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RESEARCH ARTICLE

Comprehensive Biometric Study on the Invasive Seaweed, *Caulerpa mexicana*, in the Aegean Sea

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Abstract: The present study investigates the occurrence and biometry of the invasive seaweed *Caulerpa mexicana* in the Gulf of İzmir, Aegean Sea, thereby highlighting its ecological implications in a region increasingly affected by biological invasions. The Mediterranean basin, especially the eastern and Aegean parts, is becoming a hotspot for non-native species, and the region may be a place of interest with the occurrence of the *C. mexicana* species. This research represents the first detailed assessment of its biometric characteristics in the Levant-Aegean Basin. The study's sampling was conducted from May to August 2024 at 321 stations along the Aegean coast, and *C. mexicana* was identified at only two specific sites. The species was found in shallow coastal waters at depths of 10 m and 15 m with densities of 630 shoots/m² and 469 shoots/m², respectively. Morphometric analysis revealed frond lengths ranging from 10 to 12.5 centimeters and widths from 7 to 9 millimeters, with a significant correlation between frond length and number of pinnae. Environmental parameters such as temperature, salinity, and dissolved oxygen were measured, providing a context for the species' habitat preferences. The findings underscore the invasive potential of *C. mexicana* and its capacity to perturb local marine ecosystems, highlighting the necessity for persistent monitoring and management strategies to mitigate its deleterious effects.

Anahtar kelimeler:

Caulerpa mexicana
Yerli olmayan tür
İstilacı
Biyometri
Ege Denizi

Ege Denizi'ndeki Istilacı Deniz Yosunu *Caulerpa mexicana* Hakkında Kapsamlı Biyometrik Çalışma

Öz: Mevcut çalışma, İzmir Körfezi, Ege Denizi'ndeki istilacı deniz yosunu *Caulerpa mexicana*'nın bulunmasını ve biyometrisini araştırarak, biyolojik istilalardan giderek daha fazla etkilenen bir bölgedeki ekolojik etkilerini vurgulamaktadır. Akdeniz havzası, özellikle doğu ve Ege kesimleri, yerli olmayan türler için bir sıcak nokta haline gelmektedir ve *C. mexicana* türünün görülmesi ile bölge dikkat çekebilir. Bu araştırma, Ege-Levant Havzası'ndaki biyometrik özelliklerinin ilk ayrıntılı değerlendirmesini temsil etmektedir. Çalışmanın örneklemesi Mayıs-Ağustos 2024 arasında Ege kıyısı boyunca 321 istasyonda yürütülmüş ve *C. mexicana* yalnızca iki belirli noktada tanımlanmıştır. Tür, sırasıyla 630 sürgün/m² ve 469 sürgün/m² yoğunlukta, 10 m ve 15 m derinlikteki sığ kıyı sularında bulunmuştur. Morfometrik analiz, yaprak uzunluklarının 10 ila 12,5 santimetre ve genişliklerinin 7 ila 9 milimetre arasında değiştiğini ve yaprak uzunluğu ile pinnae sayısı arasında önemli bir korelasyon olduğunu ortaya koydu. Sıcaklık, tuzluluk ve çözünmüş oksijen gibi çevresel parametreler ölçülerek türün habitat tercihleri için bir ilişki sağlandı. Bulgular, *C. mexicana* türünün istilacı potansiyelini ve yerel deniz ekosistemlerini bozma kapasitesini vurgulayarak, zararlı etkilerini azaltmak için sürekli izleme ve yönetim stratejilerinin gerekliliğini belirtmiştir.

Introduction

The Mediterranean basin, particularly the eastern basin and seas such as the Levant and Aegean, has become a significant nexus for the introduction and subsequent invasion of exotic species. These biological invasions present a substantial threat to the conservation of endangered species in natural plant and animal communities, with repercussions for seagrass on submerged algal communities (Ceccherelli and Cinelli 1998). Fish, benthic fauna, and macrophytes have been introduced intentionally and accidentally in the Eastern

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Mediterranean. The majority of these invaders are Indo-Pacific species and temperate and tropical species. Invasive organisms alter the established Mediterranean ecosystem in space and time. The dynamics of abundance and biomass of invaders, such as seaweeds, provide insights into their interaction with variations in their life history between isolated and mixed populations (Schemske et al., 1994). Clonal vegetative growth is prevalent, leading to the formation of dense aggregations, foraging, and movement to adjacent suitable areas, followed by rapid spread and low mortality (Wright, 2005).

A total of 98 species of marine algae have been introduced into the Mediterranean Sea, of which nine species were invasive and had ecological and economic impacts (Siguan & Ribera, 2002). These nine species include *Caulerpa taxifolia*, *Caulerpa racemosa*, *Sargassum muticum*, *Laminaria japonica*, *Asparagopsis armata*, *Undaria pinnatifida*, *Womersleyella setacea*, *Acrothamnion preissii*, and *Lophocladia lallemandii*. More recently, the species *Caulerpa cylindracea* and *Rugulopteryx okamurae* have also been introduced. It is noteworthy that the number of species has increased by fifty fold over the past two decades. In the western Mediterranean, sixty-seven species were identified as non-indigenous, with their geographical origins being Japanese or Pacific waters (Siguan & Ribera, 2002). Additionally, twenty-nine non-indigenous species of Indo-Pacific origin were documented in the eastern basin (Siguan & Ribera, 2002). Zenetos & Galanidi (2020) updated the list of non-indigenous seagrass species in the Mediterranean, and Cinar et al. (2021) revised the phytobenthos in the Turkish waters of the eastern Mediterranean. Of the 253 alien species that Cinar et al. (2021) found in Turkish Aegean waters, 28 were Phytobenthos. In the Turkish marine water system, 47 invasive non-indigenous macrophyte species were reported: they are mainly *Caulerpa cylindracea* Sonder, 1845, *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini, 2013, *Codium fragile fragile* Suringar Hariot, 1889, *Stypopodium schimperi* (Kützing) Verlaque & Boudouresque, 1991 and *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Çinar et al. 2025).

Caulerpa species constitute a taxonomic group of significant seaweeds, exhibiting properties such as a siphonous thallus with multinucleate cells, serving as food sources for marine organisms, and demonstrating high invasion and expansion capacity in space (Paul and Fenical 1987; Ceccherelli et al. 2002). Furthermore, these species possess a remarkable capacity for tolerating diverse temperatures and light conditions (Uchimura et al. 2000; Ljiljana et al. 2006), which enables their presence in various marine environments, each of which is adapted to its specific characteristics. These characteristics include vegetative propagules, fragmentation, and running reproduction, as well as phenotypic plasticity derived from the different environments (Ceccherelli and Cinelli 1999a, b, c; Ceccherelli and Piazzi 2001; Wright 2005). The distribution of their densities and traits in space and time provides information on the persistence of organisms, especially for a species that responds rapidly to changing environmental parameters such as seawater surface temperature and surface photon flux density in the visible spectrum (Tuya et al. 2006).

