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MORPHOLOGICAL AND ANATOMICAL INVESTIGATIONS OF THE SPECIES *FURCELLARIA FASTIGIATA* (HUDS.) LAM. FROM PUCK BAY

1. INTRODUCTION

Notwithstanding the fairly extensive achievements of floristic-ecological studies [6, 9, 10, 11, 16, 18, 19] hitherto carried out, as also of investigations conducted from the point of view of changes taking place in the biocenosis of Puck Bay [7, 8, 13, 17], there is a lack of studies referring to an analysis of morphological and anatomical characteristics of the species *Furcellaria fastigiata* (Lam.), which shows considerable plasticity depending upon the environment.

Investigations carried out over the period 1962—1969 concerning occurrence of this species showed that the quantitative state of *F. fastigiata* [8, 17] is declining. It was likewise found that pollution exerts an unfavourable influence on the growth of *Furcellaria fastigiata* in Puck Bay [7].

The aim of the present study was to investigate specific diagnostic properties of *F. fastigiata* such as the type and form of the thallus, size of cells, chemical nature of cell walls and location of reserve substances.

2. MATERIAL AND METHODS OF INVESTIGATION

Furcellaria fastigiata (Lam.) thalluses were obtained from the western part of Puck Bay on a level with the villages of Osłonino and Rzucewo (Fig. 1). Samples were taken at various depths in the autumn of 1968 and the spring of 1969.

The material for investigation consisted of the growing points of the thallus more or less 2 to 5 mm long and kept in two fixing agents — Navashin and Carnoy [5]. After fixing and washing the material was put through an alcohol-xylene-paraffin series and sealed in paraffin

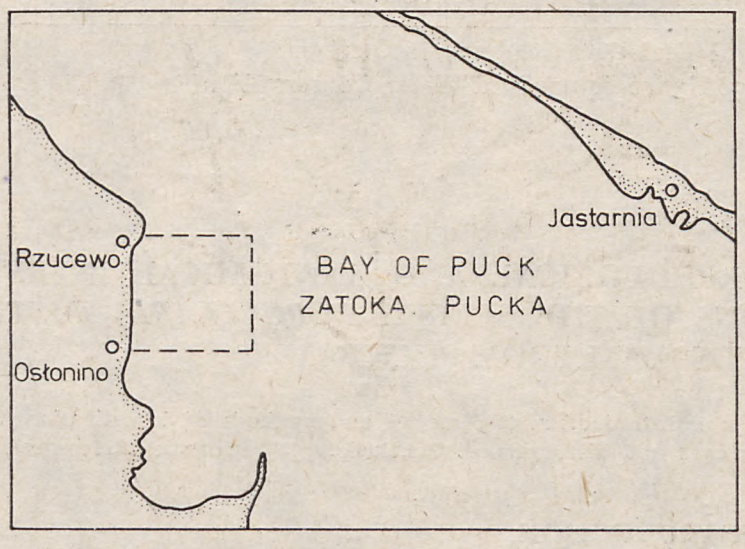

Fig. 1. Sampling region
Rys. 1. Rejon pobierania prób

with a melting point of 54°C supplied by the firm of Merc. Slices of 10 to 15 microns (after Navashin fixative) were prepared on a Zeiss Minot type microtome. Permanent preparations were dyed with gentian violet according to Newton and differentiated in a mixture of phenol at a ratio of 1:3. The smear method was also used in the investigations [14, 15]. Carnoy fluid was used as a fixing agent. Carmine dissolved in acetic acid, which gives an intensive colouring to embryos and chromatophores, was used for staining.

In order to determine the chemical nature of cell walls the following cytochemical reactions were applied:

for pectin — ruthenic red alkalized slightly with ammonia,

for cellulose — iodide in potassium with zinc chloride.

After determining that the material contained large amount of phloridine starch it was tested with Lugol fluid (J+KJ).

Observations and photographs were made by using an NfpK Zeiss microscope. Measurements of cell size and starch particles were carried out by means of a Zeiss ocular micrometer. Cell dimensions presented in the text were obtained on the basis of averages for 50 cells.

3. RESULTS OF INVESTIGATIONS AND DISCUSSION

Thalluses of *F. fastigiata* from Puck Bay have a highly-branched, bush-like form. Chondrified, cylindrical threads of the thallum branch dichotomically, and form additional, lateral „of-shoots" (Fig. 2). The branch-

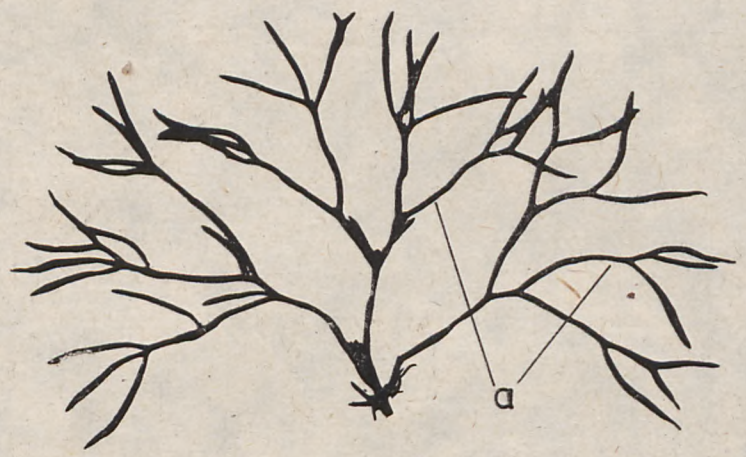

Fig. 2. *Fastigiata* (Huds.) Lam. from Puck Bay; a — lateral „off-shoots"

