THE MORPHOLOGY AND CYTOLOGY OF AUDOUINELLA SP.
(RHODOPHYTA, ACROCHAETIACEAE)

by

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We accept this thesis as conforming
to the required standard

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Audouinella sp. (Rhodophyta, Acrochaetiaeae) was collected seasonally between May 1978 and April 1980 from the North Alouette River, British Columbia and occasionally from other regions in British Columbia, Washington and Oregon states. The plants were attached to rocks, moss, or the freshwater red alga, Lemanea. In general, macroscopic specimens were collected in the winter and spring and germlings in the summer and fall. Audouinella is a uniseriate branched filament with erect and basal systems. The basal system persists most of the year. Each cell contains one parietal chloroplast which varies from spiral to irregularly band shaped; pyrenoids are absent. Large lipid bodies of unknown function are common in most cells of field and cultured material and tend to aggregate near the pit plugs. Hair cells and monosporangia are commonly found on the tips of short branches. Regeneration of the monosporangia, hair cells and filaments is common. Sexual reproduction was not observed. Growth and elongation, observed with the aid of calcofluor white ST, is limited to the apical cells of the main axes, branches and branchlets. Morphological plasticity is evident in the varying cell sizes obtained from field and cultured material at different light intensities. Four chromosomes represent the haploid condition; there are two larger (ca. 0.4 \( \mu \text{m} \)) and two smaller (ca. 0.3 \( \mu \text{m} \)) chromosomes.

Audouinella sp. has typical red algal ultrastructural features. Unicellular hairs are described ultrastructurally in the red algae for the first time. They contain a prominent nucleus, several mitochondria, endoplasmic reticulum, golgi bodies and many vesicles. Chloroplasts or proplastid type of organelles are absent.
Audouinella sp. was compared to 24 descriptions of other freshwater members of the Acrochaetiaceae and it was concluded that at present it is not possible to provide a species name for the plant observed in this study. The generic name Audouinella was chosen in agreement with current taxonomic studies.
Dedicated in loving memory to my uncle Charles Abramson
and to my aunt Ellie Hymes.
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INTRODUCTION

The red alga Audouinella Bory (1823) is a member of the family Acrochaetiaceae (Florideophyceae, Nemaliales). This family of plants comprises 200 - 300 marine and freshwater species. Genera included in the family are; Audouinella\textsuperscript{1}, Acrochaetium\textsuperscript{2} Nägeli 1861, Rhodochorton\textsuperscript{3} Nägeli 1861, Balbiania Sirotod 1876, Colaconema Batters 1896, Kylinia Rosenvinge 1909, Chromastrum Papenfuss 1945, Grania (Rosenvinge) Kylin 1944, Liagorophila Yamada 1944, Rhodothamniella Feldmann 1954 and Chantransia Desvaux 1809 (see Drew 1928; Woelkerling 1971, 1973; Garbary 1979a).

Members of the Acrochaetiaceae consist of small, uniseriate branched/unbranched filaments. Several growth forms are apparent in this group; plants may be composed of both basal and erect systems; a well developed basal system but almost no erect system; a basal system composed of a loose aggregation of filaments and a well developed branching erect portion; and a basal system consisting of one cell only and an elaborate system of erect axes. In some plants rhizoids occasionally extend downward from the bases of the erect axes.

The algae vary from ca. 20 \(\mu\)m to 10 mm in height. Their colour ranges from dark steel-blue to green to violet-red to rose. The chloroplasts may be discoid, elongate, lobed, spiral or stellate in shape and they may be either parietal or axial. There can be one to many

\textsuperscript{1} abbreviated Au, in text

\textsuperscript{2} abbreviated Ac, in text

\textsuperscript{3} abbreviated R, in text
chloroplasts per cell and they may or may not contain pyrenoids. There is always one nucleus per cell. Much confusion occurs in the taxonomy of this group because one species can have several of the morphological forms described above.

