



# Structural and ultrastructural characteristics of Rhodophyta from the Romanian Black Sea coast

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**ABSTRACT:** The present paper reports the results of our research on the morphology and ultrastructure of *Polysiphonia denudata*, *Ceramium rubrum*, *Callithamnion corymbosum*, *Phyllophora nervosa*, *Porphyra leucosticta* thus completing a series of papers on the comparative ultrastructure of some Rhodophyta from the Romanian Black Sea coast.

The experimental part of researches was accomplished during the period July 2006 – April 2009 at the laboratory of electronic microscopy of “Ovidius” University of Constantza, Romania. Strips of thalli were cut out of the algal thallus from different regions: basal, middle and apical region. In light microscopy we used fresh thalli sectioned and observed at a Novex Holland photonic microscope, without colorants. Also, thin sections from algal thalli were processed by the electron microscopic technique through transmission and was observed under the performing electronic microscope Philips CM120.

At the ultrastructural level the most distinctive features comparatively analyzed are: possession of simple plastids with unstacked thylakoids, two layered chloroplast envelope, lack of chloroplasts ER, pit-connection or pit plug, floridean starch as the storage product, which lies freely in the cytoplasm, the thallus heaving uninucleate or multinucleate cells, the nuclei being small and with one or two nucleoli and cell wall composed of cellulose with the fibrils randomly arranged.

**Key words:** *Polysiphonia denudata*, *Ceramium rubrum*, *Callithamnion corymbosum*, *Phyllophora nervosa*, *Porphyra leucosticta*.

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## INTRODUCTION

The current paper comprises the comparative cytological study of red algae in the Black Sea during the cold season. The studied species are macrophytic algae. Apart from the fundamental importance of the study of their ontogenesis, phylogenesis and cytology, many of these species can be used in human alimentation or are valorized for animal feed, while others are used as raw material in different branches of industry.

The present paper reports the results of our research of the morphology and ultrastructure of *Polysiphonia denudata* (Dillwyn) Greville ex Harvey., *Ceramium*

*rubrum* (Huds.) C. Ag., *Callithamnion corymbosum* (Ducl.) Ag., *Phyllophora nervosa* (DC) Grev., *Porphyra leucosticta* Thur. and completes a series of papers on the comparative ultrastructure of some Chlorophyta, Rhodophyta and Phaeophyta from the Romanian Black Sea Coast (BAVARU *et al.* 1995, 1999; DOROFTEI *et al.* 2000, 2002, 2004, 2007a, 2007b, 2007c, 2007d; SAVA 2006).

The number of Rodophyta inhabiting the Romanian Black Sea Coast appeared to be a large one with 69 species observed on the Black Sea coast. They belong to one class, Rhodophyceae, two subclass Bangiophycidae and Florideophycidae, and eight orders: Goniotrichales, Bangiales, Acrochaetiales, Gelidiales, Cryptonemiales,

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Gigartinales, Rhodymeliales, Ceramiales, (BAVARU *et al.* 1991). Morphology of the Rhodophyceae ranges from unicell (which are rare) to filamentous and pseudoparenchymatous forms. There is no truly parenchymatous growth, and the majority of species grow in a highly organized apical manner, with the exception of some Bangiophycidae. Some members of more advanced orders may exhibit intercalary growth (some Cryptonemiales and Ceramiales) (BOLD 1978; VAN DEN HOECK 1997).

Numerous researchers in the world have been attracted to the huge algae exploration possibilities in various domains and they have been searching for solutions not only for study but also for the exploitation of this precious marine flora and its use in different fields of activity to the use of human kind.

The Black Sea is a semi-closed sea. It communicates with the Mediterranean through the strait system Bosphorus-Marmara-Dardanelles and with the Azov Sea through the Kerch strait. It was believed that the Black Sea is a continental sea with a basin made up of a deepening in the crust, with layers above the plates. However, it was discovered that the abyssal plain leans directly on the tectonic plates; therefore it is not a continental sea. In the north-western corner of the sea, the river water flows cannot mix with the sea water as they are pushed by the north winds towards south forming the cyclonal current of the Black Sea, which leads to the covering of the sea by a layer of mixohaline water with a density lower than that of the water above. As a consequence to the difference in density in the Black Sea, there are no vertical currents, therefore, the profound waters are not oxygenized. Thus, the deep water is anoxic, improper to multi-celled life and the Black Sea is a stratified sea, multi-celled life existing down to 200 meters. The environmental factors vary brutally and amply from one place to another and from one moment to another. The specific diversity is thus quite reduced. The Black Sea is an impoverished pocket of the Mediterranean Sea. As compensation, the biomass of hydrobionts is very high, with an average of 100-200g/m<sup>2</sup>. All these properties make the Black Sea a "unicum hidrobioticum."