Rys. 2. Pokrój plechy *F. festigiata* (Huds.) Lam. z Zatoki Puckiej; a — „odrośla" boczne

ings were of various thickness along the whole length of the thallus. Their thickness ranged from 0.5 to 2 mm, with the distance between particular branchings varying. The lateral „off-shoots" did not differ in their construction from the main ones. Straight or forked parts of the thallus led, as a result of dichtomia, to the formation of bush-like forms. The colouring of the thalluses under investigation was most frequently brownish-red with a purple hue, after drying — black. The tip parts of the „off-shoots" were considerably lighter.

Retention of rhizoids in *F. fastigiata* belongs exclusively to one of the morphological adaptabilities of the species as a link with the bottom environment. *F. fastigiata* from Puck Bay has a spherical form despite the presence of rhizoids thereby adapting the thallus to float in the water body. There is no data given in literature as to whether root absorbtion is of significance for optimal growth of the thallus. Recent investigations [7] indicated seasonal changes in growth of the thallus *F. fastigiata*. Most intensive vegetative development of these algae was noted during the autumn and winter months. *F. fastigiata* communities near Aberystwyth in Wales showed optimal growth of the thallus in spring (March to May). These plants attained maturity between the fourth and sixth year of life in their natural environment.

Thalluses of *F. fastigiata* within the *Florideae* class are classified to the full, multiaxial [12] type of form. Cells of the thallus of this species were distinctly differentiated both as concerns construction, shape and size. A number of prosenchymatous cells run parallel within the thallus forming a center core from which lateral branchings separate at regular intervals. Each of these has its initial cell. The central part of the thallus branches forming a bark consisting of an internal

