The Thallus Characteristics of Some Populations of *Chaetomorpha* and *Rhizoclonium* (Cladophoraceae) from the Persian Gulf

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Abstract

Chaetomorpha and *Rhizoclonium,* two genera of the Cladophoraceae family, are rich in antioxidant, phenolic, and flavonoid compounds that have potential applications in the pharmaceutical, food, and cosmetic industries. This work examined thallus features in several populations of four *Chaetomorpha* species and one *Rhizoclonium* species. This study aimed to look into the morphological and anatomical characteristics of several populations of these taxa along the Persian Gulf and Oman Sea coasts. Good species separation in the studied species was indicated by the UPGMA dendrogram. According to a PCA-biplot of *Chaetomorpha* and *Rhizoclonium* based on equivalent morphological and anatomical parameters, the examined species can be distinguished by variables including cell wall thickness, diameter, thallus form, cell shape, and cell length. High agreement between the PCA biplot results and the heat map created using standardized morphological and anatomical features were observed. While CCA analysis of *Chaetomorpha* showed that the characteristic of cell wall thickness was affected by latitude, tallus form, and cell length were related to the longitude.

Keywords: *Chaetomorpha, Rhizoclonium,* UPGMA Dendrogram, PCA, CCA, Heatmap.

Introduction

Seaweeds are significant food, nutrient, and medicinal ingredient sources around the world. Moreover, they include significant amounts of nutritional fiber, fatty acids, vital amino acids, and vitamins A, B, C, and E. (Rajapakse and Kim, 2011). Seaweeds as functional food products that contain proteins, peptides, amino acids, polysaccharides, phenols, lipids, vitamins, and minerals (Mendis and Kim, 2011, Farasat et al., 2022). They have also been shown to have positive impacts on a variety of lifestyle disorders, including diabetes, hypertension, dyslipidemia, and obesity (Roohinejad et al., 2017). The Persian

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anatomical

Gulf is one of the strategically important aquatic ecosystems in the world, covering an area of 240,000 km2 (Tolouei, 1998). A significant quantity of green macroalgae has been found in the Persian Gulf, according to numerous studies on different marine algae (Gharanjik, 2000; Sohrabipour et al., 2004; Sohrabipour and Rabiei, 2007; Kokabi and Yousefzadi, 2015). Several of these have a high nutritional value and are employed in a variety of applications, including those in the food, feed, pharmaceutical, and healthcare sectors. Cladophoraceae is an important family of green algae (Osuna-Ruiz et al., 2016; Rani et al., 2018; Gahramzai and Taheri, 2021; Thanigaivel et al., 2021). *Chaetomorpha* Kützing and *Rhizoclonium* Kützing, two filamentous genera, exhibit antioxidant and antibacterial properties and are rich in phenolic and flavonoid compounds that are used in the pharmaceutical, food, and cosmetic industries. Due to the simplicity of the structure of these two filamentous algae and the small number of features that separate the species from each other, it is usually challenging to identify them accurately. For this reason, the thallus features of *Chaetomorpha linum* (O.F.Müller) F.T. Kützing, *C. aerea* (Dillwyn) F.T. Kützing, C. *brachygona* WH. Harvey, C. *crassa* (C. Agardh) FT. Kützing and *Rhizoclonium riparium* (Roth) WH. Harvey from selected areas of the Persian Gulf and Oman Sea were examined in this work. Since no comprehensive study has been done on these species in Iran, this work aimed to investigate the morphological traits and anatomical structure of the different populations of these genera in the coastal areas of the Persian Gulf and Oman Sea.

Materials and methods

Sampling

To *investigate* the *morphology* of the genera Chaetomorpha and Rhizoclonium, 29 populations were collected during low tide from the natural habitats of the coastal areas of the Persian Gulf. The geographical coordinates of each place were determined by GPS device (Table 1, Fig. 1). The collected algae were immediately washed with seawater to remove sand and possible epiphytic organisms. After washing and drying, the samples were identified and their morphological characteristics and anatomical structure were studied.

Anatomical and morphological analysis

Morphological and anatomical variables were examined for identification and data analysis. Characteristics like thallus form, branching type, thallus color, cell wall thickness, shape, diameter, length, and length/cell diameter (L/D) ratio were among the most crucial traits for identifying the species (Tables 2, 3). Microscopic images were acquired using an Olympus microscope and a Hund Wetzlar stereomicroscope, and Axiovision LE, Rel 4.5 software was used to measure the diameter, length, and width of the cells.