## MATERIAL AND METHODS

In view of accomplishing this paper, samples were collected from the Black Sea in March, when the water temperature was 9°C and the air temperature was between 10-13°C, from the midlittoral zone, from rocky substrate. The algae were collected from the substrate and deposited in seawater containers. Then they were brought to the laboratory for the taxonomic identification.

In the laboratory, the algae were pressed on herbarium

sheets for their determination and detailed analysis. Most times, the samples contain algae from different phyla, which is why their sorting on systematic groups is necessary. At this stage, the algae are determined as much as possible because some features are difficult to identify after drying and pressing. A piece of polished glass or a tile is used to place the paper on which the alga will be fixed.

The following procedure is used:

1. The piece of glass or tile is placed in the water container with the alga.
2. With the help of a spatulated needle or a pair of tweezers, the alga shape is adjusted and its ramifications are arranged as close as possible to their natural orientation.
3. The plate is inclined softly in order to remove the water excess. The paper on which the alga is fixed is detached from the glass plate and stuck to a vertical support in order to drain the water.

The drying and pressing stage is accomplished placing the paper with algae between sheets of newspaper or blotting paper. In order to prevent the algae from sticking to the newspaper, we protected the thalli with pieces of gauze. In order to press the algae, we placed a few kilo weights on top of the pile of sheets. The drying time interval is not fixed as it depends on the algae consistency and the rhythm of changing the gauze and newspapers between the herbarium sheets.

Transversal sections were made through the thallus of the identified algae in order to realize the microscopic preparations. The vegetal material from the Black Sea was fresh. The sections were realized with an unused shaving blade, an instrument frequently used in the laboratory. The advantage is that a considerable number of sections are done very quickly with a minimum of instruments. Transversal sections were accomplished, the blade cutting through the vegetal material perpendicularly on its axis. The piece of vegetal preparation is placed on a slide, it is covered with cover slip and then it is analyzed under the microscope. If the preparation is too thick and does not offer a clear image of the algal cells, the squash technique is employed, meaning that the slide with the preparation is pounded with a small stick in order to separate the cells.

For the ultrastructural observations the algal thalli were processed by the electronmicroscopic technique through transmission (HAYAT 1972; REYNOLDS 1963). Strips of thalli were cut out of the algal thallus from different regions: basal, middle and apical region. The samples were cut in the shape of thallus fragments of 2mm/2mm and were prefixed in tampons of sodium cacodylate (0.1N) with glutaraldehyde (2.5%). After the prefixation the specimens were washed in tampon cacodylate (0.1N) and fixed in solution of osmium tetra-oxide (2%). The specimens were washed again in tampon cacodylate (0.1N) to remove the osmium excess and dehydrated in serial baths of alcohol

of 30%, 50%, 70%, 90%, 95%, 100%, 15 -30min each. The baths are executed at the room temperature. In order not to affect the cell osmolarity all the solutions were prepared with filtered and tinalized seawater.

After the dehydration the samples were kept overnight in a mixture of propylene oxide with epoxidic resins of the type Epon 812 and DMP-30 as a hardening agent in order to introduce the warm resin polymerization. Subsequently are placed in plastic capsules, covered with Epon 812 and then placed for polymerization in sterilizers at 67°C for 48-60 hours.

The semifine and ultrafine sections were obtained with the aid of an Ultracut-R ultramicrotome. The semifine sections are plucked out of the bath with a thin wire loop and put on a degreased port-object blade. The coloration is made applying on the blade 2-3 drops of the solution of toluidine blue, then the blades are set back in the thermostat at 60°C for 10-30min, washed in flowing water, then in acetone (100%), rapidly passed through xilol, blotted, assembled and examined under the photonic microscope. The ultrafine sections of 400-600Å are placed on metallic grills and double contrasted with uranyl acetate and lead citrate. The grills were observed under the performing electronic microscope Philips CM120 and the images obtained were processed by a video camera.

## RESULTS AND DISCUSSIONS

The samples were collected from the Black Sea in the cold season. In the cold season, among the samples collected from the Black Sea, we identified five species of multicelled species of algae.