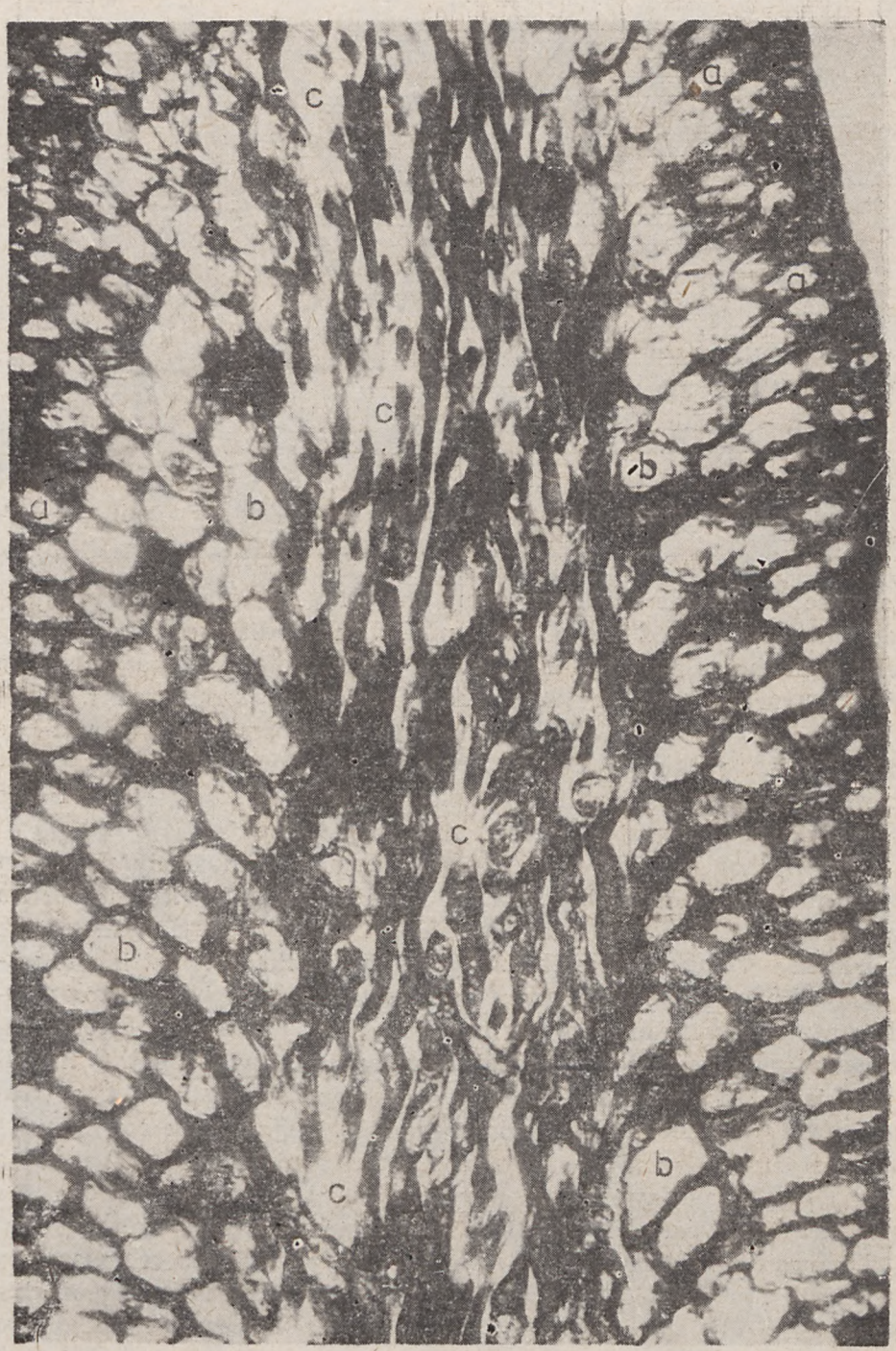

Fig. 3. Longitudinal cross-section through the top part of the *F. festigiata* thallus; *a* — cells of the external bark, *b* — cells of the internal bark, *c* — cells of the central core

Ryc. 3. Przekrój podłużny przez wierzchołkową część plechy *F. festigiata*; *a* — komórki kory zewnętrznej, *b* — komórki kory wewnętrznej, *c* — komórki rdzenia centralnego

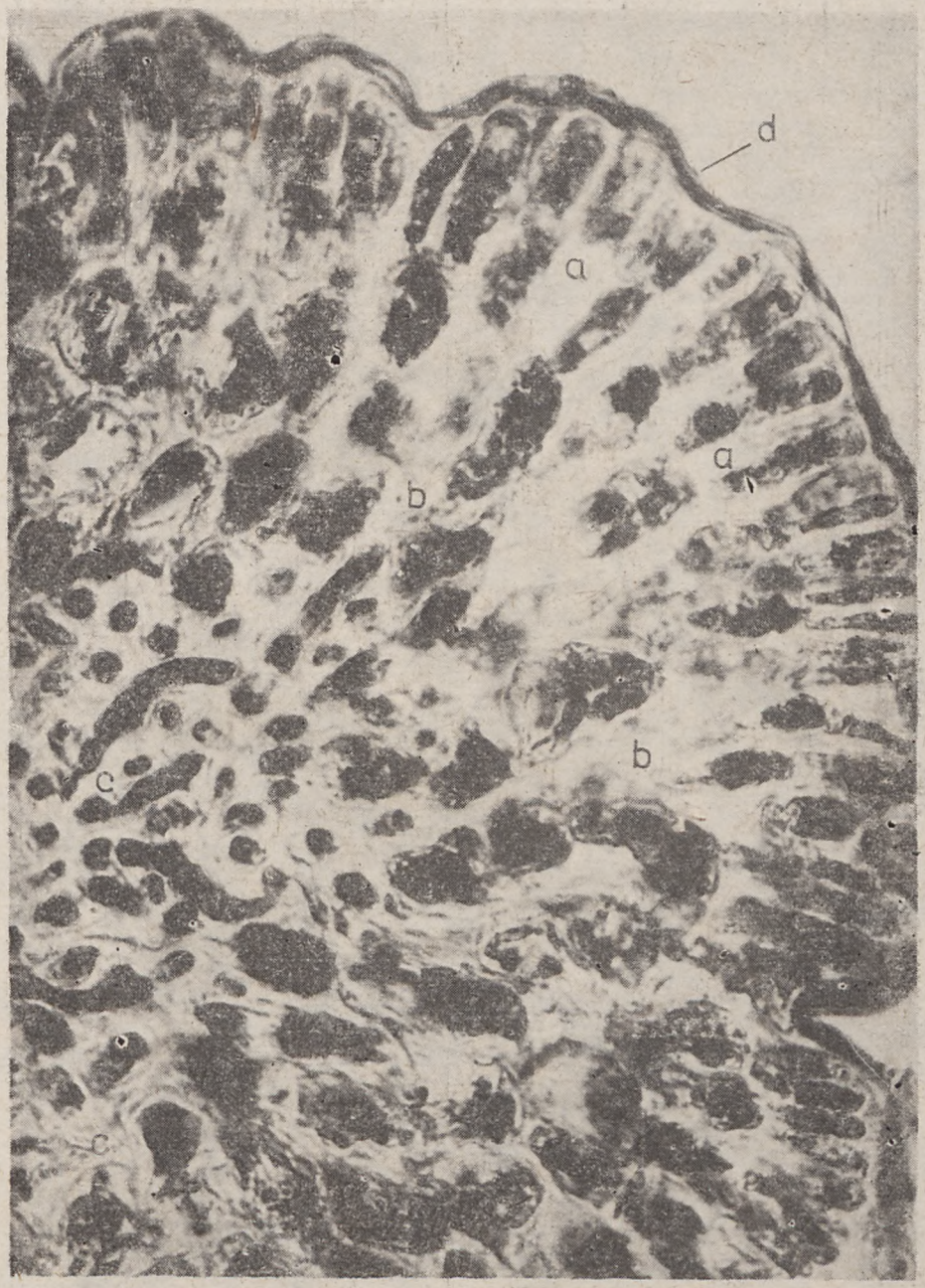

Fig. 4. Transversal cross-section of the *F. festigiata* thallus; *a* — cells of the external bark, *b* — cells of the internal bark, *c* — cells of the central core, *d* — mucous layer covering the thallus

