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CLASSICAL AND BIOCHEMICAL TAXONOMIC STUDY OF SOME CYSTOSEIRA SPECIES FROM THE SEASHORE OF ALEXANDRIA, EGYPT.

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ABSTRACT

Four species of the genus **Cystoseira** (**C. fibrosa, C. barbata, C. abrotanifolia** and **C. foeniculacea** (brown algae)) from the seashore of Alexandria, Egypt, have been studied for their taxonomy based on what the author call as cumulative taxonomic approach in which both classical and biochemical taxonomies are considered together. The four species have been compared through their morphology, anatomy, pigment composition on TLC, whole absorption spectra, five systems of their iozymes: ∝- est, Alk. ph., Ac. ph., MDH and LAP. The whole represents nine taxonomic criteria. Numerical clustering has been used to translate the isozymal electrophoretic patterns into individual and average similarity matrices and dendrograms. Based on this cumulative taxonomic approach, it was found that: 1- The four species do have enough similarity to stand together within the genus **Cystoseira**, 2- **C. fibrosa** is the one with most differentiation from other species and could stand alone as a genuine species. The same is true for **C. abrotanifolia** but to a lesser extent, and 3- **C. barbata** and **C. foeniculacea** are tied strongly together and could be considered as two varities of the same species or belonging to one sub-genus.

INTRODUCTION

Cystoseira is a perennial fucoid genus characterized by monopodial branching and a bushy habit. The primary laterals are disposed in a 2/5 spiral and grow to a much greater length than the main axis. Plants may be as short as 1-10 cm in C. abrotanifolia or as long as 60-70 cm in C. barbata. Primary laterals are flat and more or less lobed arising in one plane. Air-bladders are commonly present and usually occupy the lower parts of the ultimate laterals. Receptacles are frequently spindle-shaped (Fritsch 1935). The genus is of a worldwide distribution, though abundantly represented in the warmer seas. 80 % of the species occurring along the Mediterranean, Indian and Atlantic coasts (Roberts, 1978). Based on gross morphological criteria, like height, branching, ramuli, air-bladders and receptacles, tens of species have been assigned by different authors to the genus Cystoseira. The role of polymorphism is clear in the classification of the genus. Polymorphism has led to

situations where a number of separate taxa, usually species collected from different habitats, were later shown to be simply polymorphic forms of one and the same species. The genus, as originally described by C. Agradh (1820), contained 37 species. In the period since that date at least 114 additional entities have been assigned to genus Cystoseira, 55 out of the total of 151 remain in the genus as now understood. The remaining 55 species include only 14 of Agardh's original 37 species, and only nine of these fourteen have undergone no name change (Roberts 1978). This is not unusual in algal classification based on morphological criteria as the only taxonomic marker. Similar cases are quite common in classical classification of algal genera. Indeed, the failure to recognize morphological plasticity sugggested by Dixon (1973) is the principal cause for the taxonomic chaos found in many algal genera (Cheney and Babbel, 1978). The need to new approaches, including physiological and biochemical, to help in improving the classification of the genus is quite obvious. Pigments specially carotenoids barotenoids and chlorophyll-c have been successfully used by many authors as taxonomic markers in assigning algae to lower taxa levels (Weber and Czygan, 1972; Norris, 1980; Yokohama, 1981 a & b; O' Kelly and Floyd, 1984 and Brown and McLachlan, 1982). Comparing electrophoretic patterns of isozymes as taxonomic criteria has been proved to be great by many authors, especially on the specific and generic levels (Thomas and Brown, 1970; Holton, 1973; Klein et al., 1973; Murphy and Guillard, 1976; Fulton, 1977; Murphy, 1978; Mohamad, 1981; Shaalaan and Chapman, 1983 a, b, c; Shaalaan and Mohamad, 1985, 1986 and Mohamad and Shaalaan, 1985).

The present study is a trial to make use of every possible criterion that could serve in making the classification of genus Cystoseira more stable. So, a cumulative taxonomic approach is going to be considered in this work including morphological, anatomical, pigment analysis and isozymal patterns to express similarity and differentiation among species of Cystoseira.