A reevaluation of the global distribution of *Caulerpa mexicana* Sonder ex Kützing 1849 is necessary due to its erroneous placement within the synonymy of *Caulerpa taxifolia*. This species is widespread in warmer marine environments, especially in the western Atlantic, where it was first described from Mexico, the Caribbean, and Brazil. In contrast, in the eastern Atlantic, it has been documented in the Canary Islands and Mauritania, as well as in the Red Sea and the Indian Ocean, extending as far as Australia and the western Pacific, including regions such as Vietnam, the Philippines, Papua New Guinea, and various Pacific islands such as Fiji (Verlaque et al. 2015; Fernandez-Garcia et al. 2016) and Canary Islands and Porto Santo in the Atlantic Ocean (Pereira 2024). Notably, there have been reports of its presence in the Mediterranean, particularly in Syria, and reports from Sicily have been attributed to misidentifications of *C. taxifolia* (Rayss 1941). According to Rayss (1941), *C. mexicana* may be native to the Mediterranean. Its initial identification in the Mediterranean occurred in 1939, specifically in Palestine and Lebanon, where it was initially classified as *C. crassifolia* (Rayss 1941). Subsequent reports of the species include a 1976 finding by Mayhoub in Syria (Bitar et al. 2003) and, in 1991, a single specimen discovered in Lebanon (Bitar 1999, 2010). However, Bitar (2017) reports the disappearance of this specimen.

The following characteristics distinguish congeneric species of *Caulerpa*: the genus exhibits clonal propagation through fragmentation and frequently displays invasive tendencies when introduced outside its native range, particularly in competition with seagrasses (Varela-Alvarez et al. 2012). A distinctive anatomical characteristic of Caulerpales is the absence of internal cell membranes, which results in the nuclei being separated within the continuous cytoplasm. These algae are further distinguished by the presence of numerous internal trabeculae, which are branching ingrowths of the cell wall. Noteworthy is the observation that individuals of *C. taxifolia* have been found to reach lengths of up to 2.8 m, making it the largest known single cell of its kind (Varela-Alvarez et al. 2012). *C. mexicana*, a species of green algae in the genus *Caulerpa*, exhibits the following characteristics: it possesses a branched horizontal axis (stolon) that can reach up to 1 m in length, affixed by numerous short rhizoidal outgrowths (columns). Its photosynthetic axes (fronds) manifest a flattened, pinnately branched morphology evocative of feathers, with heights reaching up to 15 centimeters and widths ranging from 10 to 15 millimeters. The narrow midrib, which is less than the length of the ramules, is observed to be flattened in its cross-section. The branches (ramuli) are distichous, flattened, clavate-shaped and membranous, with slightly or no constriction at the base, upward curvature, and tapering, pointed tips. They are coenocytic throughout, but with slender cylindrical and branched wall projections (trabeculae) that cross the lumen. The chloroplasts lack pyrenoids, and the presence of amyloplasts is notable. Notably, the species is distinguished by its holocarpic reproduction, a characteristic that has been documented (Fagerberg et al. 2010, 2012; Guiry and Guiry 2024; Verlaque et al. 2015).

According to the published literatures, in the eastern Mediterranean basin and Turkish seas *C. taxifolia* var. *distichophylla* is a common species of non-indigenous species (Jongma et al., 2013; Mutlu et al., 2022a; Taşkın et al., 2023; Mutlu et al., 2025; Verlaque et al 2015) among *Caulerpa* genus species, expanding the western Mediterranean Sea, followed by *C. taxifolia* (Çevik et al., 2007). *C. mexicana* was reported in the Iskenderun bay, eastern Mediterranean Sea (Erduğan et al., 2009; Verlaque et al 2015), but the species could not be conspicuous. The present study was the first attempt to outline the biometric dynamics of *C. mexicana* with an emphasis on comparison with biometrics of other two species found in a survey conducted in the Aegean Sea.

Material and Methods

Specimens-environment sampling

A research cruise was conducted to investigate the distribution and species composition of seagrasses and seaweeds as submerged vegetation along the Turkish coast of the Aegean Sea from May to August of 2024 (Fig. 1). During the study, a total of 321 stations were surveyed during daylight hours, and two stations were found to contain specimens of *C. mexicana* in July 2024. These stations were situated in the Gulf of Izmir, within the Aegean Sea of Turkey (Fig. 1). At both stations, a small number of specimens were collected by SCUBA divers, who identified them within a quadrat measuring 0.4 x 0.4 m. The first station (I1) was sampled at approximately 3:00 p.m., and the second station (I2) was sampled at approximately 5:00 p.m.

Aboard the R/V "Akdeniz Su," the fronds, stolons, and rhizoids were meticulously disentangled for biometric measurements (Fig. 2). The measurements were obtained from fresh, unpreserved specimens, which were subsequently preserved in a 10%-formalin solution due to the survey's duration of 3.5 months. This constraint precluded the possibility of conducting a genetic analysis.

During the shipboard sampling process, physicochemical parameters (temperature, salinity, pH, oxygen, and total suspended solids) and optical parameters (Sechhi disk depth and photosynthetic active radiation) were measured from surface and near-bottom waters. Water samples were collected on board using a 5-liter Niskin bottle, and the physicochemical parameters were measured using multiparameter probes (AZ Combo, model 84051). Photosynthetically active radiation (PAR) was measured using an ampoule (Spherical SPQA-4671 model, Li-Cor Inc.) and a multiparameter recorder (LI-1400 model). The ampoule was poured from the surface to the near-bottom depth, and the profiled PAR values were then converted to percent values with the sea surface value as 100% over the water depth.

Biometrical measurements

The biometry of the specimens was characterized by the density (number of shoots/m2 and per quadrant; TS, number of fronds: buds; BNo) on a frond branched from the stolon and the number of paired rachis or pinnae per rachis and 1 cm of rachis. The morphometry of the specimens was characterized by rachis length; rachis 1: RL1 and rachis 2: RL2, frond length: FL, and frond width: FW) of the specimens (Fig. 2). The morphometric parameters were measured with an accuracy of µm using a capillary balance.

After a significant subsampling of the clusters of specimens, a total of 25 and 38 shoots branched from the stolon and 68 and 114 fronds were measured at I1 and I2, respectively, since a shoot contained fronds in numerous more than one frond. The number of pinnae per frond was determined by meticulously enumerating a total of 19 and 17 fronds at I1 and I2, respectively. The number of buds per frond was subsequently enumerated and measured to ascertain the biometric parameters.

Statistical interpretation of the biometry

In order to ascertain the biometric characteristics of the species, the frond length-width and frond length-number of pinnae relationships were determined using the Pearson correlation and regression model. Differences in frond length-number of pinnae relationships among bottom depths were tested using ANOCOVA. Furthermore, the normality of each biometric variable dataset was assessed using the dispersion index, randomness test, and FAO (1991) criteria, and the distribution of these variables was then tested for significance using ANOVA. The confidence level chosen for the significance of the tests was $p<0.05$. Furthermore, the number of shoots per quadrant (*c.a.* 0.16 m^2 at station I1 and I2) was calculated as shoots/m^2. All analyses were performed using the statistical tools of MatLab (vers. 20221a, Mathworks Inc.).