Rys. 4. Przekrój poprzeczny plechy *F. festigiata; d* — warstwa śluzu okrywającego plechę, *a—c* — komórki poszczególnych warstw plechy

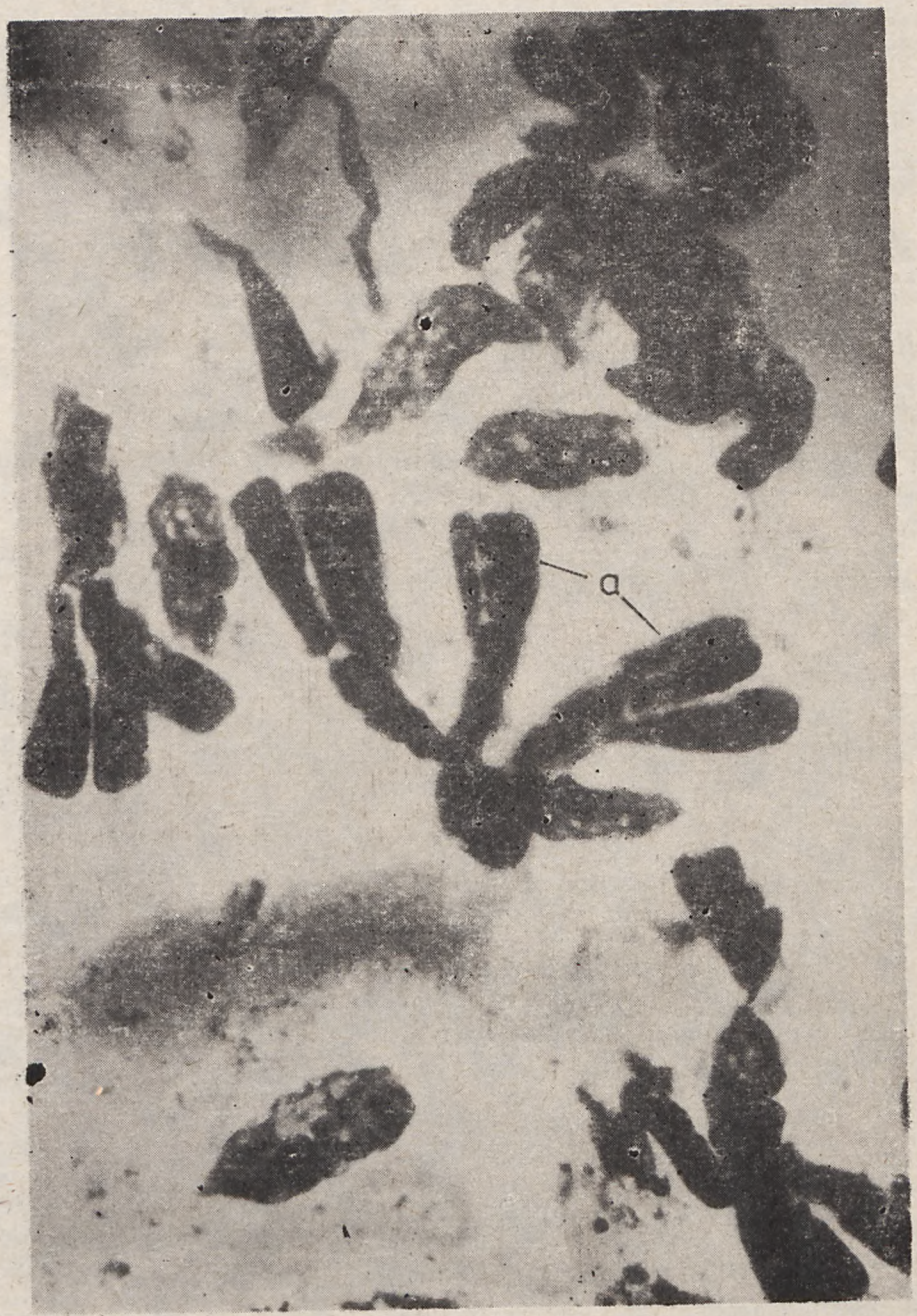

Fig. 5. Dichotomous branching of single cells of the external bark of *F. fastigiata*; *a* — shape of cells

Rys. 5. Dichotomiczne rozgałęzianie się pojedynczych komórek kory zewnętrznej *F. festigiata*; *a* — kształt komórek