*Callithamnion corymbosum* (o. Ceramiales, fam. Ceramiaceae), macroscopically, has the thallus ramified pseudo-dichotomically (Plate I, A), the filaments emerging from two basal regions, disposed alternately and ended with one little hair. It has a delicate aspect and looks like reddish-pink rounded bushes (RUENESS & RUENESS 1985; WHITTICK 1978, 1989, 1992). The attachment to substrate is done by means of small adhesive discs. The thallus cells have several nuclei and contain numerous discoid plastids without pyrenoids. The filaments emerging from two regions of the basal branches disposed alternately and ended in one little hair. It has a delicate aspect and it looks like reddish-pink round bushes. The attachment to the substrate is done by means of small adhesive discs. Carpogones are formed on the gametophyte, on the cells placed laterally on certain basal branches; also, the spermatocysts gathered in fascicles which occur at the surface of the thallus or at the ends of certain branches (Plate I, B,C,D). Under the microscope, certain antheridia can be seen on the little branches gathered in small fascicles, and disposed irregularly (Plate I, B). The cystocarps can be

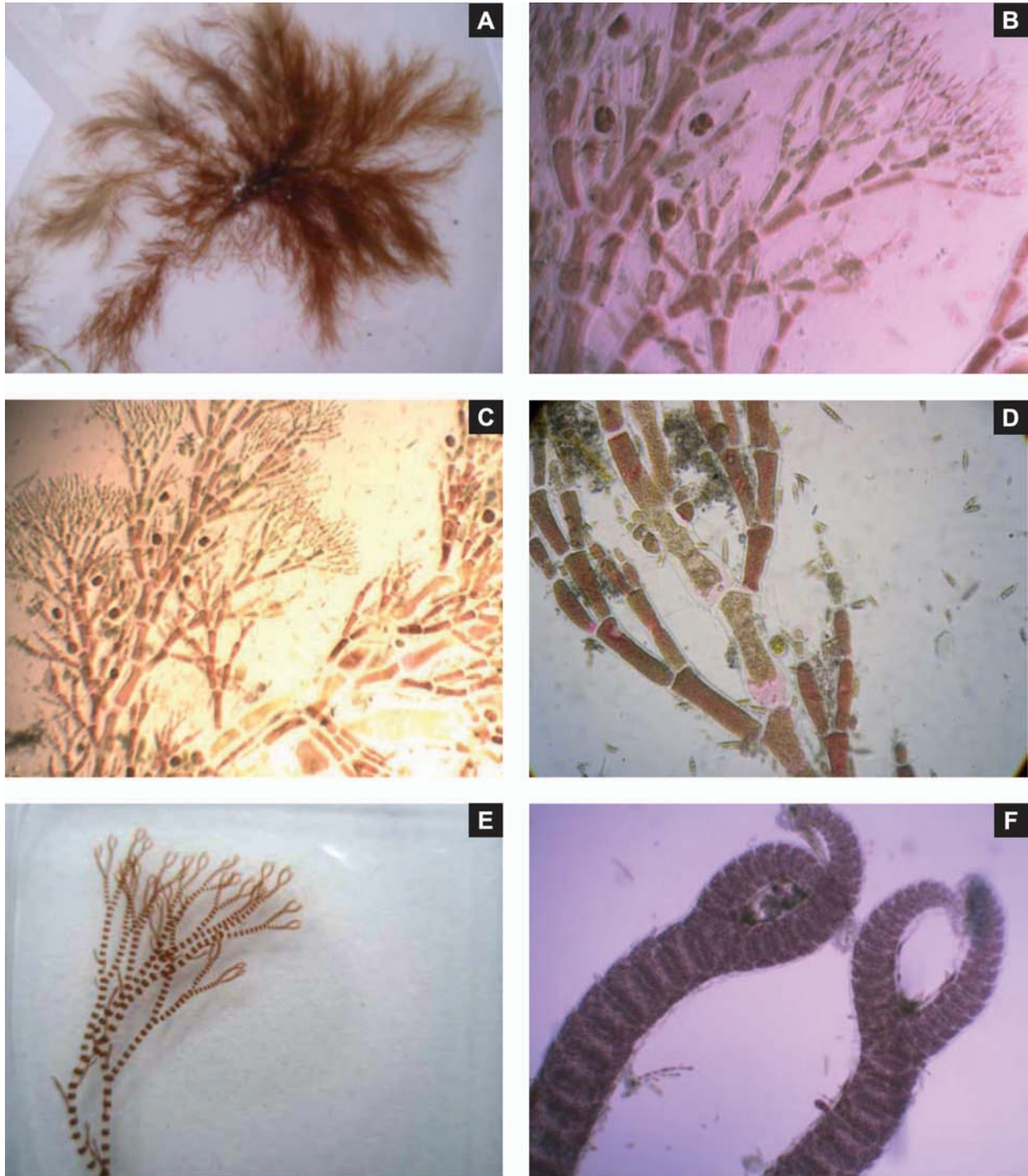
seen placed on the branches in pairs, without a membrane of their own (Plate I, B,C).

It is an annual species present in shaded areas with calm waters, from small to big depths in the infralittoral. It is present all year-round at the Romanian littoral, on hard substrate, with abundant development in spring and autumn.

