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Physiological and Structural Characteristics of Three Varieties of *Caulerpa racemosa* (Försskal) J. Agardh. From the Mediterranean Sea

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Abstract: The physiological, anatomical, morphological and ultrastructural characteristics of the three varieties *occidentiales*, *lamourouxii* and *turbinata* of the genus *Caulerpa racemosa* collected from the Mediterranean Sea at Abu Qir bay, Alexandria, Egypt were investigated. The results showed that the three varieties have similar characteristics in some physiological aspects. The urease enzyme is the constitutive enzyme and responsible for urea-degradation; the mechanism by which ^{14}C-urea was taken was the active transport; the contents of the amino and fatty acids and the composition of free and protein hydrolysates were comparable; the amount of radiations determined in each of the cell constituents was almost constant, except for carbohydrates. The morphological studies using scanning electron microscope showed a great variation among all varieties, the anatomical structure of the stolons were greatly similar. Similarity in shape and size of mitochondria, chloroplasts, nucleus and some characteristic deposits in filament and in cell vacuoles was evident among the second and third varieties. The *occidentiales* and *lamourouxii* varieties were similar in cell wall structure, while starch granules were similar in all varieties. The two varieties *occidentiales* and *lamourouxii* seems to be more related to each other than to the *turbinata* variety. Combination of morphological criteria with those of physiological, anatomical and ultrastructure aspects are more useful in plant identification and confirmation.

Key words: Morphology · Anatomy · Ultrastructure · Egypt

INTRODUCTION

Caulerpa racemosa [1] in the Mediterranean has been regarded as an Indian Ocean species immigrated via the Suez Canal since its opening in 1869 [2]. As suggested by [3] that the three different morphological varieties of *C. racemosa* which coexist in the Mediterranean are: (1) *C. racemosa* var. lamourouxii (turner), (2) *C. racemosa* var. lamourouxii f. requienii (Montagne) [4, 6] and (3) *C. racemosa* var turbinata (J.Agardh) Eubank. [5].

On the basis of morphological criteria, kinetics of spread and tendency to proliferate, it was suggested by [3] that *C. racemosa* var. *occidentalis* as an invasive taxon recently introduced to the Mediterranean Sea. According to [3], this variety differs from the two others by its upright axes with clavate, more or less inflated branchlets, which are un-crowded and radially to distichously dispose. To determine the origin of the invasive variety, [7] used the ITS1 sequences, while [8] combined ITS1 and ITS2 sequences and intron of the 18S rDNA. The ITS1 and ITS2 data confirm that the three morphological varieties of *C. racemosa* from the Mediterranean Sea are distinct taxonomic units. The 18S intron data suggest that the invasive variety could be a recent hybrid between var. *turbinata* and an unknown tropical strain.

On the Egyptian coasts, numerous publications covered the taxonomy, distribution and habitats of the genus *Caulerpa* (e.g., [5, 9-11]. Aleem [12] recorded *Caulerpa racemosa* as a recent occurrence in Alexandria and referred it as mostly belong to var. *uvifera* and var. *clavifera*, while [13] collected all the three varieties mentioned above from Red Sea and Suez Canal as a single taxon with three synonyms. They found considerable morphological variations when the same species or variety grows in habitats with different conditions of light intensities. As mentioned by [8], the question has remained open as to whether the Mediterranean

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specimens identified morphologically as *C. racemosa* var. *turbinate* f. *uvifera*, var. *lamourouxii* f. *requienii* and invasive variety illustrate the capacity of a single taxon to change, or belong to three distinct taxa. Exploration of the Mediterranean flora around the Suez Canal may provide more evidences.

The present work deals with the investigation of the physiological, anatomical, morphological and ultrastructural characteristics of the three varieties *occidentiales*, *lamourouxii* and *turbinata* of the genus *Caulerpa racemosa* collected from the Mediterranean Sea at Abu Qir bay, Alexandria, Egypt.

MATERIALS AND METHODS

Algal collection: Algal specimens were collected from shallow subtidal zone in Abu Qir Bay, Alexandria (Fig. 1) during April 2007. Ten grams fresh weight from each alga was washed thoroughly with sterilized seawater for several times and bacterial detection was carried out as described [14], positive cultures were discarded.

Algal culturing and extraction of urea-degrading enzyme: The algal specimens were transferred to a flask containing 50-ml nitrogen-free medium and kept for 10h. To be deprived from nitrogen [15]. The flasks were incubated at light intensity 9600 lux and temperature of 25°C.

The crude enzyme extract was prepared from the algal material by the method described [16] using HEPES buffer at pH 7.6. Activity measurement was made by the $^{14}CO2$ technique released after the injection of ^{14}C-urea. Radioactivity was measured by Beckman LS200 B Liquid Scintillation Counter [17]. To assess whether the enzyme present was urease or urea-amidolyase, the assay was carried out in presence of ATP (5µM / ml), avidin (100 µg/ml), hydroxyurea (10 µM/ ml) biotin |(100 µg/ml) and dithiothreitol (1mM) [14]. Protein determination was carried out as described [18].

Separation and d etermination of free amino acids: The ethanol-soluble fractions of the algal suspension were passed through a column of Amberlite 1R-120 (H^+). Free amino acids were then eluted with 1N NH_4OH; homogenized and centrifuged. The supernatant was evaporated under vacuum. The dry residue was dissolved in 0.2 M lithium citrate buffer, pH 2.2 [19], saved for analysis with the amino acid analyzer.

Preparation of protein-hydrolysates: For estimation of protein amino acids, the method described by [20] was used. Calculations were made using the equation given by [21].

Chromatography of ^{14}C-urea and radioamino assay: The TLC plates of the different treatments were prepared as described by [22]. The amount of radioactivity present in each amino acid was determined. The results were expressed as per cent of the total radiation detected under each time interval [23].

Fatty acids extraction and identification: Total lipids were extracted from algal material with chloroform / methanol mixture (2:1 v/v) according to the method [24]. Total lipids were converted into fatty acid methyl ester (FAME) using the method applied by [25]. The analyses of FAME were done by Shimadzu Gas Liquid Chromatography using GLC technique.