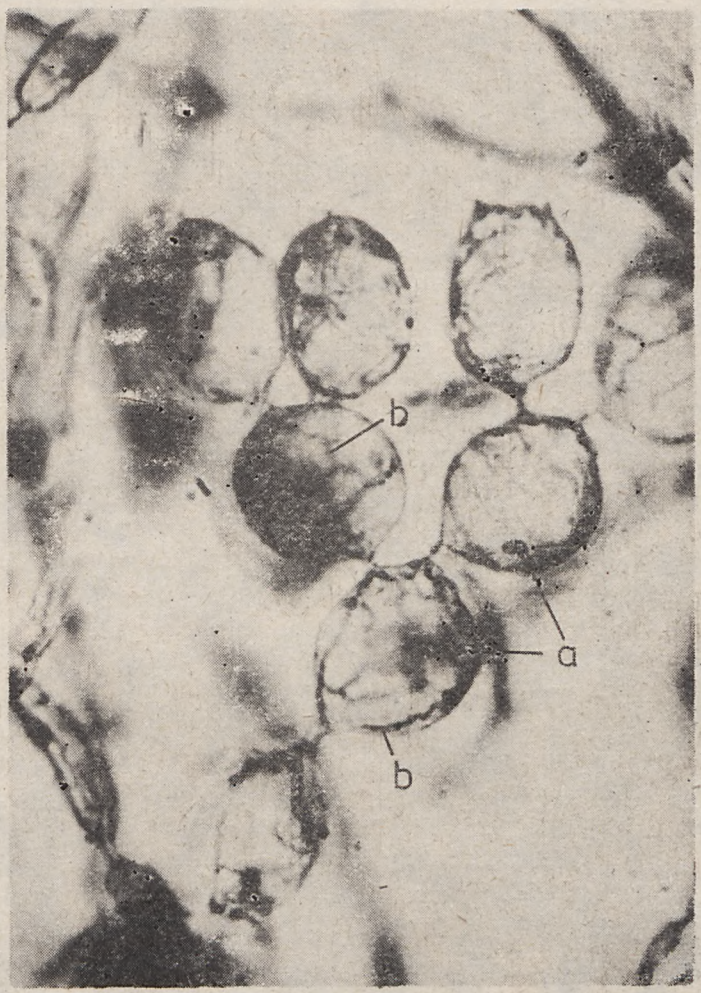

Fig. 6. Dichotomous branching and shape of cells of the internal bark of *F. fastigiata*; *a* — cell nuclei, *b* — chromatophores

Rys. 6. Dichotomiczne rozgałęzianie się i kształt komórek kory wewnętrznej *F. fastigiata*; *a* — jądra komórkowe, *b* — chromatofory

and an external part (Fig. 3, 4). Cells of the internal part branch dichotomically forming consecutive cells which, in turn, form the cells of the external structure (Fig. 5, 6). Cells of the bark layers formed in this way are linked to each other by a short protrusion of the wall (Fig. 6, 8).

As a result of investigations by the author, three types of cells were differentiated in the multi-cell thallus of *F. jastigiata:* pear-shaped cells forming the outer layer of the bark, oval cells of the internal part of the bark and thread-like cells of the central core (Fig. 5, 6, 7).

The external part of the bark is formed of one layer of pear-shaped

Fig. 7. Smear preparation of the *F. fastigiata* thallus; *a* — cells of the internal bark, *b* — cells of the central core

Ryc. 7. Komórki rdzenia centralnego (*b*) i kory wewnętrznej (*a*) *F. fastigiata*

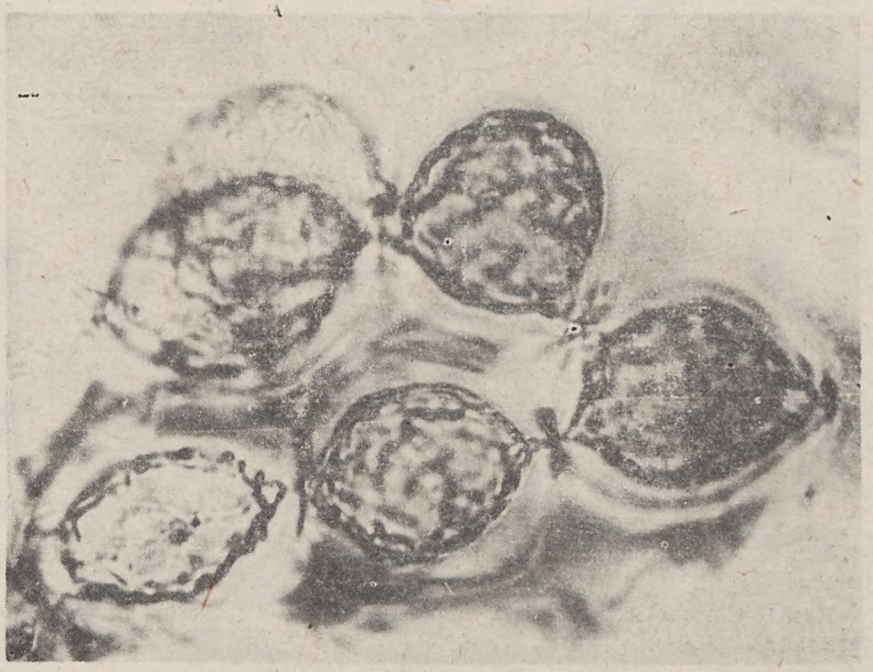

Fig. 8. Connecting of external bark cells by short out-growths of the cell wall in *F. fastigiata*