Structural analysis

Each specimen's morphological and anatomical information was collected, and quantitative features were coded according

Table 1. Specimens and related geographical and ecological data

Species	Type of substrate	Geographical location	Collection location	Code
C. aerea	Sandstone	51°53'48"E, 27°50'4"N	Bushehr: Southern Ouli	C.aSO
R. riparium var. *implexum*				R.iSO
C. aerea	Sandstone	51°53'15"E,27°50'18"N	Bushehr: Northern Ouli	C.aNO
R.riparium var. *implexum*				R.iNO
C. aerea	Sandy-mud	51°56'2"E, 27°49'56"N	Bushehr: Bandar-e Dayyer 1	C.aD1
C. aerea	Rocky-rock	51°56'9"E, 27°49'58"N	Bushehr: Bandar-e Dayyer 2	C.aD2
C. linum				C.lD2
R. riparium var. *implexum*				R.iD2
C. brachygona	Sandstone	50°53'23"E, 29° 3'40"N	Bushehr: Jazireh-ye Shif1	C.bSh1
R. riparium				R.rSh1
R. riparium	Sandstone	50°53'19"E, 29° 3'36"N	Bushehr: Jazireh-ye Shif2	R.rSh2
C. crassa	Rocky - sandy	50°50'33"E, 28°52'11"N	Hormozgan: Bandar-e lengeh, Park-e Dowlat 1	C.cSG
R. riparium var. *implexum*				R.iSg
C. linum	Sandstone	50°49'1"E, 28°58'5.6"N	Bushehr: Bandar-e Jofreh	C.lJO
R. riparium var. *implexum*				R.iJo
C. aerea	Sandy - Sandy	50°48'54"E, 28°54'36"N	Bushehr: Lian Park	C.aLP
C. brachygona	Sandstone with	50°52'44"E, 28°49'50"N	Bushehr: Halileh 1	C.bH1
R. riparium var. *implexum*	water ponds			R.iH1
C. linum	Sandy	51° 3'45"E, 28°42'41"N	Bushehr: Marjan Park	C.lMP
C. brachygona	Sandstone with	50°52'0.2"E, 28°50'30"N	Bushehr: Halileh 2	C.bH2
R. riparium var. *implexum*	water ponds			R.iH2
C. aerea	Sandy	50°48'32"E, 28°47'16"N	Bushehr: Morvarid Park	C.aMP
R. riparium var. *implexum*				R.iMP
C. brachygona	Sandy - Sandy	50°37'30"E, 29°29'6"N	Bushehr: Bandar-e Rig: Halieh1	C.bHa1
C. linum	Sandy - Sandy	50°37'32"E, 29°29'5"N	Bushehr: Bandar-e Rig: Halieh2	C.lHa2
C. brachygona				C.bHa2
R. riparium var. *implexum*				R.iH2
C. crassa	Sandy - Sandy	54°51'35.3"E, 26°32'2"N	Hormozgan: Bandar-e Lengeh: Park-e Dowlat1	C.cDP
R. riparium var. *implexum*	Clay mud	56°21'10"E, 27°11'18"N	Hormozgan: Bandar-e Abbas: Velayat Park 2	R.iV2

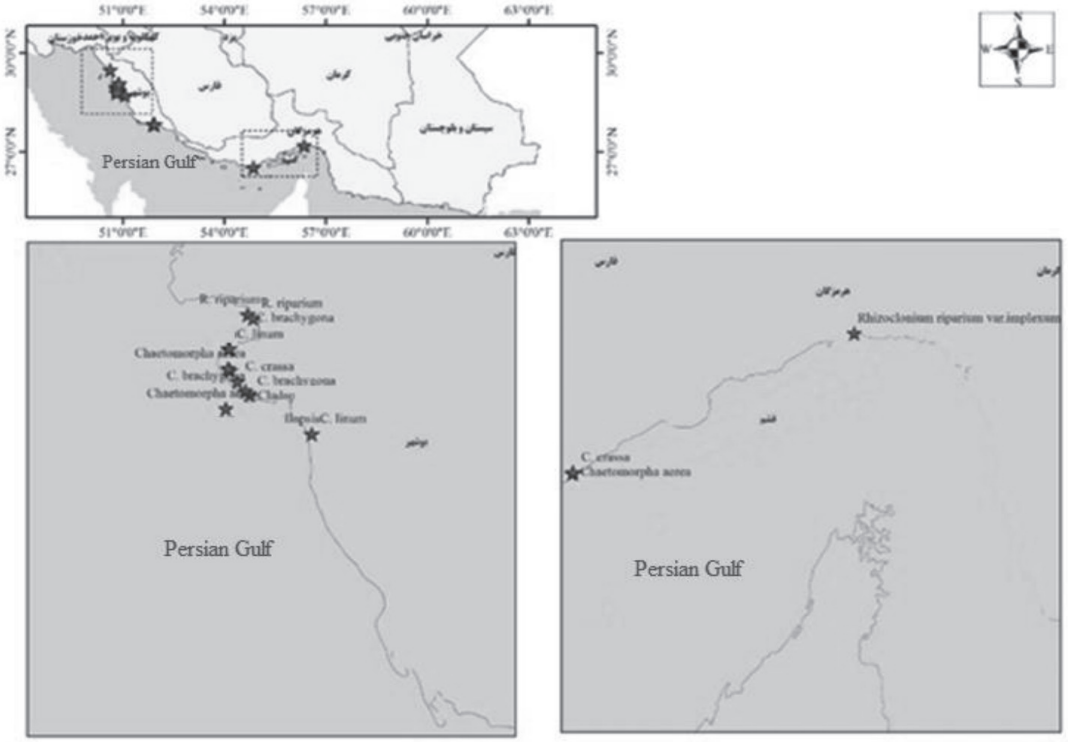

Fig. 1. Study area, sampling locations and species collected are marked on the map

to Table 2. The studied species were grouped by UPGMA (Unweighted paired group using the average method) after 1000 times bootstrap. Principal Component Analysis (PCA) was applied to investigate the species relationships and identify taxonomically discriminating features. CCA (Canonical Correspondence Analysis) was used to reveal the relationship between morphological characteristics and geographical coordinates by PAST version 2.0 software (Hammer et al. 2012). A heatmap was produced based on standardized morphological and anatomical characters by Heatmap software.

