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Growth of thalli and reproduction of the red alga *Hildenbrandtia rivularis* (Liebm.) J. Ag.

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The fresh-water red alga *Hildenbrandtia rivularis* occurs on scattered sites almost all over the world. It is known from Africa (river Congo basin), Asia (Java, Sumatra, Krakatau), America (Venezuela, Jamaica, State of Pennsylvania, USA), and nearly the whole of Europe, where it chiefly occurs in the upland parts. Its distribution in the Scandinavian countries and in the Baltic Lake District was best investigated by Luther (1954). In Poland it occurs on scattered sites in Pomerania, Mazuria and in the district of Suwałki, in the Poznań province, in the Sudetes and Carpathians. Data on its occurrence are continuously increasing in number as algological investigations cover new regions of this country.

In spite of so wide a distribution this alga is by no means common; its occurrence is irregular, being limited above all to shaded places in streams with pure, neutral or slightly alkaline water, rich in electrolytes. In Carpathian streams with a variable bottom eroded or covered with rock-rubble it occurs almost exclusively on the walls of waterfalls and on larger blocks set in the bottom. It often forms there extensive concentrations of a surface area of several dm². In Pomerania, the Poznań province, and Mazuria it also occurs on smaller stones on the bottom of streams, when the bottom is not mobile, the water sufficiently deep and shaded, or slightly turbid. It also occurs in lakes and their out flows. In the Boden Lake it was found by Zimmermann (1927) at a depth of 8—45 m.

The kind of substratum on which *Hildenbrandtia* grows seems to be of little significance, however, the opinion prevails that it settles most readily when the substratum contains a certain quantity of calcium. Thus, Isrealson (1942) reported that in Sweden *Hildenbrandtia* does not occur in streams flowing on a granite substratum, appearing only lower where the quantity of electrolytes is higher and the water has become more eutrophic. On the other hand, it was found on granite and quartzite rocks, on volcanic tuffs, on sandstones (e.g. in the Carpathians), on dolomites and limestones, as well as on cement blocks, on mollusc shells (chiefly dead shells of *Margaritana*), and on the glass of old bottles lying in the water.

The opinion also frevaded that this alga of such a cosmopolitan distribution is not uniform as regards systematics. The present author has described the differences in the width of threads of *Hildenbrandtia* from Pomerania and from the Carpathians, as well as from France and Java, which he examined (Starmach 1928). Later, however, more detailed analyses showed that similar differences in the width of cells (from 4 to 12 μ) can be found in thalli from any site if they are collected at various seasons or if a larger number of samples are examined. It seems therefore that it is a species forming little variable thalli even when its sites lie very far apart. This is probably due, on the one hand, to the great similarity of the character of the environment in which it occurs, and on the other, to the exclusively vegetative mode of its reproduction.

There are no certain data on the origin of *Hildenbrandtia*. Skuja (1938) presumed that it originates from at least the Tertiary or even from an earlier period. Tarnavschi (1943), after investigating the occurrence of this alga in Roumania, observed that it always appears on sites to which the Sarmatian and Pannonian Sea once reached; it would therefore be a Tertiary relict. In North Europe *Hildenbrandtia* does not occur at altitudes exceeding 100 m. That is why Luther considered that this alga could have settled only after the Glacial period. It would be difficult to assume that *Hildenbrandtia* is a relict in the Baltic countries. Neither is it clear how it could have migrated from the sea or brackish waters to rivers and streams when it reproduces only vegetatively by means of aid of diaspores unable to migrate upstream. Or else, one has to accept De-Toni's (1893) observations that this alga is carried by leeches, which, attracted by its red colour, eat the tetraspores (gemmae).

The occurrence of *Hildenbrandtia* seems to be determined above all by the environmental conditions. There where it occurs, it finds as a rule no competitors, being thus able to develop freely, since few algae require so much shade and even diatoms do not fórm larger aggregates there. Moreover, apart from the species *Audouinella violacea*, it is the only fresh-water red alga which has retained a blood-red colour. All the other species have more or less distinct violet, brown, or olive-green shades. Lingelsheim (1922) reported that thalli of *Hildenbrandtia* have a smell similar to that of marine algae, this, however, does not seem easy to ascertain.