Rys. 8. Połączenia komórek kory wewnętrznej krótkim uwypukleniem ściany komórkowej u *F. fastigiata*

cells which lie with their narrowing facing the mid-part of the thallus (Fig. 3, 4). Cells of the external bark adhere to each other and are surrounded by a layer of mucous. The close formation of this layer of cells is similar to the epidermis in higher plants. Length of the cells of the external bark ranged from 17.4μ to 37.8μ, whereas the width at the broadest part of the cells was from 14.4μ to 26.4μ.

The internal part of the bark in thalluses consisted of 3 to 5 — and in some of the durable slices 6 — layers of large oval cells (Fig. 3, 6, 7). This forming of layers is the result of periodic increase in thickness. Investigations showed that the increase in thickness of *F. fastigiata* is most evident in the layer of cells in the internal bark. Taking this fact into consideration it can be stated that apart from the elongational growth observed by other authors [1, 7] the mentioned growth in thickness constitutes a second sphere of *F. fastigiata* growth. The thickness of this layer has a direct influence on the amount of pectin substances.

Cells of the internal bark were fairly loosely arranged in the smear preparations, enabling more detailed observation. The cells of this layer and those of the external bark were connected by means of characteristic thin outgrowths due to which neighbouring cells were in direct contact (Fig. 6, 8). It is cited in literature that the wall separating two

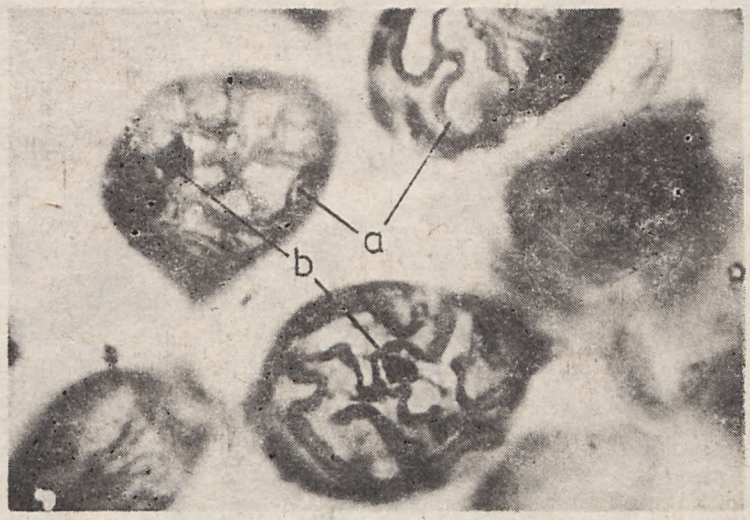

Fig. 9. Cells of the internal bark in *F. fastigiata*; *a* — chromatophores, *b* — cell nuclei

Rys. 9. Chromatofory (*a*) i jądra komórkowe (*b*) komórek kory wewnętrznej *F. fastigiata*

outgrowths is perforated and it is assumed that this type of linkage may be the result of genetic connection between cells of the complex, multicell thallus. The pores and plasmodesms in *Pheophyta* representatives have been described in detail by Bisalpurta [4]. This author is of the opinion that linkages between cells took place by plasmodesms passing through cell walls.

The oval cells of the internal bark observed on permanent preparations formed a compact layer of bark similar to the perenchyma in higher plants. The size of these cells varied. Larger cells were to be found nearer the central core and each particular layer consisted of cells decreasing in size towards the surface of the thallus. The mean length of the cells of this layer ranged from 38.1μ to 60.0μ, their width from 36.0μ to 54.0μ.

The part making up the core of the *F. fastigiata* thallus was formed of cells of various length, highly elongated, tubular in shape. These cells were connected to each other and enclosed in mucous-like surround. This part of the thallus, both in the permanent and the smear preparations was of a more compact structure than cells of the internal and outer bark (Fig. 3). The mean length of cells of this part of the thallus ranged from 108.0μ to 289.2μ, their width from 6.6μ to 22.2μ.

The interior of the living cell of *F. fastigiata* thallus was colourless. The cytoplasm of the cells under observation was not stained and adhered closely to the walls. Ribbon-shaped chromatophores without pyrenoids were arranged in the cytomplasm, their main parts branching

