A taxonomic analysis of the *Ectocarpaceae* (*Phaeophyta*) from the Gulf of Gdańsk

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Ectocarpaceae
Numerical taxonomy

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Abstract

Three species of *Ectocarpaceae* are cited in the literature as present in the Gulf of Gdańsk: *Pilayella littoralis*, *Ectocarpus siliculosus* and *E. confervoides*.

The morphological characters were analysed using the numerical taxonomy; method the life cycle was also studied. Material was collected with a dredge from 4 regions of the Gulf of Gdańsk at monthly intervals from April to October.

Cluster analysis showed that there are no satisfactory morphological characters the distinguishing *Ectocarpaceae* spp. from the Gulf of Gdańsk. In addition, observations of their life cycle indicated that *Ectocarpus siliculosus* and *Pilayella littoralis* are two generations of the same species.

1. Introduction

The family *Ectocarpaceae* currently contains the simplest and probably also the most primitive members of the *Phaeophyta*. The thallus form of every species is a heterotrichous filament with little or no structural modification. Sporangia and gametangia are formed from vegetative cells and occur either as intercalary structures within filaments or as modified branch apices (Russell and Garbary, 1978).

Ectocarpaceae spp. confirmed in the Gulf of Gdańsk are *Pilayella littoralis* (Lyngbye) Kjelm. – the dominant species, *Ectocarpus siliculosus* Dyllwyn and *Ectocarpus confervoides* (Roth) Le Jolis (Lakowitz, 1907; Pliński and Giebułtowska–Mindak, 1974; Pliński and Florczyk, 1984). During the past 20 years, the Gulf has been subjected to strong pressure by man's activities, and these have brought about far-reaching changes in the composition and distribution of the phytobenthos. One of the most important changes has been the more than doubling of the biomass of brown algae from the family *Ectocarpaceae* (Pliński *et al.*, 1989).

The aim of this work was to carry out a taxonomic analysis of the *Ectocarpaceae* from the Gulf of Gdańsk. A systematic investigation was carried out using numerical taxonomic methods, and the life cycle of the algae was observed.

2. Materials and methods

2.1. Analysis of morphological characters

The material was collected every month from April to November. The samples were taken at 4 stations (Fig. 1) in the western part of the Gulf of Gdańsk with a drag-net from a depth of 3–4 m, and manually from shallow water down to 1 m. All material was preserved in 4% formalin. The algae were initially classified according to orthodox taxonomy and the main morphological forms were distinguished.

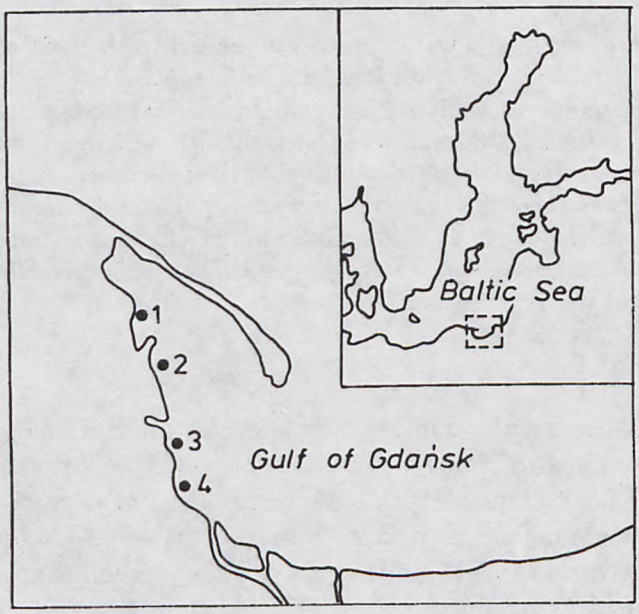

Fig. 1. Distribution of sampling stations in the Gulf of Gdańsk

Most of the material consisted of unattached thalli; that is why the OTU (Operational Taxonomic Unit) was taken to be a thallus composed of tangled and morphologically similar filaments, readily removed from the sampled material without breaking the filaments. The OTUs were described by a set of morphological characters (Tab. 1) which included the principal taxonomic features given by various authors and specific features recorded in the studied thalli.

Table 1. List of morphological characters of the *Ectocarpaceae* from the Gulf of Gdańsk used in cluster analysis

No.	Name of character
1.	Length of main axis cells
2.	Width of main axis cells
3.	Distance between branchings
4.	Length of plurilocular sporangia
5.	Width of plurilocular sporangia
6.	Diameter of unilocular sporangia
7.	Number of unilocular sporangia in chains
8.	Percentage of fertile sporangia
9.	Percentage of dichotomous branchings
10.	Percentage of opposite branchings
11.	Order of branchings
12.	Frequency of sporangia ⋆
13.	Branching angle
14.	Presence of longitudinal divisions in unilocular sporangia
15.	Occurrence of longitudinal divisions in main axis cells
16.	Presence of sporangia terminating in a hair
17.	Occurrence of ECT-type sporangia. ⋆⋆
18.	ECT-type sporangia dominant
19.	Intercalary chains of unilocular sporangia dominant
20.	Apical chains of unilocular sporangia dominant
21.	Sub-apical chains of unilocular sporangia dominant
22.	Intercalary plurilocular sporangia present
23.	Presence of apical, long plurilocular sporangia
24.	Presence of apical, short plurilocular sporangia
25.	Arc-shaped plurilocular sporangia present
26.	Branch verticils present
27.	Soft thalli present
28.	Long branches present
29.	Short branches present
30.	Presence of characteristic 'brushes' consisting of short branches at the end of long ones
31.	Presence of vegetative branches longer than generative ones
32.	Presence of branches terminating in a hair
33.	Occurrence of rhizoidal filaments
34.	Occurrence of main axis filaments with fused ends

⋆ – the following scale was applied: 0 – no sporangia, 1 – sporangia seldom present, 2 – sporangia abundant.