MATERIAL AND METHODS

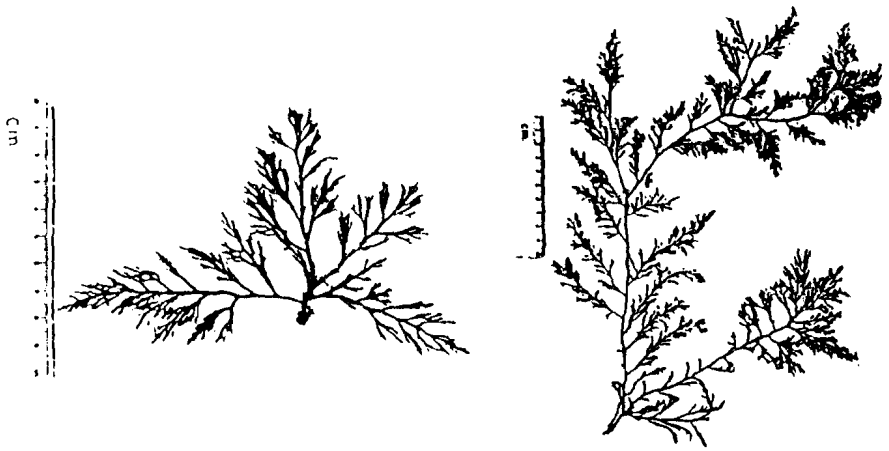
Four marine algal species were picked up for this work from the seashore of Alexandria, Egypt in August 1989: Cystoseira fibrosa (Ag.), Cystoseira barbata (Ag.); Cystoseira abrotanifolia (C.Ag.), and Cystoseira foeniculacea (Ag.). They were identified according to the external morphological features described in the literature and compared with specimens in herbarium Nasr (Botany Department, University of Alexandria). Characters that have been used in identification and considering morphological relationships among species are: shape of primary laterals, mode of branching, vesicles, and receptacles. For the anatomical study, transverse sections made near the bases of primary laterals of the four species have been compared. For pigment and isozymal analyses, algae have been washed thoroughly and equal weights have been taken from the fresh material including all the parts of thalli (main stalk,

primary laterals, ramuli, vesicles, and receptacles). For pigment analysis, thalli were ground with quartz sand in a mortar. Small equal volumes of acetone have been added to the homogenates for extraction. The extracts were centrifuged at 7000 g for 20 min. Centrifugation was repeated if the supernatant was still turbid. Equal volumes of the clear pigment extracts were applied on a silica gel plate. The run was made using developing solvent as follows: 45 distilled benzene, 15 petrolium ether and 20 acetone (v/ ratio). After the run was finished, chromatogram was photographed immediately. For absorption spectra, equal volumes of the extracts were scanned on the spectrophotometer for all the visible range 380-800 nm. For isozymal analysis, thalli were ground in the same way but extracted with equal volumes of 0.7 M Tris-HCl buffer pH 8.0. Extracts were centrifuged as mentioned above and then concentrated in previously activated dialyses tubings on a bed of sucrose. Samples were transferred into small tubes to which few drops of bromophenol blue (1 %) were added as an electrophoretic marker. Samples were kept in a deep-freezer for immediate gel runs. Electrophoretic gel preparations were the same as described by Scandalios (1969). All chemicals used were reagent grades. For isozymal developing the recovered gels were transferred directly to the appropriate staining reaction mixture as described in the original literature as follows: 1-α-esterase, alkaline phosphatase, malate dehydrogenase (Scandalios, 1969); lencine aminopeptidase (Smith and Van Frank, 1975); acid phosphatase (Shaw and Prasad, 1970). Numerical clustering was applied according to Sokal and Sneath (1963).