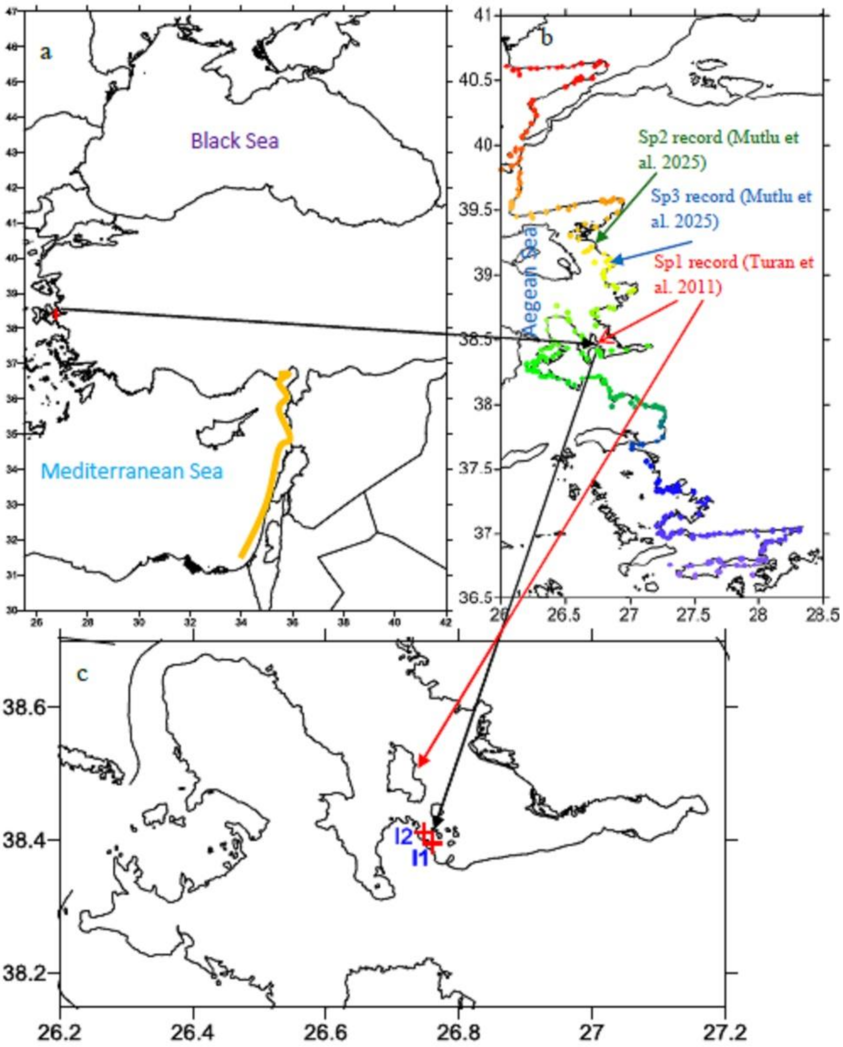

Figure 1. Study area (a, b) and sampling stations (b) and stations where *C. mexicana* occurred (+, c). Previous Mediterranean records of *C. mexicana* (brown line from Verlaque et al 2015) (a), and sampling stations colored with the latitudes during the present study (b). Red arrow denotes the fist occurrence of Sp1: *C. taxifolia* in the Aegean Sea (Turan et al., 2011), dark green Sp2: *C. taxifolia* var. *distichophylla* (Mutlu et al., 2025) and blue Sp3: *C. taxifolia* found during the present survey (Appendix Fig. A1, A3).

Results

Study environment

The hydrograph of the entire study area was described by (Mutlu et al., 2025) as follows: A total of 321 sampling stations were surveyed, and two of these stations provided evidence for the presence of *C. mexicana* specimens (Fig. 2). The stations were located in coastal waters around Urla province in the Gulf of Izmir (station I1 at 10 m and station I2 at 15 m, Table 1). The area is frequently visited by recreational boats and tourists. Notably, the Gulf of Izmir is home to one of Turkey's largest seaports, Alsancak Harbor, which serves as a gateway for international maritime traffic.

The sea surface temperature ranged from 20.5 to 28.5°C, while the temperature of the near-bottom waters exhibited a range of 18 to 28°C (Fig. 2). The Gulf of Izmir was found to have the highest temperatures of the two layers. The salinity levels exhibited a range from 30.5 to 38 parts per thousand (PSU) at the sea surface and from 33 to 37.5 PSU in the near-bottom waters (Fig. 2). The pH values demonstrated a comparable range at both stations, both at the sea surface and in the near-bottom waters (Fig. 2). The salinity levels exhibited a decreasing gradient from south to north within the study area, a trend that was more pronounced in the near-bottom waters. Conversely, oxygen content and pH exhibited a slight increase from south to north within the study area, contrasting with the variation in total suspended solids (Fig. 2). The Gulf of Izmir was characterized by the highest pH value, the lowest oxygen levels, and moderate total suspended matter values in the near-bottom waters. The Black Sea and the Sea of Marmara experienced cold water in the Dardanelles Strait exit within the Aegean Sea, while the northern part of the Aegean Sea was marked by warmer water. The influence of the river Meriç resulted in less saline water in this specific region.

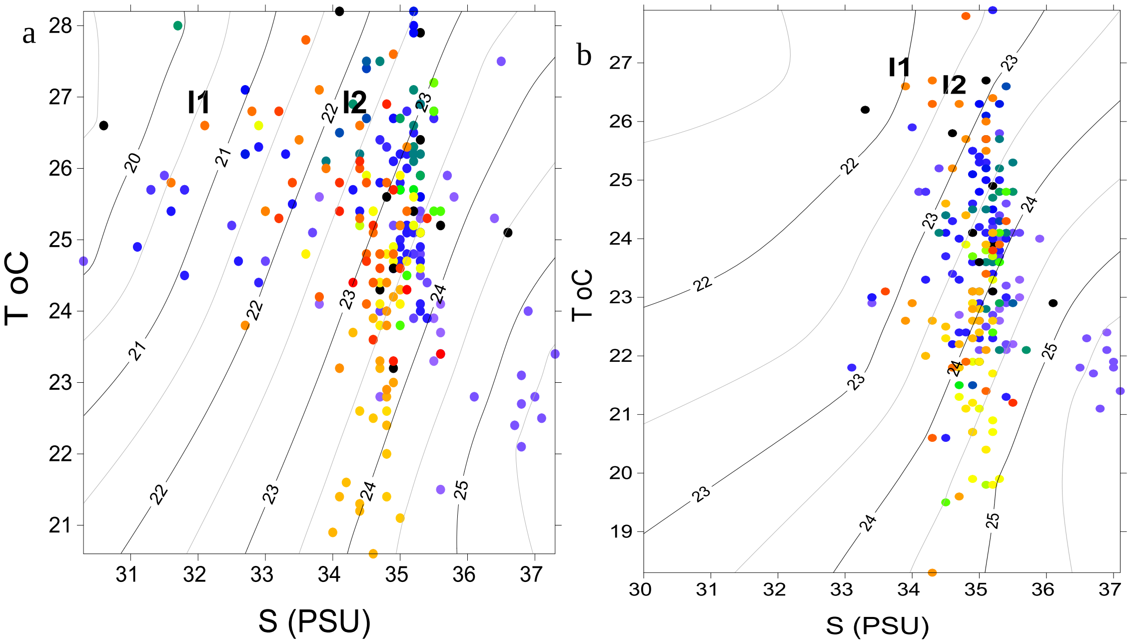

Figure 2. Sea surface water and near-bottom water T-S diagram with isoline of density, σ_t at the stations colored with geographical latitudes (see Fig. 1b for the station locations).

In contrast to the sea surface water, the TS diagram revealed that stations I1 and I2 exhibited distinct characteristics compared to the other nearby stations in the Gulf of Izmir and the study area. These two stations were characterized by lower salinity and higher temperatures compared to the other stations (Fig. 2, Table 1). Water temperatures were measured as warm, ranging from approximately 26°C at the sea surface to near bottom waters at both stations (Fig. 2). However, a notable disparity in salinity was observed between the two stations, with I2 exhibiting a lower salt content compared to I1 (Fig. 2). Furthermore, the dissolved oxygen levels varied significantly, with I1 recording a maximum of 8.5 mg/L in the near-bottom water and a minimum of 10 mg/L in the surface water (Fig. 2, Table 1).