In spite of the great interest taken in this species and of numerous works dealing with it, there are still many details concerning the development and structure of the thalli, and above all the mode of reproduction, which should be investigated. The data presented below on their growth and reproduction were collected in the course of a number of years of observations of this alga, carried out both on natural sites and on laboratory cultures.

STRUCTURE AND GROWTH OF THALLI

Young *Hildenbrandtia* thalli are built of threads trailing on the substratum and developing radially from the initial cell. Adult thalli are composed of short threads growing vertically from the trailing ones. These threads are stuck together laterally by an intercellular substance staining distinctly with ruthenium red and methylene blue, therefore containing

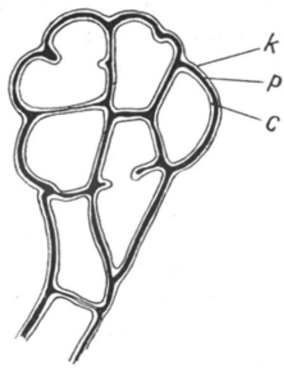


Fig. 1. Young thallus developing at the top of a rhizoid: *k* — cuticle, *p* — pectin membrane, *c* — cellulose membrane

pectins. The coalised threads stuck form a nematoparenchymatous tissue, i.e. one in which the run of single threads is well visible. The sticking is of varying stability; thalli pressed by a cover-glass disintegrate in varying degree into single threads. The least readily disintegrating are threads of thalli trailing flatly on the substratum. Vertical threads disintegrate more easily whereas the most readily and often spontaneously disintegrating are threads of mature gemmae (diaspores).

In principle, the cellular membrane is composed of two layers: an interior one giving a distinct reaction to cellulose and an exterior, pectin layer. In addition, membranes of marginal cells have a thin cuticle (fig. 1, photo. 14). During division of the cell the membrane grows from the exterior to the interior; its tip entering into the interior of the cell stains strongly with methylene blue and ruthenium red.

The vertical threads are composed for the most part of 6—12 cell layers; their length ranges from 35 to 120 μ (seldom more). At about half way of their length the threads ramify forming 2—4 branches stuck together and directed upwards. That is why the threads at the top are always thinner than near the base, this has to be taken into account in determining the dimensions of the threads or cells. The width of cells in the basal parts amounts to 8—11.6 (—12) μ, and at the tops of threads

Plate I

1 — Border of a young, flatly growing thalus;

2 — marginal part of a thallus growing irregularly;

3 — young thalli in the form of round spots;

4 — irregular growth of the thallus; beginning of formation of vertical threads;

5, 6 — threads growing out from the border of the thallus; they were broken off in the course of preparation. Actually they are long, ramified stolons forming new thalli at the apex.

Plate II

7, 8 — Growth of marginal cells of a thallus; the protoplasm is concentrated at the top of cells, which bifurcate and then divide with their oblique walls;

9 — rim of mucus at the top of growing cells, well visible after staining;

10 — the growing zone of the thalus is composed of dividing top cells and of a layer of strongly vacuolized cells with still weakly developed chromatophores.

Plate III

11, [illegible] - Groups of more intensely growing cells differentiate on the border of the thalus, this leading to an irregular growth of thalli;

13 — Peeling off surace of the thallus groups of cells called diaspores fall off from it;

14 — young thallus at the top of a rhizoidal outgrowth from a diaspore cell;

15, 16, 17 — young thalli formed at the tops of rhizoidal threads; living top cells of threads are visible, containing nuclei and chromatophores, as well as empty cells dying in the oldest parts of threads.