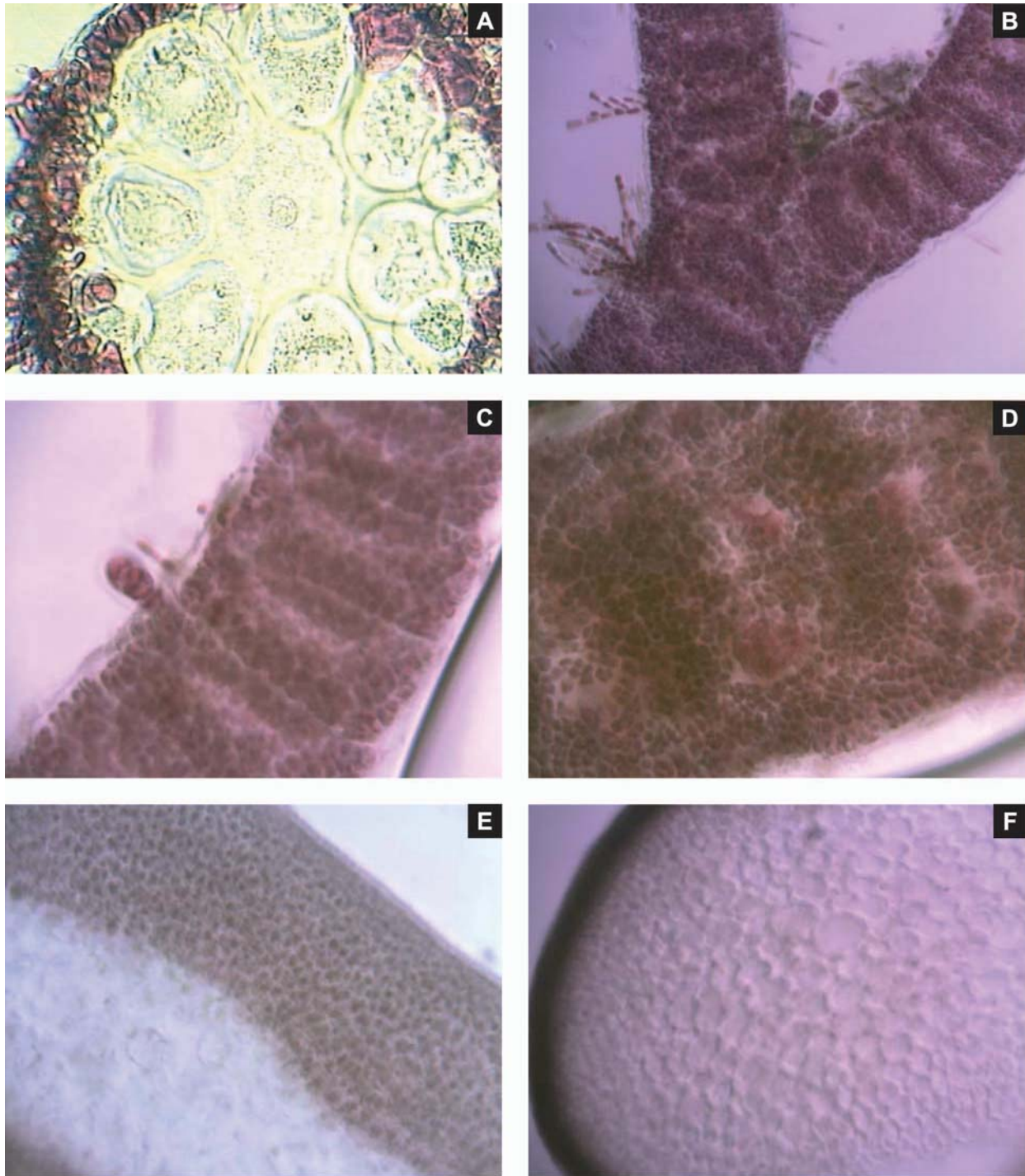
*Ceramium rubrum* (o. Ceramiales, fam. Ceramiaceae) looks like a dark-red filamentous bush made up of erect filamentous fronds with dichotomic ramification (Plate I, E, F). It attaches to a substrate by means of rhizoids. The growth occurs from an initial cell (which can be masked) present at the apex level. The thallus filaments, observed under objective 20x or 40x are made up of a string of cells, their heads meeting at the level of nodes. The thallus appears covered on its entire surface by a cortex represented by a layer of small cells, fused together, resulted from the continuous division of the periaxial cells. The length of the cells at the base of the filaments is twice as much as their width. Each ramification ends with two short arms that form a small pair of pincers (Plate I, E, F). The thallus filaments are made up of a string of cells, their heads meeting at the level of the nodes (Plate I, E, F; Plate II, B, C, D). The thallus is covered on its entire surface by a cortex represented by a layer of small cells, fused together, and resulted from the continuous division of the periaxial cells (Plate I, F; Plate II, A, B, C, D). The length of the cells at the base of the filaments is twice as much as their width. The carpogonial branches are formed in a pericentral cell located toward the top of the frond. The tetrasporocysts are shaped sub-spherically, with tetragonal division, located externally at the level of nodes (Plate II, B, C). The cystocarps without a membrane of their own are surrounded by a rosette of short and thin branches (Plate II, D). In transversal section through the median area of the thallus, there is a big cell in the center, surrounded by pericentral cells, usually eight, and themselves surrounded by the layer of cortical cells (Plate II, A). In the axial cell, the plastids are big, shaped like ribbons, while in the peripheral cells the plastids are small and discoid.