⋆⋆ – the characteristic unilocular sporangia occurring on some thalli are thus described (Fig. 2c).

A total of 30 OTUs were distinguished and covered all the morphological forms of *Ectocarpaceae* found in the Gulf of Gdańsk.

A cluster analysis was done using the 'Cluster' program (Florczyk, 1989); 6 dissimilarity coefficients and 7 clustering methods were applied.

Coefficients:

Squared Euclidean Distance, Manhattan Distance, Canberra Distance (Abbott *et al.*, 1985), Sajzy Distance

$$SD = \sum_{n=1}^{k} 1 - \frac{\min(x_i, x_j)}{\max(x_i, x_j)}, \tag{1}$$

where

k – number of characters,
x_i – value of character in item i,
x_j – value of character in item j.

Queue Coefficient

$$QC = \sum_{n=1}^{k} Fr_i - Fr_j, \tag{2}$$

where

k – number of characters,
Fr – cumulative frequency of given state of character,
i, j – compared items.

Queue Distance

$$QD = \sum_{n=1}^{k} 2(QC_{ij} - QC_{ii} - QC_{jj}), \tag{3}$$

where

k – number of characters,
QC – queue coefficient,
i, j – compared items.

Formulas 4–6 are given according to Batko and Zakryś (in press). The following clustering methods were used: UPGMA, WPGMA, Flexible sorting, Centroid sorting, Ward method, Single link, Complete link (Abbott *et al.*, 1985).

The squared Euclidean Distance and Manhattan Distance were calculated on standardized and normalized data. The formula for data standardization was

$$x' = \frac{x - X}{SD}, \tag{4}$$

and for normalization

$$x' = \frac{x - X_{\min}}{X_{\max} - X_{\min}}, \tag{5}$$

where

x' – new value of character,
x – value in data matrix,
X – mean,
SD – standard deviation,
X_{min} – minimum value of character,
X_{max} – maximum value of character.

In the Canberra Distance and Sajzy Distance, calculations were also done on basic data; Queue Distance and Queue Coefficient were calculated only on basic data. 84 dendrograms were obtained which were then scanned for repeating clusters.

2.2. Life cycle

Ten thalli, collected at four stations in the Gulf of Gdańsk (Fig. 1), were transferred to the laboratory. The plants were then cultivated in sterilized Erlenmeyer flasks at 15°C in a 16 h light ($\sim$ 30 $\mu Em^{-1}s^{-1}$) and 8 h dark cycle. The algae were grown on a liquid mineral medium (von Stosch, 1964) which was changed weekly.

Plurilocular sporangia were noted after 2 weeks of acclimatization. 5 isolated sporangia, free of epiphytes and other bodies visible in the optical microscope, were placed in 5 separate Petri dishes with the same medium. Observations were made every 2–3 days during the course of new thallus development. The experiment was repeated using 5 newly-formed sporangia.

3. Results

3.1. Analysis of morphological characters

Ectocarpaceae thalli occur in the Gulf of Gdańsk from April to October, mainly as unattached forms, although in April they were noted in small numbers only at stations 2 and 3. Attached forms were observed only in the off-shore zone (down to 1 m) at stations 1 and 3. They were present in the Gulf till the end of summer (September).

Three main morphological forms were found to occur (Fig. 2):

1. Plants with opposite branchings of the main axis, forming chains of unilocular sporangia on the side branches. These chains were mostly located sub-terminally or terminally and sometimes in an intercalary position (Fig. 2a). This form was classified as *P. littoralis.*
2. Plants with dichotomous branchings of the main axis, forming long or short plurilocular sporangia, sessile or pedicellate, with terminal in lateral branches or, as in a few cases, in an intercalary position. Some