Results

Anatomical and morphological analysis

In this work, four species of *Chaetomorpha* and one species and one variety of *Rhizoclonium* were identified by the analysis of morphological and anatomical traits. While Table 4 lists the morphological and anatomical traits of each species, Figures 2–6 display the thallus

Table 2. Qualitative and quantitative morphological and anatomical traits of *Chaetomorpha* species were used in statistical analyzes

No	Traits	Type of traits	Code	No	Traits	Type of traits	Code
1	thallus form	straight, slightly curled skewer-like and straight, more or less twisted curly, cushion-like, rough to the touch twisted and tangled, cushiony, to the touch soft	1 2 3 4	5	cell shape	cylindrical swollen and barrel-like square to elongated rectangle	1 2 3
2	branching type	unbranched grouped	1 2	6	cell diameter	-	-
3	thallus color	light green dark green yellowish green	1 2 3	7	cell length	-	-
4	cell wall thickness	the length equal to the width the length 1.5-2 times the width the length less than the width	1 2 3	8	length/cell diameter (L/D) ratio	-	-

Table 3. Qualitative and quantitative morphological and anatomical traits of *Rthizoclonium* species were used in statistical analyzes

No	Traits	Type of traits	Code	No	Traits	Type of traits	Code
1	thallus form	filamentous in the form of felt or mass	1 2	5	cell diameter	-	-
2	thallus color	light green dark green yellowish green	1 2 3	6	length/cell diameter (L/D) ratio	-	-
3	cell wall thickness	-	-	7	Rhizoid	With rhizoid Without rhizoid	1 2
4	cell diameter	-	-			few rhizoid branches	3

Table 4. Morphological and anatomical traits of the examined species

Species	Thallus form	Branching type	Thallus color	Cell wall thickness (µm)	Cell length (µm)	Cell diameter (µm)	Cell shape	Length/cell diameter (L/D) ratio	Other charactristics
C aerea	straight, slightly curled	unbranched grouped	light green to slightly dark green, sometimes yellowish	(-5) 7-24 (-38)	(-42) 63-215 (-255)	(-52.5) 64-171 (-338)	cylindrical, in some cases oval	0.5-1.5	the height of the thallus is up to 9 cm, with needle crystals in some cells, compressed in the place of the transverse wall
C. linum	skewer-like and straight, more or less twisted	unbranched	light dark green	(-7.2) 9-24 (-33)	(-67) 84-240 (-440)	66-138 (-297)	Rectangular	0.5-0.75	cells slightly compressed in the transverse wall
C. brachygona	curly, cushion-like, rough to the touch	unbranched	light green to slightly dark green, sometimes yellowish	(-5)8-17 (-20)	(-40) 75-143 (-203)	84-166	square to rectangular	often the length is equal to the width, sometimes more than the width and rarely less than it	thallus is twisted and tangled
C. crassa	twisted and tangled, cushiony, soft to the touch	unbranched	light green	(-15)18-31(-38)	111-249	196-274	slightly swollen and barrel-like and cylindrical	often the length is less than the width, sometimes the length is more	cell wall often thick and bladed
R. riparium	filaments intertwined or involved with other algae	unbranched	light green to yellowish	1.5-4.4	19.3-75.8	12-37	rectangular	the length is usually 1.5-2 times the width, sometimes up to 3 times	with short and scattered irregular rhizoid branches or without them

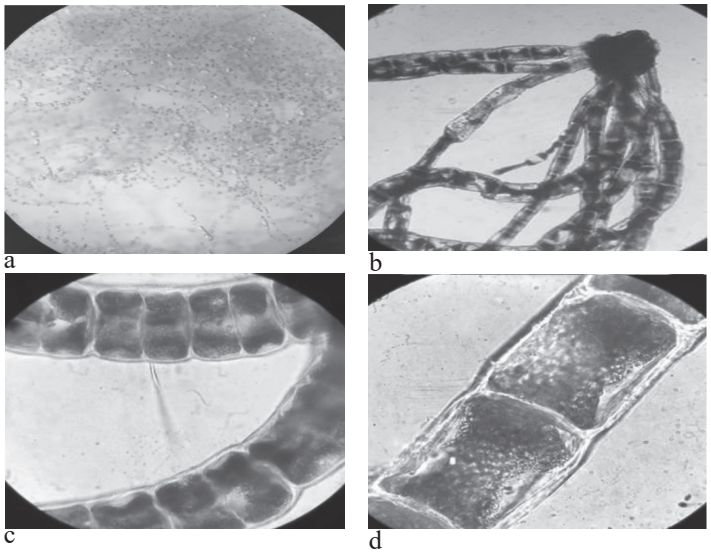

Fig. 2. *Chaetomorpha aerea* (a, b) Alga general view, scale bar=10 mm(a), 20 µm (20X) (b), (c, d) Cell structure, scale bar=10 µm (40X) (c), 10 µm (100X) (d)