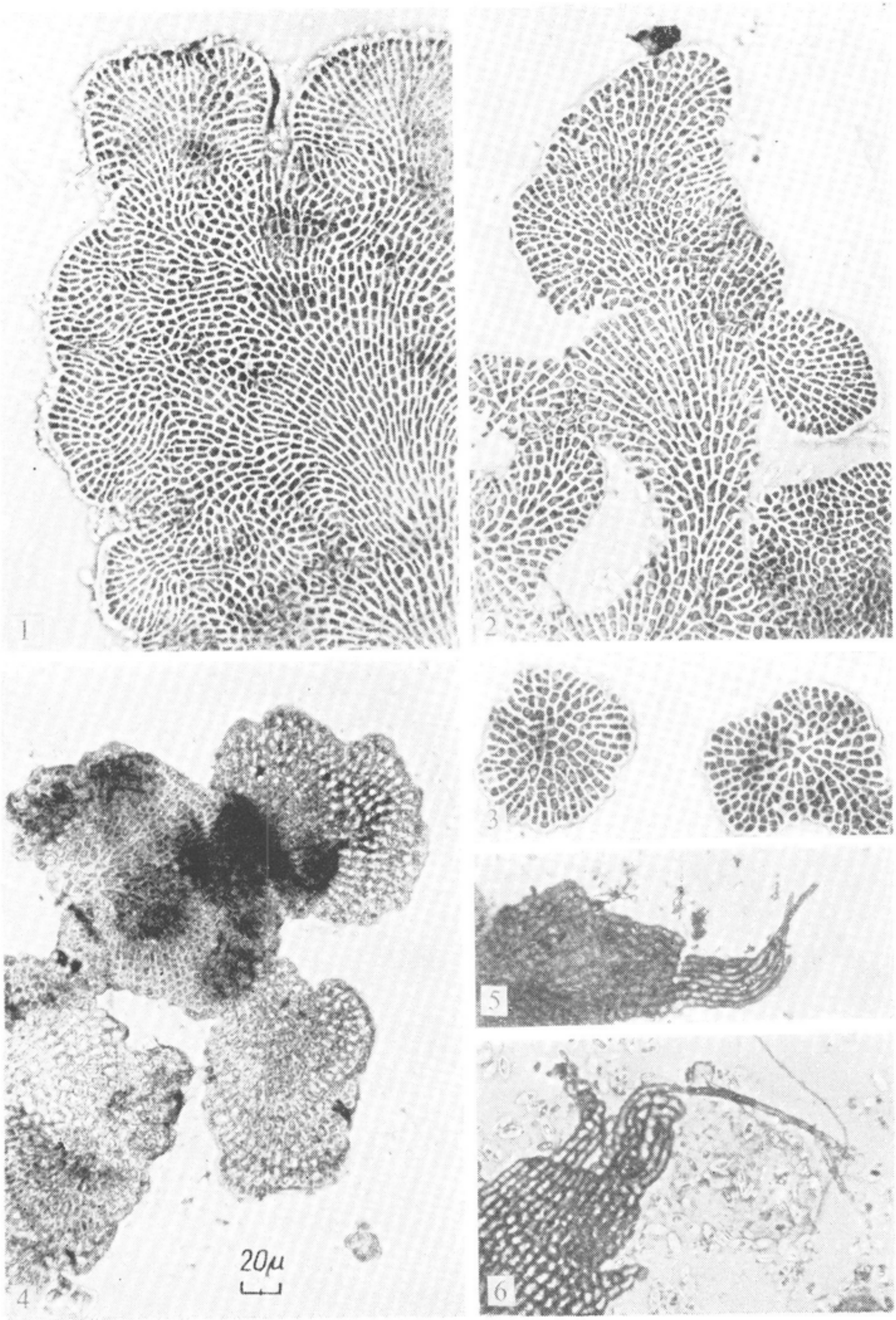

1
2
3
4
5
6
20μ

Plate II