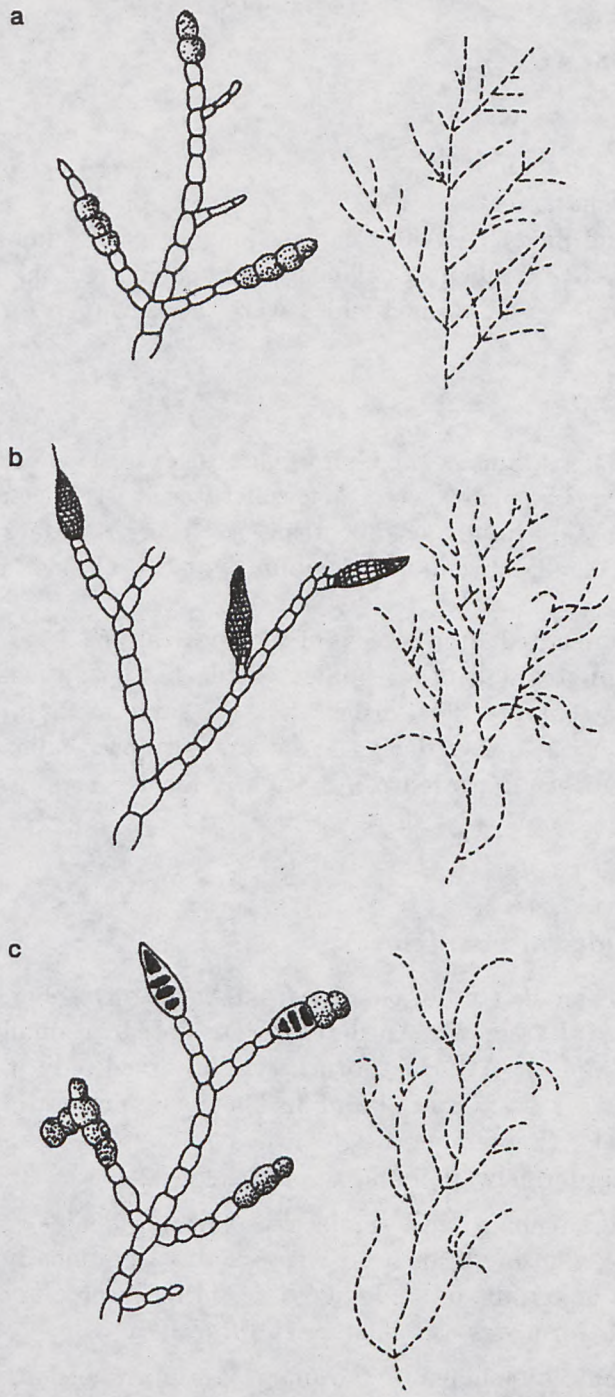

Fig. 2. Morphological forms of *Ectocarpaceae* in the Gulf of Gdańsk: a – form **1.** Mag. 160×, b – form **2.** Mag. 160×, c – form **3.** Mag. 160×

sporangia terminated in a hair (Fig. 2b). The thalli belonging to this form were classified as *E. siliculosus* or *E. confervoides.*

3. Plants with opposite and dichotomous branchings of the main axis, forming chains of unilocular sporangia at the ends of the branches. These chains were often branched while the initial stages of their formation were similar to those of the young plurilocular sporangia of form (Fig. 2c). Although these thalli show dichotomous branching of the main axis, they were classified as *P. littoralis.*

Besides these, many forms combining the characters of the above-mentioned groups were observed:

a thalli with dichotomous branchings of the main axis and chains of unilocular sporangia,

b thalli with dichotomous branchings of the main axis, forming chains of unilocular sporangia and long apical plurilocular ones,

c thalli with dichotomous branchings of the main axis, chains of unilocular and plurilocular sporangia in an intercalary position,

d thalli with opposite branchings of the main axis and plurilocular sporangia at branch ends or in an intercalary position,

e thalli without sporangia but with dichotomous and opposite branchings of the main axis.

In some cases the mature unilocular sporangia were divided lengthways (especially in form **3**); similar divisions were noted in the vegetative cells. Although some thalli occurred as unattached forms, they possessed rhizoidal filaments along the main axis (Fig. 3). Many plants, particularly those collected in spring and summer, had lateral branchings terminating in a hair. There were no marked differences in chromatophore morphology between the different forms and the thalli.

Numerical analysis of the data yielded three main clusters of OTUs in most (60%) of the dendrograms (Fig. 4). **Cluster A** contains OTUs typical of plants classified as *P. littoralis* and covers the material collected at stations 1, 2, 3 from August to October. Morphologically, these plants corresponded to the description of forms 1 and 3. They were additionally characterized by short branches, hard thalli and a large (90°) branching angle.

Cluster B comprises OTUs collected during the whole sampling period at station 4, and from April till July at the other stations. The OTUs from station 4 basically corresponded to form **1.** They were classified as *P. littoralis*, although in some of them individual characters accepted as typical of *E. siliculosus* were observed. Taxonomic identification of the other OTUs

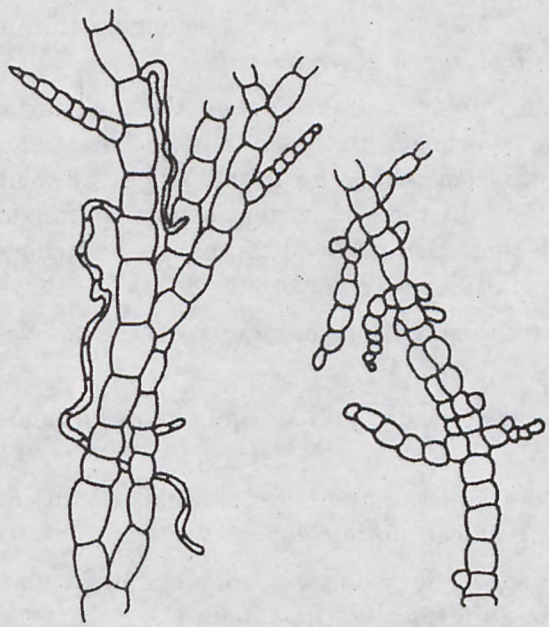

Fig. 3. Unattached thalli of *Ectocarpaceae* with rhizoidal filaments (right) and longitudinal divisions of cells (left). Mag. 250×

(from stations 1, 2 and 3) was difficult for lack of sporangia, or because they possessed characters typical of different species. These OTUs corresponded to intermediate forms **a**, **b**, **c**, **d**, **e** and had soft cottony thalli. **Cluster C** contains OTUs similar to thalli classifiable as *P. littoralis* forms with plurilocular sporangia, *E. siliculosus* and *E. confervoides*. They corresponded to form **2** and intermediate forms **b** and **c** and, in addition, had the following characters in common: a soft thallus, long branches with characteristic brushes at the end of them. All the OTUs were collected in June and July at stations 1, 2, 3, except for *Ectocarpus* sp. which was taken at station 1 in October.

