SCINAIA COMPLANATA (COLLINS) COTTON VAR. *INTERMEDIA* FROM THE MEDITERRANIAN SEA OF ALEXANDRIA, EGYPT.

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Abstract

Scinaia complanata (Collins) Cotton var. intermedia (Rhodophyta) was newly recollected from Abu-Qir locality along Alexandria seashore. The alga showed multiaxial construction, dichotomously branched thallus, attached to the rocks by multicellular rhizoids. The female gametophyte carried unilocular, uninucleate carpogonia produced on carpogonial branches. Constitutive urea-amidolyase was the enzyme responsible for ^{14}C-urea-degradation in this alga; ^{14}C-urea transported via passive mechanism. This alga was characterized by its high protein, amino acids, fatty acids, Ash and Mg contents. It was also characterized by absence of mercury, low Ni, Mn, Zn and Co contents; as well as low iodine value.

Key words: Alexandria, Cell contents, ^{14}C-uptake, Mediterranean, Scanning, *Scinaia complanata*.

Introduction

Until this decade relatively little was known about *Scinaia* species from Alexandria (Aleem, 1945). The genus *Scinaia* (Rhodophyta) was firstly detected in Sweden by Agardh (1822). It was classified as a member of family Chaetangiaceae of order Chaetaginales from the Rhodophyceae (Tseng, 1941). This family comprised only one genus; *Scinaia*. Chaetangiaceae family differed morphologically and anatomically from family Nemalionaceae of order Nemalionales (Feldmann, 1942). In 1989; Wynne classified *Scinaia* as a member of family Galaxaureaceae of order Nemaliales. *Scinaia* species were recorded from most of the Mediterranean basin countries: Egypt, France, Spain, Britain, Italy, Portugal, Sweden, Irland, Tunisia, Greece, Dublin, Corsica, Morocco, Turkey, Norway …etc (Aleem, 1945; Stegenga *et al*., 1977; Menez and Mathieson, 1981; Wynne, 1989; Silva *et al*. 1996; Vroom and Abbott, 2004; Brands, 2007).

Recent studies indicated that the genus *Scinaia* is a world-wide, comprises approximately 49 species, subspecies, varieties, forms, and cultivars in this genus most of the publication were concerned about identification and morphological characters (Huisman, 1985; 1986; 2004; 2006; Wynne, 2005; Guiery and Guiery, 2007).

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Two species from *Scinaia* were recorded in Alexandria (Aleem, 1945 and 1993):

-*Scinaia pseudocrispa* (Clemente), 1807 = *Scinaia forcellata* Bivona-Bernardi in Svedilius 1915.

-*Scinaia complanata* (Collinis) Cotton var. intermedia. Boergesen, 1916.

Scinaia complanata (Rhodophyta, Florideophycae, Nemaliales, Galaxauraceae) was detected in the Mediterranean for the first time by Agardh (1822). Also, the specimens found in Alexandria washed ashore during August, 1944 for the first time and were fertile (Aleem, 1945). Since that date no detection for this species in other check lists of the marine algal flora of Alexandria (Savigny and Audowin, 1809; Delile, 1813; Areschoug, 1870; Muschler, 1908; Nasr, 1940 a and b; Khalil, 1987 and Aleem, 1993).The present specimens were obtained from Mandra, near Abu Qir, from the depth 2m. It was easily distinguished from *S. pseudocrispa* by its polygonal epidermal cells and its ovoid cystocarps.

The aim of this study is to shed a light on this unknown species from physiological and morphological point of view.

Materials and Methods

Abu-Qir locality is an estuary, located at the east of Alexandria (north-eastern the Mediterranean basin) between longitudes 30º 5´ and 30º 22´ E and latitudes 31º 16´ and 31º 28´, with average depth of 12m (Nessim and El-Deek, 1993), with a temperatures minimum of 18º C in April and maximum of 32º C in July. It is mainly an exposed rocky site at its north-eastern edge but sandy towards the west.

Algal collection and identification

Scinaia complanata was firstly collected in April 2001, from Abu-Qir locality as drifted specimens; it appeared again on Abu-Qir seashore by the end of March, and during April 2006; washed ashore from deep water. It was fertile, heavily covered with carpogonia.

The systematic revision of *S. complanata* species based on the comparative morphology described by Holmes (1873); Howe (1914); Yoshida (1998); Huisman (2004 and 2006).

Algal culturing and extraction of urea enzyme:

Ten grams fresh weight was washed thoroughly with sterilized seawater for several times and bacterial detection was carried out as described by Bekheet and Syrett (1977).

The crude enzyme extract was prepared from the algal material by the method described by Thomas and Syrett (1976) using HEPES buffer at pH 7.6.

Radioactivity measurements:

Activity measurement was made by the $^{14}CO_2$ technique released after the injection of ^{14}C-urea. Radioactivity was measured by Beckman LS200 B Liquid Scintillation Counter as described by Price (1983).

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) (Bekheet and Syrett 1977).

Separation and determination 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 (Benson *et al*., 1969), saved for analysis with the amino acid analyzer.