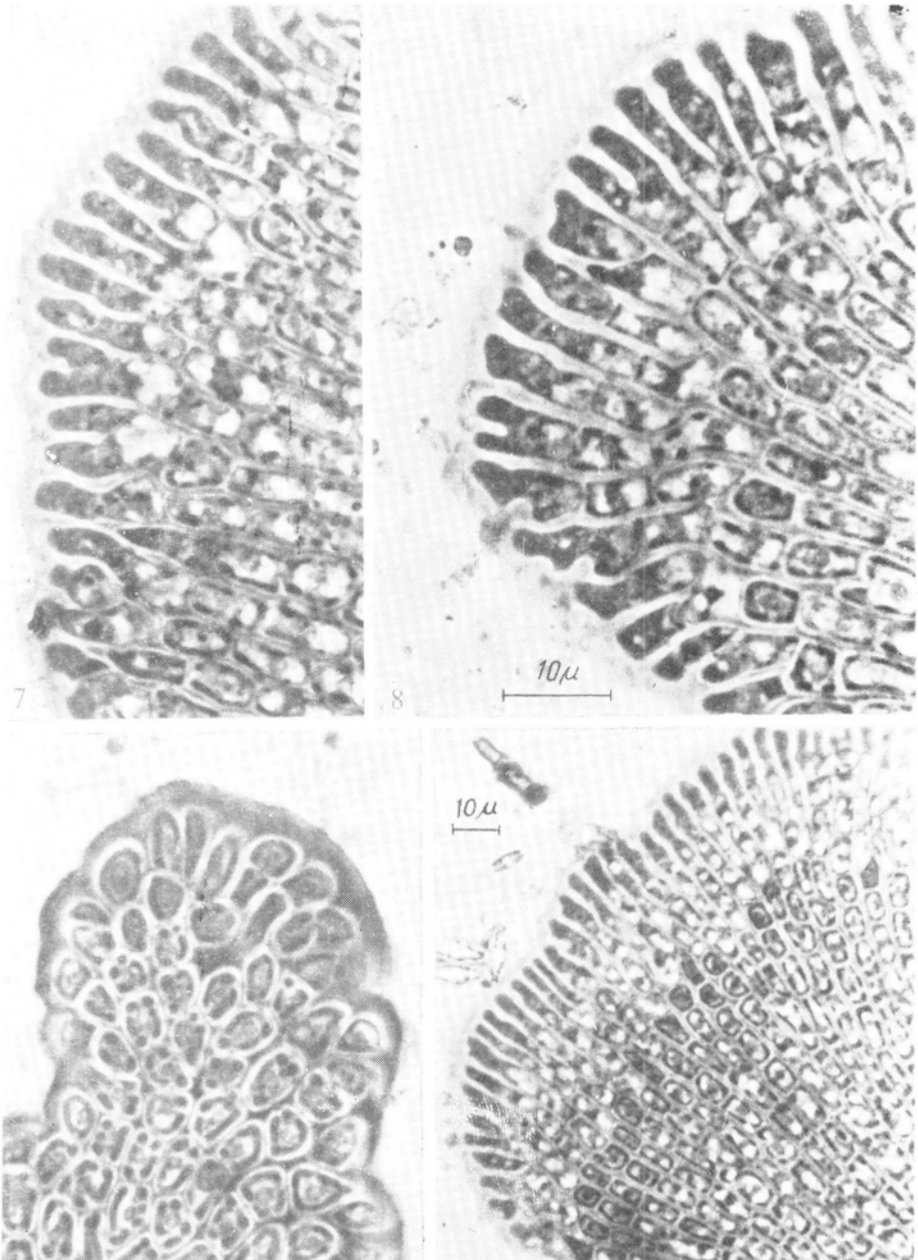
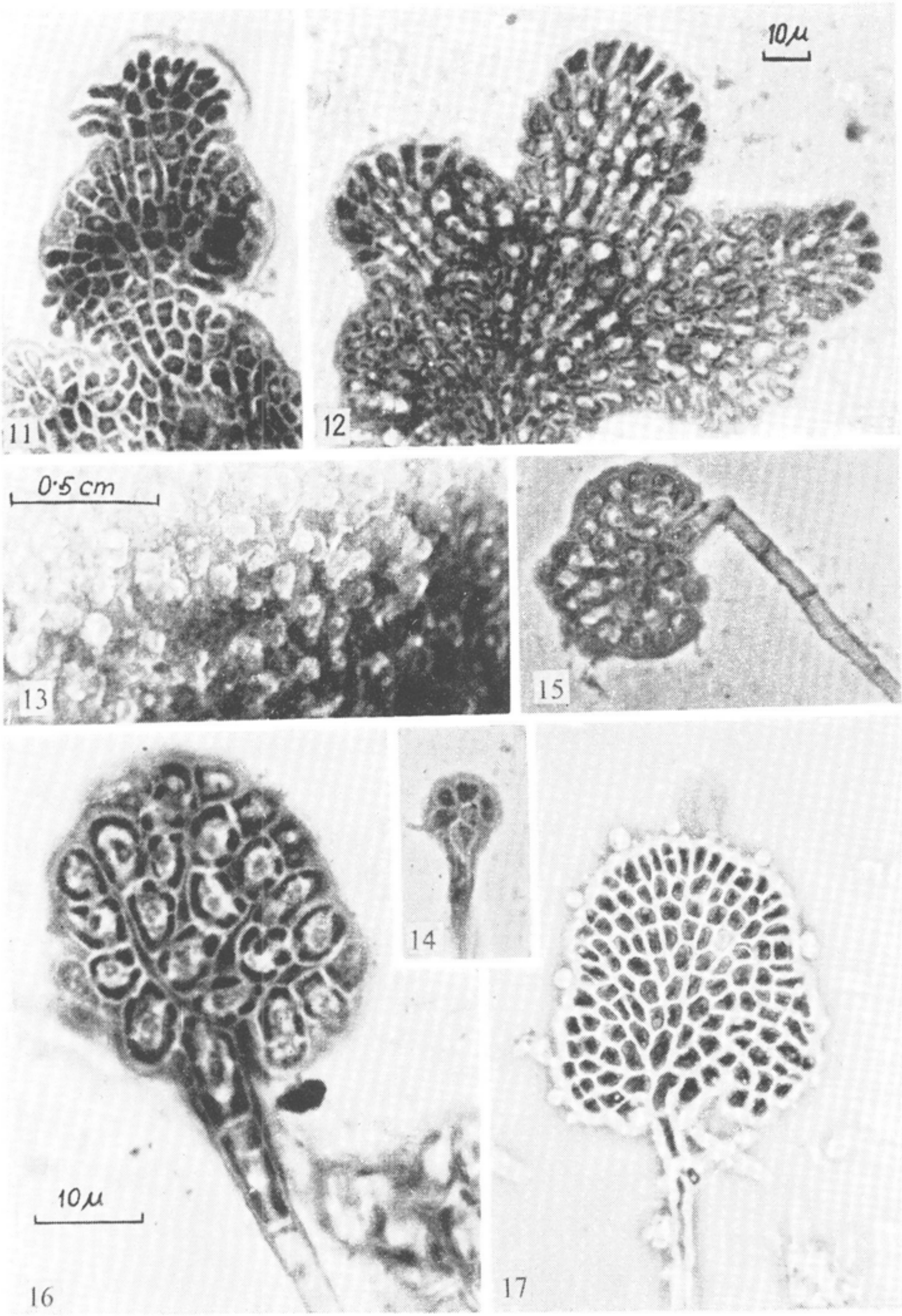