Table 1. Physicochemical properties of the sea surface and near-bottom waters at station I1 and I2.

Sta.	WD	T (ºC)	S (ppt)	Ph	TSM (mg/l)	DO (mg/l)	C (ms)	SD (m)	BD (m)
I1	SS	26.6	34.4	8.23	25.4	10	50.7	9	10
	NB	26.3	34.7	8.31	25.6	8.5	51.1		
I2	SS	26.6	32.1	8.34	23.9	9.2	47.2	13.5	15
	NB	26.6	33.9	8.39	25.1	8.8	50.1		

The Secchi disk recorded a reading of 9 meters at I1 and 13.5 meters at I2. Due to the discrepancy in the hourly sampling times between the I1 and I2 stations, the PAR values were measured in units of μmol photons/cm^2/s (Fig. 3a). However, the percentage of light reaching the near-bottom waters was estimated to be approximately 99.9% of the surface PAR (Fig. 3b).

Plant traits

The following materials were examined: unpreserved specimens; one stolon for each station of I1 and I2; 25 shoots at I1, 38 shoots at I2 for rachis length measurements; 68 fronds at I1 and 114 fronds at I2 for measurements of frond length and width; and 19 fronds. at I1 and 17 fronds at I2 for the enumeration of pinnae on the fronds, collected at location of 38.395003 N and 26.759612 E (I1), and 38.412348 N and 26.746 E (I2). The samples were collected from depths of 10 and 15 meters by SCUBA diving on July 9, 2024, by Yaşar Özvarol and Barış Akçalı at locations designated as I1 and I2, respectively. The specimens were identified by Erhan Mutlu and Barış Akçalı.

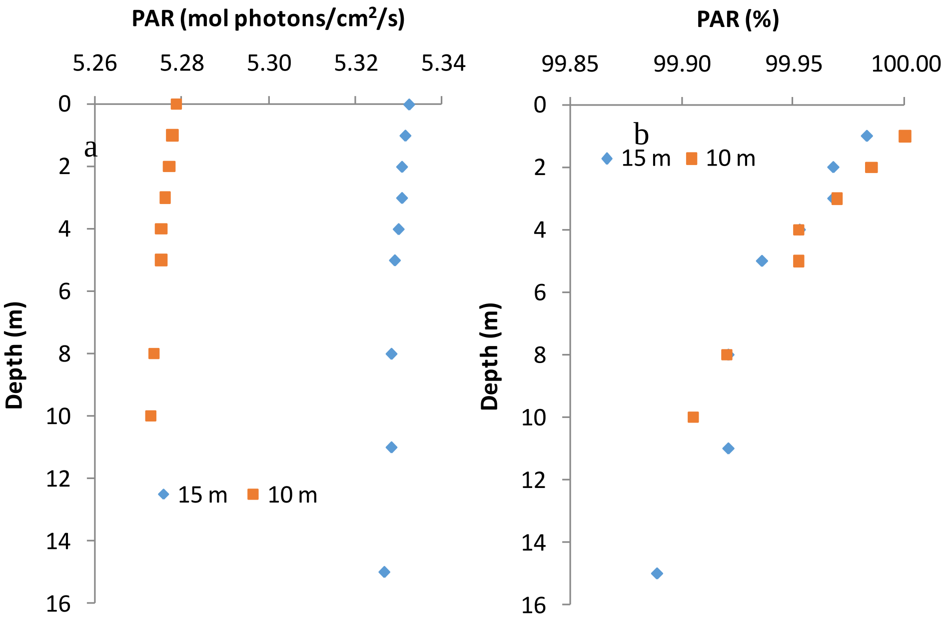

Figure 3. PAR profile in mol photon/cm^2/s (a) and percent based on sea surface PAR (b) along the water depth from the surface to bottom at 10 and 15 m.

Figure 4. *C. mexicana*: appearance of entire specimens (a and b) and close-up view of fronds (c and d). F: frond, St: stolon, Rh: rhizoid, FL: frond length, FW: frond width, P: pinnae, R1: rachis 1, and R2: rachis 2

Description

C. mexicana is characterized by its erect fronds, which are distinguished by their flattened branches. These fronds emerge from a creeping stolon, a specialized root-like structure, that is attached to the substrate by rhizoidal branches, which are specialized structures that facilitate nutrient uptake. The branchlets of *C. mexicana* are typically grassy green in color and are arranged in opposition along the midrib of the frond. This midrib is flat, and the branchlets are positioned in a manner that appears to be overlapping and pointing upward, contributing to the frond's distinctive feathery appearance. The length of frond 1 varied from 0.3 millimeters to 8.3 millimeters, with a maximum frond length ranging from 10 to 12.5 centimeters and a width ranging from 7 to 9 millimeters. The number of branchlets exhibited a range from 10 to 28 and from 120 to 194, with an average of 12 to 15 to 20 to 27 branchlets per 1 cm frond length (Fig. 4).

Remarks

C. mexicana exhibited structural similarities *to C. taxifolia*, yet *C. taxifolia* was distinguished by branchlets with constricted bases and a compressed midrib (Loos et al., 2023).

This species possesses a substantial, branched horizontal axis (stolon) that can attain lengths of up to 1 m, anchored by a multitude of short rhizoidal outgrowths (columns).Fronds: The photosynthetic axes (fronds) are characterized by a flattened structure, with lengths reaching up to 15 centimeters (6 inches) and widths ranging from 10 to 15 millimeters (0.4 to 1.5 inches). These fronds exhibit a pinnate branching pattern, akin to feathers, and possess a narrow midrib that is flattened in its cross-section. Branchlets (ramuli): The branchlets are distichous (arranged in two rows), flattened, and membranous in nature. The samples were found to be slightly or not constricted at the base and were observed to be curved upward, tapering to pointed tips.

The slope of the relationship between frond length and number of pinnae was estimated to be higher (2.15) than 2 for *C. taxifolia* var. *distichophylla* and more or less (0.91-1.19) than 1 for *C. taxifolia* (Mutlu et al., 2025) (Fig. A2).

Distribution

In addition to previous records in the easternmost Mediterranean, *C. mexicana* has been found in two different locations in the shallow and coastal waters of the Aegean Sea (Gulf of Izmir, Turkey). The species is primarily found in subtidal marine environments and has been observed to thrive in the following habitats: Depth range: It is typically found at depths ranging from 0.5 to 33 meters. Substrate: This species demonstrates a marked preference for rocky substrates and sandy bottoms, where it can anchor itself with its rhizoids. Its ecological role is to form lush underwater communities that provide shelter and grazing for various marine organisms, making it an important component of its ecosystem.

Biometrics

The presence of *C. mexicana* specimens at each station was evidenced by their location in wispy bundles of rachis, exhibiting a reticular stolon organization attached to the bottom substrates (Fig. 4). This occurrence was analogous to that of the species found at the bottom, contrasting with the highly elongated stolons characteristic of *C. taxifolia*. The biometric parameters of the species were identified as density and morphometric variables to characterize the recent measurements made from the living specimens that occurre for the first time in the Aegean Sea.

Density

Following the conversion of the number of shoots (fronds) to abundance, it was determined that the shallow station exhibited a higher abundance (630 shoots/m^2 at 10 m bottom depth) in comparison to the deep station (469 shoots/m^2 at 15 m bottom depth). Among the density variables, the number of shoots per sample (TS) and the leaf area index (frond length*width in cm^2 per square sampling area) exhibited a significant relationship with bottom depth, contrasting with the number of pinnae per 1 cm frond length (BNo) at $p < 0.05$ (Fig. 5). Analogous to abundance, the number of shoots per sample demonstrated a higher value in the shallower water (Fig. 5).