The second dendrogram (Fig. 5) is the result of clustering based on all the characters; the clustering method and coefficient were the same as in Figure 4, but in contrast to the latter, a different method of initially transforming the data (*i.e.* normalization) was applied.

The third dendrogram (Fig. 6) illustrates the results of cluster analysis based exclusively on reproductive characters. As in the case of Figure 4, the same clustering method, coefficient and initial transformation of the data were used. Two clusters were distinguishable at a similarity level of 70%. One of them, the cluster **c**, comprises all the OTUs from cluster **C** (Fig. 5) and one OTU from the cluster **B**, *P. litt.* 23/2. All of them possess

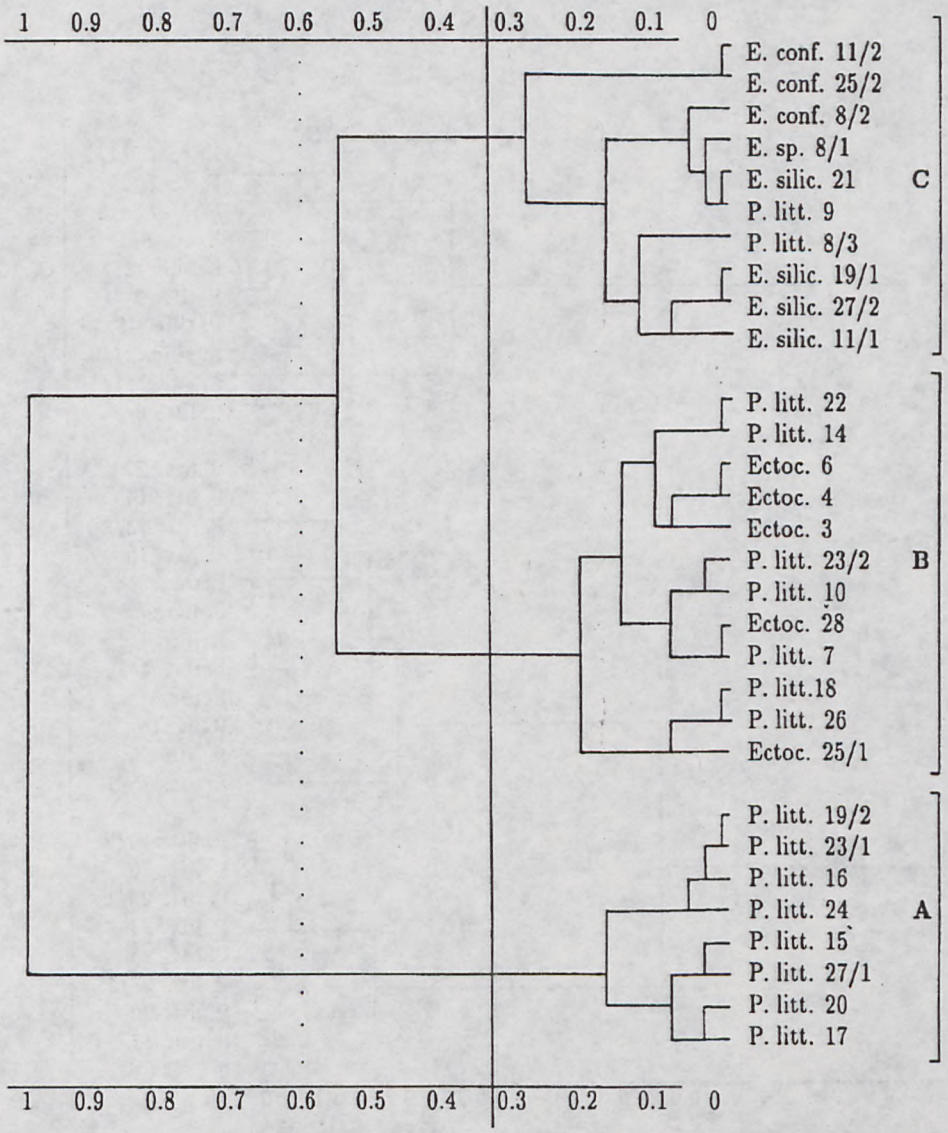

Fig. 4. Clustering of *Ectocarpaceae* – all characters; Squared Euclidean Distance; Ward Method; standardized data

E. conf. – *Ectocarpus confervoides*,
E. silic. – *Ectocarpus siliculosus* ,
E. sp. – *Ectocarpus sp.*,
Ectoc. – *Ectocarpaceae* (undetermined thalli),
P. litt. – *Pilayella littoralis*,
Figures – numbers of samples