RESULTS AND DISCUSSION

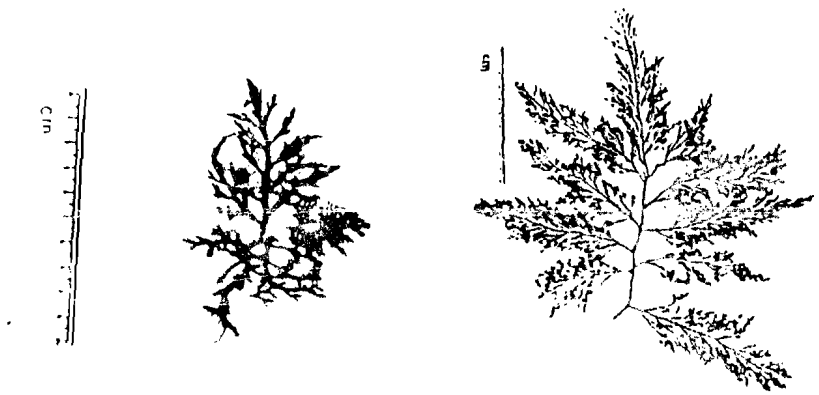
Morphology of the four species studied is shown in Fig. 1. Anatomical features of the bases of their lateral branches are shown in Fig. 2. Fig. 3 represents a TLC chromatogram, while Fig. 4 includes the absorption spectra for their pigment extracts. The electrophoretic patterns of their isozymal analysis for Ac. ph., α-est, Alk. ph., MDH and LAP are represented in Fig. 5. Tables 1-6 are the individual and average similarity matrices of the zymograms and Fig. 6 shows their individual and average similarity dendrograms. Table 7 is a an overall conclusion for the species grouping and classifcation with points of similarity and dissimilarity. Figure 7 is a representation of the similarity bonds tying the four species with each bond representing one of the taxonomic characters studied.

All species studied are morphologically differentiated based on criteria of branches and ramuli. However, a degree of similarity is observed between Cystoseira barbata and C. foeniculacea, though the later could be differentiated by its air bladders and also mode of branching. Anatomically, all species look alike in Fig. 2 except for C. foeniculacea which is characterized by a wider medullary region.



C. fibrosa

C. barbata



C. abrotanifolia

C. foeniculacea

Fig. 1

Morphology of **Cystoseira** species.

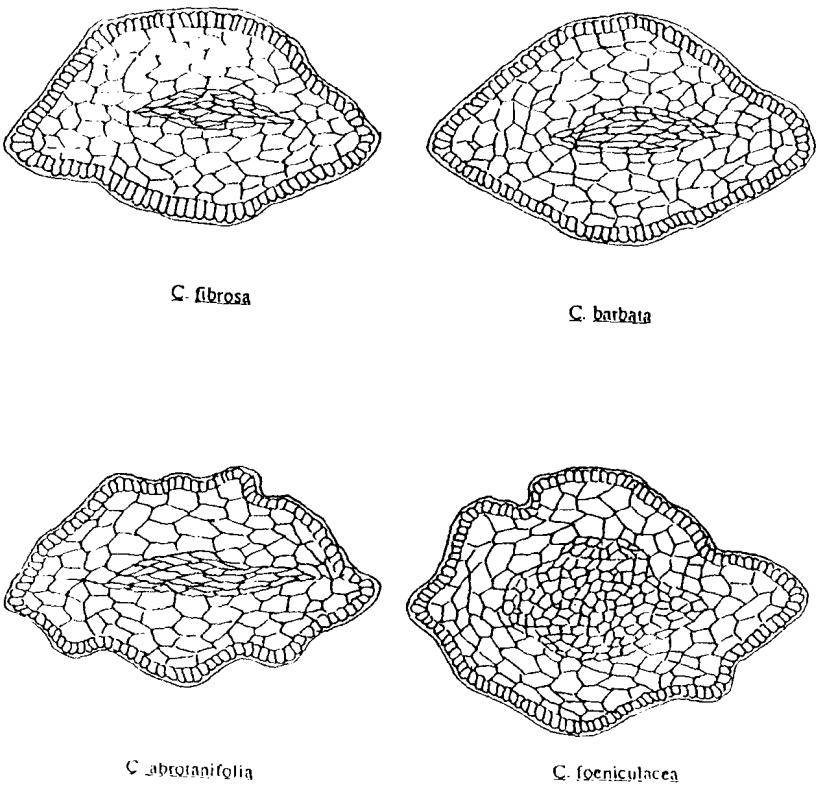


Fig. 2

Anatomy of primary lateral basis of **Cystoseira** species.