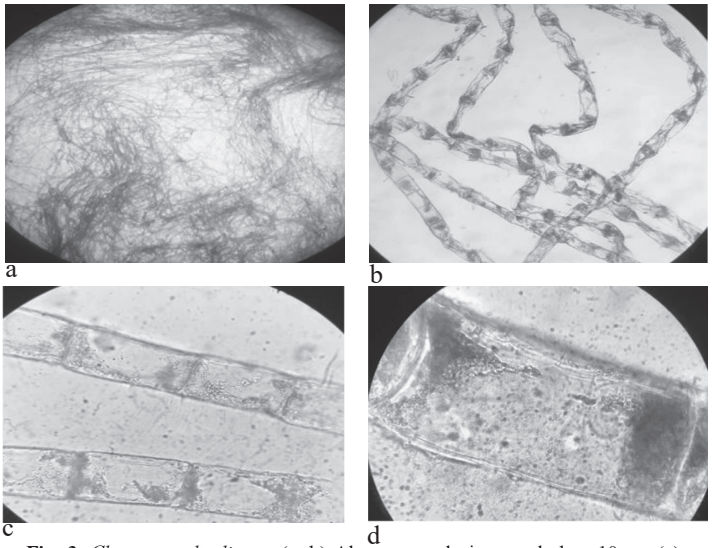

Fig. 3. *Chaetomorpha linum* :(a, b) Algae general view, scale bar=10 mm(a), 20 µm (20X) (b), (c,d) Cell structure, scale bar=20 µm (40X) (c), 10 µm

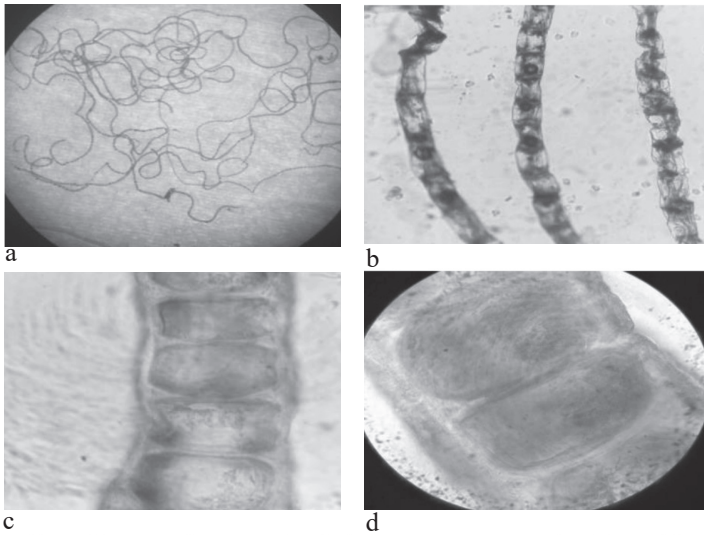

Fig. 4. *Chaetomorpha crassa* : (a, b) Algae general view, scale bar=15 mm (a), 20 µm (20X) (b), (c, d) Cell structure, scale bar=20 µm (40X) (c), 10 µm (100X) (d)

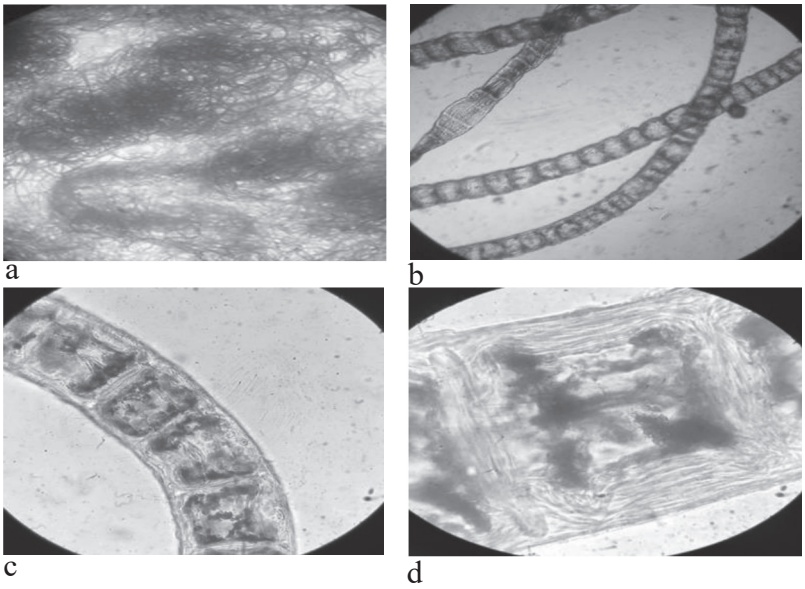

Fig. 5. *Chaetomorpha brachygona*:(a, b) Alga general view, scale bar=10 mm(a), 20 µm (20X) (b), (c,d) Cell structure, scale bar=20 µm (40X) (c), (10 µm (100X) (d)