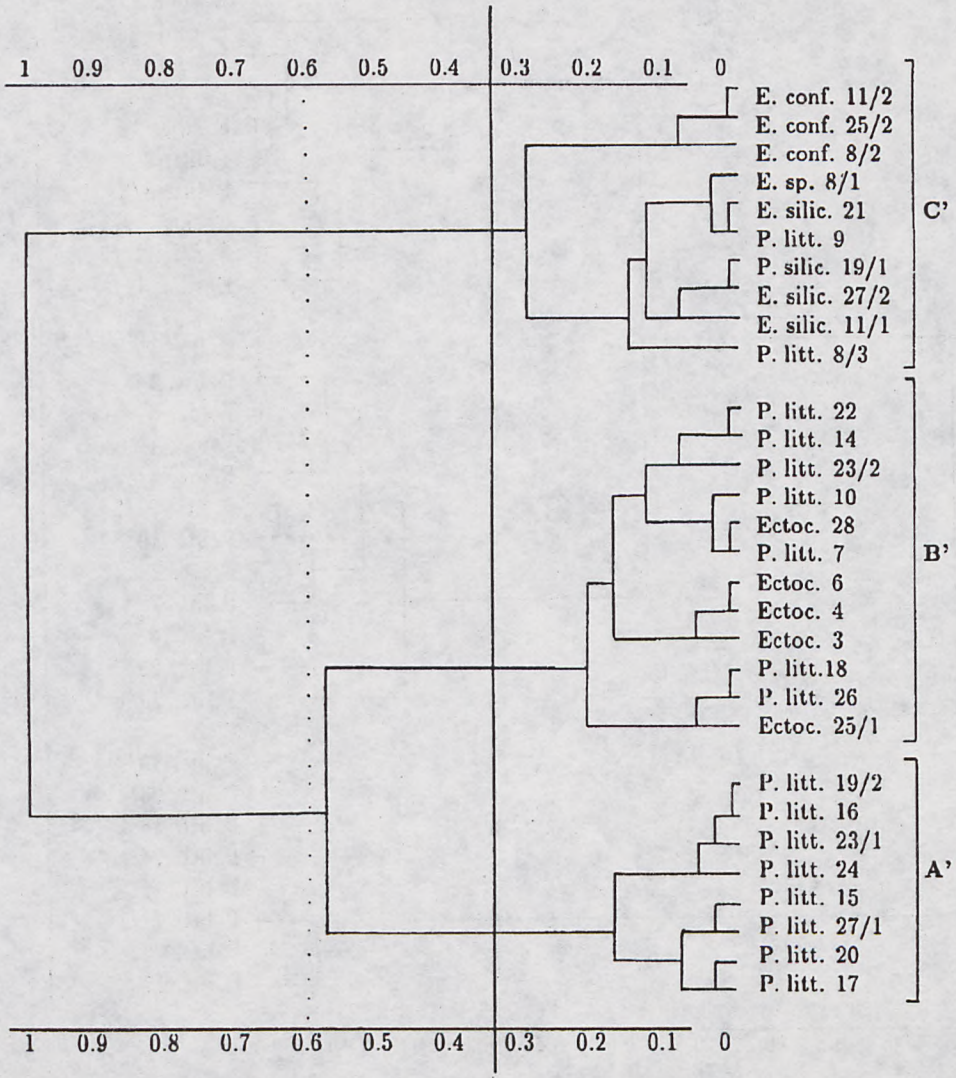

Fig. 5. Clustering of *Ectocarpaceae* – all characters; Squared Euclidean Distance; Ward Method; normalized data

E. conf. – *Ectocarpus confervoides*,
E. silic. – *Ectocarpus siliculosus*,
E. sp. – *Ectocarpus sp.*,
Ectoc. – *Ectocarpaceae* (undetermined thalli),
P. litt. – *Pilayella littoralis*,
Figures – numbers of samples