*Ceramium rubrum* (o. Ceramiales, fam. Ceramiaceae) is an annual species which colonizes the rocky substrates in mid- and infralittoral, in the zones exposed to wave activity. It is often epiphyte (on large algae). *Ceramium* is a cosmopolitan genus characterized by great specific diversity with more than 191 species described all over the world (BOO 1993; FUJII *et al.* 2001; CHO *et al.* 2003; MAGGS & HOMMERSAND 1993). At our littoral, it can be found all year-round, with better development in spring and summer.

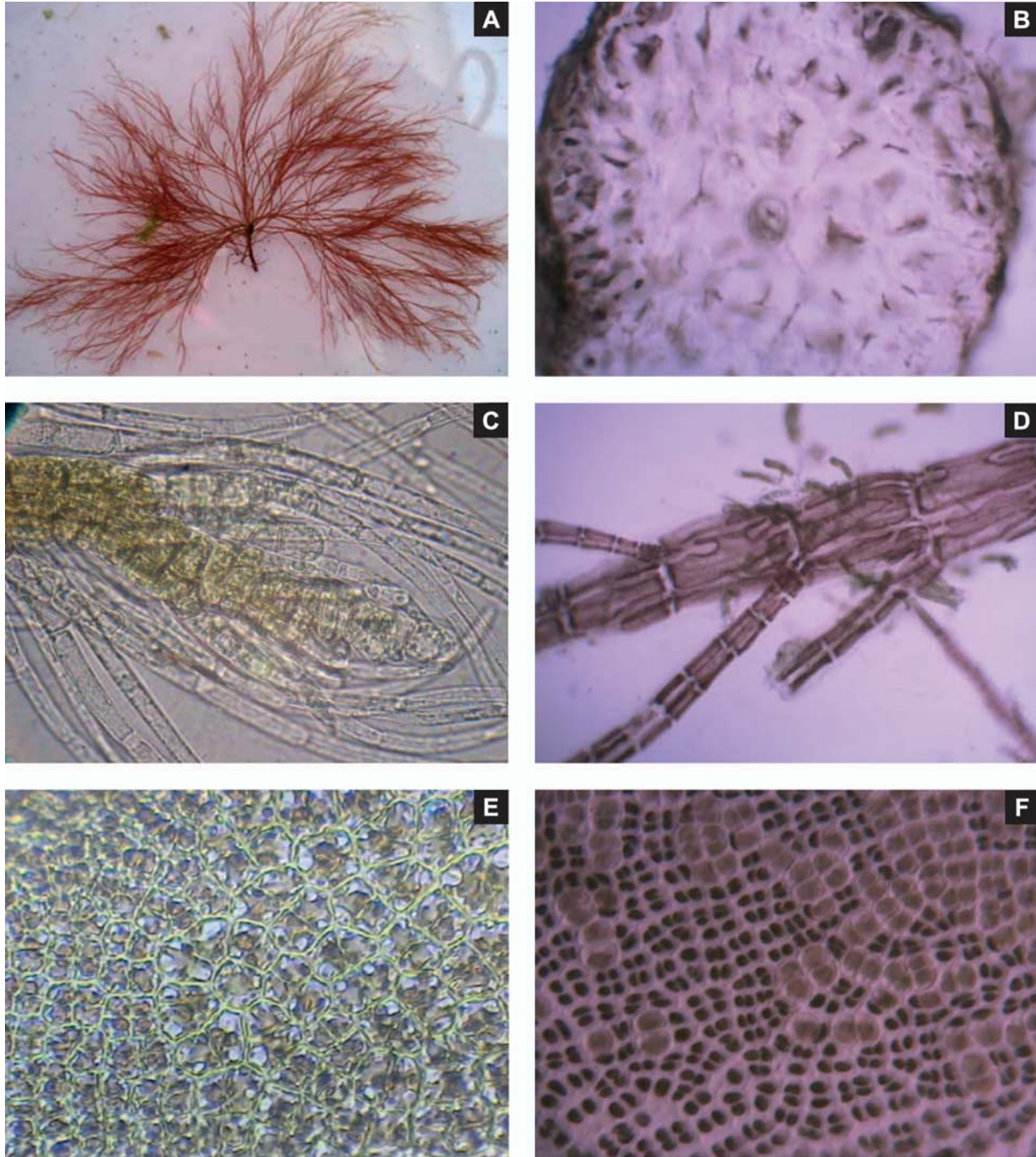
*Phyllophora nervosa* (o. Gigartinales, fam. Phylloporaceae) is an alga with ribbon-shaped thallus which ends with a discoid sole used to get attached to the substrate.