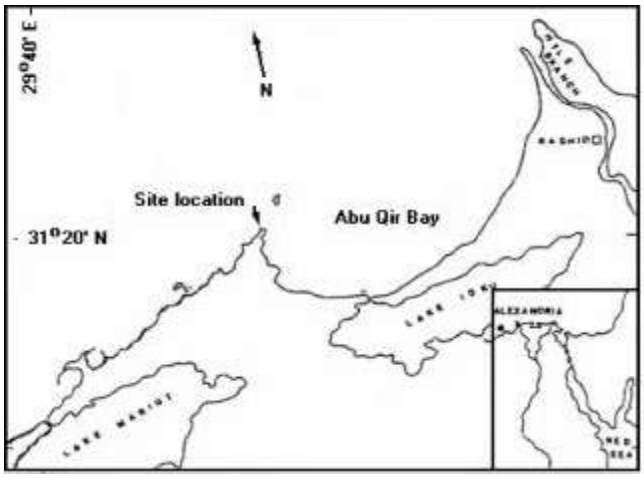


Fig. 1: Shows the site location and the position of Alexandria with respect to the eastern Mediterranean and Red Sea

Identification of each fatty acid was estimated by comparing its retention time with those of standards.

Electron microscope: A-For scanning electron microscopy (SEM), 2-5 samples per spacemen were washed in water, air-dried, investigated by the stereomicroscope, mounted on brass stubs and coated with a thin layer of gold using JEOL -JFCL 1100 E ion sputtering. Coated samples were examined and photographed on a JEOL-JSM 5300 SEM with an accelerating voltage of 15 Kv at the Electron Microscopic Unit, Faculty of Science, Alexandria University. Close-up views were always taken from the lateral view from the algal material. The characters evaluated were that of leaf (length, shape, apex. surface), surface sculpture, overall surface pattern, curvature of the outer walls, boundaries epicuticular wax. Diatoms type invading the algal surface and their morphology (polarity, summetry, shape, diamentions, aperture and exine) Terminology followed [26-28] was adopted to describe the thallus.

B-For Transmission electron microscope (SEM), stained sections were examined with a JEOL 1010 Transmission Electron Microscope at 80 Kv in the RCMR [29].

RESULT AND DISCUSSION

Enzyme activity: The enzyme activity in the investigated varieties is shown in Table 1. In absence of ATP or in presence of both ATP and avidin, the enzyme activity was higher than in presence of ATP alone or in presence of both adivin and biotin and was very low in presence of hydroxyurea. These results may indicate that the enzyme responsible for urea-degradation by all varieties was urease enzyme and not urea-amidolyase.

Bekheet and Syrett [14] proposed a new system for algal classification based on the enzyme responsible for urea-degradation. Different varieties or strains of the same algal species may possess different enzymes activities, but single variety appears to have one enzyme or the other but not both. For instance, Al-Houty and Syrett [30] found urease enzyme in strain 335/1a of *Klebsormidium flaccidum*, whereas strain 335/1b of the same species contained amidolyase. F-test (Table 1) indicates no significant difference among the enzyme activities of the present varieties and suggests a higher similarity among them. According to [31], urease in these varieties is a constitutive enzyme since there was an activity in alga which did not receive urea.

Table 1: Radioassay of urea-degrading enzyme in three varieties of *Caulerpa racemosa* from Abu Qir, Alexandria. Activites are μ mol $^{14}CO_2$. g^{-1}protein.h^{-1}. Enzyme activity in presence (*) or absence (**)

Algal varieties	*C. racemosa* var.I	*C. racemosa* var.II	*C. racemosa* var.III
Boiled extract	0	0	0
-ATP**	1530	1632	1576
+ATP*	1201	1197	1204
+ATP+Avidin*	1547	1529	1522
+Adivin+biotin*	1238	1205	1186
+Hydroxy urea*	90	81	87
F-test	Var I&II	Var I&III	Var II&III
	0.9651	0.9984	0.9666

Table 2: Incorporation of ^{14}C-urea into the cells of different varieties and the remaining in the medium calculated as percent of total radiation injected. Var1, *occidentiales*; var2, *lamourouxii*; Var3.

	Algal varieties		
Treatment	Varity I	Varity II	Varity III
^{14}C in cells	57.00	61.00	56.00
^{14}C in medium	43.00	39.00	44.00
Accumlation ratio C/M	1.33	1.56	1.27

Urea uptake and transport: The accumulation ratio of ^{14}C-urea in *Caulerpa racemosa* varieties is shown in Table 2. The accumulation ratios in variety 1, 2 and 3 were 1.33, 1.56 and 1.27 respectively. These ratios indicated that the active transport was the mechanism by which ^{14}C-urea was taken up these varieties, since the ratios exceeded one.

In *Saccharomyces* and *Phaeodachytulum* urea transport occurs by two pathways. The first via an active transport system and is sensitive to nitrogen repression and the second passive transport system occurred at relatively high external urea concentration [2, 15]. This did not agree with our results, since the enzyme was a constitutive enzyme and insensitive to nitrogen depression.

Urea metablism

Protein amino acids: The three varieties of *C. racemosa* utilized urea (either radioactive or non-radioactive) in the primary formation of amino acids, variety 1 and 2 contained the highest total protein amino acids.

In protein hydrolysate most of the amino acids determined were common in urea metabolism especially the essential amino acids. Arginine, phenylalanine, leucine, isoleucine and histidine were the dominant amino

Table 3: Amino acids content of protein hydrolysate (P) and free amino acids of *Caulerpa racemosa* varieties, collected from Abu Qir, Alexandria. Values in mg g^{-1} dry wt