Conversely, the leaf area index (frond length*frond width in cm per square sampling area) exhibited a contrasting relationship with the number of shoots, suggesting that the LAI was elevated at greater depths (Fig. 5).

The mean BNo remained at approximately 2.57 and 2.82 pinnae/cm frond length (Fig. 5). No statistically significant differences in BNo were observed between the two sampling depths. The number of pinnae exhibited variability, ranging from 28 to 194, with an average of 18 ± 3 pinnae/cm FL at 10 m and 10 to 130, with an average of 16 ± 2 pinnae/cm FL at 15 m (Table 2).

Morphometry

The characterization of species morphometry was conducted by measuring rachis length (R1 in millimeters) and frond length (R2 in millimeters), frond width (in centimeters), and unilateral leaf area (in square centimeters), independent of surface area per sampling unit (Fig. 6). A statistically significant difference was observed among all morphometric variables between the two sampling depths of 10 and 15 meters at a $p < 0.05$ significance level.

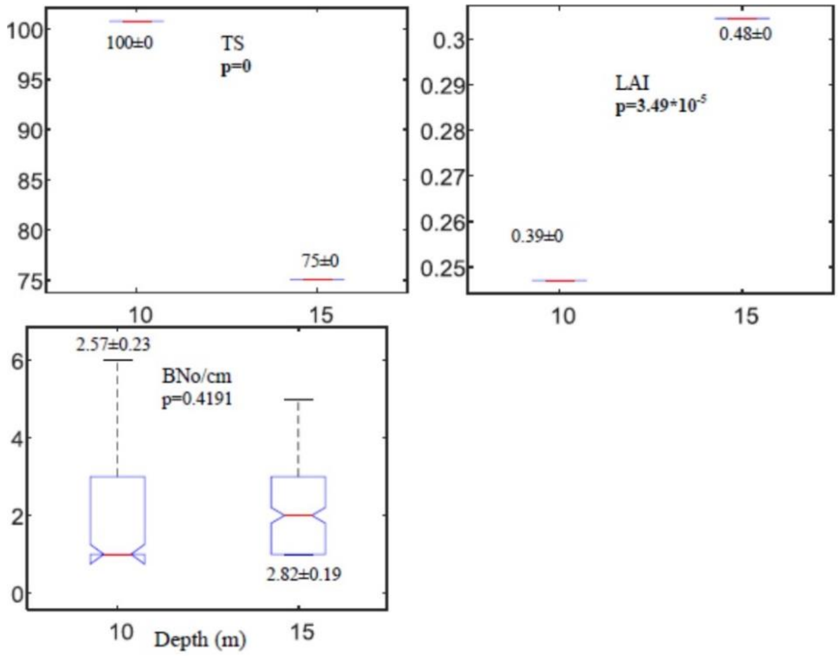

Figure 5. Notch plots of density variables of *C. mexicana* at the sampling depths. TS: number of shoots per sampler, LAI: single-sided leaf area index (cm^2/m^2), and BNo: number of pinnae per 1 cm frond length. The bold p value denotes a significant difference in the variable between two bottom depths, and the average value ± SD at each bottom depth.

Table 2. Minimum, maximum, mean and standard deviation values of density variables of *C. mexicana* at the sampling depths. TS: number of shoots per sampler, LAI: single-sided leaf area index (cm^2/m^2), and BNo: number of pinnae per 1 cm frond length.

	10 m (I2)			**15 m (I1)**		
	FL	**NP**	**BNo**	**FL**	**NP**	**BNo**
Min	1.7	28	15	0.5	10	12
Max	12.4	194	27	7.9	130	20
Mean	5	90	18	4	70	16
SD	3	48	3	2	42	2

Rachis 1 length exhibited a range from 0.3 mm to 8.3 mm, with an average of 3.5 mm at 10 m and 1.9 mm at 15 m. Frond length demonstrated a range from 3.6 to 91 mm at 10 m and from 3.9 to 81.6 mm at 15 m, respectively. The frond length at 10 m was 3.7 mm greater than that at 15 m (Table 3, Fig. 6). Similarly, the mean frond width was narrower at 10 m than at 15 m (Table 3, Fig. 6).

Table 3. Minimum, maximum, mean and standard deviation values of morphometric variables of *C. mexicana* at the sampling depths. RL: Rachis 1 length in mm, FL: frond length in mm, and FW: frond width in mm.

	10 m (I2)			**15 m (I1)**		
	R1	**FL**	**FW**	**R1**	**FL**	**FW**
Min	1	3.6	1.8	0.3	3.9	3
Max	7.4	91	7	8.3	81.6	9
Mean	3.5	31.1	4.37	1.9	34.8	5.76
SD	21.3	19.6	7.32	22.3	20.2	10.16

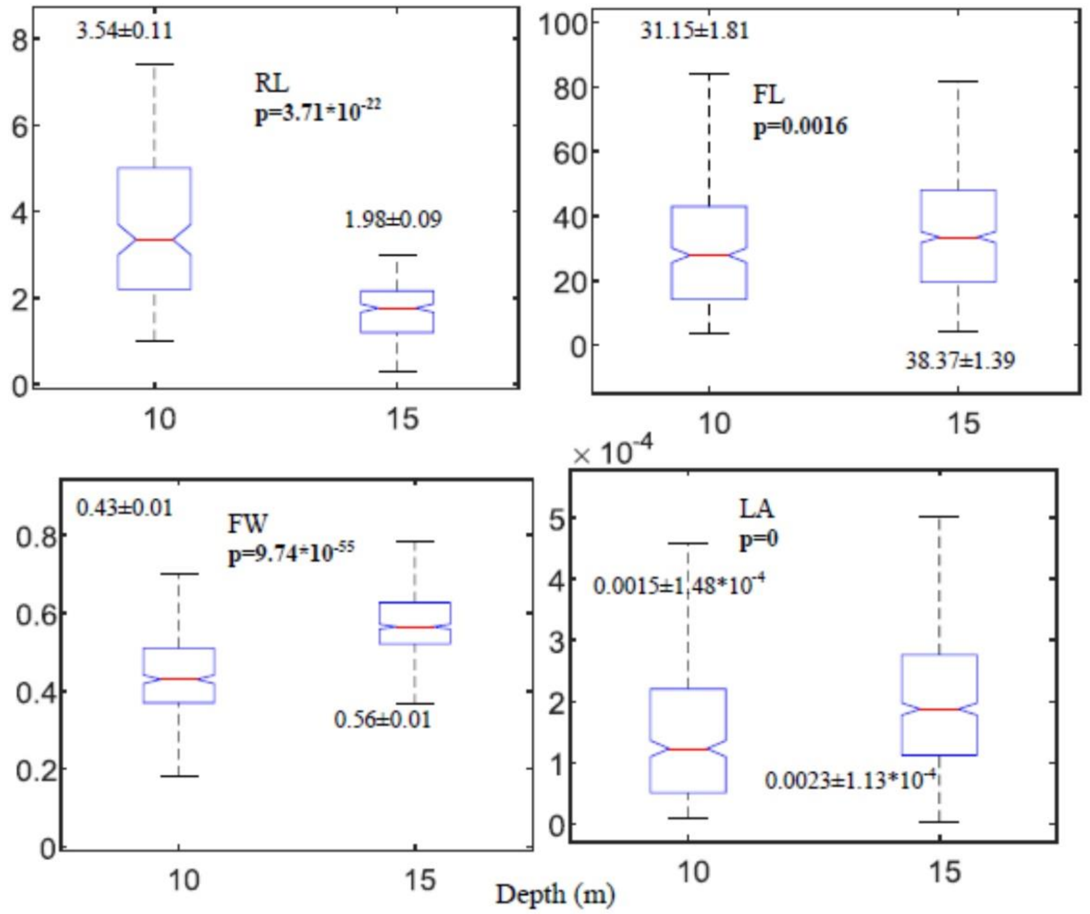

Figure 6. Notch plots of morphometrical variables of *C. mexicana* at the sampling depths. RL: Rachis 1 length in mm, FL: frond length in mm, FW: frond width in mm, and LA: leaf area in m^2. Bold p value denotes significant difference in the variable between two bottom depths, and average value ± SD at each bottom depth.