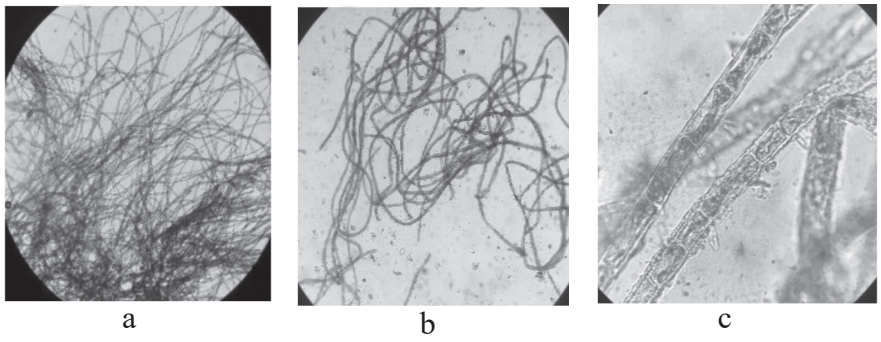

Fig. 6. *Rhizoclonium riparium*: (a,b) Alga general view, scale bar=20 mm (10X)(a), 20µm (20X) (b), (c) cell structure, scale bar= 10 µm (100X)

structures of each species.

Figures 7 and 8, display the UPGMA dendrograms based on combined morphological and anatomical data in the populations of the studied species. The UPGMA dendrogram demonstrated adequate species-level differentiation. In the UPGMA dendrogram of *Chaetomorpha* species, two completely distinct cluster was created. In the first group, there were *C. aerea* and *C. linum*, and second group including *C. brachygona* and *C. crassa.* However, due to the overlapping of some characteristics, some degree of interference of traits was also observed.

Based on morphological characteristics, a PCA-biplot of the *Chaetomorpha* species showed traits like X1; thallus form, X7; cell length, X3; thallus color, and X4; cell wall thickness, respectively, are diagnostic traits that can be used to distinguish the species from one another. According to PCA analysis, the first axes account for around 70% of the total variation. Characteristics of X1 and X7 have the highest positive correlations to this axis, with r values of 0.98 (Fig. 9).

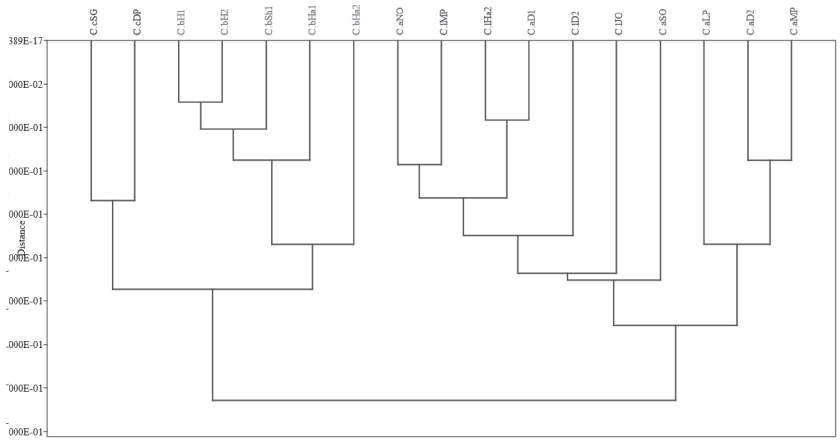

Fig. 7. UPGMA dendrogram of *Chaetomorpha* species based on morphological and anatomical features

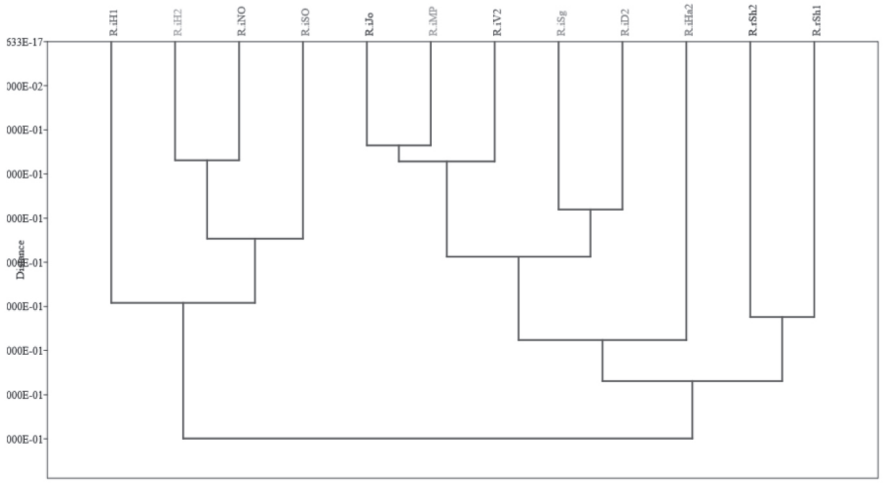

Fig. 8. UPGMA dendrogram of *Rhizoclonium* populations based on morphological and anatomical features