	Algal varieties					
	C. racemose var.I		*C. racemosa* var.II		*C. racemosa* var.III	
Amino acid	P	F	P	F	P	F
Asp	3.40	0.85	0.24	0.73	2.83	0.81
Thr	8.34	0.7	7.14	0.69	7.64	0.48
Ser	2.74	0.83	2.62	0.91	2.09	0.59
Glut	2.50	0.74	2.43	0.50	2.16	0.27
Prof	2.73	1.54	2.49	1.32	3.1	1.55
Gly	1.81	1.24	2.54	1.01	2.31	0.94
Alan	2.46	1.13	2.53	1.09	1.79	1.04
Cyst	3.10	0.68	2.85	0.84	2.82	0.75
Val	4.60	1.74	3.91	1.6	3.53	1.47
Meth	4.49	0.02	5.23	0.12	8.41	0.24
Isoleucine	20.60	0.14	17.76	0.26	20.52	0.59
Leucine	23.66	1.98	20.33	1.37	21.58	1.09
Tyro	1.74	0.65	2.01	1.39	2.13	1.11
Ph.al	27.48	2.11	23.09	2.58	22.54	2.63
Hist	18.87	2.21	19.41	1.05	18.36	0.74
Lys	13.94	1.73	11.38	1.59	11.17	2.04
Arg	31.62	3.40	33.07	3.66	32.95	2.95
Ammonia	2.30	0.70	3.17	1.08	3.02	1.2
F-test	Var. I&II	Var. I&III	Var. II&III	Var. I&II	Var. I&III	Var. II&III
	0.82	0.83	0.99	0.95	0.66	0.70
Total(F+P)	202.32		183.99		189.44	
Lysine value	7.74		7.04		6.97	

Table 4: Radioactivity detected in both protein (P) and fee (F) amino acids of *C. racemosa*. Values are per cent of amino acid contents determined by the analyzer.

	Varieties					
	Var.I		Var.II		Var.III	
Aminoacide	P	F	P	F	P	F
Asp		0.32		0.28		0.33
Thrs		0				0
Glut						
Prol		0.11		0.13		0.12
Gly		0.48		0.24		0.49
Alon		0.36		0.5		0.44
Cyst		0		0.01		0
Val	0		0	0		0
Meth	0		0	0	0	0
Isoleu	0.26		0.24		0.19	
Len					0	
Tyro		0		0		0
Ph.al	1.05		0.51		0.49	
Hist	0.21	0	0.19	0	0.18	0
Lys	0.31		0.37		0.35	
Ang	0.34		0.23		0.28	
Ammonium	0.57	0.27	0.68	0.31	0.7	0.22
Total a.a	2.74	1.54	2.22	1.47	2.19	1.60
Total (F+P)	4.28		3.69		3.79	

acids in the protein hydrolysate of the three investigated taxa. Tyrosine, glycine, threonine and alanine were the least common amino acids.

Free amino acids: Arginine, histidine and phenylalanine were also present with high concentration while methionine, isoleucine and threonine were in low amounts. Some amino acids were found in both free state and as a building stone of proteins. These acids were: arginine, phenylalanine, histidine and leucine. However, most of the postulated conversions followed the pathways of reductive ammination, transamination and biosynthesis [33].

Lysine value was almost equal in the three varieties of this investigation, ranged between 6.97 and 7.74.

Radio assay

Radioactivity in protein amino acids: Values of amino acids as estimated of total urea (radioactive and non-radioactive) using the data given by the Amino Acid Analyzer are shown in Table 3. Total radioactivity determined in protein and free amino acids are shown in Table 4. Only 2.74, 2.22 and 2.19% from the total protein amino acids was radioactive, i.e due to ^{14}C-urea metabolism, in variety 1, 2 and 3, respectively. Almost, constant amount of ^{14}C- urea was detected in each of the individual amino acids of protein and also in total protein hydrolysate. Radiation was detected in five amino acids: phenylalanine, arginine, isoleucine, histidine and lysine in addition to ammonia in the three varieties of *Caulerpa racemosa*. Valine and methionine were present in minute amounts. The increase in phenyl alanine was greater in variety than the other two varieties (0.5%).

Radioactivity in free amino acids: The total radiation determined in free amino acids (as a per cent of total urea taken) ranged between 1.47-1.6%. The increase in individual free amino acids in the three varieties was due to alanine, ammonium, aspartic, glycine and proline,whereas the least was cystien, valine, methionine, tyrosine and histidine. The results indicated that there was a coincidence in the metabolic pathway of ^{14}C-urea in both protein and free amino acids which confirmed that, the three varieties are related to the same species.

Radiation in cell constituents: In cell constituents, the total radioactivity (Table 5) in carbohydrates was higher in var.2 (7.38%) than in var.1 (4.92%) and var.3 (6.31%). Other cell constituents showed approximately equal percentage of the total radiation and this may indicate a similar physiological trend in the metabolic pathways of all varieties.

Table 5: Percentage of radiation in each of the cell constituents of *C. racemosa* from Alexandria

	Var.I	Var.II	Var.III
Protein a.a's	2.74	2.22	2.19
Free a.a's	1.54	1.47	1.60
Carbohydrates	4.92	7.38	6.31
Fatty acids	1.97	2.05	1.87
Total radiation	11.17	13.12	11.97

Table 6: Fatty acids contents of *Caulerpa racemosa* varieties. Values are µg.ml^{-1} and per cent of total fatty acids

	Variety					
	Variety I		Variety II		Variety III	
Fatty acids	µg	% total	µg	% total	µg	% total
Saturated fatty acids						
C6:0	9.7432	36	0	0	0	0
C8:0	0.0003	0	3.7298	9.84	0	0
C10:0	4.8714	18.2	0	0	0	0
C11:0	6.0715	22.69	1.5545	4.1	8.2223	16.16
C12:0	6.0502	22.61	1.4093	3.72	2.0357	4
C14:0	0.0009	0	0.0002	0	0.0002	0
C15:0	0.0001	0	2.0089	5.3	0	0
C16:0	0.0095	0.03	0.0029	0	0.0012	0
C17:0	0.0002	0	6.9364	18.31	7.9311	15.58
C18:0	0.0008	0	0.0003	0	0.0001	0
C20:0	----	-----	2.9924	7.9	0	0
C23:0	0.0009	0	0.0002	0	0	0
C24:0	0.0006	0	0.0002	0	0	0
Monounsaturated						
C14:1	0.0001	0	2.0258	5.34	5.6119	11.03
C15:1	0.0001	0	3.1304	8.26	3.0983	6.09
C16:1	0.001	0	0.0002	0	5.8821	11.56
C17:1	0.0003	0	5.1132	13.5	0	0
C20:1	0.0001	0	3.0806	8.13	2.2482	4.41
Polyunsaturated						
C18:2	0.0017	0	0.0015	0	7.1129	13.98
C20:2	0.0002	0	---	-----	----	------
C18:3	0.0005	0	0	0	0.0002	0
C20:4	0.0001	0	5.8772	15.51	8.7306	17.16
C20:5	0.0001	0	0	0	0	0
Total	26.7538	99.53	37.873	99.91	50.8748	99.97

Fatty acids contents: Fatty acids composition may solve some taxonomic problems depending only on the morphological bases.