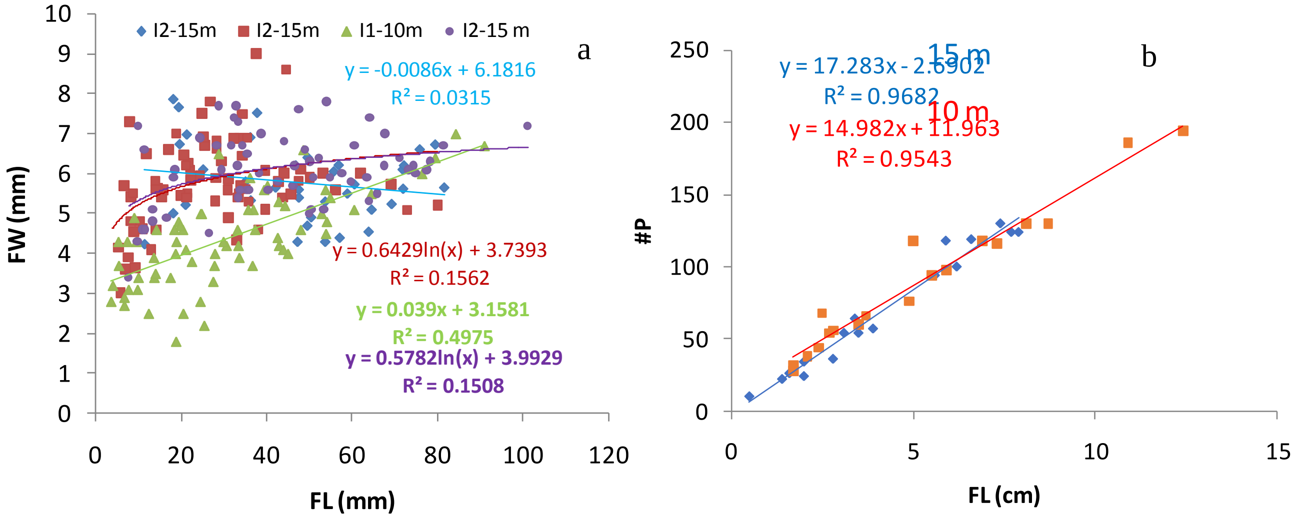

Figure 7. Relationships between frond length (FL) and frond width (FW) at 10 and 15 m depth (a) and between frond length and number of pinnae (#P) at 10 m and 15 m bottom depth (b). It is noted that the marked diamond showed the relationship of the fronds without buds, square with buds, and circle with a new measurement data set with and without buds after irregular relationship obtained at 15 m.

Biometrical relationship

The relationship between frond length and frond width, as well as frond length and number of pinnae, is demonstrated in Figure 7.

The FL-FW relationship proved to be significant for specimens found at 10 m, yet it was non-significant at 15 m. At 15 m, the relationship was plotted separately for either the main frond or buds, and the main frond exhibited a decreasing trend in the number of pinnae with frond length. However, buds demonstrated a bifurcated, non-significant linear relationship between frond length and number of pinnae. Given the irregular relationship between FL and #P at the 15-m depth, the re-established relationship with repeated measurements of the variables exhibited a similar insignificant logarithmic regression line as the previous fitting lines (Fig. 7).

The FL-#P relationship was evaluated significantly for samples taken at both 10 m and 15 m (Fig. 7). A subsequent analysis revealed no statistically significant variation in the FL-#P relationship between the 10-m and 15-m bottom depths (p = 0.0648).

Figure 8. Schematized frond budding (see Fig. 5 for real appearance of budding reticulated with branches) of *C. mexicana* found at 10 m (a) and 15 m (b).

Budding

The stolons of this species manifested in a reticulated form with budding (see Fig. 4). A total of 26 shoots were examined at the 10 m, and 40 shoots at the 15 m to assess the budding patterns of *C. mexicana* specimens. Regardless of the reticulated stolons, the number of buds and buds of buds appeared to be more pronounced at the 15-meter compared to the 10-meter (Fig. 8). Furthermore, the branching of the buds was found to be more forked at 15 m. The maximum number of buds of buds observed varied between 3 buds found at 10 m and 8 buds found at 15 m (Fig. 8). Additionally, 38% of the fronds were found to be bifurcated at 10 m, while it was more doubled at 15 m, with 60% of the fronds examined showing this characteristic

Discussion

Intra/inter-biometric relationships have the potential to assist taxonomists in identifying species at the lower taxonomic level. Depending on the differing growth conditions in various environments, such as the water mass characteristics depicted in the T-S diagram, these relationships can be distinguished by the specific water mass. Consequently, variations in these relationships were observed across different depths of the Izmir Gulf, the study area, which exhibited oligotrophic-eutrophic characteristics in its various sectors. Throughout the year, the near-bottom and sea surface water salinity maintained a consistent value of approximately 39 PSU, with the exception of a decline to 33 PSU in February, where sea surface salinity registered at around 19 PSU. This observation indicates that sea surface salinity exhibited a decrease in February when compared to other months.

Additionally, the water temperature fluctuated seasonally, ranging from a minimum of 15 °C in February to a maximum of 27 °C in July. The maximum recorded temperature in the near-bottom water was 23 °C, while the sea surface temperature ranged from 14-15 °C in February to a peak of 27 °C in July, subsequently dropping to 18 °C in November. A study by Mutlu (2021) and Mutlu and Akçalı (2022) revealed that the temperature of the sea surface and bottom waters were equivalent in November.

In relation to the nutrients necessary for the rapid growth of *C. mexicana*, the levels of surface dissolved inorganic nitrogen fluctuated between 0.5 and 0.7 µM, maintaining a consistent level of 0.5 µM in July and November (extreme 10 µM), and ranging from 0 to 0.5 µM (8 µM) in February. The levels of PO_4 in the outer gulf remained consistent throughout the year, ranging from 0 to 0.1 µM. However, in the inner and middle gulf, PO_4 levels increased to 2 µM in April, reaching a maximum of 3 µM in the inner gulf in July and November, and in the inner and middle gulf in February 2010 (Yelekçi et al. 2021). Furthermore, the 15-meter depth zone served as an intermediate zone for the adaptation of seagrasses to shallower and greater depths, exhibiting high variability in biometric measurements. This suggests that there were irregular and insignificant relationships between frond length and width measured at the 15-meter depth. In this transition zone, the fundamental environmental requirements (temperature, photoperiod, and light intensity, among others) exert a pivotal influence on biometric disparities and variations, as well as on the presence or absence of species (Catucci and Scardi 2020; Gnisci et al. 2020; Mutlu et al. 2022b).