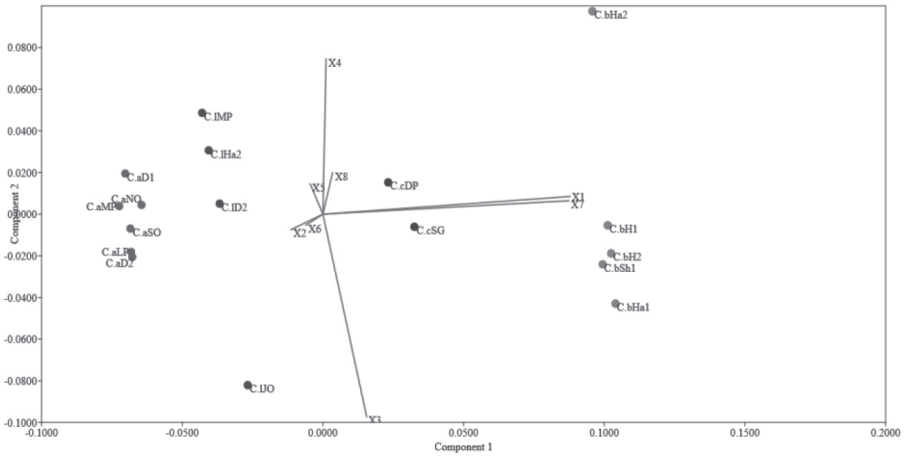

Fig. 9. PCA-biplot of *Chaetomorpha* species based on morphological and anatomical data. X1-thallus form,X2- branching type, X3-thallus color, X4- cell wall thickness, X5- cell shape, X6- cell diameter,X7- cell length, X8- length/cell diameter (L/D) ratio

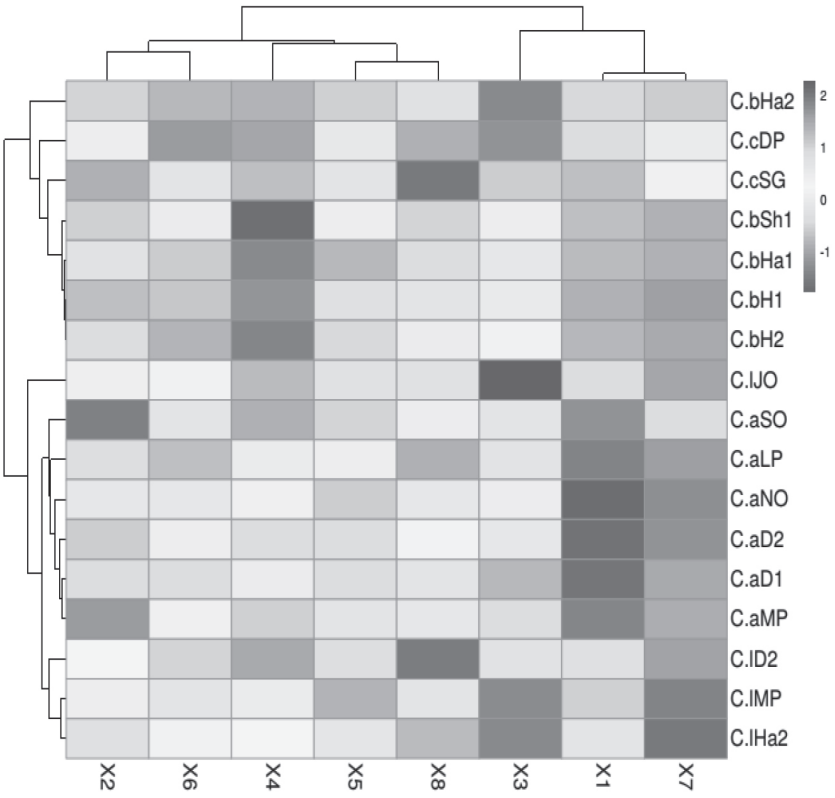

Fig. 10. Heat map of *Chaetomorpha* species based on standardized morphological characters. X1-thallus form,X2- branching type, X3-thallus color, X4- cell wall thickness, X5- cell shape, X6- cell diameter,X7- cell length, X8- length/cell diameter (L/D) ratio

The PCA biplot of the *Rhizoclonium* populations showed that features such as length/cell diameter, L/D ratio, cell length, and thallus form, more than other characteristics, can be differentiated the populations.