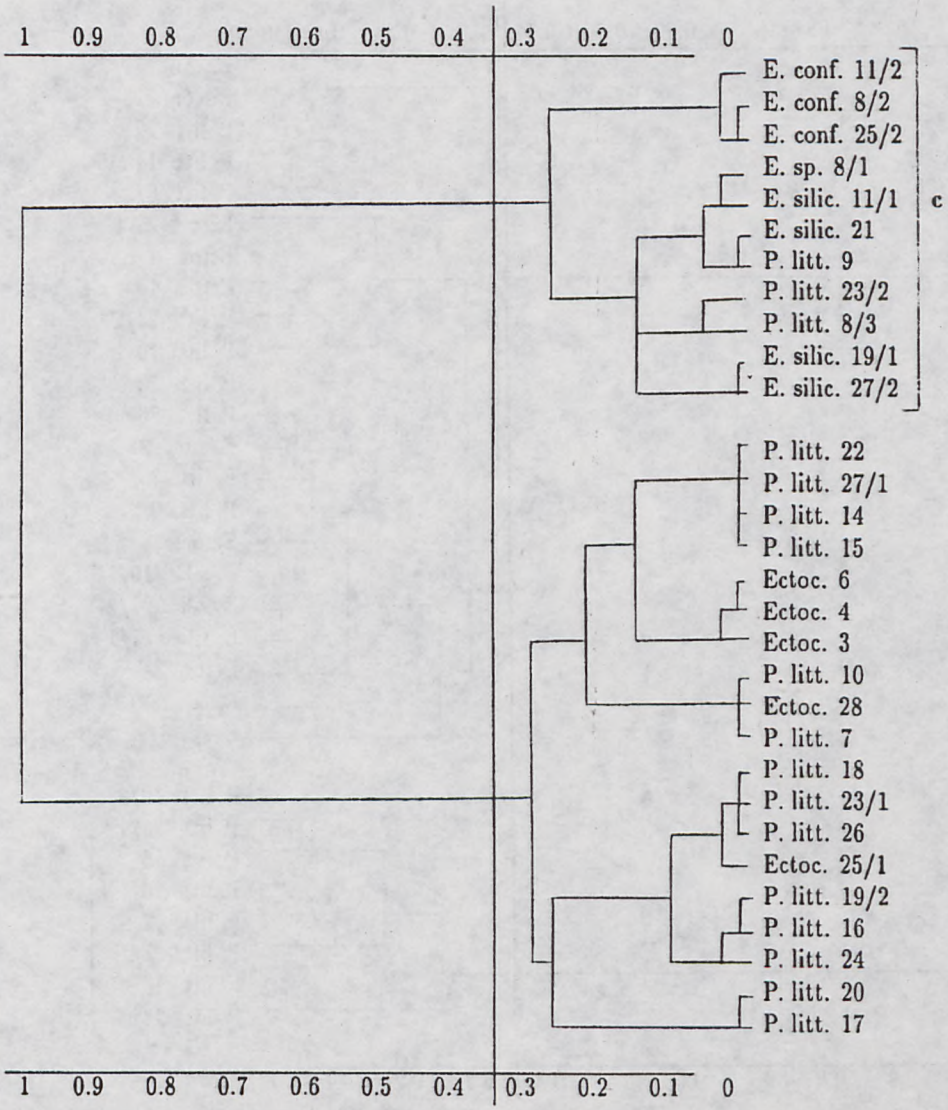

Fig. 6. Clustering of *Ectocarpaceae* – characters connected with sporangia; Squared Euclidean Distance; Ward Method; standardized data

E. conf. – *Ectocarpus confervoides*,
E. silic. – *Ectocarpus siliculosus*,
E. sp. – *Ectocarpus sp.*,
Ectoc. – *Ectocarpaceae* (undetermined thalli),
P. litt. – *Pilayella littoralis*,
Figures – numbers of samples

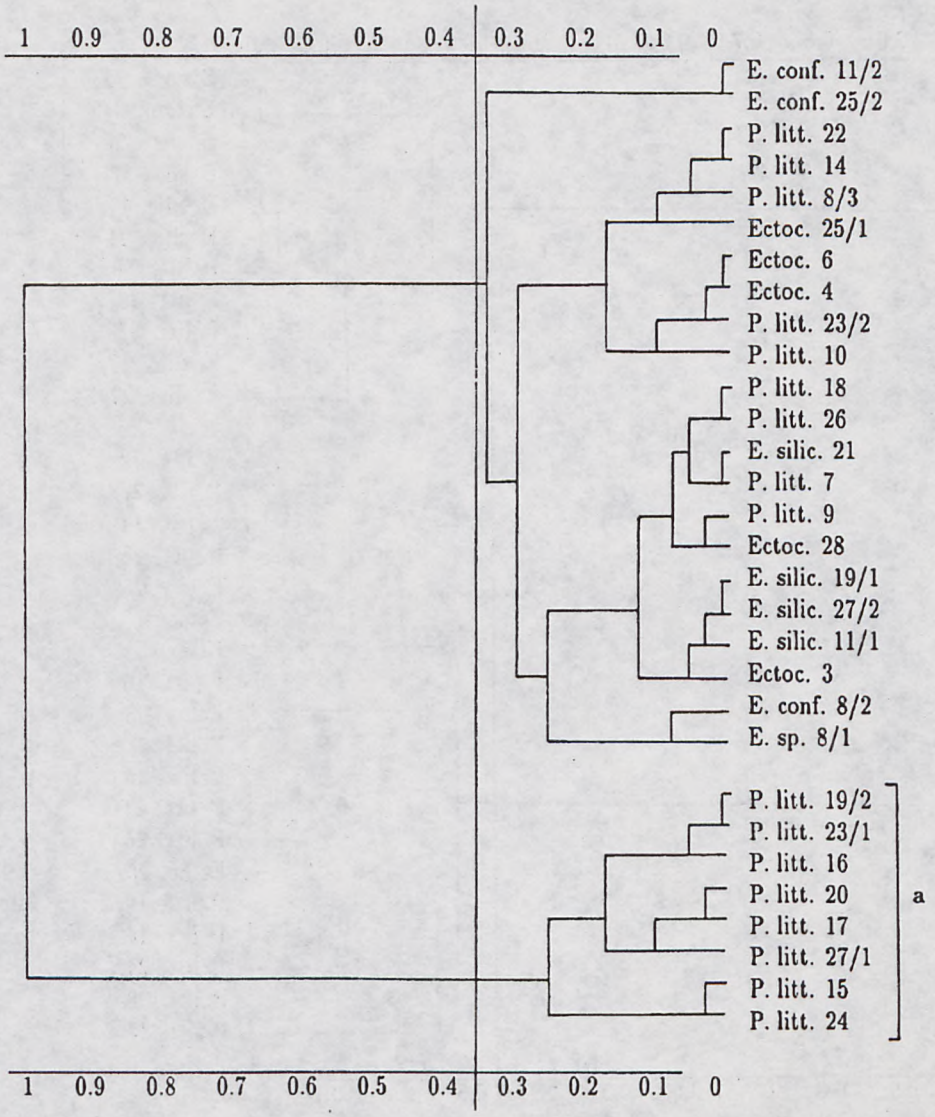

Fig. 7. Clustering of *Ectocarpaceae* – characters not connected with sporangia; Squared Euclidean Distance; Ward Method; standardized data

E. conf. – *Ectocarpus confervoides*,
E. silic. – *Ectocarpus siliculosus*,
E. sp. – *Ectocarpus sp.*,
Ectoc. – *Ectocarpaceae* (undetermined thalli),
P. litt. – *Pilayella littoralis*,
Figures – numbers of samples

plurilocular sporangia, whereas *P. litt.* 23/2 and *P. litt.* 8/3 also form unilocular sporangia typical of *P. littoralis.* The second cluster consists of OTUs with unilocular sporangia only or without any sporangia. However, none of its clusters corresponds to cluster **A** in Figure 4. The second cluster therefore comprises items from clusters **A** and **B.**

The final dendrogram (Fig. 7) presents the results of clustering based on all the characters except those involved in reproduction. The coefficient, clustering method and initial transformation of the data were the same as in Figure 4. As before, two phenones were recognizable: the first one, cluster **a**, was composed of the OTUs from cluster **A** (Fig. 4), and the second cluster comprised the OTUs from clusters **B** and **C** in Figure 4.

