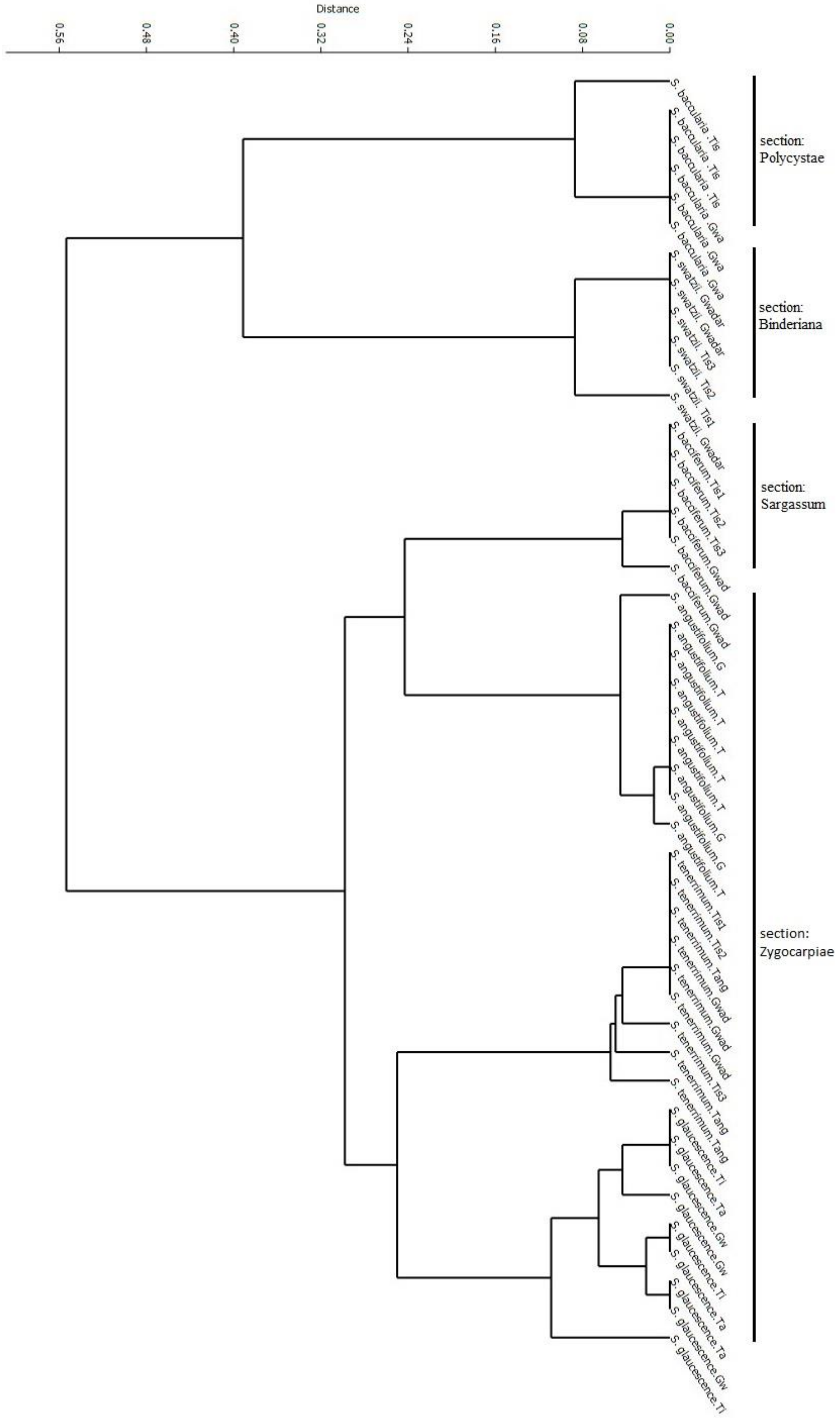

Fig. 1. UPGMA tree of morphometric data of populations of *Sargassum.*

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

Variables	PC1	PC2	PC3
Alga color	0.34511	-0.12643	0.33302
The height of thallus	0.13888	0.086907	-0.24532
Presence of stolon-like horizontal branches	-0.06522	0.1407	-0.17159
Holdfast shape	-0.04566	-0.20093	0.062014
Main axis shape	0.17061	-0.21984	-0.03463
Main axis Processes	-0.01146	0.087742	0.044873
Primary branches type	-0.13663	0.26796	0.2299
Secondary branches type	-0.18099	0.24242	0.007913
Leaf shape	0.11071	0.39139	0.31153
Leaf margin	-0.11578	0.10172	0.1795
Leaf apex	0.27795	0.52124	-0.37063
Leaf base	-0.24677	0.12985	-0.12265
Leaf width	0.14714	-0.13431	-0.17071
Midrib shape	0.10329	-0.13953	-0.1549
Number of Vesicle	-0.18099	0.24242	0.007913
Vesicle apex	-0.19626	-0.10714	0.041945
Vesicle shape	-0.24677	0.12985	-0.12265
Vesicle stalk size	-0.18458	0.20936	-0.0161
Vesicle stalk type	-0.10099	0.04242	0.00711
Vesicle stalk Processes	0.11578	-0.10172	-0.1795
Receptacle type	-0.11578	0.10172	0.1795
Branches of receptacle	0.53441	0.13919	-0.19825
Receptacle appendage	0.31596	0.26286	0.53274