According to the heat map created using standardized morphological and anatomical characteristics (Fig. 10), *Chaetomorpha* species can be distinguished based on morphological characteristics such as thallus form, cell length, thallus color, and cell wall thickness, and *Rhizoclonium* populations can be separated based on length/cell diameter, L/D ratio, cell length, and thallus form. These findings were in high accordance with the PCA biplot findings.

According to the CCA analysis, latitude had an impact on the parameter of cell wall thickness, whereas longitude factor affected thallus form and cell length in *Chaetomorpha* species (Fig. 11). The CCA analysis in *Rhizoclonium* populations showed that thallus form, thallus color, rhizoid, and the cell length, were affected by latitude (Figures are not shown for *Rhizoclonium riparium*).

According to a PCA-biplot of *Chaetomorpha* and *Rhizoclonium* based on equivalent morphological and anatomical parameters, the species under study can be distinguished by variables including cell wall thickness, cell diameter, thallus form, cell shape, and cell length (Figure is not shown).

In general, the two species, *Chaetomorpha linum* and *Chaetomorpha aerea* showed

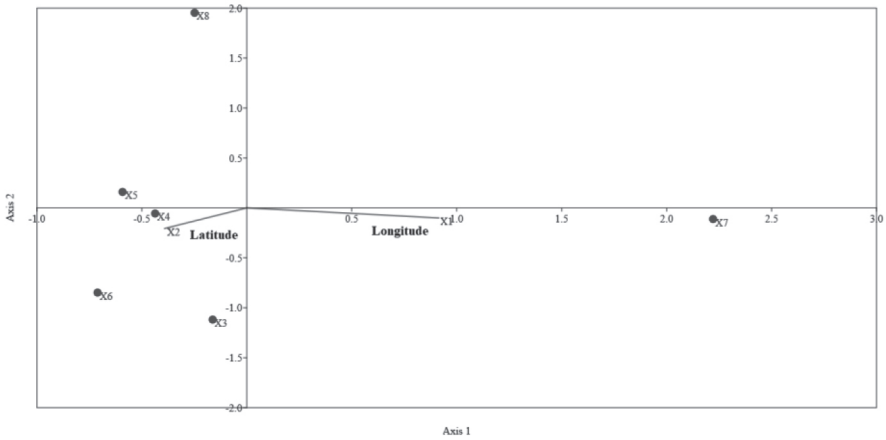

Fig. 11. CCA plot of the geographical distribution of *Chaetomorpha* species based on their morphological and anatomical data. X1-thallus form, X2- branching type, X3-thallus color, X4- cell wall thickness, X5- cell shapeX6- cell diameter, X7- cell length, X8- length/cell diameter (L/D) ratio

similarities in different characters, though the overlap of some traits was observed. However, *C. aerea* is light green to slightly dark green, sometimes yellowish, and *C. linum* is often light dark green. However, some variation was found in different populations. In this way, some thalli of *C. linum* in the Jofreh area (C.lJO) were yellow-green and slightly brown. For instance, in the Marjan Park population (C.lMP), the thallus was slightly twisted and light green whereas in the Dayyer population (C.lD), the filaments were observed as intertwined, bright green, and sometimes yellowish green.

In some *C. aerea* populations, such as Morvarid Park (C.aMP) and Lian Park (C.aLP) populations, some thalli were observed in a cluster, consisting of 2 to several thalli, attached to a large and distinct holdfast. The thallus color of *C. aerea* in Morvarid Park and Southern Ouli (C.iSO) populations were light green, sometimes dark green, and in the Northern Ouli population (C.iNO), was yellowish green and some filaments were springy. In the Lian population, the thallus was light or dark green, and some filaments were springy and twisted. In this population, mineral crystals on some thalli were observed. In some thalli, cells with a diameter greater than or equal to the length were also observed. In the Dayyer population, dark green filaments with yellow tendencies were seen. Most species were slightly compressed at the junction of the walls, and in some populations, the cells were slightly swollen.

For *C. aerea*, the highest length and diameter of the cell belonged to the northern Ouli population (255 and 337.7 µm, respectively), and the lowest length and diameter of the cell belonged to the southern Ouli population (41.6 and 52.5 µm, respectively). Also, the highest cell wall thickness was related to the Park Dowlat2 population (38 µm), and the Lian population

(5 µm) was the smallest in size. For *C. linum*, the highest cell length, and diameter related to the Marjan Park population (239.4 and 138.2 µm, respectively) and the lowest cell length was seen in Halieh 2 population (67 µm). The lowest cell diameter was observed in Dayyer 2 population (64.3 µm). However, a few cells with a length of up to 441.3 µm and a thickness of up to 33 µm were also seen in the Marjan Park population. The highest cell wall thickness was related to Halieh 2 population (24.7 µm), and the lowest cell wall thickness was recorded for Dayyer 2 population (7.2 µm).

Both *C. crassa* and *C. brachygona* are cushion-like. While *C. crassa*, is curly and rough to the touch and is usually free floating or involved with other algae, C. brachygona is soft and smaller in terms of cell dimensions and cell wall thickness. In addition, *C. crassa* cells are swollen and barrel-shaped but the cells of C. brachygona, are often square or rectangular, and the cell length is equal to or less than the cell diameter, and sometimes cell length reaches half the diameter. In Halileh 1 population, the thalli were yellowish green to light or dark green, some filaments were seen as skewer-like and smooth, and most of the cells had a cell diameter highest than the cell length.