Plate III



to 5—6.5 μ. The cells are of varying length. In the basal parts of threads they are shorter than the width, being often square in the middle of the threads and shorter again at their tops.

Thalli grow in width in the marginal parts. On their border there appear trailing threads growing radially. These threads have cells longer than the width, ramifying at the tops. The width of cells usually amounts to 4—5 μ and their length to 8—13 μ. Young thalli have the form of roundish spots owing to the radial growth of the threads (photo. 3). This, however, is not the rule. Very often young thalli grow unidirectionally or irregularly (photos 1, 2, 4). From the old thalli, frequently occupying a large space on the substratum, tongue-shaped strands of threads separate on the border, or even single threads trailing on the substratum and forming finally new thalli (photos 5 and 6).

The threads trailing on the borders of thalli adhere much stronger laterally than the erect ones and it is rather difficult to separate them under pressure. The growth of these threads can be observed only in cultures, in a fluid medium, after the gemmae have been set on cover-glasses giving a hard basis. On a soft agar basis the threads germinating from diaspore cells do not form thalli. In the material from natural growths the author succeeded in examining the marginal parts of growing thalli only when the alga grew on calcareous stones. This material derived from the vicinity of Dijon (France) and was brought to the author in 1927 by Professor R o u p p e r t. After embedding the stones in acetic acid it was easy to transport the almost undamaged threads detached from the substratum on a microscopic slide. The scraping off of thalli with a knife from a substratum insoluble in acid gives only fragments of broken threads.