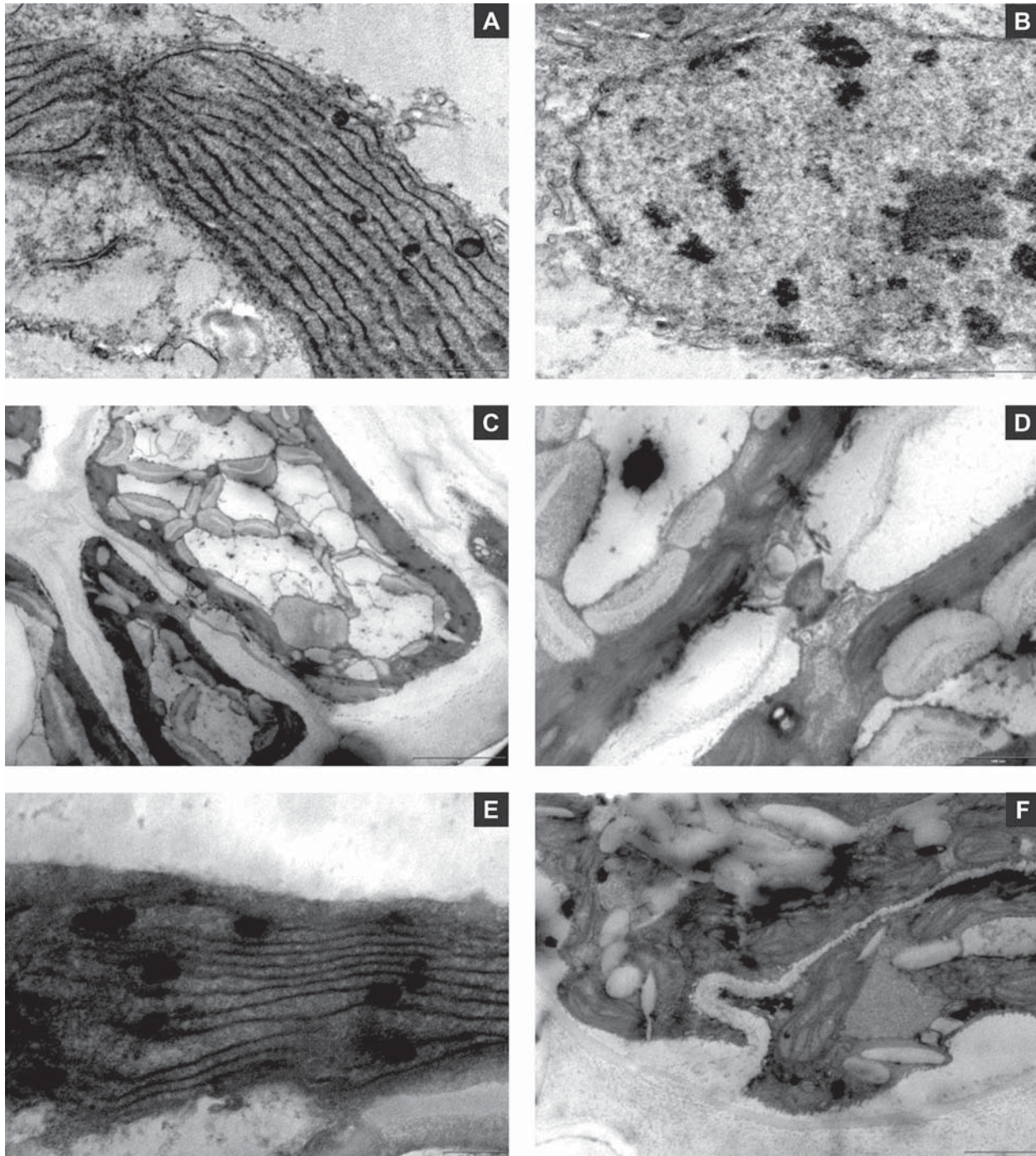
**Plate I.** A. *Callithamnion corymbosum* macroscopic aspect of the thallus. B. *Callithamnion corymbosum* apical region with carpagones. C. *Callithamnion corymbosum* apical region with carpagones. D. *Callithamnion corymbosum* medial region of the thallus with tetraspores. E. *Ceramium rubrum* macroscopic aspect of the thallus with dichotomic ramification. F. *Ceramium rubrum* microscopic aspect of the thallus with dichotomic ramification.