Reports of sexual reproduction in the Acrochaetiaceae are rare but asexually developed monosporangia are common. Cytological behaviour during sporangial formation is poorly known. Tetrasporangia can be formed meiotically, apomeiotically or possibly mitotically. Gametangial plants, when present, can be either monoecious or dioecious. Spermatangia are formed singly or in clusters. The carpogonial branch is unicellular and the carpogonia are terminal, lateral or intercalary. After fusion the carpogonium does not associate with an auxiliary cell; it develops directly into a carposporophyte. Some of the species produce a "carpotetrasporophytic" generation.

Members of the Acrochaetiaceae appear to have exploited a very wide range of the environment and have adapted to many different niches. Plants grow in fresh, marine or brackish water; epiphytic on moss, water plants or other algae; endophytic; endozoic; epilithic or epipelic. Some of the free-living marine species grow high in the intertidal region, on rocks in damp well shaded areas; the endozoic, endophytic, and epiphytic marine species are found throughout the intertidal and subtidal regions (Dixon and Irvine 1977). The freshwater species are usually found in cold, clear, fast-running streams and rivers in shaded areas attached to rocks, submerged mosses or other species of red algae _Lemanea_ and _Batrachospermum_ (Skuja 1938). Occasionally these species grow in more open, lighted areas in muddy brown, slower moving streams (Dillard 1966).
Complete circumscriptions of this family have been recorded by Drew (1928), Papenfuss (1945), Kylin (1956), Woelkerling (1971, 1973) and Garbary (1979a).
A. TAXONOMIC STUDIES

Some of the first descriptions of plants in the Acrochaetiaceae involved the freshwater members. The generic name *Chantransia* was first used in 1805 by De Candolle in the Flore Francaise (p. 49) (in Drew 1928). However it was shown later by other workers (for details see Drew 1928) that all eight species he described belonged to other groups of algae and hence they were renamed. Desvaux (1809) and Fries (1825) both used *Chantransia* as a generic name to describe other species and therefore the name still exists.

Desvaux (1809) listed *Chantransia hermanni* (Roth) Desvaux (=*Conferva hermanni* Roth) growing on *Lemanea incurvata* Bory. Fries (1825) referred to two freshwater forms, *Chantransia hermanni* (Roth) Desvaux and *Chantransia chalybea* (Roth) Fries. The name *Chantransia* has been considered by Drew (1928) and Papenfuss (1945) to be nomenclaturally invalid "since all the species originally included in the genus *Chantransia* have been removed permanently to other genera" (Drew 1928). Today the word chantransia is often used to denote the alternate phase of the life history of *Batrachospermum*, *Lemanea* and other freshwater red algae.

Recently, Starmach (1977) used the genus *Chantransia* in his Flora of Poland (Flora Slodkowodna Polski) and described all the freshwater members under this name.

The genus *Audouinella* was first described by Bory (1823) for the freshwater red alga, *Audouinella miniata* [=*Audouinella hermanni* (Roth) Duby, 1830]. This genus has been accepted today by several workers as being the oldest valid name that can be applied to members of the Acrochaetiaceae (Papenfuss 1945, Woelkerling 1971, Dixon and Irvine 1977)
and Garbary 1979a). Nägeli (1861) described the marine genera *Rhodochorton* and *Acrochaetium*, the former reproducing by tetraspores and the latter by monospores. Balbiania was introduced by Sirodot (1876) for the freshwater species that reproduced sexually. The other genera in the Acrochaetiaceae include names that have been applied to marine species only and their origins will not be discussed in detail.

Drew (1928) proposed that all members in the Acrochaetiaceae be included under the generic name *Rhodochorton*, the older name described (Nägeli 1861). She considered that the morphological characteristics used to separate the different genera were unsatisfactory and caused confusion. However, *Rhodochorton* was not the oldest epithet, as previously stated.