Preparation of Protein-hydrolytes

For estimation of protein amino acids, the method described by Tempst and Jozef (1983) was used. Calculations were made using the equation given by El-Mahdy and El-Sebaiy (1985).

Fatty acids extraction and identification

Total lipids were extracted according to Blight and Dyer (1959) by extracting the algal cells with chloroform – methanol (2:1). The total lipids were then converted into fatty acids methyl esters using the procedure described by Radwan (1978). The fatty acids methyl esters were identified as fatty acids using Gas Chromatography, model 4cm- Schimadzu equipped with a flame ionized detector. Identification of individual fatty acid was carried out by comparing its retention time with those of standards.

Iodine value

Iodine number of lipids was determined by using the method described by Yasuda (1931): I.N. = a-b/c x 1.27/5

Where a = volume for blank titration.

b = volume for 0.02 N $Na_2SO_2O_3$ titration.

c= weight of lipid in g.

Biochemical analysis

Protein was measured according to the method of Hartree (1972). The phenol – sulphoric method (Dubois *et al*., 1959) was applied for carbohydrates estimation, using glucose as a standard. Trace metals contents were carried out as described by Harold *et al*. (1981).

Electron micrograph:

For scanning electron microscopy (SEM), 2-5 samples per specimens 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 (Barthlott, 1981).

Results and Discussion

Scinaia complanata (plate 1) showed erect, cylindrical multiaxial, glittering dense tufts; brownish-red in color. The thallus is dichotomously branched, arise from multicellular rhizoids, branching up to 7 times. The thallus is 9 cm long and about 1-2 mm thick. Branches are 3-6 mm long. Female gametophyte showed dense growth of carpogonia, no detection for male plant.

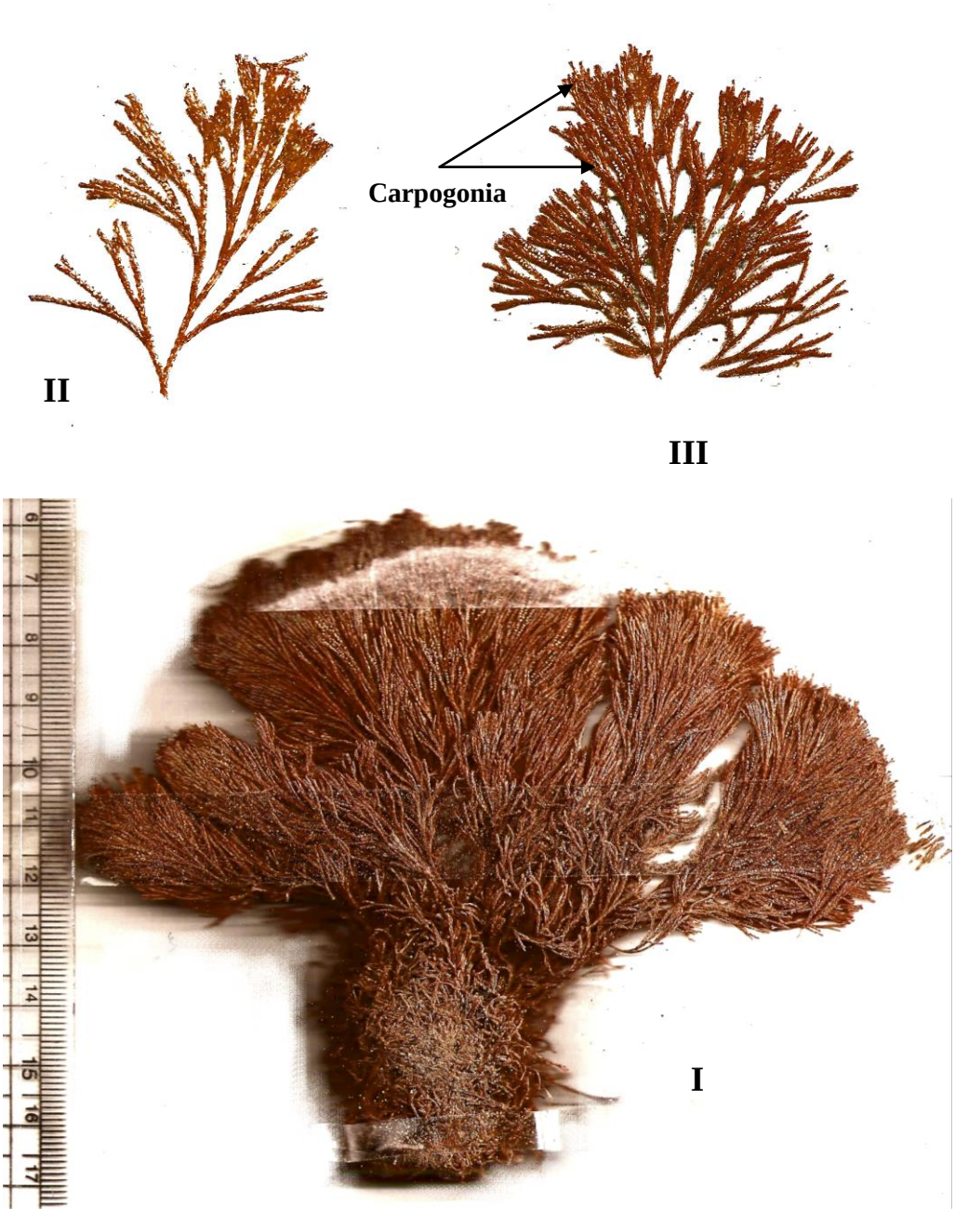
On using scanning electron microscope (SEM), multiaxial construction was obtained (Plate 2a). The carpogonia were produced on a segmented carpogonial branches. These branches showed wrapped-edge, inside which the carpogonia were born on long stalks (Plate 2b). Transection of cell surfaces showed soft texture with mall wax depositions (Plate 3a), the enlarged section showed wrinkled surfaces characteristics to these alga (Plate 2b).