The establishment of a suitable environment by such invasive algae could facilitate their colonization and subsequent spread in space. This species is one of the Lessepsian species in the Mediterranean Sea and could most likely have been introduced to the Aegean Sea via the ballast water of commercial ships that often visit the Gulf of Izmir (Çinar et al (2025). However, the species has not been observed along the Turkish Mediterranean coasts on the path of species introduction first observed at several loci, including the Israeli, Lebanese, and Syrian waters (Rayss 1941; Mayhoub 1976).

In the Mediterranean basin, *C. mexicana* was first documented in the Aegean Sea, following prior records that were exclusively reported in the easternmost Mediterranean waters (Fig. 1a). A comparison of *C. mexicana* and *C. taxifolia* reveals several distinguishing characteristics. The rachis (ramuli) of *C. taxifolia* is flattened and arranged with pinnules in a sickle shape, while the branchlets are characterized by flat blades with broad, flat marginal pinnae (Verlaque et al. 2015). In contrast to *C. taxifolia*, the ramuli of *C. mexicana* are wide and overlap one another, and the blades are clavate in shape. The ramuli were abruptly tapered towards the curved end spine (Coppejans and Beeckman 1990; South and N'Yeurt 1993; Aysel and Dural 1998), as seen in our materials (Figs. 4, 1A). However, the basal part of the ramuli of the specimens published by Erduğan et al. (2009) exhibited no contraction, and the frond blade was sickle-shaped. According to the descriptions made by Verlaque et al. (2015), the species could be identified as *C. taxifolia* var *distichophylla*. In contrast, the specimen reported from the Turkish coast of the Aegean Sea by Turan et al. (2011) was identified as *C. taxifolia* due to the contracted basal part of the ramuli. However, both studies did not establish the biometric relationship. The rachis with frond exhibited an almost ellipsoid shape in both *C. mexicana* and *C. taxifolia*, while in *C. taxifolia* var *distichophylla*, it was more or less cylindrical (Verlaque et al. 2015). The specimen of *C. mexicana* exhibited a unique morphology, characterized by a hybrid form between a clavate structure and a club-shaped structure (Fig. 1A). The latter component of the specimen was wider than the tip parts of the ramuli.

The budding structure of the three species was found to vary significantly. *C. mexicana* exhibited a reticulate structure (Figs. 4, A3) as determined by Verlaque et al. (2015). Çevik et al. (2007) conducted a molecular analysis to identify specimens found in Iskenderun Bay (Turkey) by comparing them with specimens from other regions and aquariums in Izmir. This analysis led them to recognize the Iskenderun specimens as *C. taxifolia*. The Iskenderun specimens exhibited an absence of a contracted structure in the basal part of the ramuli attached to the rachis, suggesting that this species differed from *C. taxifolia* collected from the aquarium. Indeed, both locus specimens (Iskenderun and Izmir) exhibited biometric differences during the description performed by Çevik et al. (2007) who performed molecular analyses for identification of the specimens. Therefore, subsequent analysis by Jongma et al. (2013) addressed the genetic misidentification of the Iskenderun Bay specimens, emphasizing the challenges and limitations of integrating genetic and morphological studies in determining species identity. They concluded that the Australian endemic green alga, *Caulerpa distichophylla*, was present along the Sicilian coast of the Mediterranean Sea. The slender *Caulerpa* previously reported as *C. taxifolia* from southeastern Turkey (Gulf of Iskenderun) also belongs to *C. distichophylla*. Morphologically, *C. distichophylla* clearly differs from *C. taxifolia* in its slender thallus and the lack of large rhizoidal pillars. However, genetic data do not provide undisputed evidence that the species are distinct. The analysis of the tufA cpDNA gene and the cp16S rDNA intron-2 sequences revealed a single nucleotide mutation that demarcated the two taxa, while the ITS rDNA sequences did not provide unequivocal distinction between them.

Biometric measurements have been identified as a primary factor in distinguishing the identity of closely related species and monitoring growth patterns. Consequently, the size and dimensions of three congeneric species of *Caulerpa* vary, and the location or sea-based location affects its biometry (density and morphometry) (Benzie et al., 2000). The potential for species *C. taxifolia* to exhibit ecomorphic variants of *C. mexicana*, or vice versa, as a result of misidentification, has been observed in different environments (Chisholm et al. 1995; Olson et al. 1998). However, there is a paucity of detailed studies examining the occurrence of these species with biometric variation in space and time for the Mediterranean Sea. The present study could be important to provide their density and morphometry for the Mediterranean Sea for future studies, since the species has been restricted to the easternmost corner of the Mediterranean Sea in terms of its distribution. The density of *C. taxifolia* species has increased very rapidly, reaching the number of fronds up to 5000 fronds/m^2 (Pereira et al. 2016; Loos et al. 2023). The length-width relationship of the frond of *C. taxifolia* var. *distichophylla* specimens collected from Antalya Gulf of the Turkish Mediterranean coast (Mutlu et al., 2022a) exhibited a comparable relationship with specimens of the Aegean Sea (Fig. A2). The relationships between the seas were not significantly different at $p < 0.05$ (ANOCOVA, p: 0.457).

In addition to environmental measurements (Tuya et al. 2006), the depth of the seabed has affected the size of the algae and seagrasses. For example, the photosynthetic activity of *C. mexicana* was at its highest in the coldest season and preferred low photosynthetic irradiance (Robledo and Freile-Pelegrín 2005). However, *C. taxifolia* has been observed to exhibit a preference for higher light intensity in comparison to *C. mexicana* (Gayol et al. 1995; Chisholm and Jaubert 1997). Furthermore, the shallower waters have been found to harbor denser shoots of various seaweed and seagrass species compared to those at greater depths. The length of the rachis (the part that holds the leaves or shoots) of these algae or the vertical rhizome length (the root-like structure that helps the plant take in water) of the seagrass was measured to be longer in the shallow waters. Conversely, the frond length, leaf length, and leaf width exhibited a decrease with increasing depth. The environmental parameters exhibited a variation with respect to bottom depth, with the density and size measurements of two species of seaweed, *Caulerpa prolifera* and *C. taxifolia* var. *distichophylla*, demonstrating distinct variations. These measurements varied in both time and space along the Turkish Mediterranean coast (Mutlu et al., 2022a). For instance, in contrast to *C. mexicana*, *P. oceanica* biometrics exhibited a response to numerous predicted environmental variables in the Mediterranean. The shoot density was predicted in response to geographic coordinates and seafloor depth, followed by prevailing wind, bottom gradient, and species in order of importance (Catucci and Scardi 2020; Gnisci et al. 2020; Mutlu et al. 2022b). Additionally, the seagrass *Cymodocea nodosa* exhibited distinct density and plant characteristics between the cold and warm water months, contingent on the physical, optical, chemical, and sedimentary characteristics of the environment (Mutlu et al. 2022c).

The number of pinnae on the frond increased with frond length; however, it remained constant with frond length per 1 cm of frond or rachis length. Furthermore, it did not differ with bottom depth, a phenomenon that has also been observed in the number of leaves of certain seagrass species. The measured frond length reached higher (15-25 cm) in the national Spanish waters (Canary Islands) fed with the riverine environment (Pereira, 2024) than our estimates.