Papenfuss (1945) suggested that chloroplast type could be used as the chief criterion for distinguishing the genera. The group was then resolved into four clearcut genera: *Rhodochorton*, containing a few to many small, discoid chloroplasts per cell; *Acrochaetium*, containing one parietal or laminate chloroplast per cell; *Audouinella* containing one or more spiral chloroplasts per cell, this includes marine and freshwater genera; and *Chromastrum* containing one or more stellate chloroplasts per cell. The remainder of the genera were synonymized with above four genera. Papenfuss (1947) subsequently replaced *Chromastrum* with *Kylinia*, the older epithet containing one or more stellate chloroplasts.

Kylin (1956) separated the marine and freshwater genera and recognised *Audouinella, Kylinia, Acrochaetium, Grania, Rhodochorton, Colaconema, Liagorophila* and *Conchocelis*. Each was separated by habit, chloroplast type, means of reproduction (tetraspores vs. monospores; some sexual reproductive features) and habitat. *Audouinella* was recognised as the only freshwater genus containing parietal chloroplasts which were more or
less band-shaped with irregular margins.

Feldmann (1962) also separated the marine and freshwater genera, and in agreement with Chemin (1937), two families were recognised, the Acrochaetiae and the Audouinelliae, and eight genera were accepted. The families were circumscribed based on several features: the development of the gonimoblast, characteristics of the chloroplasts, and either a haplobiontic or diplobiontic life cycle. The different genera were separated based on post-fertilization characteristics of the carpogonium. Bourrelly (1970), who did not agree with Drew (1928), chose to follow this classification scheme and he recognised three freshwater genera, Audouinella, Balbiania and Acrochaetium. Audouinella contained a few parietal ribbon-like chloroplasts, and reproduced both sexually and asexually. Balbiania was separated from Audouinella because it had a protothallus growth phase, several parietal chloroplasts and a transverse division in the carpogonium following fertilization. Acrochaetium was separated from Balbiania and Audouinella because it contained only one parietal band-shaped chloroplast and sexual reproduction was unknown.

Woelkerling (1971), Dixon and Irvine (1977) and Garbary (1979a) all agreed with Drew that it is not valid to separate the plants into different genera based on morphological features. Woelkerling (1971) placed all species with sexual reproduction in the genus Audouinella and those without in the genus Colaconema. Kylinia and Liagorophila were not examined and their distinction as genera remained questionable.

Dixon and Irvine (1977) and Garbary (1979a), recognizing only the family Acrochaetiae, assigned all members into the genus Audouinella. Garbary (1979a, b) reached his conclusions by using numerical taxonomy, culture studies and a literature review. As Drew (1928) and Woelkerling
(1970, 1971) he found that at the generic level morphological and reproductive characteristics were unreliable for use in taxonomy. He did not include details of post-fertilization because they are not known for most of the genera in this group.

In this thesis I am following the generic concepts of Dixon and Irvine (1977) and Garbary (1979a) and have classified the freshwater plant that I have studied into the genus Audouinella.

B. PREVIOUS STUDIES OF THE ACROCHAETIACEAE AND INTENDED RESEARCH

All taxonomic names given in the remainder of this thesis will be those used by the author of the paper quoted. For any emendations of the names see Woelkerling (1971, 1973), Dixon and Irvine (1977) and Garbary (1979a).

The first freshwater genera described in the Acrochaetiaceae were from field collected and preserved samples. Murray and Barton (1891) described sexual reproduction from fixed material of Chantransia boweri Murray and Barton. Drew (1935) did seasonal collections of Rhodochorton violaceum (Kütz.)Drew and described its morphology and sexual reproduction. Subsequently Drew (1936) considered C. boweri and R. violaceum as synonymous (both=Audouinella hermanni).

Jao (1940, 1941) described the freshwater members from field collections in China and reported four new species (see Appendix Table I). New freshwater species of Acrochaetium and Rhodochorton have been reported from field collected material in India, Venezuela and Japan (Raikwar 1962; Khan 1970; Patel 1970; D'LaCoste and Ganesan 1972; Kumano 1978). Swale and Belcher (1963) were the first to describe the life history of
a freshwater member from field and culture material (*Rhodochorton investiens* (Sirodot) Swale and Belcher).