In Plate 4, the carpogonial branches carried numerous carpogonia, produced on each branch (a). Longitudinal sections showed flower-like hair protecting the carpogonium (b). These carpogonia were unilocular, uninucleate structure with single ovum surrounded by very thick wall (Plate 5 a and b). Most of these characters agree with that described by Vroom and Abbott (2004) and Huisman (2006) for the genus *Scinaia*.

Moisture content of *Scinaia complanata* was similar to other red algae (Table 1). Ash contents was higher than the corresponding values of *Palmaria* sp., *Porphyra* sp. and *Porphyra yezoensis*, 40.2 compared to 15-30, 8-16 and 7-8, respectively. Protein was also higher than the first alga but less than *Porphyra yezoensis* and *Porphyra* sp. (Table 1). Carbohydrates concentration was low compared to the other three algae, it was amounted to 1/4 of that of *Porphyra* sp. and 1/5 *Porphyra yezoensis*. Total fat contents were high compared to other red algae (Arasaki and Arasaki, 1983).

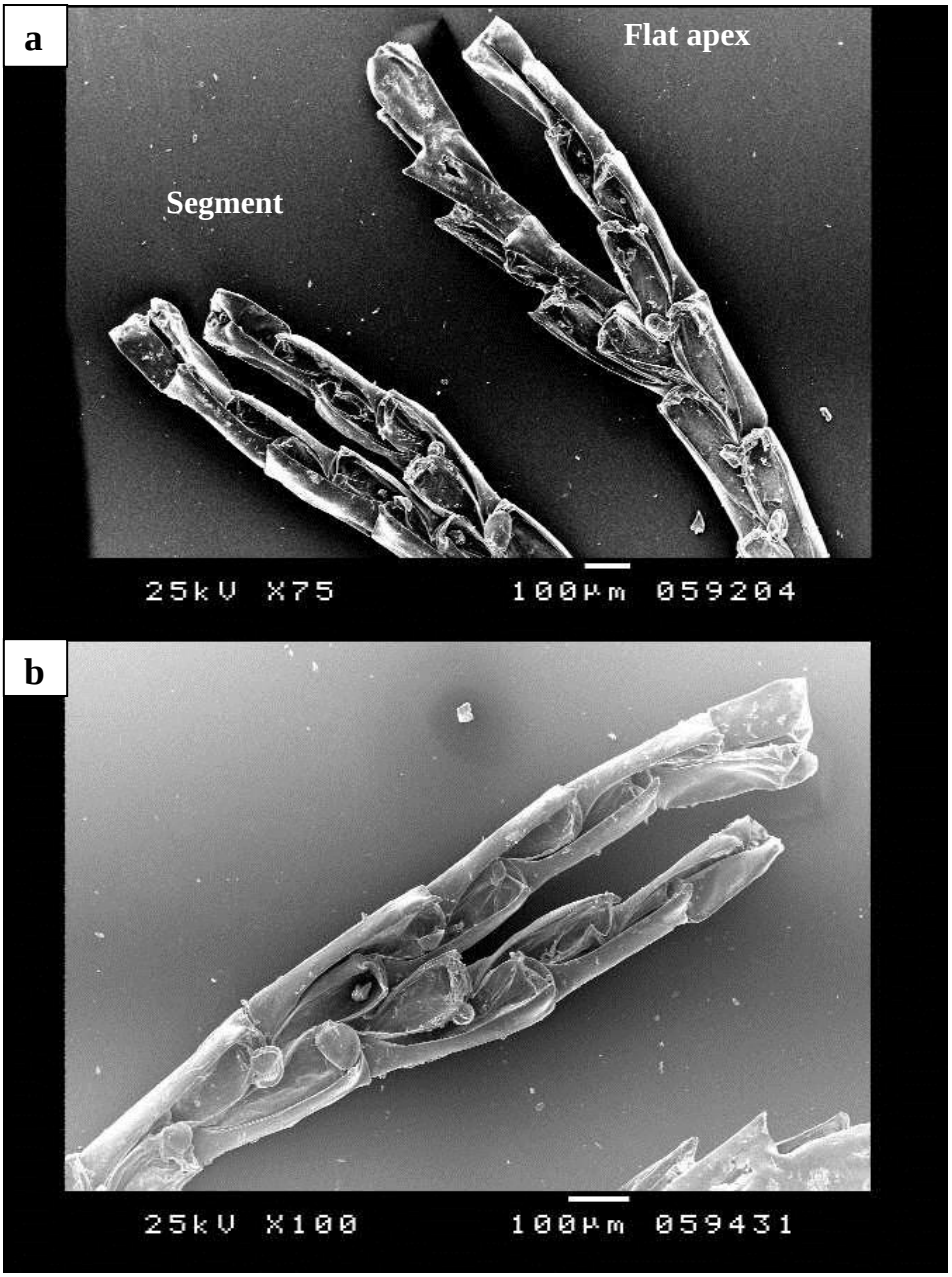
Table 2 showed the metal contents of *Scinaia complanata* compared to other red algae used as food adjuvant for their high nutritive value (Harold *et al*., 1981). Metal analysis of *Scinaia complanata* showed high Mn, Cd and Cr; low iron contents while arsenic showed normal concentration.