Among the three varieties investigated for their fatty acids contents it was shown that the carbon chain lengths ranged between C6 and C24 (Table 6). Saturated fatty acids comprised 13 fatty acid, while unsaturated comprised 10 only.

The most widely distributed fraction among saturated and unsaturated fatty acids were C20 and C18.

Long chain fatty acids C23 and C24 fractions were present in minute amounts in saturated fatty acids of the three varieties, Saturated fatty acids (C6-C20) comprised 99.5% from the total fatty acids determined in variety 1; became 49.17% in variety2 and decreased to 35.74% (of the total fatty acids) in variety 3. Unsaturated fatty acids (C14-C20) showed opposite results to the saturated fatty acids among the varieties studied in this investigation. Monounsaturated fatty acids C14-C2o were present in trace amounts in variety 1, formed 35.23 and 33.09% of the total fatty acid contents in variety 2 and 3 respectively. Also, polyunsaturated fatty acids were present in minute amounts in variety 1 and comprised 15.51 and 31.14 per cent in varieties 2 and 3.

Concerning the individual fatty acid contents one can conclude that: there was a good coincidence between variety 1 and 2 in some fatty acids contents and between variety 2 and 3 for another fatty acids, which confirm the interrelationship between the three varieties but not coincide, i.e. they are three separate taxa.

C20 was the dominant fraction in this investigation especially in the unsaturated fraction of *C.racemosa* varieties. C20:0 was absent from saturated fatty acids of variety, while C20:2 was absent from the unsaturated fatty acids of the other two varieties. Frequent distribution of C20 fraction in both saturated and unsaturated fatty acids was accompanied with increased concentrations (31.54 and 21.57 per cent) in both variety 2 and 3. On the contrast C18 was also frequently distributed but found only with minute amounts.

According to Graeve *et a*l. [34], Chlorophyta comprise the most modern group due to the frequency of C18 unsaturated fatty acids which were typically of the vegetative tissues of higher plants. According to Graeve bases, *C..racemosa* varieties companied an advanced systematic position through Chlorophyta. C17:0 was present with a high concentration in varieties 2 and 3 (18.31 and 15.58%). Traces of C17:1 were detected in 1 and 3, although high concentration was determined in variety 2 (13.5%).

The dendrogram of the fatty acids of the three varieties investigated (Fig. 2), supported the above mentioned results (page 20). The highest similarity value was 86% between varieties 2and 3 while it was 85% between 1 and2 and between 1 and 3. The dendrogram confirmed the interrelationship between the three varieties, but still dissimilar in 14-15% i.e.; according to varieties specific properties. Variety 2 and 3 seemed to be more advanced than variety, 1 on the bases of fatty acids and ^{14}C-urea metabolism.

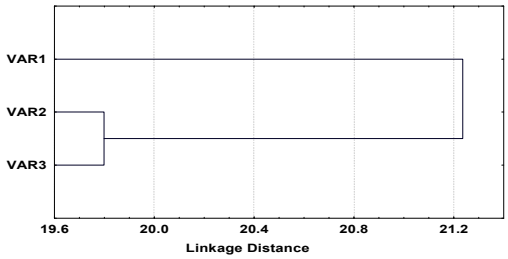


Fig. 2: Dendrogram of three varieties of *C. racemosa* from Alexandria based on fatty acid contents

Table 7: Branchlet characteristics of the three study varieties

Branchlet criteria	Var. *lamourouxii*	Var. *turbinata*	Var. *occidentalis*
Density	Rare to frequent	More frequent	Dense
Arrangement	Irregular to		
Sub-opposite	Alternate to spiral	Radial	
Stalk	Sessile	Sessile	Stalked
Shape	Clavate to		
Sub-spherical	Clavate	Spherical	
Apex	Rounded	Flattened	Rounded
Length	4-7 mm	2-3 mm	2-3(4) mm

Habit and morphology: *C. racemosa* var. *lamourouxii* forms large bluish green turf on consolidated sandy bottom of subtidal zone; stolon densely branched; branches spread out on the bottom, 3-4 mm thick; erect branches are mostly simple, rarely branched, 4-8 cm long; branchlets are rare to frequent, irregular to sub-oppositely arranged, long clavate with a rounded apex,, 5-9 mm long. *C. racemosa* var. *turbinate* forms bluish green dense cushion in shallow subtidal zone; stolon is densely branched, 2-4 mm thick; erect branches are 3-4 cm long; branchlets are more frequent, alternate to spirally arranged, clavate shape with a flattened apex,, 2-3 mm long.

C. racemosa var. *occidentalis* forms grass-green, living with other taxa in shallow subtidal zone; stolon branched, 3-4 (5) mm thick; erect branches are 8-10 cm long; branchlets are dense, radially arranged, spherical shape with a stalk, 2-3 (4) mm in diameter.

The materials of the three varieties mentioned above seen to be comparable with those previously collected and identified [13] from the Suez Canal and Red Sea (Table 7). The authors suggested light intensity as a major factor affecting the gross morphology of C. racemosa. *C. racemosa* var. *turbinata* (J. Agardh) Eubank has been known since 1926 in Tunisia [35], *C. racemosa* var. *lamourouxii* (Turner) Weber van Bosse has been known since the 1950s in the Levantine basin [36], while *C. racemosa* var. *occidentalis* has been suggested as a recent hybrid spreading quickly since the early 1990s throughout most of the Mediterranean Sea [8]. In Egypt, Aleem [12] recorded *Caulerpa racemosa* as a recent occurrence in Alexandria and referred it as mostly belong to var. *uvifera* and var. *clavifera*.

Ultrastructure

Scanning Electron Microscope (SEM): The SEM figures of the investigated varieties are shown in Plat 2 and their details are presented in Table 8. This description indicated that the three algae were three different taxa. Scanning Electron Microscope of the rhizome showed similarity in the anatomical structure of the three taxa i.e. they belonged to the same species.