The remaining life history and culture studies have been on marine species only. Knaggs (1965a, b, 1966a, b, 1967a, 1968) and Knaggs and Conway (1964) examined *R. floridulum* (Dillwyn) Näg. and *R. purpureum* (Lightf.) Rosenvinge from Britain using field and culture material. The life histories of both species were ascertained and the morphology of *R. purpureum* was studied in relation to its environment (Knaggs 1966a, b).

On the west coast of North America several culture studies of marine species in the Acrochaetiaceae were done by West. He observed the morphology and reproduction of *Acrochaetium pectinatum* (Kylin) Hamel (West 1968); *R. purpureum* and *R. tenue* Kylin (West 1969 1970a); *R. concrescens* Drew (West 1970b) and *R. membranaceum* Magnus, West (1979). In culture the algae were subjected to different temperatures and photoperiods. Using light microscopy West also studied the environmental control of the development of hair cells and sporangia in *Ac. proskaueri* West (1971) and the regulation of asexual reproduction in *R. purpureum* (1972).

Stegenga (1978), Stegenga and Borsje (1976, 1977), Stegenga and Vroman (1976) and Stegenga and VanWissen (1979) observed the life histories of several European marine species of *Audouinella*. Plants were grown at different light and temperature conditions and observations were made using the light microscope. Garbary and Rueness (1980) described the life history of a new species *Audouinella tetraspora* Garbary and Rueness in culture.

A few other morphological studies on marine members have been done. Pearlmutter and Vadas (1978) observed regeneration of thallus fragments of
R. purpureum in culture, and Garbary (1979b) studied patterns of cell elongation and the effects of temperature on the growth and morphology of some Audouinella spp.

In the Acrochaetiaceae there have been few ultrastructural and cytological studies. Lichtlé (1973a, b) investigated the fine structure of the chloroplast and its degeneration in R. purpureum. Observations of the pit plugs of four species were reported by Lee (1971) and others by Pueschel and Cole (1981 pers. comm.). A few cytological observations were done by Magne (1964), Woelkerling (1970) and West (1969, 1970b).

Because there is a dearth of cytological information and chromosome counts in the Acrochaetiaceae, plants were collected and fixed in order to study cell structure and count the chromosomes. Cell measurements of field and cultured material were recorded for the determination of morphological plasticity. Experiments were also set up to observe the effects of different light intensities on the growth of Audouinella, as previous workers had done (West 1972, Garbary 1979b).

The complete description of Audouinella obtained in this study was compared to 24 other descriptions of freshwater algae in the Acrochaetiaceae in order to provide an accurate species name for the plant.
II. MATERIALS AND METHODS

A. NATURAL POPULATIONS

Audouinella sp. from the lower portion of the North Alouette River in the municipality of Maple Ridge, British Columbia; 49° 122° SW was collected in a shaded area close to the river bank at the point where the river meets 232nd Street (Table I). Wehr (1979) described the climate of this area as a warm maritime-mesothermal type which is humid to rainy. Snow is infrequent although rain is common during the winter months. The river is located in the Coastal Western Hemlock Biogeoclimatic zone (Wehr 1979). The North Alouette River is a fast flowing river and there is little ice cover during the winter. The river is slightly acidic with pH ranging between 6 and 7 (Wehr 1979).

Specimens were collected from natural populations fairly regularly between May 1978 and April 1980 (Table I) and the temperature and the pH of the river were also recorded each time (Table I). In the North Alouette River Au. sp. was attached only to rock surfaces. Wehr (1979) noted that the rocks in the streambed are acid granitic and consist largely of quartz diorite, diorite and gabbro. No preference to rock surface was noted but plants were found on rocks in shaded areas only. The plants were carefully removed from the rocks; some were fixed immediately for electron microscopy and cytological work and others were brought back to the laboratory on ice for examination and culturing.