Plate 1



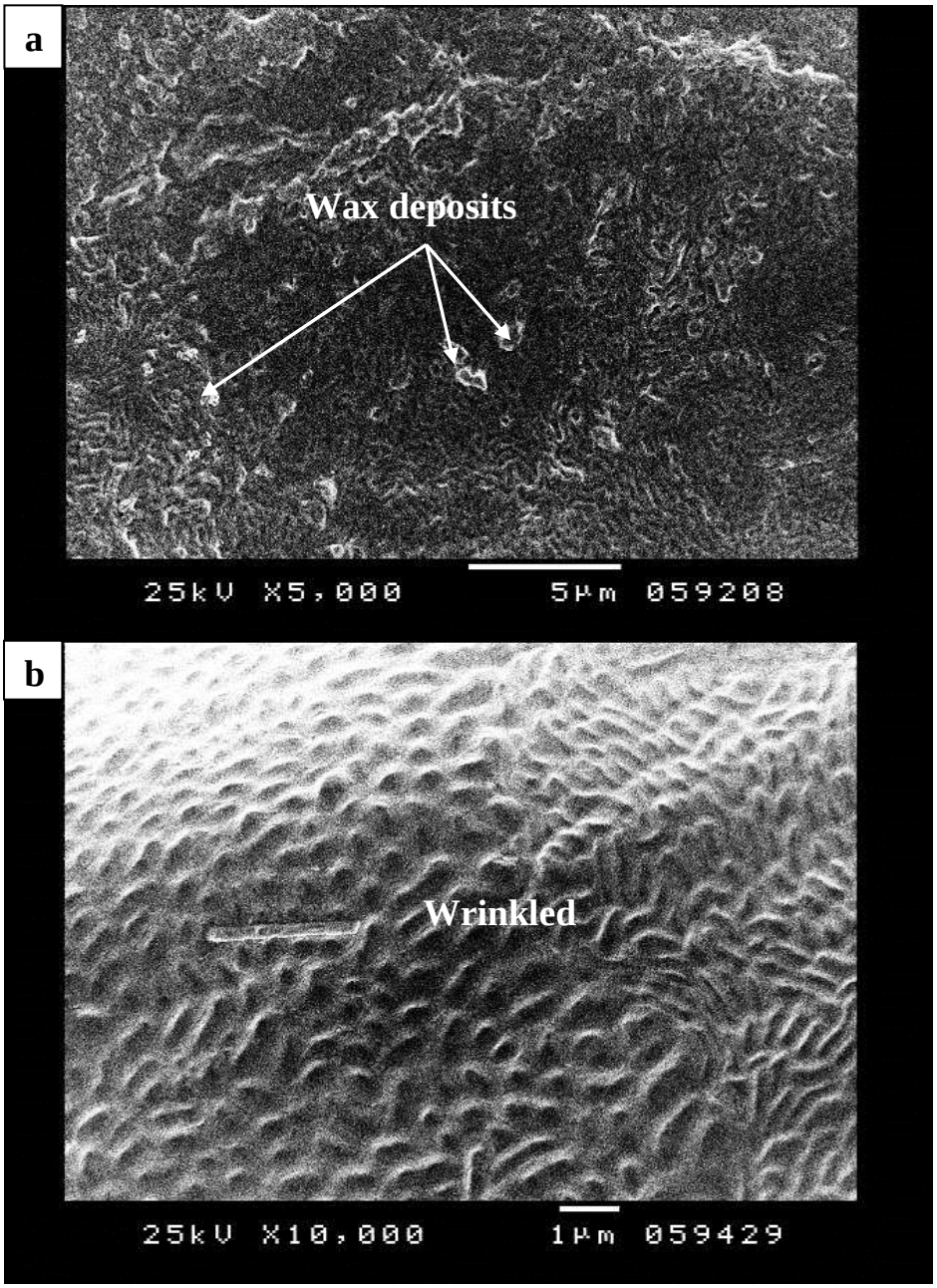
Morphology of *Scinaia complanata*. I) habit of gametophytic plant; II) mode of branching; III) heavily covered thallus with carpogonia