For *C. brachygona*, the highest cell length and diameter belonged to Shif 2 population (C.bSh) around 203.2 and 166 µm, respectively. However, the lowest length and diameter of the cells were found in Halileh 1 population (C. bH1) about 53.2 and 60 µm, respectively. The highest and lowest thickness of the cell wall was found in the filaments of the Halileh 2 population (22.4 and 7 µm, respectively). In *C. carssa*, the highest cell wall thickness was seen in the population of Shegab Park (C.cSP) (8.26 µm), and the lowest one was observed in the Park-eDowlat1population (C.c DP) (3.8 µm).

The results showed that in *C. aerea*, cell wall thickness has an inverse relationship with latitude, whereas, in *C. linum*, *C. brachygona*, and *C. crassa*, this relationship is direct, that is, with increasing latitude, cell wall thickness increases in *C. aerea*. In *C. crassa*, the highest cell length was related to Shegab Park population (202.6 µm), and the lowest was seen in the Park-e Dowlat 1 population (81µm). The highest and lowest cell diameter belonged to the Park-e Dowlat 1 population (236.1 and 88 µm, respectively).

Discussion

Chaetomorpha and *Rhizoclonium* are unbranched filamentous algae, closely related to each other and belonging to Cladophoraceae, Cladophorales, and Chlorophyta (Zhao et al., 2018). *Chaetomorpha* is a common and widespread filamentous green macroalgae known as Spaghetti algae (Novaczek, 2001). Some species including *C. crassa*, *C. linum*, and *C. brachygona* are edible and consumed as salad or dessert in East Asian countries (Apaydin-Yagci and Turna, 2002). Also, this genus is currently the most popular macroalgal group

in saltwater aquariums (Odom and Walters, 2013). There are 141 species names in the genus *Chaetomorpha* including subspecies, variations, and forms, 78 of which are acknowledged taxonomically (Guiry, 2022). This genus includes filamentous, unbranched, connected by a holdfast, cylindrical or barrel-shaped, sometimes with lamellar walls, with reticulate chloroplast, multinucleate with abundant pyrenoids (Lawson and John, 1982; Teo and Wee, 1983). The genus *Rhizoclonium* was named by Kützing (1845), and *R. riparium* is considered the type species of this genus (Zhao et al., 2018). Thirty of the 97 species names for the genus *Rhizoclonium* including subspecies, variations, and forms are accepted taxonomically. This alga has a single-cell layer, a strong wall, and unbranched filamentous cells with many pyrenoids, nuclei, and chloroplasts with reticulation. The length/cell diameter (L/D) ratio of a cell is 4/1 (less than 6) (Lawson and John, 1982; Blair, 1983).

In some references, *R. riparium* var. *implexum* is known as R. implexum (Kützing, 1845). The length is two to five times the width, cell wall thickness from 1.5 to 2 µm, and few or no rhizoid branches are the characteristics that can be applied to identify this variety/species (Lawson and John, 1982; Farasat et al., 2013). Several populations of the examined species differed in cell wall thickness and the length/cell diameter (L/D) ratio.

The majority of the diagnostic characteristics used to identify the species demonstrated a high degree of agreement with those which were listed in the earlier reliable references (Borgesen, 1939; Lawson and John, 1982; Teo and Wee, 1983; Tseng, 1984; Ruangchuay et al., 2007; Alves et al., 2009; Milchakova, 2011). However, cell dimensions in some populations differed from earlier studies. For instance, Rath and Adhikary (2006) stated that the cell width in *C. linum* species obtained from India was smaller (15 to 30 µm) than our findings. Additionally, compared to the samples from the present study, Hinson and Kapraun (1991) found that the cell width of *C. brachygona* and *C. aere*a from the western Atlantic was lower (55 and 80-90 µm, respectively). They showed a correlation between cell dimensions and genome size so that species with a diameter of less than 100 µm, such as *C. brachygona* and *C. aerea* have almost half of the genomes of *C. antennina* and *C. melagonium* species with a diameter of over 400 µm. Contrary to these studies, some reports have stated larger cell dimensions for *Chaetomorpha* species. For example, Satpati and Pal (2016) reported greater cell width (400-620 µm) in samples of *C. aerea* from India.

Due to the morphological flexibility of the genera and the small number of species, *Chaetomorpha* and *Rhizoclonium* species generally create numerous systematic issues. The most variable characters that are measured to distinguish between various species include basal cell size, thallus form, cell diameter, and length/width ratio. Additionally, variations of the morphological

characters in these two genera are evident in the investigated populations due to changes in environmental conditions at different latitudes and longitudes.

The findings of this study are applicable for researchers looking for active compounds in these species because, with changes in plant morphology, it is not unlikely that biologically active compounds in populations from various areas will also change.

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