3.2. Life cycle

Plurilocular sporangia were isolated from thalli phenotypically similar to the OTUs from cluster **C** (Fig. 4) and were designated as *E. siliculosus.* Sporangia were then transferred to separate Petri dishes and after about 4 weeks young plants attached to the bottom by rhizoids were observed. After a further month the thalli had formed two kinds of reproductive structure: plurilocular sporangia of different shapes, often terminating in a hair (Fig. 8), and chains of unilocular sporangia (Fig. 9) located in various parts

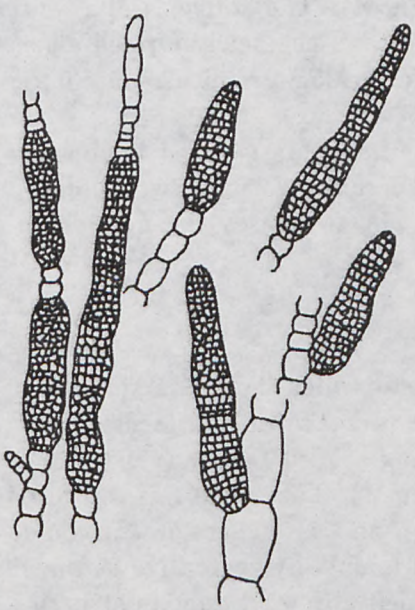

Fig. 8. Plurilocular sporangia obtained in culture. Mag. 250×

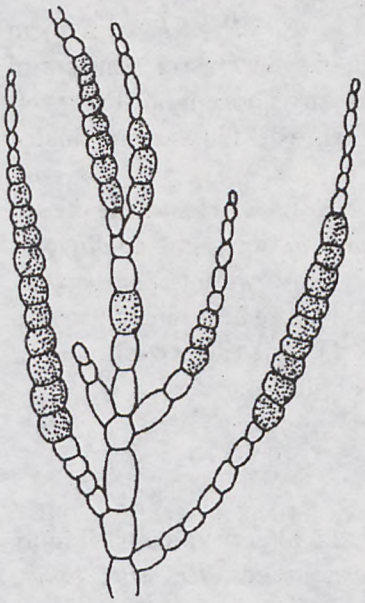

Fig. 9. Unilocular sporangia obtained in culture. Mag. 250×

of the branches. Moreover, algae with two kinds of sporangium were noted. Morphologically, the thalli with unilocular sporangia corresponded to the OTUs from cluster **A** (Fig. 4), while those with two kinds of sporangia were similar to the OTUs from cluster **B** (Fig. 4). The above results were obtained in four out of five replicates. In one case, the newly-formed algae produced exclusively plurilocular sporangia, intercalary or apical, sometimes terminating in a hair.

Similar results were obtained in the experiment on isolated unilocular sporangia cultivation. However, the new generation formed two kinds of sporangium in three out of five replicates. In two cases the new thalli resembled the initial ones.

4. Discussion

The basic features of the three main clusters identified at a similarity level of 70% (Fig. 4) are the following: the presence of plurilocular sporangia (cluster **C**); a large branching angle and a hard thallus (cluster **A**); morphologically indeterminate forms (cluster **B**). These features are also confirmed by two other dendrograms (Figs. 6 and 7). Therefore, it could be said that cluster **A** (Fig. 4) is determined mainly by vegetative features whereas cluster **C** (Fig. 4) is represented chiefly by reproductive structures. In Figures 6 and 7, a cluster corresponding to cluster **B** from Figure 4 was not distinguished. This fact upholds the view that the OTUs located

in this cluster are of an intermediate nature. Within this cluster the OTU *P. litt.* 23/2 deserves some attention. Although its plurilocular sporangia determine its assignment to cluster **c** (Fig. 6), in Figure 4 it occurs in cluster **B**. These differences are evidence for the significant influence of the other morphological features and support the morphologically indeterminate character of the thallus. In addition, they indicate that the presence of plurilocular sporangia is not necessarily dependent on the occurence of the other morphological features.

It is interesting to compare the dendrograms in Figures 4 and 5. Both comprise analogous clusters – **A** and **A′**, **B** and **B′**, **C** and **C′**. The main difference between the dendrograms lies in the fact that there are two clusters at a similarity level of 40% (dotted line). However, in Figure 5 one of them is cluster **C′** corresponding to cluster **C**, and the other is a combination of clusters **B′** and **A′** corresponding to clusters **A** and **B**. Figure 4 shows the opposite. The fact that a different method of data transformation facilitates various linkages of cluster **B** with the other clusters confirms the intermediate character of the OTUs and indicates a lack of clear and unequivocal differences between the OTUs from clusters **A** and **C**. This is also evident in the other dendrograms (Figs. 6 ad 7). If the criterion of classification were sporangium morphology, it would be possible to distinguish a cluster corresponding in OTU composition to cluster **C**; in this case, however, cluster **A** in the form in Figure 4 does not exist. On the other hand, if the other characters were applied as a criterion, a cluster corresponding in OTU composition to cluster **A** would be obtained. But the OTUs from clusters **B** and **C** in Figure 4 are mixed. This means that (i) it is difficult to obtain a specific set of features unequivocally distinguishing the OTUs from different clusters (**A**, **B**, **C**), and (ii) morphological differentiation of sporangia does not result in the differentiation of other features, and vice versa. From the present data it may be concluded that (i) as the OTUs investigated do not possess distinct and unequivocal morphological characters, they are indistinguishable from one another, and (ii) cluster **C**, mainly comprising thalli of the genus *Ectocarpus*, was distinguished on the basis of the presence of plurilocular sporangia.