Transmission Electron Microscope (TEM): Careful observation was conducted on the *Caulerpa racemosa* varieties from the Mediterranean Sea. Interrelationships between the three varieties in some aspects confirmed that they belong to the same species but with some variations due to varieties specific criteria. The cell wall of the three algae has a fribriller structure highly stratified in var. III. It was formed from thick outer layer composed of several strata with electron dense deposits in variety III. The cuticler cover of the wall surface is very thin in variety I; moderate in variety II; very thick in var. III. These aspects related to the ultrastructure of the members of order Caulerpales. In *Caulerpa sertularioides* and *Derbsia tenuissima*, the cell wall has also a fibrillar structure but has a thick inner layer and an outer electron dense layer [37].

Historical review: The genus *Caulerpa* is easily distinguished from the other algae as its thallus consists of a rhizome which creeps over the sand or mud, rooted into them by means of numerous rhizoids terminated with a group of fixative filaments. The stolon gives rise to numerous erect assimilators or fronds. The frond consists of a central axis with lateral branchlets or ramuli, in arrangement which varies according to varieties or forms [13].

Caulerpa racemosa (forsskäl) J.Agardh is shown in plate 1 (I, II, III). Records under other synonyms are *C. racemosa* var. lamourouxii (turner) [4, 5], *C. racemosa* var. lamourouxii f. requienii (Montagne) [4, 6] and *C. racemosa* var turbinata (J.Agardh) Eubank. [5].

As concluded by [2,28,35] that *Caulerpa racemosa* reaches the northern limits in the shores of the Suez Canal and overrunning the Eastern Mediterranean from the Indian Ocean, as they were known along the costs of East Africa. Aleem [2] proposed that these species came

Table 8: Morphological criteria describing the scanning electron micrograph (plate 2) of three varieties of *Caulerpa racemosa* from the Mediterranean Sea in Alexandria

	Varieties		
Morphological criteria	Variety I	Variety II	Variety II
-Branchlets			
-Arrangement	Alternate	Alternate	Alternate
length	560-600 µm	360 µm	760-800 µm
shape	oblancealate	abovate	oblancelate
apex	truncate	rounded	truncate
surface	wrinkled	wrinkled	nearly smooth
-Surface sculpture	cuticular deposition	cuticular deposition	
overall surface pattern	regulate	nearly smooth to finely regulate and sparsely punctate surface	nearly smooth
epidermal cells shape	elongated		
curvature of the outer peirclinal walls	convex	concave	Almost flat
boundaries (anticlinal walls)	undulate (sunken)		
epiculicular wax	present in the form of plates	Granulas	irregular (Blocks)
-Diatoms on the thallus	isopolar	isopolar	heteropolar
polarity	radially symmetrical	radically symmetrically elliptic	bilateral symmetrical
symmetry	prolate, elliptic to broodly elliptic	prolate, elliptic to broodly elliptic	obovate
shape	p(polar axis) = 1.1 µm	P = 12.0 µm	P = 11.3 µm
diamentions	E (equatorial axis = 0.73 µm	E = 8.57 µm	E = 6.66 µm
	P/E = 1.5 (prolate)	P/E = 1.4 (prolate)	P/E = 1.7 (prolate)
aperture	Monocolpate, colpus length approximatly 0.68 µm. not extending to the poles. colpus with pointed ends and margin smooth	Absent	Absent
axine	Sexine are scralariform to scrobiculate, lumina are transversally elongated	Sexine microretculate to tectate perforate, lumina, circulan at the poles, to irregulan cross in shape in the center.	Sexine microretculate to tectate perforated, lumina are circular at the poles and at the center

to the Mediterranean through Arab navigation in the Indian Ocean.

Varieties or forms of *Caulerpa racemosa* vary considerably in thallus characteristics according to the substrate, depth, light, nutrient level and exposure to waves. Aleem [12] pointed out to the two varieties of *C. racemosa* in the Mediterranean Sea of Alexandria: var. unifera and var. clavifera as of recent occurrence in the Mediterranean Sea of Alexandria, Egypt. These varieties were very small and dilect according to the rocky habitats, shallow water and exposed situations. Varieties of Suez Canal and Red Sea were characterized by their large size; stolon may reach two meters or more [12].

Argyrou *et al*. [39] found that *Caulerpa racemosa* of the Red Sea expanded to Moni Bay of Cyprus; between 1992 and 1997. This expansion was due to the increase of water temperature associated with global warming of nutrient inputs or with the differences in the life history characteristics of this migrant vs. native algal species. Also in 1999, [40] studied the dynamics of the tropical algal population *C. racemosa* in the Mediterranean, while [41], studied the fragmentation as a strategy for *Caulerpa* species migration. They described *C. racemosa* as invasive weed.

In 2000; [13] pointed out to the previous three varieties described by [4, 5]. They collected and identified them from the Suez Canal and Red Sea, but they recorded them as a single taxon with three synonyms. Also, [7] used the sequencer analyzer to determine the origin of the invasive variety. They confirmed that they were three distinct taxonomic units.

Again, in 2001; [42] described the dispersal of *C. racemosa* fragments in the, Mediterranean. The study of [43] showed the effect of introduced *C. racemosa* in the Mediterranean. Also, [44] investigated the sexual reproduction of invastive *C. racemosa* var. occidentalis from the Mediterranean Sea.