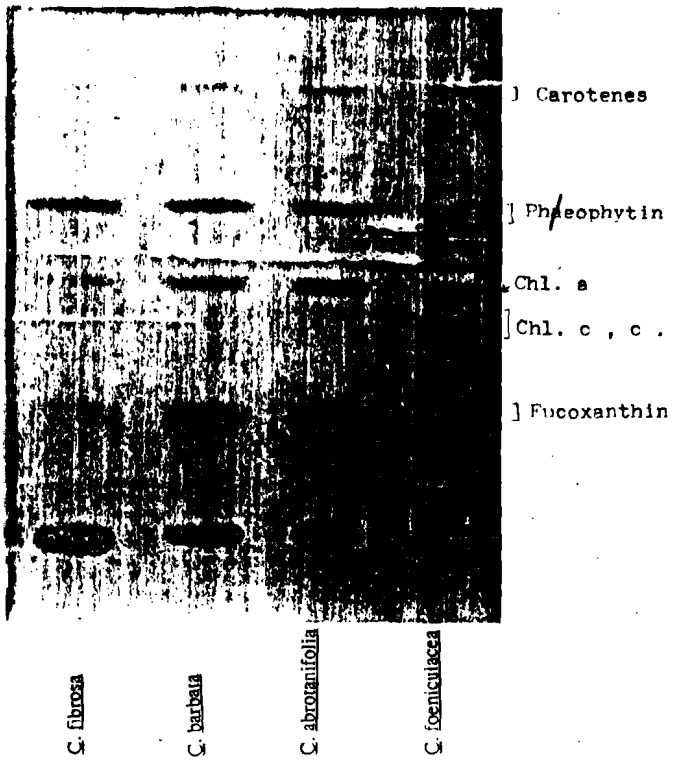
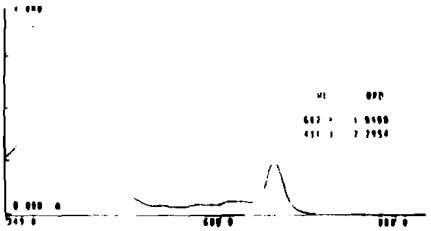
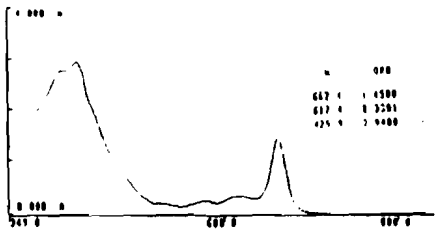


Fig. 3

Thin layer chromatogram of pigment extracts of **Cystoseira** species.

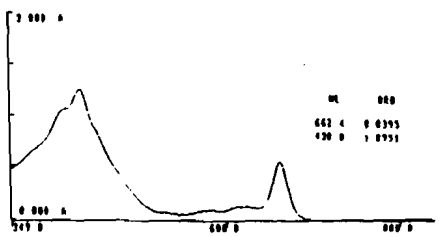


C. fibrosa



C. barbata

C. abrotanifolia



C. foeniculacea

Fig. 4

Whole absorption spectra of pigment extracts of **Cystoseira** species.