Plate 2



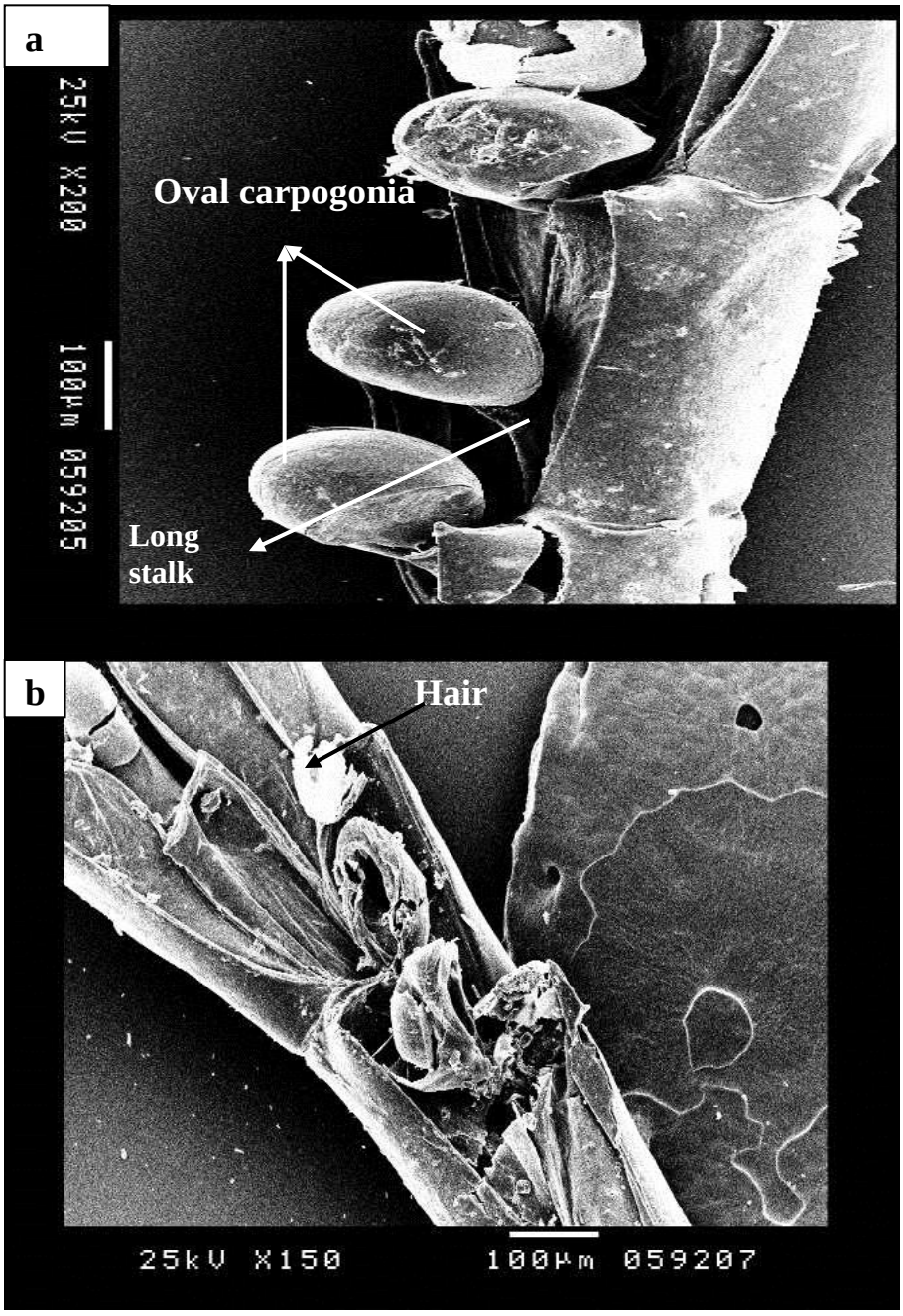
Scanning electron micrograph (SEM) of *Scinaia complanata*
a) dichotomously – branched thallus;
b) wrapped – edged segmented thallus with carpogonia