**Plate II.** **A.** *Ceramium rubrum*, transversal section with a big cell in the center, surrounded by pericentral cells, usually eight, and themselves surrounded by the layer of cortical cells. **B.** *Ceramium rubrum* apical region of the thallus with carposporophyte. **C.** *Ceramium rubrum* - carposporophyte. **D.** *Ceramium rubrum* - on the surface of the thallus are observed tetrasporocysts (light red coloured). **E.** *Phyllophora nervosa*, transversal section through the thallus with a cortical layer made up of 1-2 strings of small cells and an internal layer with cells much bigger in diameter. **F.** *Phyllophora nervosa*, transversal section through the basal region of the thallus, the cortex cells from the base of the thallus have thickened walls with role in supporting the thallus.



**Plate III.** A. *Polysiphonia denudata*, macroscopic aspect of the thallus. B. *Polysiphonia denudata*, transversal section through the apical region of the thallus, with one axial cell surrounded by four periaxial cell and two or three layers of cortical cells. C. *Polysiphonia denudata*, apical region of the thallus with trichoblasts. D. *Polysiphonia denudata*, medial region of the thallus with articular cells and spermatocysts. E. *Porphyra leucosticta*, the medial region of the thallus with cells which contains one stellate chromatophore. F. *Porphyra leucosticta* the medial region of the thallus with less colored areas representing the sporocysts developing on the same area of the thallus with strongly pigmented carpogones.



**Plate IV.** **A.** *Callithamnion corymbosum*, plastids with parallel thylakoids. **B.** *Callithamnion corymbosum*, the nucleus with nucleolus and blocks of heterochromatin. **C.** *Ceramium rubrum*, section through the medial region of the thalli. It can be observed the pit connection or "pit plug" formed between two adjacent cells. **D.** *Ceramium rubrum*, section through the medial region of the thalli. It can be observed the pit connection or "pit plug" formed between two adjacent cells. **E.** *Polysiphonia denudata* plastids with parallel thylakoids. **F.** *Polysiphonia denudata*, section through the medial region of the thalli. It can be observed the pit connection or "pit plug" formed between two adjacent cells.

A short flattened "stalk" emerges from this sole. This is continued with numerous dark-red lamellate ramifications. The edges of these ramifications are undulated with an axis in the middle imitating a nervure. In transversal section through the thallus (objective 20x), there is a cortical layer made up of 1-2 strings of small cells, while the internal layer is made up of an approximately equal number of cells with a much bigger diameter compared to the cortex cells (Plate II, E, F). The cells (objective 40x) contain numerous plastids located in parietal position, without pyrenoids. The cortex cells from the base of the thallus have thickened walls with role in supporting the thallus (Plate II, F).

*Polysiphonia denudata* (o. Ceramiales, fam. Rhodomeleaceae), marine alga, with ramified thallus which varies over the year, being reduced to the main axes in autumn, which are covered by numerous small ramifications in spring. The thallus cladonia are made up of a caulid looking like a rod, covered in cortical pleurids with phylids shaped like leaves, reduced to trichoblasts (Plate III, A, C). The trichoblasts are ramified hyaline hairs which usually occur in the growing tip. The thallus appears articulate because of the numerous horizontal strings that surround the central filament. In transversal section through the median area of the thallus, the multiaxial structure of the thallus can be observed and it appears in the following way: the apical cells (growth cells) produce a central axial cell which elongates and divides forming periaxial cells (Plate III, B), which in turn elongate them. There are approximately 5-8 cells which form the pericentral layer, but they are less developed in the apical growth area (Plate III, B). Under objective 40x, it can be observed that there are several plastids without pyrenoids in the cells. It is a perennial alga. It lives in infralittoral on rocks from depths of 5-6m, between spring and autumn.

*Porphyra leucosticta* (order Bangiales, fam. Bangiaceae) has a reddish-purple thallus, represented by a monostromatic blade, foliaceous and fragile. This blade narrows at the base and forms a short stipes which continues with an attachment formation. It is an arctic alga which develops abundantly at the Black Sea in cold seasons, early in spring till the beginning of June, on rocks at the shore down to depths of 4-5 meters. Under microscope (objective 40x), the thallus cells contain one stellate chromatophore with one colorless pyrenoid, one nucleus as well as the assimilation product, the floridean starch which can be found outside the plastids as large discoid granules which surround the chromatophore (Plate III, E, F). Under objective 20x, small cells can be seen in the median area, with thick cell walls, as well as frequent division processes and a visible stellate chromatophore (Plate III, F). The characteristic of the median area of the thallus is the presence in its center of a growth zone with small cells resulted from repeated divisions, the *Porphyra*

thallus growing in surface by alternative longitudinal and transversal bipartitions. Also in the median area of the thallus there are less colored zones representing the sporocysts developing on the same area of the thallus with strongly pigmented carpogones, with thick cell walls represented by diads and tetrads (Plate III, F). The fecundation occurs by the union between a spermatia nucleus and the feminine nucleus as the former enters the oosphere through the carpogone papilla.