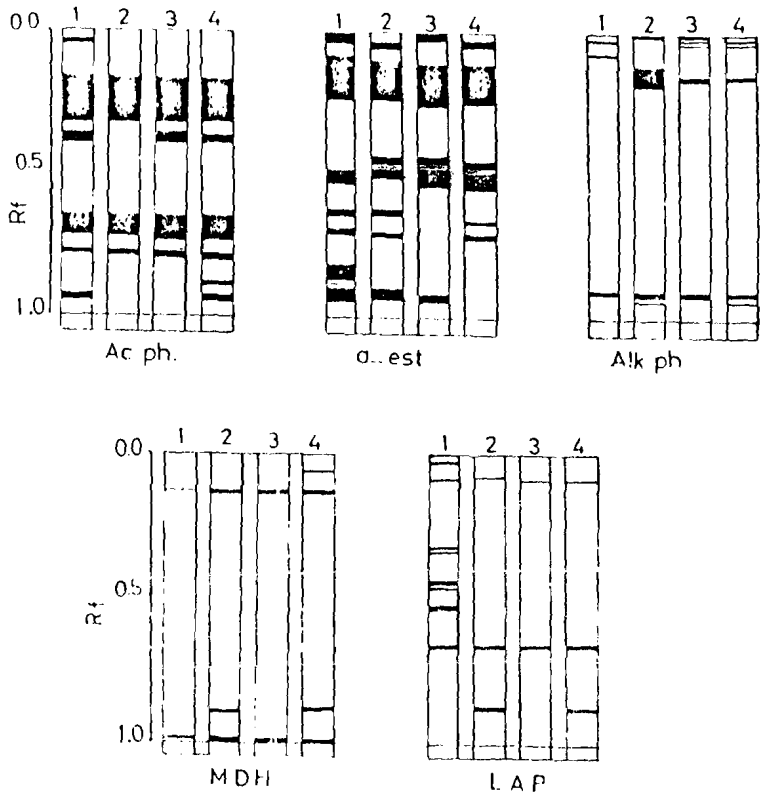
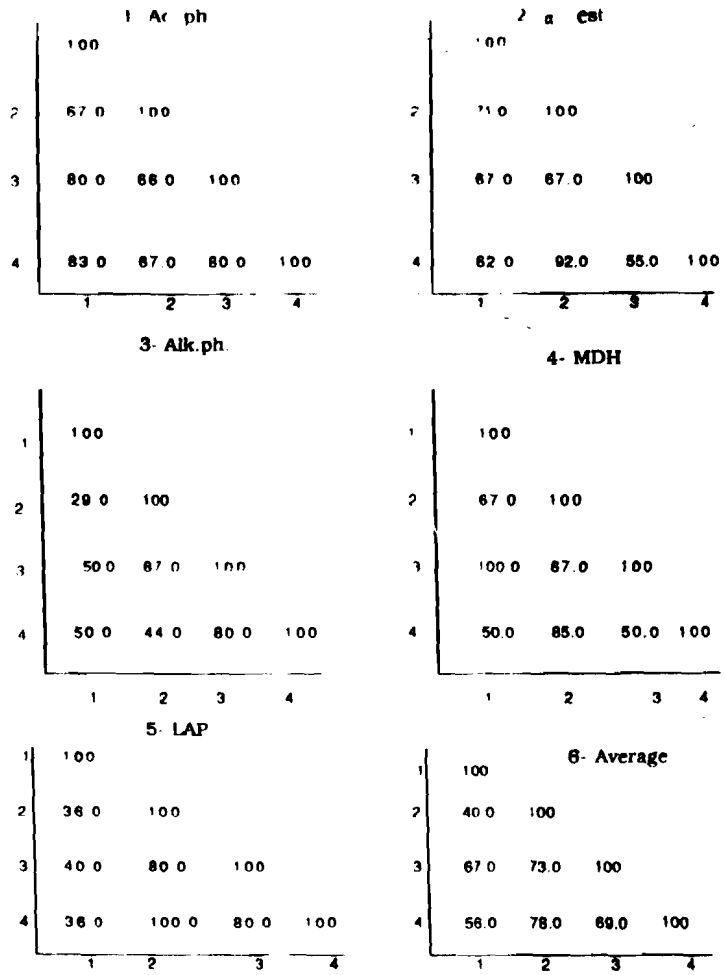


Fig.5

Zymograms of **Cystoseira fibrosa (1), C. barbata (2), C. abrotanifolia (3),** and **C. foeniculacea (4).**