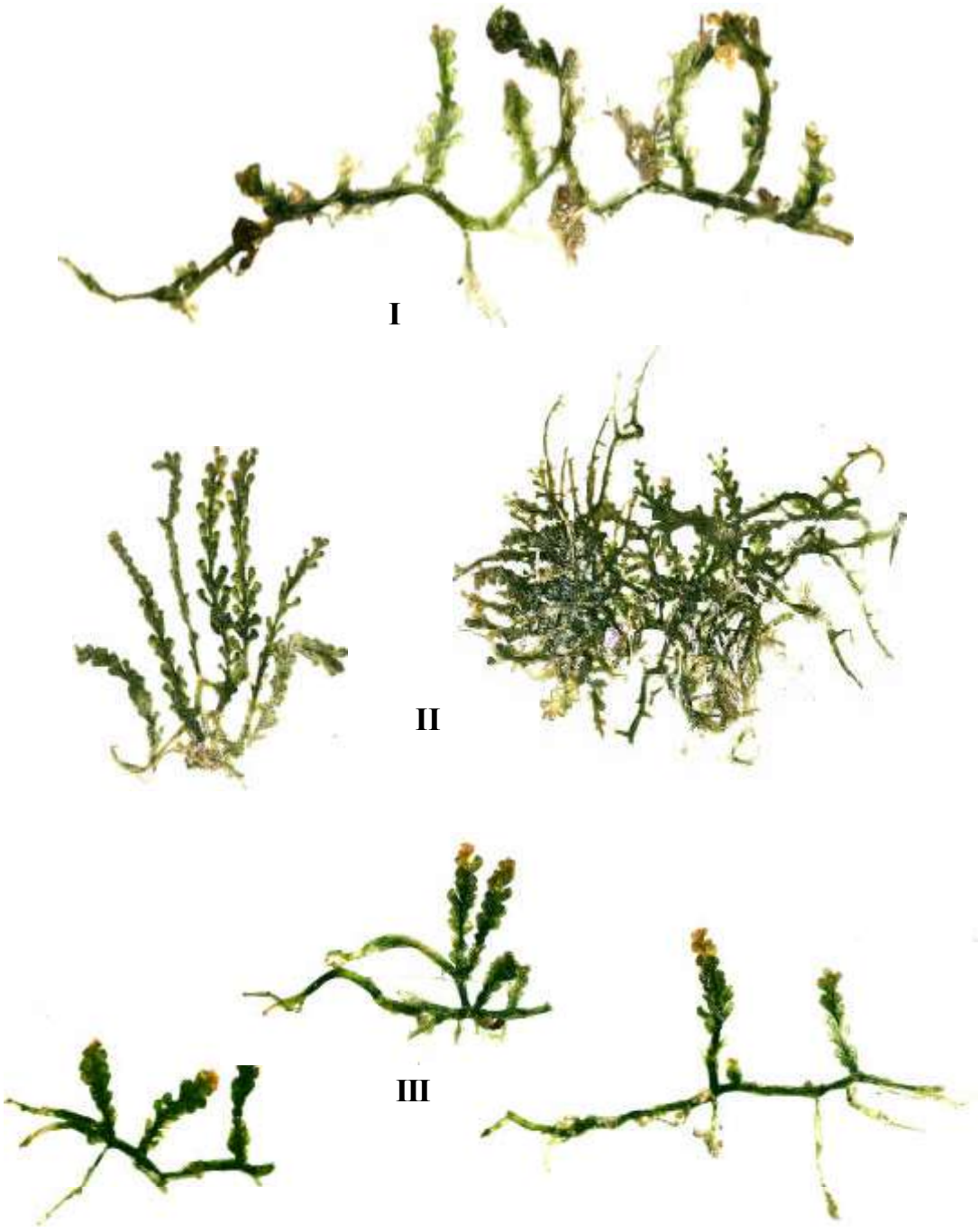
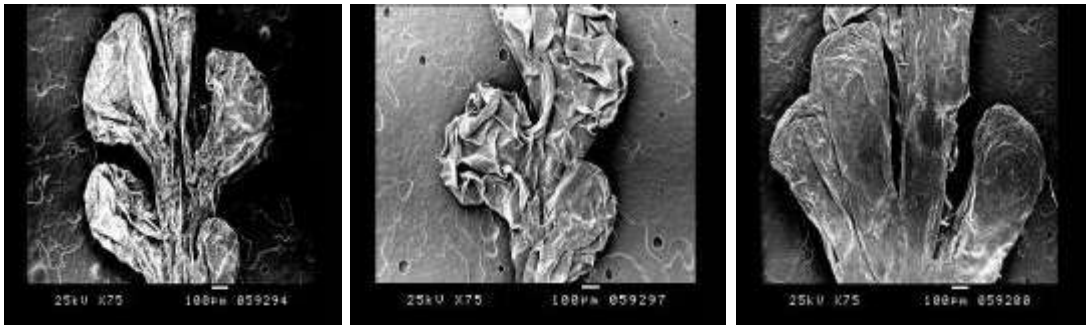
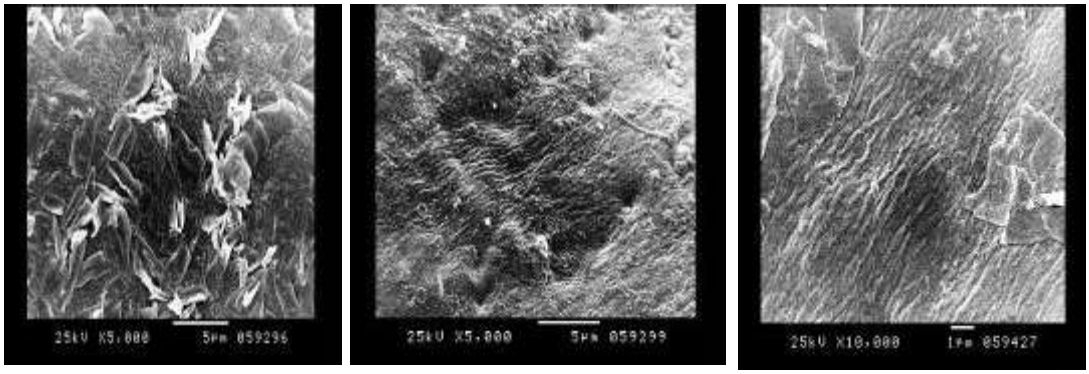


Plate 1: *Caulerpa racemosa* habit from the Mediterranean sea in Alexandria , Egypt. I= var. occidentiales, II= var. lamourouxii.f. requienii and III= var. turbinata-unifera. Materials collected during April 2007 from Abu Qir Bay.