At the ultrastructural level, we can observed, on the fine sections, that the structure and the organization of the rodophyte thylakoids show a primitive homology with those of blue-green algae, with parallel thylakoids whose margins abut against the inner membrane of the plastidial envelope (Plate IV, A, C, D, E, F). The chloroplasts have an envelope comprises two parallel membranes (Plate IV, A, E). Inside the chloroplast there are many thylakoids which contain photosynthetic pigments and significant arrangements. Red-algal thylakoids typically are one per lamella and lamellae are parallel to each-other and with the longitudinal ax of the chloroplasts (Plate IV, A, E).

Plastids are usually found in the peripheral cytoplasm of algal cells and are characterized by the presence of portions of a peripheral thylakoid and coiled lamellar bodies that range in size up to 0.5 micron. One type of coiled lamellar body occurs in the peripheral region of the plastid and is continuous with the peripheral thylakoid, while the other type is found in the central portion of the stroma. These coiled lamellae separate and expand, adding membranes to both thylakoid systems, thereby functioning as thylakoid-forming bodies. As each coiled lamella unravels, it forms an undulated double-membraned structure having the same width as a thylakoid. After substantial expansion, the developing thylakoids begin to straighten and assume a parallel orientation to each other, thus becoming mature thylakoids. Small-coiled lamellae often persist in mature chloroplasts, and are utilized in additional thylakoid formation (Plate IV, A, E).

The storage product is floridean starch, which lies freely in the cytoplasm. Starch, when stored, is held in the cytoplasm, outside the chloroplast (Plate IV, C, D, F). The cortical cells of *Callithamnion* and *Porphyra* thallus are uninucleate, the nucleus has medium size, with a double nuclear envelope, a ground substance or nucleoplasm, chromosomes and one or two nucleoli, which are densely staining concentrations of basophilic material rich in ribonucleoprotein (Plate IV, B). There are numerous mitochondria in apical region and a small number in the basal region of the thallus, while the endoplasmic reticulum seems to be well developed in middle and especially in apical region of the thalli. There are also microbodies (ca 0.2  $\mu\text{m}$  in diameter). The cell wall surrounds the cells and is comprised of electron-dense reticulated cellulose



microfibrils embedded in an amorphous matrix, with a randomly arrangement. Cell wall thickness decreased from very thick in discoid base and basal region of the thallus to thin in apical region of the thallus (Plate IV, C, D, F).

A specialized feature is the pit connection or “pit plug” which has been linked with intercellular transport, structural strengthening in filamentous and pseudoparenchymatous thalli (Plate IV, C, D, F). In the rhodophytes primary pit plugs are formed between two cells during division and secondary pit plugs develop between laterally adjacent cells. The plugs are usually biconcave, with a central constriction (Plate IV, D).

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## Strukturne i ultrastrukturne karakteristike Rhodophyta Rumunske oblae Crnog Mora

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Uradu su dati rezultati morfoloških i ultrastrukturnih istraživanja crvenih algi *Polysiphonia denudata*, *Ceramium rubrum*, *Callithamnion corymbosum*, *Phyllophora nervosa* i *Porphyra leucosticta* sa obala Crnog mora u Rumuniji.

Eksperimentalni deo istraživanja obavljen je od juna 2006. do aprila 2009. u laboratoriji za elektronsku mikroskopiju univerziteta „Ovidius” u Konstanci. Preseci različitih delova talusa su posmatrani pod svetlosnim i Novex Holland fotonskim mikroskopom bez bojenja. Takođe je korišćen i elektronski mikroskop Philips CM120 za posmatranje delova talusa.

Na ultrastrukturnom nivou su komparitivno analizirani izraziti karakteri: plastid sa jednostavnim tilakoidima, dvoslojna membrana koja obavija hloroplast, nedostatak hloroplastnog ER, pit-konekcija ili pit-plagovi, floridni skrob kao rezervni materijal slobodan u citoplazmi, talus sa jednojedarnim ili višejedarnim ćelijama, jedra sa jednim ili dva jedarca, kao i ćelijski zid koji se sastoji od neravnomerno raspoređenih celuloznih fibrila.

**Ključne reči:** *Polysiphonia denudata*, *Ceramium rubrum*, *Callithamnion corymbosum*, *Phyllophora nervosa*, *Porphyra leucosticta*