The growth of the threads in length is distinctly apical. The protoplasm concentrates in the top part of the cell; also the nucleus shifts towards the top, whereas the posterior part of the cell is strongly vacuolized (photo. 7 and 8). The growing cells also secrete much mucus, which surrounds their tops as well as the whole border of the growing thallus with a layer reaching in width sometimes nearly half the length of the top cell (phot. 9). The top cells divide longitudinally and sligthly obliquely, while from the top there develops a cavity of the membrane shifting gradually towards the back of the cell and dividing it into two forked outgrowths. The formation of the cavity is preceded by division of the nucleus. The transverse walls separating the two forked sections of the cell from its vacuolized basis form at the end. These walls grow in from the exterior to the centre, similarly as in cells of blue-green algae. The protoplasm at the top of the dividing cell is greyish-pink, but no chromatophores are visible in it. They become distinct only in the cells lying further away from the top.

The growth of *Hildenbrandtia* thalli takes place according to the multiaxial type (Springbrunnentypus—O l t m a n n s, 1922), characteristic of many species of red algae. It is pronounced above all in the marginal parts of thalli growing flatly on the substratum (e.g. photos 11 and 12), but is also visible, although to a lesser degree, in thalli growing in thickness. The growth takes place rhythmically; not all parts of the thallus grow at the same time and after a period of rapid growth there occurs a break followed by a further growth. This is visible e.g. from photo. 4. In the case of a rapid growth the layer of the growing marginal cells is accompanied by a layer of 2 to 4 strongly vacuolized cells with weakly developed chromatophores; this layer separates the growing border from the thallus which has already grown (photo. 8 and 10). Most frequently, however, only some groups of cells grow more rapidly on the border of the thallus, owing to which there develops an irregular, indented and folded border (photo. 2, 4, 11, 12).

Sometimes already the third cell from the top of the flatly growing thread divides longitudinally in a plane parallel to the substratum, giving rise to a thread growing vertically. However, in the case of strong peripheral growth the vertical threads from much further from the border of the thallus. The growth of thalli in height is always limited. It is of interest that when one examines mature and unquestionably multiannual *Hildenbrandtia* thalli, occupying sometimes an area several dm^2, one observes no annual layers in the vertical section. It seems therefore that after having formed some 12 layers of cells thalli cease to grow in thickness. The author encountered in the examined material only pinching layers originating from the growth of one thallus over the other. Such cases are not uncommon either under natural conditions or in artificial cultures when several young thalli grow close to each other, or when a damaged thallus regenerates.

REPRODUCTION

The growth of thalli in height is inhibited by their peeling off at the surface, which is distinctly visible in phot. 13. The peeling off groups of threads have as a rule thicker and paler cells filled with reserve material. They form in small packets and produce around themselves a small layer of mucus, loosening in this way their connection with the rest of the thallus. Grey, slightly protruding on the flat thallus groups of threads can easily be removed with a small needle or scalpel. They are composed of thick, pale threads, often producing when still joined to the mother thallus characteristic rhizoidal outgrowths. These groups of threads having the form of scales falling off from the thallus were at one time termed by the author gemmae (diaspores) (S t a r m a c h 1952). After having carefully set them on cover-glasses embedded in a medium (K n o p's medium strongly diluted with well water can be used, among others, for this purpose) a growth of new thalli was observed through the medium, consisting of long, sometimes ramified outgrowths having the appearance of rhizoids forming from cells of diaspore threads. The growth of rhizoids is distinctly apical. Similarly as in threads growing on the border of the thallus, the protoplasm concentrates at the top of the apical cell. It is there that a division takes place, leading to the ramification of the rhizoid, or else by means of oblique divisions new, flat thalli are formed. Around the growing top of threads a small layer of mucilage appears. Rhizoidal threads can also divide into cells containing chromatophores similar in shapte to *Chantransia* threads. Most frequently, however, the multicellular rhizoid is composed of thin, very elongated cells, empty in the oldest parts and alive only at the growing end. This kind of continuous apical growth and dying of the oldest parts, a progressive unidirectional growth, is also observed in shreds of fungi.