Plate 3



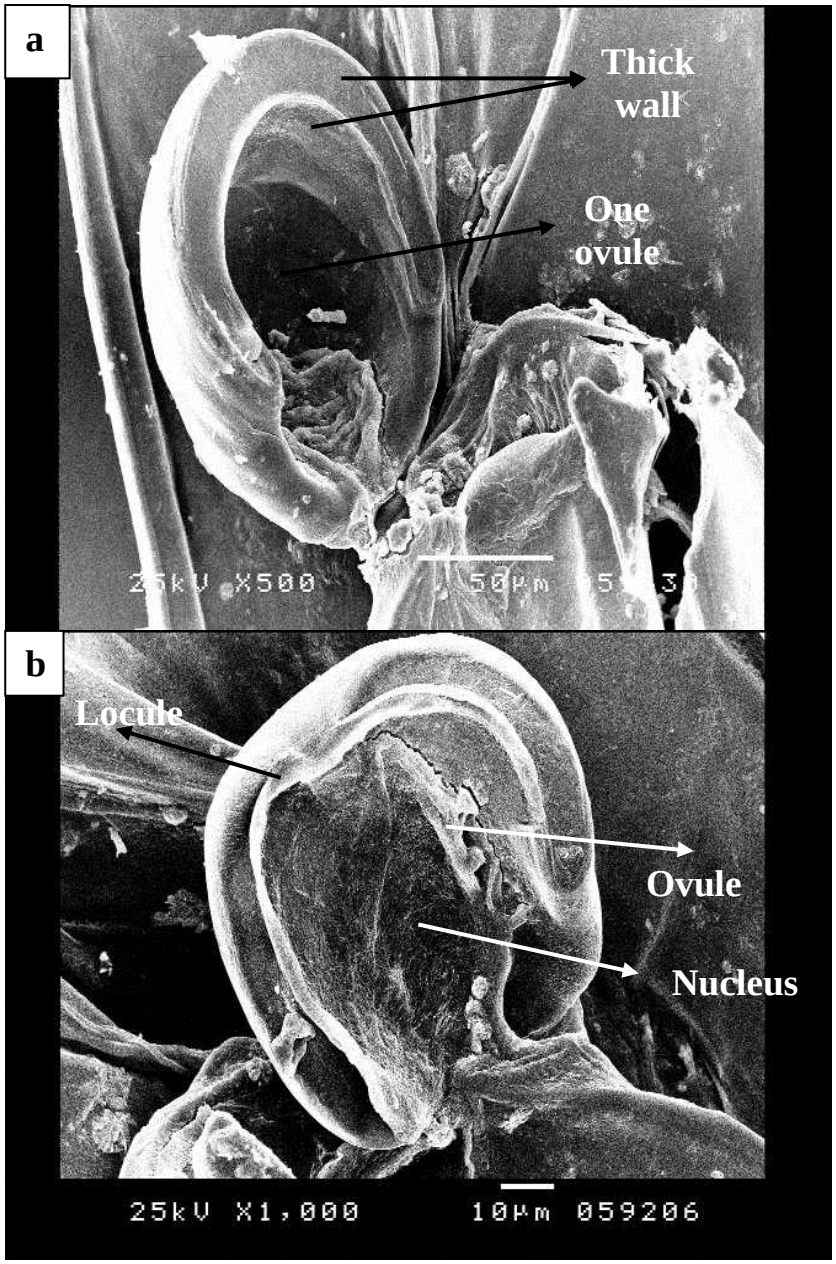
Scanning electron micrograph (SEM) of *Scinaia complanata*.
a) wax deposition b) wrinkled cell surface.

Plate 4



Scanning electron micrograph (SEM) of *Scinaia complanata*
a) carpogonial branch with flower – like hair, b) closed carpogonom

Plate 5



Scanning electron micrograph (SEM) of *Scinaia complanata*
a) longitudinal section showing thick – walled unilocular carpogonium arise from the segment, b) carpogonium with uninucleate ovum.

Table (1): Cell constituents of *Scinaia complanata* compared to some other red algae. Values are g/100g dry wt.

Algal Species	Water	Ash	Carbohydrates	Protein	Fats (total)	References
Scinaia complanata	87.50	40.2	9.55	31.0	10.5	-
Palmaria polmater	79 – 88	15 – 30	N.D.	8 – 25	7 – 9	Morgan *et al*. (1980)
Porphyra sp.	86.00	8 – 16	40.0	33 – 47	3.3	Arasaki and Arasak (1983)
Porphyra yezoensis	N.D.	7 – 8	49.4	43.6	2.4	Nisizawa *et al.* (1987)

Mercury was completely absent; nickel and manganese comprised about 5% of the total metal content. Also, zinc and cobalt were present with equal concentrations.

Table (2): Metal contents of *Scinaia complanata* from the Mediterranean sea of Alexandria, Egypt. Values are µg.g^{-1} dry wt % dry wt.

Metals	*Scinaia complanata*	
	µg . g^{-1} dry wt	% from dry wt
Fe	739.23	0.074
Ni	21.63	0.0021
Zn	39.72	0.0039
Cd	5.01	0.005
Cu	57.25	0.0057
Cr	3.63	0.0003
Co	17.26	0.017
Pb	79.06	0.0079
Mn	69.42	0.0069
Mg	29663.7	2.9
Hg	--	--
As	79.31	0.0079

The data in Table 3 indicated that the enzyme responsible for urea-degradation in *Scinaia complanata* from the Mediterranean Sea of Alexandria was urea-amidolyase, since no inhibition occurred in presence of hydroxyurea and

also avidin. Stimulation for cleavage occurred when adding ATP; and inhibition by avidin, it was reversed by prior addition of biotin to the incubation mixture. Urea-amidolyase of *Scinaia complanata* was a constative enzyme since the uptake mechanism in this alga did not affected by nitrogen starvation (Table 4).

Table (3): Urea degrading enzyme in *Scinaia complanata* collected from Abu Qir Alexandria. Extraction with HEPES buffer (pH 7.6). Activities as n mol $^{14}CO2$. protein^{-1}.hr. ^{14}C-urea conc. 100nmol.mL^{-1}.