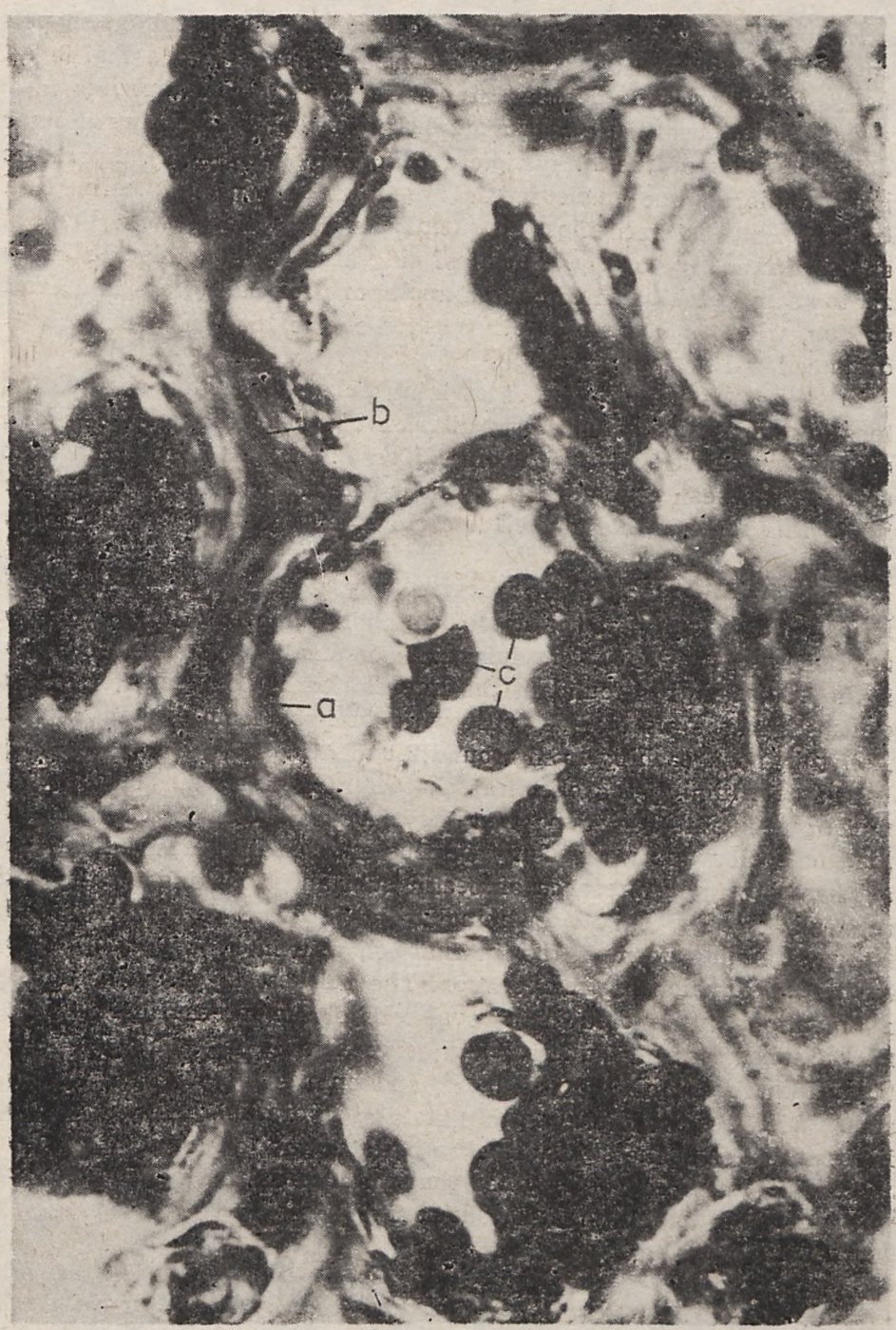

Fig. 10. Two-layer cell walls in *F. fastigiata* in cells of the internal bark; *a* — cellulose, *b* — pectin, *c* — starch in storage cells

Rys. 10. Skrobia w komórkach magazynujących kory wewnętrznej i budowa ścian komórkowych *F. fastigiata*; *a* — celuloza, *b* — pektyna, *c* — skrobia w komórkach magazynujących

partially and forming an irregular net work. Small oval or shapeless protuberances formed on some parts of the chromatophores (Fig. 6, 7, 9). Easiest to observe for detailed study were the chromatophores in the cells of the internal bark. Chromatophores of similar shape were observed in the cells of the *F. fastigiata* thallus by Austin [2]. Apart from chromatophores, one nucleus was noted in *F. fastigiata* cells. Nuclei observed in cells were most frequently oval in shape, with their size ranging from 2.1μ to 5.7μ. Nuclei of this alga were not located in one and the same position in cells. The nuclei of peak cells were in a central position, whereas in cells of other parts of the thallus they were next to the cell wall.

Despite the use of various staining substances, numerous observations of preparations showed no division of nuclei. On the basis of investigations carried out by Austin [2] on *F. fastigiata* from the Danish straits it has been concluded that this species is a diploid form. According to this same author nuclei of diploid cells contain 68 chromosomes [2n=68].

Floridine amylum is the reverse polysaccharide. It was found in *F. fastigiata* directly in the cytoplasm in the form of small, colourless amylum discs (Fig. 10). Amylum discs of smaller diameter than those in the external were found in the internal bark. The diameter of amylum discs in the algae under observation ranged from 4.5μ to 7.9μ with their thickness reaching from around 2.1 to 2.4μ.