In PCA biplot, the characters that separate the species are well illustrated. For example, leaf shape, leaf apex and receptacles characters, distinguish *S. bacciferum* from *S. glaucescens.* The main axis shape and vesicle stalk process also distinguish *S. swartzii* and *S. baccularia.* According to this plot, the characters of midrib shape and vesicle apex are valuable for identification of *S. tenerrium*.

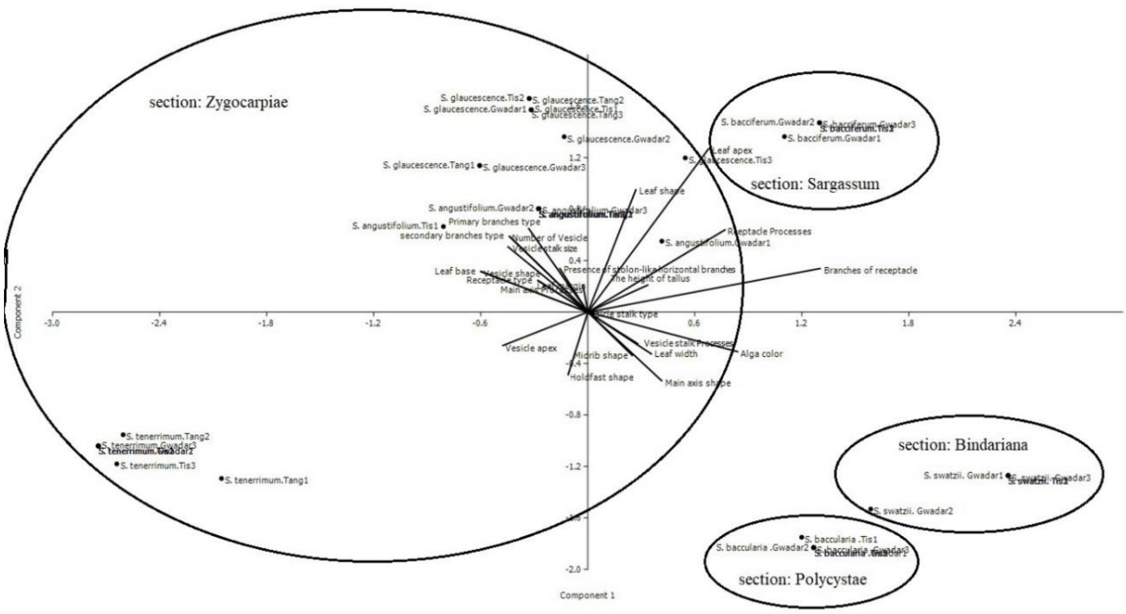

Fig. 2. PCA plot of *Sargassum* species.

DISCUSSION

The UPGMA tree was mostly in consistent to the existing classifications of the genus with minor difference, but the PCA analysis was well able to show the relationships between species and populations. In the classifications of *Sargassum*, three species including *S. angustifolium*, *S. tennerium* and *S. glaucescens* are placed in Sect. *Zygocarpiae*. *Sargassum swartzii* is placed in Sect. *Binaderiana*. *Sargassum baccularia* and *S. bacciferum* are placed in Sect. *Sargassum* and *Polycystae*, respectively. As you can see in figs. 1 and 2, the tree and the PCA analysis in this study also clearly have separated these sections. In the previous studies on species morphology in this genus (Noormohammadi & al. 2011; Mattio & Payri 12010), several morphological characters were used to show the relationships of the species in this genus. In the study of Noormohammadi & al. (2011) qualitative characters were more effective in identifying the species and quantitative characters did not separate the species well. In the current study, choosing both qualitative and quantitative characters helped in getting a better and more effective method for identification of species.

It should be admitted that regardless to the attempt for finding the most useful morphological characters for identifying species, there are still much left to be done reaching to a comprehensive data to be used for identification of *Sargassum* species which are important elements in the flora of coasts of the Oman Sea and the Persian Gulf.

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