Boild extract	-ATP	+ATP	+ATP +Avidin	+Avidin +Biotin	+ Hydroxy urea
0.36	367	824	453	904	821

It should be mentioned that an individual alga may posses one enzyme or the other but not both. Different varieties or strains of the same algal species may show different enzyme activities. It was reported (Syrett and Leftley, 1976) that one difference between the members possessing amidolyase might be that urease is a constitutive enzyme but amidolyase is an inducible one. Bekheet and Syrett (1977) claimed that since there was no known example of an inducible urease in algae. Shafik (1992) proved that *Eichhorrnia crassipes* plant contained inducible urease. Again, Shafik in 1993 proved that the marine algae *Ulva lactuca*, *Cystoseira larbata*, *Dictyota dichotoma*, *Sargassum linifolium*, *Pterocladia capillaceae* and *Enteromorpha intestinalis* contained inducible urease enzyme.

The rate of appearance of radioactivity in the cell was taken as a measure of the uptake; it was measured by the Scintillation counter (Table 4). The uptake of ^{14}C-urea by this alga is a passive mechanism, since the accumulation ratio did not exceeded one, under all time intervals. In the marine algae studied by Shafik (1993), urease enzyme was transported by active mechanism (i.e. accumulation ratios exceeded 1). Williams and Hodson (1977) described two transport systems in *Chlamydomonas reinhardii* operating in different urea concentration ranges. Also in *Saccharomyces*, *Phaeodactylum* and *Chlorella* two transport systems had been postulated (Shafik, 1993) the first via an active transport system and is sensitive to nitrogen repression, the second passive transport occurs at relatively high external urea concentration.

The amount of amino acids determined by amino acid analyzer was shown in Table 5. Essential and non-essential amino acids were listed, to point out to the nutritive value of this red alga. One of the characteristic features for this alga was its high amino acid contents.

Table (4): Incorporation of ^{14}C-urea into *Scinaia complanata* marine alga after 10h of nitrogen starvation. ^{14}C-urea. 100nmol.ml^{-1}. values nmol.ml^{-1}. Acc. Ratio= radioactivity in cell / radioactivity in medium.

Time of incubation	1 min		5 min		10 min		30 min	
	Starved	Non-starved	Starved	Non- starved	Starved	Non- starved	Starved	Non- starved
^{14}C in cells	51.00	53.0	64.0	62.0	87.0	83.0	98.0	99.0
^{14}C in medium	60.70	64.60	68.10	68.13	87.88	92.20	89.09	100.0
Accumulation ratio C/M	0.84	0.82	0.94	0.91	0.99	0.90	1.10	0.99

Total essential amino acids comprised only about 20% from the total amino acid contents of this alga. Total free amino acids represented 19% from the total amino acid contents of *S. complanata*.

Table 5: Amino acid contents of *Scinaia complanata* from Abu Qir Alexandria. Values are µg a.a. g^{-1} dry wt and as % of total protein amino acids.

Amino acids		Protein	Free	Protein % from total protein a.a.
Essential amino acids	Isoleucine	1.87	0.44	2.26
	Leucine	2.89	0.68	3.49
	Lysine	2.59	0.61	3.14
	Methionine	0.89	0.21	1.08
	Phenyl alanine	1.70	0.40	2.06
	Threonine	3.61	0.85	4.36
	Valine	3.36	0.79	4.06
Total		16.91	3.98	20.45%
Non-Essential amino acids	Alanine	7.83	1.84	9.47
	Arginine	8.42	1.98	10.18
	Aspartic	9.27	2.18	11.21
	Cystine	1.36	0.32	1.64
	Glutamic	14.13	3.32	17.07
	Glycine	12.64	2.97	15.28
	Histidin	3.61	0.85	4.36
	Proline	5.02	1.18	6.07
	Tyrosine	--	--	--
	Serine	3.57	0.84	4.32
Total		65.85	15.48	79.55
Total (Es + Non-Es)		82.76	19.46	100%
Total (F +P)		102.22		

Total protein hydrolysate amino acids formed about 81% from the total amino acids determined in this alga. About 65% from the total non-essential (80%) were detected in total protein amino acids, the other 15% were in total free.

Concerning the individual amino acids, some amino acids were present in both the free state and as building stones of proteins. These acids were: glutamic (17%), glycine (15%) and aspartic (about 11%), they form together about half of the protein hydrolysate. Least amounts in both protein and free amino acids were recorded for methionine, cystine, phenyl alanine and isoleucine. Tyrosine was absent from both free and protein amino acids. Proline formed 6% from the total amino acid contents of this alga.

All the amino acids normally occurring in protein were present also in the free state. The exact balance between amino acids is so dependent on the nutrition prevailing before harvesting the alga. The free amino acid pool in this alga composed of individual amino acids similar to those found in flowering plants (Tempst and Jozef, 1983).

Comparing the results of this investigation with some other red algae (Table 6) indicated that, the amino acids composition of *Scinaia* sp. coincidence mainly with the other red algae, but the total amino acid content of *Scinaia* exceeded greatly that of *Pterocladia*, *Chondrus* and *Microcystis* (Table 6). In general, it was observed that *Scinaia complanata* resembling *Chondrus* alga in its individual amino acids.