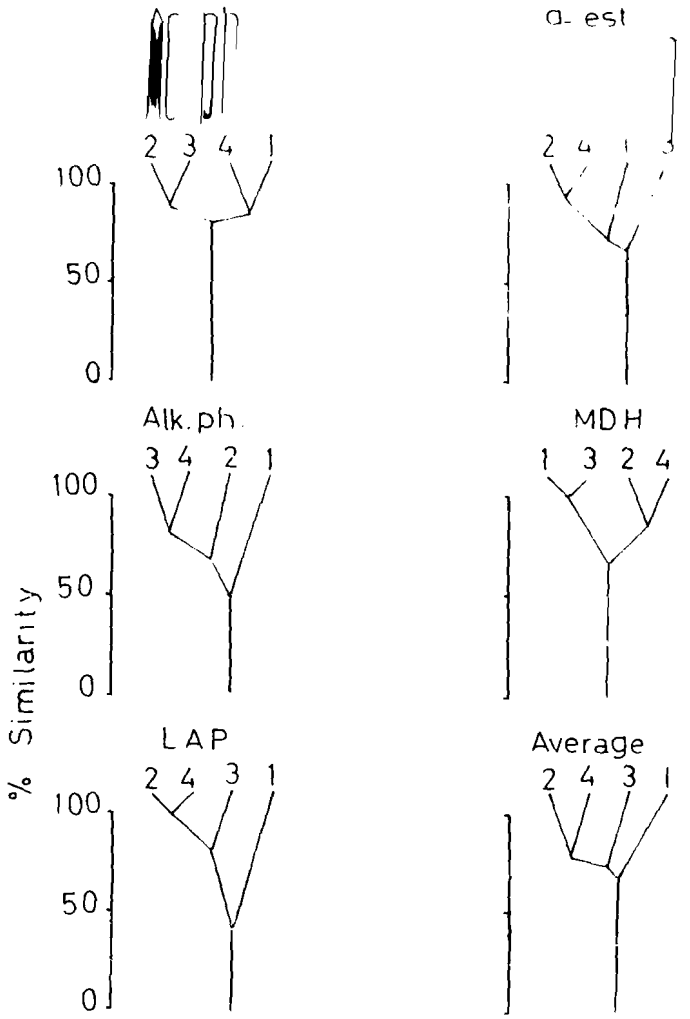
(1) Cystoseira fibrosa

(2) C. barbata

(3) C. abrotanifolia

(4) C. foeniculacea

Tables (1-6) individual and average similarity matrices



(1) Cystoseira fibrosa

(2) C. barbata

(3) C. abrotanifolia

(4) C. foeniculacea

Fig. 6

Individual and average similarity dendrograms.

Species studied		Similarity criteria	Dissimilarity criteria
A	C. barbata	6	3
	C. foeniculacea	6	3
B	C. abrotanifolia	5	4
C	C. fibrosa	3	6

Table 7

Points of similarity and dissimilarity among Cystoseira species based on the nine criteria studied.

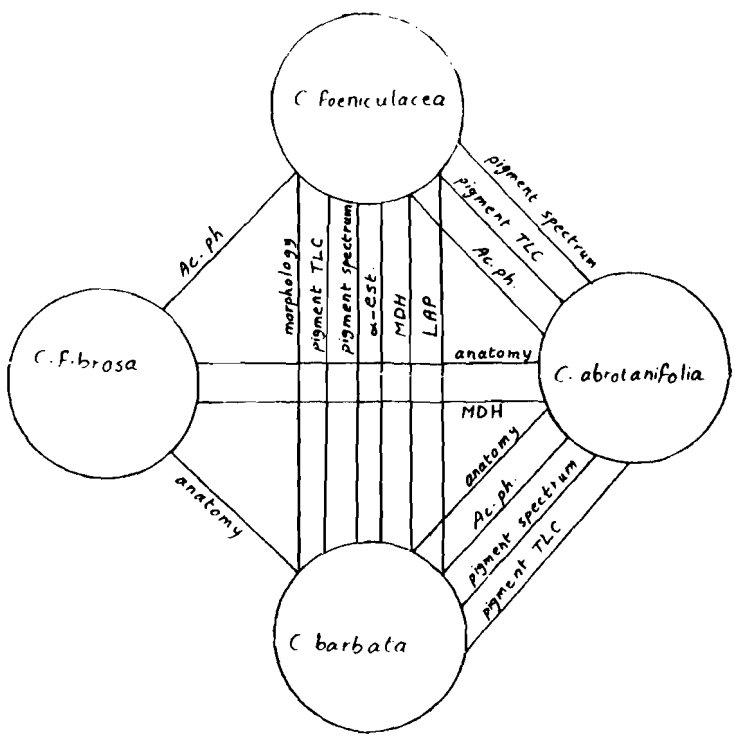


Fig. 7

Taxonomic stereogram with classical and biochemical similarity bonds for **Cystoseira** species.

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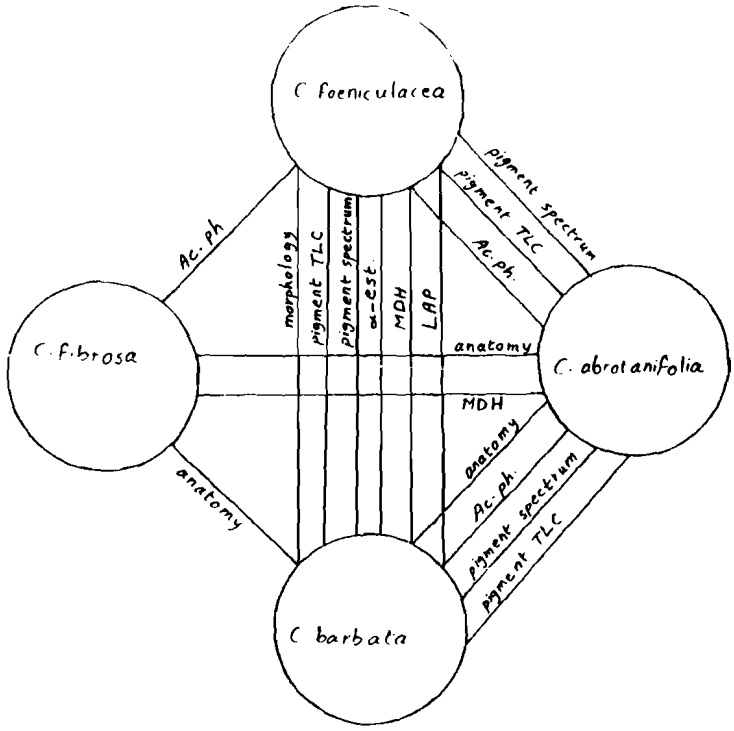