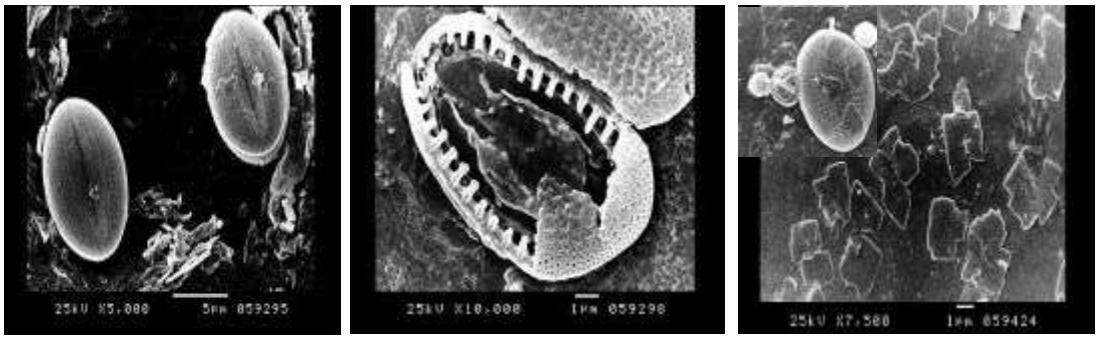
Var. I Var. II Var. III



(a)

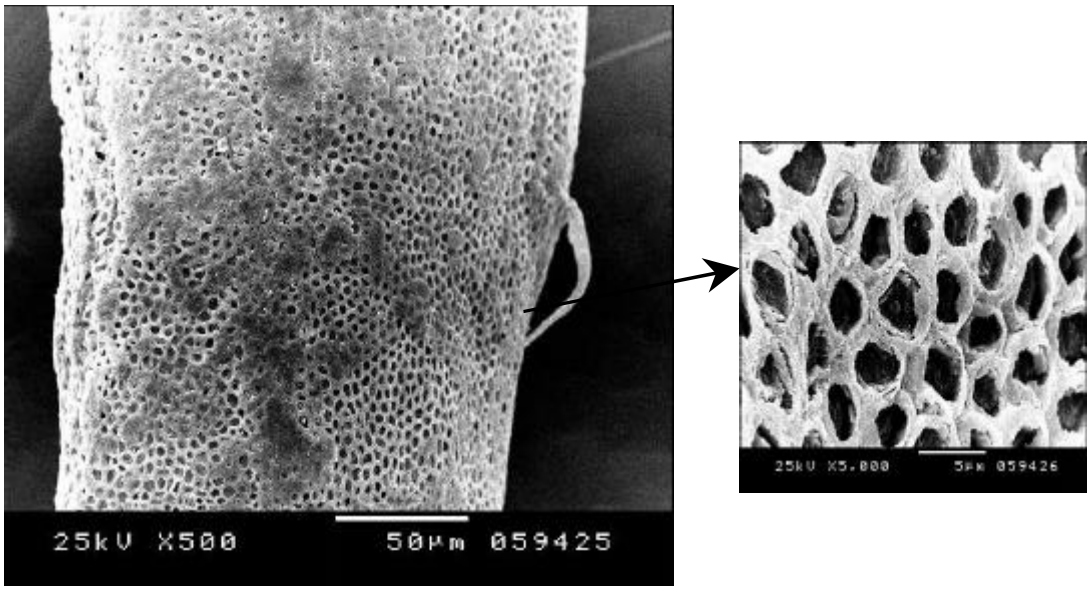


(b)

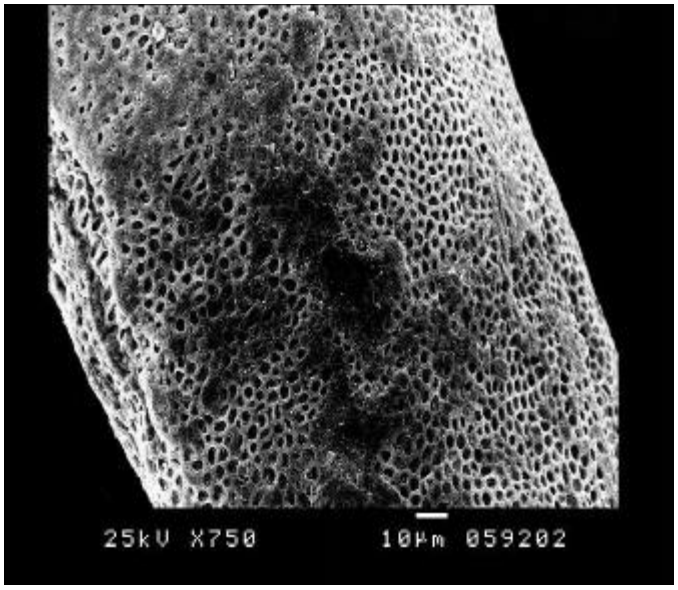


(c)

Plate 2: Scanning electron micrograph showing the morphology of *C. racemosa*. (a): thallus (leaf) morphology; (b): surface sculpture; (c); diatom invading the surface



Var. I+II



Var. III

Plate 3: Anatomy of *C. racemosa* stolon by Scanning Electron Micrograph (SEM)

In 2002; [45] described the invasive *C. racemosa* taxa as "invastive strain" in the Mediterranean. Durand *et al*. [8] confirmed that the three morphological varieties of *C. racemosa* from the Mediterranean Sea are distinct taxonomic units.

The three taxa of *C. racemosa* [3] occurred in the Mediterranean Sea, one of them is the invastive variety, provisionally regarded as close to *C. racemosa* var.occidentalis (J. Agardh), is currently spreading spectacularly throughout the Mediterranean. They identified the invastive variety as *Caulerpa cylindracea* (Sonder), endemic to South-West Australia and currently known as *C.racemosa* var.laetevirens f.cylindracea (Sonder). They postulated that *C. cylindracea* differed from the tropical north Australian *C. laetevirens* (Montagne) by its slender thallus, lack of large rhizoidal pillars, the slight inflation of the basal part of the upright axes immediately above the attachment to the stolon by