In the light of the modern knowledge of *Ectocarpaceae* biology and taxonomy, plurilocular sporangia are an inadequate criterion for distinguishing the species *P. littoralis* and *E. siliculosus* or *E. confervoides* (Cardinal, 1964; Hamel, 1939; Müller, 1979; Russell, 1966, 1967, 1973; Russell and Garbary, 1978). This means that the literature data on the occurrence of these three *Ectocarpaceae* species in the Gulf of Gdańsk is of rather doubtful value.

Russell and Garbary (1978) have suggested that different genera and species of *Ectocarpaceae* have been distinguished owing to inconsistently applied criteria of division. The studies of Ravanko (1970) show that the criteria for *Ectocarpaceae* division are not satisfactory. In some cases, two generations of the same species or two forms (attached and unattached) have been classified as two distinct species because of apparently large morphological differences (Russell, 1966, 1967).

Cluster **C** in the first dendrogram (Fig. 4) comprises the OTUs with plurilocular sporangia collected at stations 1, 2, 3 in June and July, as well as one collected in October. On the other hand, cluster **A** consists of items also collected at stations 1, 2, 3 but from August till October. Together with the previous conclusions, this fact could suggest that the life cycle follows a characteristic pattern. To test the above hypothesis, observations of the life cycle were made. They show that a thallus with morphological characters typical of cluster **C** OTUs (form 2) could produce a thallus with morphological features typical of cluster **A** OTUs (form 1). Different morphological forms and kinds of sporangia during life cycles were also found by Müller and Stache (1989), who studied *P. littoralis* from other regions. In their experiments thalli with sporangia typical of form 3 and thalli with only plurilocular sporangia were not observed. It is possible that the formation of this kind of sporangia and the occurrence of plants with only plurilocular sporangia is determined by environmental factors.

The results of the present studies indicate that in the Gulf of Gdańsk there is only one species of *Ectocarpaceae* with a characteristic life cycle, *i.e. Pilayella littoralis.* The differences between the two generations resolve themselves mainly into the kind of sporangium formed, the length of the branches, and the angle and degree of branching pattern. Similar differences have been observed in two generations of *E. siliculosus* (Müller, 1964) and *P. littoralis* (Müller and Stache, 1989) from other regions.

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References

Abbott L. A., Bisby F. A., Rogers D. J., 1985, *Taxonomic analysis in biology. Computers, models and databases*, Columbia Univ. Press., New York.

Batko A., Zakryś B., *The numerical proof of Euglena classification*, J. Cryptogamic Botany, (in press).

Cardinal A., 1964, *Etude sur les Ectocarpacées de la Manche.* Beih. zur Nova Hedwigia, 15, 1–87.

Florczyk I., 1989, *The cluster analysis package 'CLUSTER'. Software for IBM PC XT/AT,* Inst. Oceanogr. UG.

Hamel G., 1939, *Phaeophycees de France,* Paris.

Lakowitz K., 1907, *Die Algenflora der Danziger Bucht,* Gdańsk.

Müller D. G., 1964, *Life cycle of Ectocarpus siliculosus from Naples, Italy,* Nature, 203, 1402.

Müller D. G., 1979, *Genetic affinity of Ectocarpus siliculosus (Dillw.) Lyngb. from the Mediterranean, North Atlantic and Australia,* Phycologia, 18 (4), 312–318.

Müller D. G., Stache B., 1989, *Life history studies on Pilayella littoralis (L.) Kjellman (Phaeophyceae, Ectocarpales) of different geographical origin,* Bot. Mar., 32, 71–78.

Pliński M., Florczyk I., 1984, *Analysis of the composition and vertical distribution of the macroalgae in the western part of the Gulf of Gdańsk in 1979 and 1980,* Oceanologia, 19, 101–115.

Pliński M., Florczyk I., Manasterska M., 1989, *Long-term changes in the composition of the phytobenthos from the Gulf of Gdańsk,* Proc. 21 Symp. EMBS, Gdańsk, 381–385.

Pliński M., Giebułtowska-Mindak A., 1974, *Some aspects of the biology of chosen species of the family Ectocarpaceae from Puck Bay,* Zesz. Nauk. Wydziału BiNoZ UG, Oceanografia, 4, 79–96, (in Polish).

Ravanko O., 1970, *Morphological, developmental and taxonomic studies in the Ectocarpus complex (Phaeophyta),* Nova Hedwigia, 20, 179–252.

Russell G., 1966, *The genus Ectocarpus in Britain. I. The attached forms,* J. Mar. Biol. Ass. U. K., 46, 267–294.

Russell G., 1967, *The genus Ectocarpus in Britain. II. The free-living forms,* J. Mar. Biol. Ass. U. K., 47, 233–250.

Russell G., 1973, *The Phaeophyta: a synopsis of some recent developments,* Oceanogr. Mar. Biol. Ann. Rev., 11, 45–88.

Russell G., Garbary D., 1978, *Generic circumscription in the family Ectocarpaceae,* J. Mar. Biol. Ass. U. K., 58, 517–525.

von Stosch H., 1964, *Wirkungen von Jod und Arsenit auf Meeresalgen in Kultur,* [In:] *Davy de Virville,* Feldmann (ed.), J. Proc. Fourth International Seaweed Symposium, Biarritz, September 1961, MacMillan, New York.