Fig. 7

Taxonomic stereogram with classical and biochemical similarity bonds for Cystoseira species.

Concerning the pigment analysis, four characteristic zones are destinguished for each species on the TLC (Fig. 3). These are fucoxanthin (orange), chlorophylls c_1, c_2 and a (green), pheophytin-pheophorbide (grey), carotenes (yellow) with increasing RF respectivey. The lack of Mg by chlorophyll results in pheophytin with grey color and reduced absorption spectrum in the blue zone from about 430 to about 400 nm, while the lack of phytyl chain would result in chlorophyllide with no change neither in color nor in absorption spectrum. So the lack of both Mg and phytyl gives rise to pheophorbide with same color and absorption specturm like pheophytin. A good degree of similarity is clear on the chromatogram among the four species. However, the ratio of pheophytin to chlorophyll differs from the left to the right side of the chromatogram. Though the chlorophyll content is clearly higher than that of pheophytin-pheophorbide in C. foeniculacea to the right, the reverse is true in C. fibrosa to the left. The later species is also characterized by very little or absence of c chlorophylls, especially c_1.

Parallel to the above mentioned meaning, it is clearly translated on the absorption spectra of the species studied in Fig. 4. All species have a red zone band at about 662 nm which is thin and short, a blue to green zone band which is high and wide, and an optical window in between except for two little domes at the left side of the red peak in C. barbata, C. abrotanifolia and C. foeniculacea. The thin band at 662 nm represents chlorophyll a only and it is quite similar in all species of Cystoseira. The two little domes refere to types of chlorophyllc. The wide blue-green band is an overall representation of all chlorophyll and chlorophyll products and carotenoids. The highest peak in this band at about 429 nm in all species but for C. fibrosa represents chlorophylls (a c_1 c_2). The clear shoulder on its left represents the pheophytin-pheophorbide, while the right little one at about 450 nm referes to carotenoids (fucoxanthin and carotenes). All the absorption spectra of C. barbata, C. abrotanifolia and C. foeniculacea are very similar, and have the head of their blue bands with the highest peak to the right and a shoulder to the left. C. fibrosa is characterized from other species studied by having the head of its blue band turned over i.e., the peak to the left and the shoulder to the right, and the disappearance of the little domes on the left of the red band. This is in quite agreement with the chromatogram as this species has more pheophytin and less chlorophyll and this would make the peak at about 410 nm for pheophytin-pheophorbide, and a shoulder at 428 nm for chlorophylls. The disappearance of the two little domes characterizing chlorophyll c_1,c_2 in the red zone is explained by the very poor content of these pigments in C. fibrosa comparable to the rest of species.

Looking at the zymograms, individual and average similarity matrices and dendrograms for the five isozymal systems analysed for our species of Cystoseira, we find that there are two species out of four (C. barbata and C.

foeniculacea) that are tied together on one branch at the left side of the average dendrogram at the level of about 80.0 % similarity. Thes could be traced back to high characteristic individual similarities in the electrophoretic patterns of ∝-est, LAP, and MDH systems. Still in the other two systems (Ac. ph. & Alk. ph.), these two species are not far away from each other down the dendorgrams. C. fibrosa is again, well differentiated in the isozymal analyses and has the least similarity towards any other species on the average dendrogram. C. abrotanifolia has a moderate case and stands in between the barbata-foeniculacea branch and fibrosa. The characterization of C. fibrosa could be traced back to the individual systems of LAP, Alk. ph., and∝-est, although this is not the case concerning MDH and Ac. ph. patterns. This adds to the significance of applying multiple isozymal systems pointed out by Mohamad(1981); Mohamad and Shaalaan (1985) and Shaalaan and Mohamad, (1985 & 1986) for studying genera and species relationships, and the weakness of using one or two systems only, especially when taxonomic implications are to be considered.