In conclusion, the distribution of the invasive non-indigenous seaweed *C. mexicana* has extended its range northward from its initial occurrence in the Aegean Sea to the eastern basin of the Mediterranean Sea. This phenomenon is a consequence of the ongoing tropicalization resulting from global warming in the Mediterranean Sea, which has led to the proliferation of tropical species into previously unoccupied ecological niches. Consequently, the introduction of non-indigenous and invasive species, such as *C. mexicana*, poses a significant threat to the endemic biodiversity of the eastern Mediterranean basin. The biometry of *C. mexicana* can serve as a baseline for comparison with the characteristics of other congeneric species of *Caulerpa* throughout the Mediterranean basin and their native localities. Given its reproductive characteristics comparable to *C. taxifolia* and the elevated nutrient levels characteristic of the Urla region where it was identified, *C. mexicana* is likely to spread rapidly in this area. The turbidity resulting from high nutrient levels and the degradation of sediment quality leads to the removal of seagrasses, which can act as a barrier to invasive species, thereby providing an advantage to invasive species. In this study, we seek to elucidate the significance of biometric variations among organisms in facilitating species identification, in addition to their structural and plant characteristics. However, molecular analysis was not conducted on the specimens of all three *Caulerpa* species encountered in various locations (Fig. 1) during the present study, as they were preserved in formalin solution following measurement. Biometric measurements and analyses are more expeditious and economical than genetic analysis and can swiftly identify species (Figs. A1-A3). These relationships or morphometric analyses and ratios have been applied to the identical characters of many organisms, e.g., fish (e.g., Geladakis et al., 2017; Currie et al., 2024), zooplankton (e.g., Mutlu et al., 2020; Duman et al., 2025), and zoobenthos (e.g., Strafella et al., 2021) across various marine environments. The genetic analysis can discern slight variations in specimens exhibiting plasticity in their plant traits. However, the practical application of genetic analysis to each specimen during the survey, whether in the same or different area, was not feasible. Further studies are recommended to explore the potential of genetic analysis. Nonetheless, this comparative study underscores the significance of biometric relationships and plant traits among the three species examined in this study (Figs. A1-A3).

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Conflict of Interest

On behalf of all authors, the corresponding authorstates that there is no conflict of interest.

Author Contributions

Erhan Mutlu: Onboard works, Project administration,Supervision, Software, Data analyzes, Writing, Funding acquisition. Yaşar Özvarol: Onboard works, Laboratorial works, Measurements, Data entry. Barış Akçalı: Onboard works, Laboratorial works, Measurements, Data entry, Writing. Berivan Elif Aslan: Onboard works, Laboratorial works, Measurements, Data entry. Zeynep Narlı: Onboard works, Laboratorial works, Measurements, Data entry. Zeynep Zabun: Onboard works, Laboratorial works, Measurements, Data entry.

Ethics Approval

Not applicable.

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Appendix

Fig. A1. Fronds, rachises and pinnae of *Caulerpa mexicana* (a, d), *C. taxifolia* (b, (unpublished material) and *C. taxifolia* var. *distichophylla* (c) from the Turkish Aegean waters obtained during the present study (Mutlu et al. 2025).

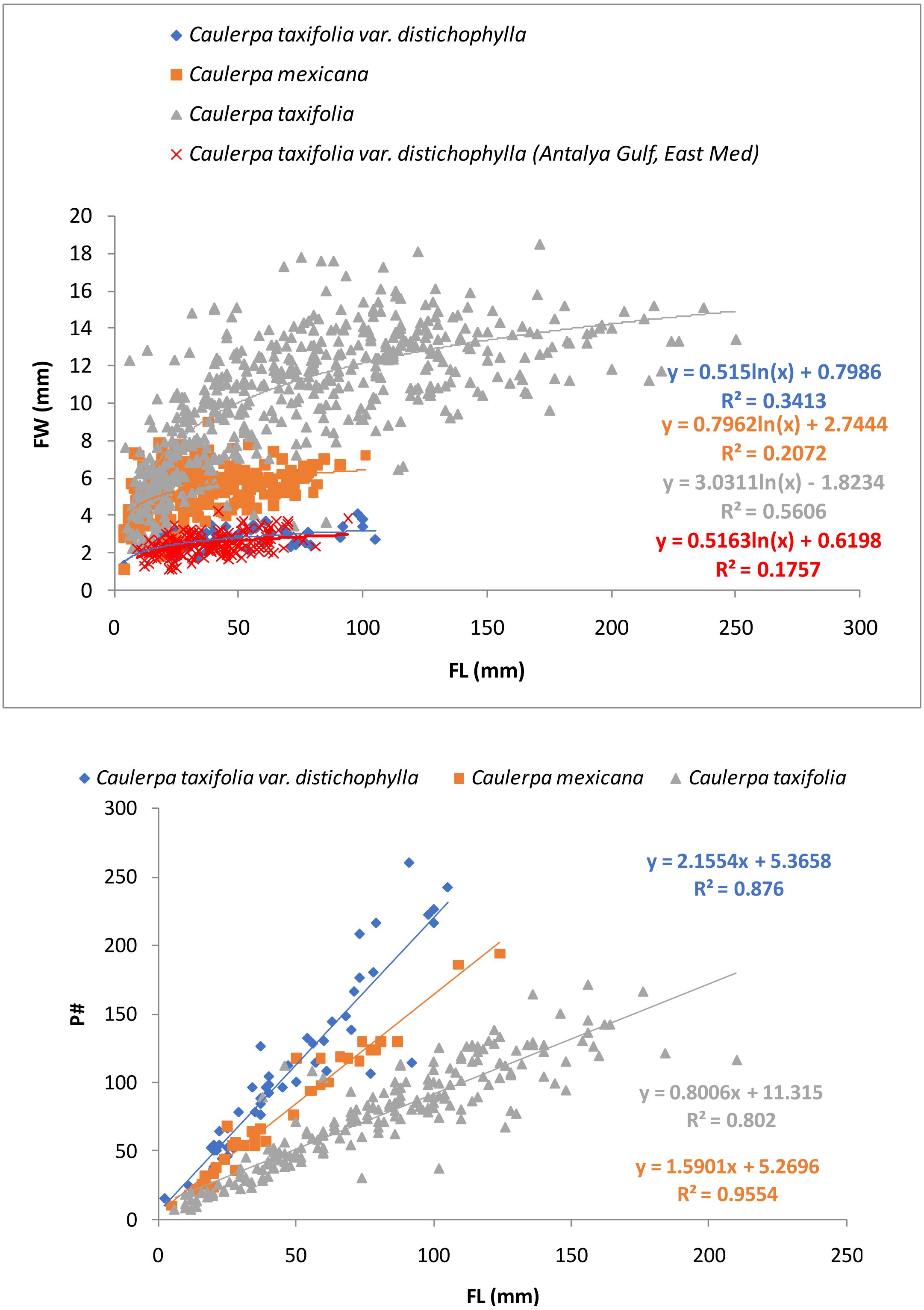

Fig. A2. Frond length (FL)-width (FW) and frond length (FL)-number of pinnae (P#) of *Caulerpa mexicana*, *Caulerpa taxifolia*, *C. taxifolia* var. *distichophylla* specimens collected during the present study conducted in the Turkish coast of the Aegean Sea (Mutlu et al. 2025) and *C. taxifolia* var. *distichophylla* from Antalya Gulf, Eastern Mediterranean Sea (data from Mutlu et al. 2022a).

Fig. A3. Plant traits of fresh specimens of *Caulerpa taxifolia* (a, b), *C. taxifolia* var. *distichophylla* (c, Mutlu et al. 2025) and *C. mexicana* (d) collected during the present study (See Fig. 1 for locations of the species occurrence)