Worth noting is the fact that in the development of all kinds of spores in red algae, as e.g. carpospores, monospores, tetraspores, and polyspores, there always develops a thread which either directly produces at the top a new thallus, or else again forms spores, and only from these, by the intermediary of threads, produces new thalli. A similar case occurs during the development of fresh-water *Hildenbrandtia* diaspores, From this the conclusion can be drawn that cells giving rhizoidal outgrowths developing new thalli at the tops are an analogue of the spores of other red algae. The author showed in an earlier work (l.c) that there exists a similarity between the diaspores of *Hildenbrandtia rivularis* and the tetraspores of marine species of this genus. Diaspores and tetraspores form therefore from vegetative cells, they are similarly disposed on the mother thalli and develop similarly into new thalli. To be exact, neither one nor the other species were investigated cytologically, but basing on the fact that the developing tetraspores of marine *Hildenbrandtia* always give thalli similar to the mother ones, we assign them, according to Svedelius's (1935) thesis, to the type of spores the formation of which is not accompanied by a reduction division. With regard to their development they are modified vegetative cells, or in other words constitute a specific type of gemmae not uncommon among algae.

The difference in the development of organs of vegetative reproduction in marine and fresh-water *Hildenbrandtia* could be attributed to the conditions of the environment. Marine red algae, as known, cease to reproduce sexually, developing instead to a high degree vegetative reproductive organs. For example: some species of red algae growing in the waters of the Baltic Sea of low salinity, as e.g., *Polysiphonia nigrescens* and *Rhodomela subfusca*, form normal reproductive organs in the North Sea but no longer in the Baltic. Some species of red algae by means of vegetative reproduction produce besides tetraspores also polyspores and paraspores. The latter, representing muticellular fragments of apical thallus threads changed into chains of spores (e.g. in *Seirospora Griffithsiana*), closely resemble the thickened threads of *Hildenbrandtia rivularis* diaspores.

Therefore, it is not so very surprising that in fresh-water *Hildenbrandtia* one finds no sexual reproduction, organs, or even tetraspores similar to those in marine *Hildenbrandtia*. On the other hand, there have appeared for a long time in the literature mentions and even descriptions of alleged trichogynes and antheridia, however, after a closer examination this was not confirmed. The first to describe these organs in *Hildenbrandtia rivularis* were Borzi (1880) and Petit (1880). Borzi supposedly found antheridia looking like slightly paler, raised points on the surface of a thallus, which probably were peeling off diaspores. Petit described well and drew typical rhizoids growing out of cells of diaspore threads, considering them to be trichogynes. Not long ago Palik (1957) described trichogynes and antheridia, and even drew a fertillized trichogyne. However, neither the description nor the drawings are convincing. The trichogynes described by Palik are merely young rhizoidal threads growing out of a diaspore cell. Moreover, they are badly drawn. These threads actually grow out of the interior of the cell after breaking its membrane, whereas in Palik's drawings (e.g. drawings 7, 10, 15, 16, 18) they look as if they were outgrowths of the membrane. The drawing of a fertilized trichogyne (drawing 18) is quite improbable. The spherical, colourless cell (presumably of a bacteria or fungus) adhering to the outgrowth (of the membrane ?) is no evidence at all. These spherical, colourless cells (presumably of a fungus) are always visible when thalli of *Hildenbrandtia* are kept in aqueous cultures for a month or longer, this being the case with the material examined by Palik. Similar cells are visible in my photo 15, taken from a diaspore culture on a microscopic slide. The process of fertilization, or if only of fusion of the spermatozoid protoplasm and trichogyne was not observed. The empty (without plasmic content) outgrowth of the membrane with a colourless cell at the top is not a fertilized trichogyne. Neither does the empty cell open at the top over which the alleged spermatozoid is hovering, correspond to the notion of antheridium in red algae. These open cells are always visible when one examines thalli scraped off from stones; they are simply damaged cells. Thus, the mentioned author could not have seen spermatozoids forming in antheridia.

If organs of sexual reproduction do form in red algae they cannot be so uncommon as to make it impossible to observe the stages of their development. In any case, as long as the development of antheridia, the formation and liberation of spermatozoids, as well as the development, formation, fertilization, and further development of carpogonium are not exactly described, one cannot admit that sexual reproduction has been demonstrated in *Hildenbrandtia rivularis*. It would be very strange if in the course of over 120 years which have passed since the first description of this alga, none of the large number of algologists, who in various countries carried out not unfrequently investigations on the fresh-water *Hildenbrandtia*, had not observed it in organs of sexual reproduction, and failed to examine their development and functions.