In determining the chemical character of cell walls of the *F. fastigiata* thallus, it was found that they had two layers of cells (Fig. 10). External cell walls were highly viscous and capable of swelling. Staining has shown the presence of pectin substances making up the external walls of cells. They were found to be in direct contact with a thin layer of cellulose forming the internal cell wall in *F. fastigiata*, preventing its deformation or translocation. The thickest layer of the substances mentioned was noted in the oval cells of the internal bark (Fig. 10). Pectin substances are adhesive and serve as a linkage between cells of the internal bark on the one hand, and on the other, constitute a system which keeps the thallus in a state of hydration. This last property is connected with the ability to absorb water and ions of compounds dissolved therein. It can be assumed that the internal bark is most exposed to the effect of environmental pollution.

4. CONCLUSIONS

Results obtained permit the formulation of the following conclusions:

1. The thallus of *Furcellaria fastigiata* shows distinct morphological differentiation accompanied by corresponding differences in its anatomy.

2. Three types of cells make up the complex of cells forming the thallus of *F. fastigiata:* cells making up the bark, oval cells of the internal bark, and elongated cells of the central core.

3. Apart from the sphere of elongational growth of the thallus, a second sphere of growth was found in the internal layer of bark.

4. It was found that the cell walls consist of two layers — an external peptide layer and an internal cellulose one.

5. The thickness of the layer of cells making up the internal bark has a direct influence on the amount of pectin substances.

6. The presence of starch and the amount of chromatophores constitutes proof that the internal and external bark of the *F. fastigiata* thallus perform the principal role in physiological processes.

7. Morphological and anatomical characteristics can constitute an indication of the ecological state of *F. fastigiata*.

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BADANIA MORFOLOGICZNO-ANATOMICZNE GATUNKU *FURCELLARIA FASTIGIATA* (HUDS.) LAM. Z ZATOKI PUCKIEJ

Streszczenie

Praca niniejsza oparta została na materiale zebranym jesienią 1968 r. i wiosną 1969 r. z różnych głębokości Zatoki Puckiej. Plechy *Furcellaria fastigiata* (Huds.) Lam. poławiane były na wysokości Osłonina i Rzucewa (rys. 1). Materiał do badań stanowiły wierzchołki wzrostu plechy długości 2—5 mm (rys. 2). Podczas analizy laboratoryjnej do utrwalania i barwienia preparatów zastosowano znane metody cytochemiczne stosowane dla roślin wyższych.

W toku morfologiczno-anatomicznych badań ustalono: typ budowy, kształt plechy, wielkość komórek, wielkość jądra komórkowego, chemiczną naturę ścian komórkowych oraz lokalizację podstawowej substancji zapasowej.

Obserwacje dotyczące struktury wewnętrznej plechy *F. fastigiata* Lam. wykazały, że wewnątrz plechy biegnie równolegle szereg prozenchymatycznych komórek, tworząc wiązkę rdzeniową, z której regularnie odchodzą odgałęzienia boczne. Centralna część plechy rozgałęzia się, tworząc okorowanie składające się z poszczególnych komórek kory wewnętrznej, a następnie przechodzi w rozgałęziające się dichotomicznie komórki kory zewnętrznej (rys. 3, 4). W strukturze wewnętrznej powtórzona została dichotomiczna zewnętrzna budowa plechy (ryys. 5, 6). Tworzące się w ten sposób komórki warstw korowych łączą się ze sobą krótkim uwypukleniem ściany (rys. 6, 8).

W zespole komórek tworzących plechę *F. fastigiata* stwierdzono trzy ich typy: komórki tworzące warstwę okorowania, komórki owalne kory wewnętrznej i wydłużone komórki rdzenia centralnego (rys. 5, 6, 7). Obok strefy wzrostu elongacyjnego stwierdzono obecność drugiej strefy wzrostu w warstwie kory wewnętrznej. Ta część korowa plechy składała się z 3—6 dużych owalnych komórek (rys. 3, 6, 7). Grubość tej warstwy ma bezpośredni wpływ na ilość substancji pektynowej w plesze widlika. Cytoplazma obserwowanych komórek nie była zabarwiona i ściśle przylegała do ścian. W cytoplazmie rozmieszczone były chromatofory i jądra komórkowe (rys. 6, 7, 9). W licznych preparatach mimo zastosowania barwień substancji jądrowej nie obserwowano podziałów jądra komórkowego.

Określając naturę chemiczną ścian komórkowych plechy *F. fastigiata* stwierdzono ich dwuwarstwową budowę. Zewnętrzne ściany zbudowane były z grubej warstwy substancji pektynowych, które bezpośrednio stykały się z cienką warstwą celulozy (rys. 10). Najgrubszą warstwę substancji pektynowych stwierdzono w owalnych komórkach kory wewnętrznej. W komórkach plechy, bezpośrednio w cytoplazmie, zlokalizowana była skrobia w postaci drobnych dysków skrobiowych (rys. 10). Obecność skrobi i ilość występujących chromatoforów świadczy o tym, że główną rolę w procesach fizjologicznych plechy pełni kora zewnętrzna i wewnętrzna.

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