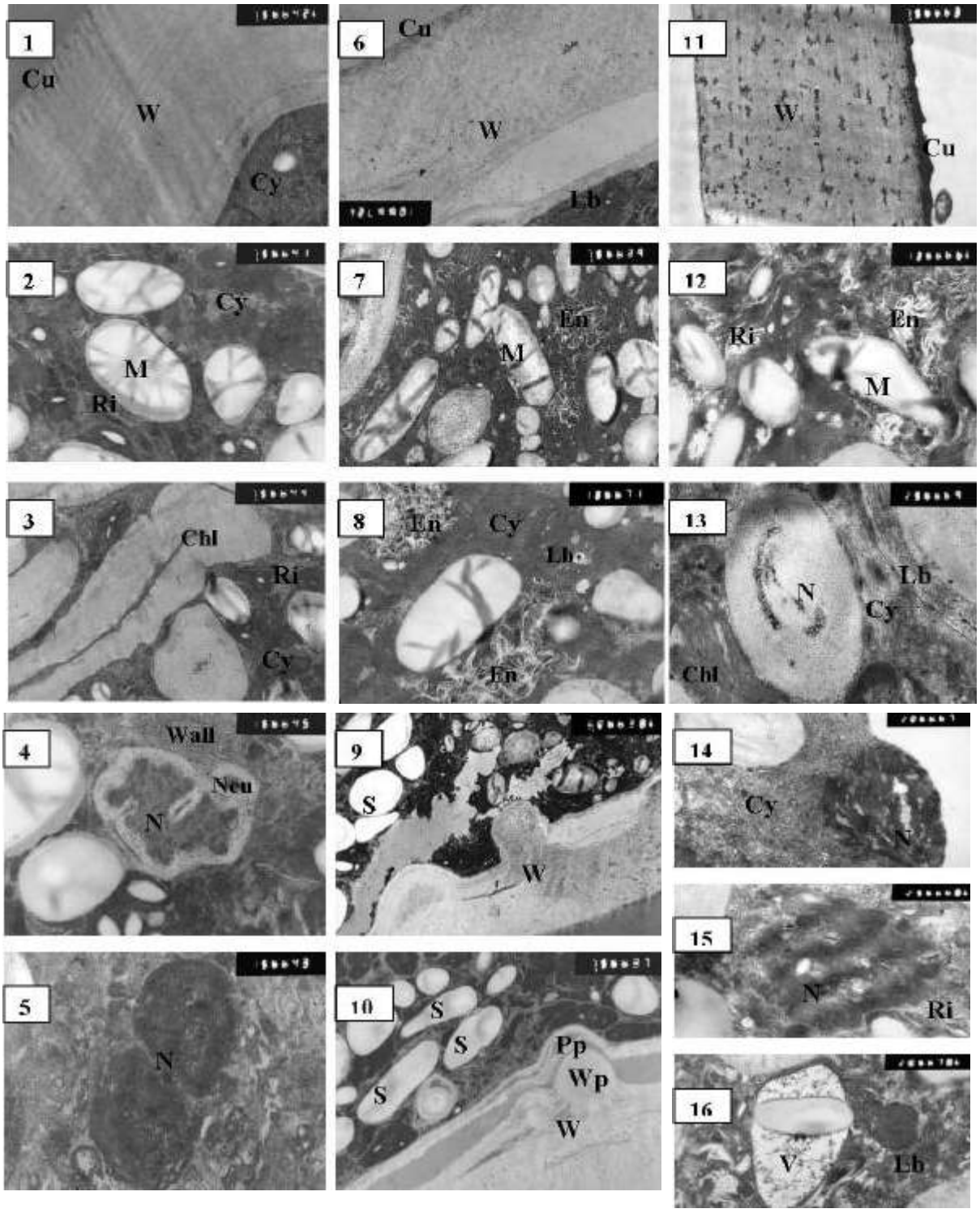


Plate 4: *Caulerpa racemosa* transmission electron micrographes . (1-5): var. I; (610): var.II; (11-16): var. III; W: cell wall; M: mitochondria; N: nucleius; Neu: neucleolusV: vacule; Chl: chloroplast; S: starch grains; En: endoplasmic reticulum; Lb: lipid body; Pp: plasma papilla; Wp: wall papilla Cy: cytoplasm; Cu: cuticle; Ri: ribosome.(1,2,3) x 7500;(4,5) x 1500; (6,7) x 7500;(8) x 1300; (9) x 5000; (10,11) x 7500; (12) x 1300; (13,15) x 2500; (14,16) x 2000.

the range of morphological variations (branchlets clavate to cylindrical but never trumpet-like or shield-like). According to [3], a new combination in 2003, *C. racemosa* var. *cylindracea* was therefore proposed.

In 2004, [46] observed that *C.racemosa* var. *cylindracea* reached the Canary Islands of the North-East Atlantic. They identified this variety as a member of Bryopsidales and not Caulerpales as recorded by most of the taxonomists [47]. Nishikawa *et al*. stressed on similarities existing in many of the Caulerpales, including a "giant mucleus" present in several young members. Order Bryopsidales was found to be completely differed from order Caulerpales, either morphologically or cytologically [48].

If [3] introduced *C. racemosa* var. *cylindracea* as a member of Bryopsidales, this variety must be omitted from the *C. racemosa* varieties and introduced as a new species or genus of Bryopsidales. The proposed systematic position of [46] must be rejected and replaced by [3] and the preceding investigators; maintaining its taxonomic position as *C. racemosa* var. *occidentialis*.

CONCLUSIONS

According to the present investigation and to the reviewed previous studies, we can conclude that three varieties of *C. racemosa* occurred in the Mediterranean Sea of Alexandria, Egypt; var. *occidentiales* (*cylindracea* J. Agrdh Borgesen), previously known in Australia as var. *laetevirens* f. *cylindracea* (Sonder); var. *lamourouxii* f. *requienii*, previously also known as var. *lamourouxii* f. *requienii* and var. *turbinata*–unifera, previously known as var. *turbinata*. We propose that var. *occidentiales* is the variety known as *lamourouxii* according to the physiological and cytological results obtained. The old reported variety occidentiales (recently invaded the Mediterranean) is a tropical taxa took a long journey from the Indian Ocean and Australia to the Red sea and Suez Canal. It quickly spreaded to the Mediterranean, since the early 1990s, starting from Egypt through Suez Canal then to Libya, Cyprus, Tunisia, Balearic Islands, France, Western and Southern Italy, Sardinia, Sicily, Greece, Grete, Turkey, Lampedusa and recently (in 2004) reached the Canary Islands of the north-east Atlantic. This is coincided with Aleem [49] who pointed out to the role played by Suez Canal which allowed free interchange of Mediterranean Sea and Red Sea algal flora. Arab navigation transferred *Caulerpa racemosa* varieties from the Indian Ocean to the Mediterranean through Suez Canal.

The results culminated into the suggestion of using the physiological and ultrastructural criteria as a tool for taxonomic separation between taxa. Collection and identification of the third variety *C. racemosa* var. *occidentiales* from the Mediterranean Sea in Alexandria, Egypt is a new record.

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