Neither can P a l i k's conception on the formation of tetraspores in *Hildenbrandtia* threads be upheld. Thalli of *Hildenbrandtia* squashed on a microscopic slide give such varied images of threads and of their fragments that one can find not only four-cell sections but also others composed of two to many cells. Moreover, tetraspores do not form in this way in red algae; their disposition and outward appearance are perfectly determined.

There are cases, however, when in some cells of threads there form as if 2 or 4 autospores. These cases occur very rarely and as yet have not been elucidated. The author regards them as an anomalous course of cell division. The infrequency of the appearance of this kind of pattern in preparations and their merely sporadic occurrence with no relation to the time of the year, the water stage, or the age of thalli, indicate that they are not organs of reproduction.

Stolon threads also serve the expansion of algae. They grow out from the borders of the thallus singly or several together (photos. 5 and 6), trailing on the substratum and forming new thalli at some distance from the mother thallus. These threads have longitudinal cells similar to those of *Chantransia* threads. B u d d e (1926) wrote about them giving them the name of *Chantransia hildenbrandtiae*. Also the present author observed them many times both in a material from natural sites and in cultures (S t a r m a c h 1928, 1952). These threads have to be regarded as stolons, since they do not proceed from germinating diaspores. Sometimes also rhizoids growing out of diaspores develop into threads similar to *Chantransia*.

Hildenbrandtia can also reproduce vegetatively with the aid of detached fragments of thalli. This was demonstrated experimentally by the author in a work published in 1952. Fragment of thalli are capable of forming new thalli without the intermediary of threads as is the case with diaspores.

Summing up the author wishes to stress once more that as yet no one succeeded in establishing any other mode of reproduction of the red alga *Hildenbrandtia rivularis* as by propagation with the aid of

a) fragments of thalli (rarely!),
b) threads growing out from thalli in the form of stolons (not often!),
c) groups of cells of diaspore type peeling off from the surface of

thalli, which possibly can be recognized as homologous with tetraspores of marine *Hildenbrandtia*, or paraspores of other red algae.

Any other modes of reproduction of this alga are considered by the author as not sufficiently proved.

SUMMARY

The growth of the thalli of a fresh-water red-alga *Hildenbrandtia rivularis* is described. Flat crust-like thalli increase their width trough the division of the marginal cells. The thalli have a limited growth in thickness instead. In some periods on the surface of the thalli rise the diaspores which are curried out with the curent of water and on a new substratum form the germs of the young thalli. Other means of propagations of the red-alga were not stated.

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Wzrost plech i rozmnażanie się krasnorosta Hildenbrandtia rivularis (Liebm.) J. Ag.

Streszczenie

Słodkowodny krasnorost *Hildenbrandtia rivularis* jest glonem o kosmopolitycznym rozmieszczeniu, przy czym pod względem morfologicznym jest bardzo jednolity. Tworzy on nawet na bardzo od siebie odległych stanowiskach i w różnych klimatach na kuli ziemskiej podobne plechy. W Polsce krasnorost ten znany jest z wielu stanowisk; występuje zawsze w wodach dość czystych, dobrze ocienionych i raczej chłodnych. W artykule niniejszym przedstawiono nie dość dokładnie znane szczegóły budowy i wzrostu plech oraz uwagi o rozmnażaniu się.

Płaskie, skorupiaste plechy mają ograniczony wzrost na grubość, rozrastają się zaś silnie w częściach obwodowych. Nici rosną szczytem i rozchodzą się promieniście. Rosnące komórki szczytowe nie mają z początku chromatoforów i oddzielone są od reszty plechy warstwą 2—4 komórek posiadających duże wodniczki. Komórki te, po wyróżnicowaniu się chromatoforów przechodzą stopniowo w normalne komórki plechy (Tabl. I, II).

Hildenbrandtia rivularis rozmnaża się tylko na drodze wegetatywnej za pomocą: 1. fragmentów plech (rzadko), 2. nici wyrastających z brzegów plech na kształt rozłogów, 3. diaspor złuszczających się z powierzchni plechy (tabl. III, fot. 13). Diaspory można porównać z tetrasporami lub parasporami krasnorostów morskich. Innego sposobu rozmnażania się nie stwierdzono, a spotykane tu i ówdzie w literaturze opisy organów rozmnażania płciowego nie znajdują powierdzenia.