In this taxonomic study, based on cumulative approach, the author considered nine miscellaneous criteria, all of them could be used as taxonomic markers; these are: 1) morphology) 2) anatomy of lateral branch base, 3) pigment chromatogram separating the individual pigments of every species, 4) shape of the absorption spectrum of these species expressing the absorption of all pigments together, and 5-9 electrophoretic patterns and their corresponding dendrograms of Ac.ph., alk. ph.,∝-est, MDH,and LAP, respectively.

C. barbata and C. foeniculacea showed to be tied together; i.e. identical to highly similar in six criteria out of nine (moropholohy, pigment chromatogram, absorption spectrum, LAP,∝-est, and MDH) and differentiated in three criteria (anatomy, Ac. ph. and Alk.ph). The reverse is true concerning C. fibrosa which proved to be characterized from the other species in six criteria (morphology, pigment chormatogram, absorption spectrum, LAP, ALK. ph. and∝-est.) and similar to other species in three criteria (anatomy, MDH and Ac.ph.). C. abrotanifolia showed to have good degree of similarity in five criteria and differentiated by four ones. The above mentioned argument is summarized and represented in Table 7 and Fig. 7, respectively.

Finally, based on our cumulative taxonomic approach, an over-all conclusion that we may come out with is that:

1- The four species studied do have enough degree of similarity to stand together within the genus Cystoseira. None of them showed to be unique in all criteria.

2- C. fibrosa could stand alone as a genuine species. The same could be true as for C. abretanifolia as its similatities are not steady towards one and the same species.

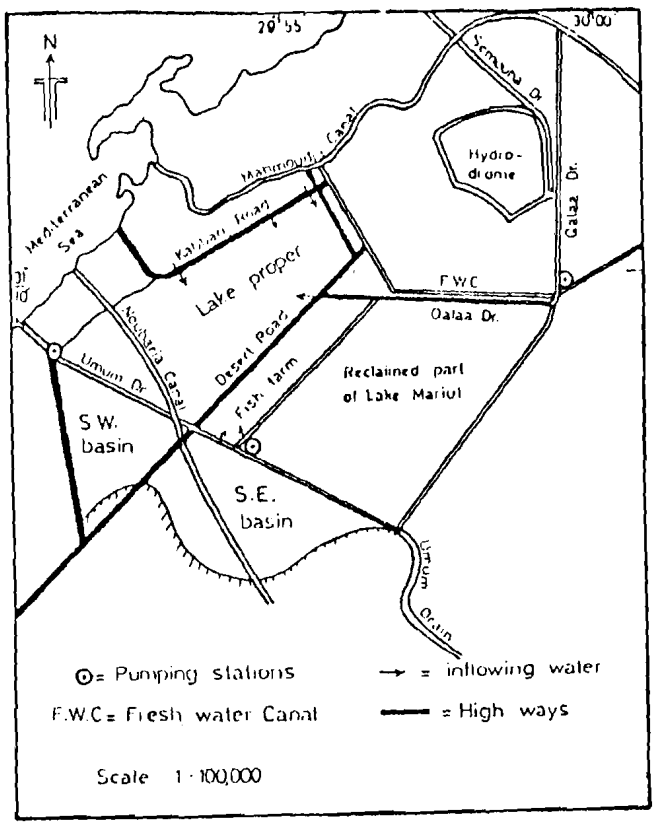

Fig. 1

Morphometry of Lake Mariut.

mainly from El-Qalaa Drain which flows at a rate of 482-893 x 10^3 m^3/day (Samaan and Abdelmoneim, 1986). Its water is a mixture of agricultural runoff and sewage effluents contaminated with industrial wastes discarded from Alexandria drainage system. The Lake proper receives also at its northern margin considerable amounts of sewage, flowing through Karmous and El-Kabbari sewers at a daily average of 25 x 10^3 and 19 x 10^3 m^3/day respectively. An industrial waste disposal pipe is located at the eastern corner of the Lake proper and it pours untreated wastes of several industrial plants at a rate of 18-23 x 10^3 m^3/day. The aim of the present work is to evaluate the Lake eutrophication in relation to phytoplankton and some physical and chemical parameters.

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3- C. barbata and C. foeniculacea could be considered as two varieties of one species or two species belonging to one sub-genus. The present work emphasized the significance and success of including as much taxonomic criteria as possible (considering both classical and biochemical) for making an algal classification on a more solid basis.

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