The carbon chain lengths of *Scinaia complanata* ranged between C6 and C22 (Table 7). Saturated fatty acids comprised 12 fatty acid, while unsaturated comprised only 8. The most widely distributed fraction among saturated fatty acids were C16 and C18. Long chain fatty acids C23 and C24 were absent from the fatty acids of this alga.

The total saturated fatty acids formed about 85% from the total fatty acid contents in this alga, while unsaturated formed only the other 15%, fractionated into monounsaturated (9.9%) and polyunsaturated (5.1%). Total unsaturated fatty acids was 3.72 $\mu g.g^{-1}$ dry wt, monounsaturated fraction represented 66% of this amount. Only three polyunsaturated fatty acid were detected in this alga. Highest unsaturated acids were C14:1, C15:1 and C20:1.

Fat analysis of *Chlorella pyrenoidosa* grown on nitrogen-starved media indicated close agreement with the results of this investigation (Schlenk *et al*., 1960).

Iodine value determines the quality of fats when used as food or food additives, low iodine value indicated high fat quality. The value obtained in this alga was within the normal range (120-200).

On the light of the above mentioned results, one can conclude that *Scinaia complanata* resembling typically the vegetative tissue of higher plants (Graeve *et al.*, 2002).

Our knowledge of the marine algal flora of the eastern Mediterranean is still scantly, although the western shores have been fully investigated (Huisman *et al.*, 2005). During the study of algal flora on the shores of eastern Mediterranean of Egypt, number of species appeared to be invasive, introduced to the sea through shipping activities or ballast water; others are newly collected. *Scinaia complanata* was newly collected from Alexandria after 60 years of the first collection. Cystocarpic plants were collected in April 2006 instead of August of 1944 due to the raising in temperature which allowed early reproduction. No indication that invasion occurred to this algal species; the future work will confirm or reject this proposal.

Table 6: Comparison between *Scinaia complanata* aspects of this investigation and some red and brown algae.

Alga	*Scinaia* sp.	*Chondrus* sp.	*Microcystis* sp.	*Pterocladia* sp.
Total a.a.	102.22	54.70	47.70	80.57
Highest content	glu, gly, asp.	arg, glu, proline, leucine	glu, alanine	hist, arg, isolu, glu
Least amount	meth, cyst, ph.ala, isoleu	meth, ph. ala, cyst, trypt	meth, isoleu	cyst, meth, val.
Absent a.a.	tyrosine	Isoleu, serine	---	---
Urea-enzyme	Amidolyase	---	---	Urease
Category	Inducible	---	---	Constitutive
Transport system	Passive	---	---	active
referance		Clement *et al.* (1968)	Fowden (1962)	Shafik (1993)

Table 7: Fatty acids contents of *Scinaia complanata* from Alexandria. Values µg fatty acid.ml^{-1}.

Fatty acids		µg .mL^{-1}
Saturated	C 6:0	0.17
	C 8:0	0.96
	C 10:0	0.07
	C 11:0	0.20
	C 12:0	0.36
	C 13:0	0.28
	C 14:0	0.11
	C 15:0	0.37
	C 16:0	9.31
	C 17:0	0.90
	C 18:0	5.34
	C 20:0	0.20
Total		18.27
Monounsaturated	C 14:1	0.57
	C 15:1	0.56
	C 17:1	0.24
	C 20:1	0.47
	C 22:1	0.29
Total		2.13
Polyunsa-turated	C 18:2	0.24
	C 22:2	0.44
	C 18:3	0.42
Total		1.10
Total (unsaturated)		3.23
Total fatty acids		21.50

Iodine value = 120.56

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طحلب سينايا كومبلاناتا من البحر المتوسط بالإسكندرية ـ مصر

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ينتمي طحلب السينايا لمجموعة الطحالب الحمراء والتي قد تم تجميع الطحلب من على شواطئ منطقة أبي قير بالإسكندرية في شهر ابريل 2006، و الطحلب من الطحالب عديدة المحاور وحيدة الجنس، له تفرعات ثنائية. والنباتات التي تم الحصول عليها كانت نباتات مشيجية مؤنثة، وعند استخدام الميكروسكوب الماسح وجد أن عضو التكاثر المؤنث المسمى بالكربوجونة يخرج من عند العقد الموزعة على الأفرع، وان كل كربوجونة تحتوي على حجرة واحدة بداخلها بويضة وحيدة النواة. وعند دراسة الطحلب من الناحية الفسيولوجية وجد أنه يحتوي على إنزيم اليوريا اميدوليز المحلل لليوريا وان هذا الإنزيم هو من الإنزيمات التركيبية، ولقد وجد أيضا أن اليوريا مشعة الكربون تنتقل خلال خلايا الطحلب بميكانيكية الامتصاص السلبي، تميز هذا الطحلب بمحتواه العالي من الماغنسيوم و البروتين والأحماض الامينية والأحماض الدهنية ذات الرقم الايوديني المنخفض، كذلك تميزت تحاليل المعادن بغياب عنصر الزئبق و قلة محتواه من النيكل والزنك والكوبالت والمنجنيز، وكلها تعطي قيمة غذائية عالية.