Other collections of Au. sp. were made in June 1978 by Dr. R. Sheath during a field trip in the states of Washington and Oregon (Table II). The plants in the streams were epiphytic on mosses and the freshwater
alga *Lemanea*. Two populations of *Au*. sp. were collected from other areas in British Columbia (Table II). All of these plants were observed and compared to *Au*. sp. from the North Alouette River; some were fixed for electron microscopy and others were grown in culture (Table II).

Cell measurements were obtained from most of the natural populations collected throughout the year. The majority of the measurements were done in April through June. Measurements of cells in different plant parts were taken in order to compare this plant with descriptions of other freshwater red algae in the Acrochaetiaceae and to show the size range that can be obtained for one species. Most of the data were replicated a minimum of ten times. Plant parts were designated as follows:

a) main axis = a row of cells greater than ten in number, not terminating in hair cells or monosporangia.

b) secondary axis = lateral branches, less than ten cells, sometimes terminating in monosporangia or hair cells.

c) monosporangia.

d) hair cells.

The size range, mean and standard deviation were calculated for each part.

B. CULTURES

The medium which produced the best growth of this alga consisted of a combination of various artificial salts, soil water and vitamins (Table III) (Sheath and Cole 1980). Several clumps of plants (the number varied depending on the collection) were teased apart and placed into plastic petri dishes, 100 x 20 mm (Optilux 1005) containing ca. 35 ml of medium and were then placed on a shaker (91 shakes/min., each shake = 5.6 cm)
in a 5°C lighted incubator. The growth chamber was maintained on a 12:12 L:D cycle. In order to reduce irradiance, paper towelling was placed on top of the petri dishes. Hence the algae were exposed to ca. 6 μEin-m⁻²-s⁻¹. All irradiances were recorded using a LiCor Quantum Meter Model L.I.-185A. Plants that were removed from the shaker and kept on a shelf in the chamber grew poorly and began to senesce.

In order to observe regeneration and to initiate faster growth, plants in culture (collected 18 May and 13 July 1978 from the North Alouette River and 24 June 1978 from the river north of Humptulips, WA; see Tables I and II) were removed, chopped with a razor blade into small pieces 5-10 cells long and then placed into petri dishes containing fresh medium. The cultures were maintained under the same conditions as outlined above.

C. LIGHT MICROSCOPY

Living material from the North Alouette River and cultures were observed using a regular dissecting microscope and a compound microscope. In order to detect some of the cellular details an Olympus microscope with the Hoffmann modulation contrast system was also used.

Samples of cultured material were fixed for light microscopy using the J.B.-4 methacrylate embedment technique (Polysciences Inc. Warrington PA 18976). Filaments were fixed in 2.5% glutaraldehyde buffered in phosphate (pH 7.2) for two hours at 4°C, rinsed in buffer and distilled water followed by dehydration in methanol plus 0.5% NaCl in increasing increments from 25 to 100% alcohol. Infiltration was done using the technique outlined in the J.B.-4 kit. Sections 4.0 μm thick were cut
using glass knives and a J.B.-4 Porter Blum microtome. Sections were stained with periodic acid-Schiff's (PAS) (Jensen 1962), counterstained with aniline blue (PAS/AN) and mounted permanently with Euparal (supplied by Gurr, England).

D. ELECTRON MICROSCOPY

Samples of *Au.* sp. to be examined with the electron microscope were fixed for two hours in 2% glutaraldehyde/phosphate buffer (pH 7.2) at 4°C. The plants were then washed with phosphate buffer 3-4 times at ten minute intervals. Post-fixation followed using 1.5% osmium tetroxide/phosphate buffer at 4°C. The material was then washed again in several changes of buffer. The buffer was washed out with distilled water in three stages. Dehydration followed using a graded series of alcohol-acetone or just acetone. All specimens were embedded in a 4-step infiltration of Spurr's low viscosity resin and hardened for 12 h at 70°C (Spurr 1969).

Sections were cut using glass knives or a Guido Sasso diamond knife on a Reichert Ultramicrotome OmU 3. Sections were picked up on carbon coated copper grids of various mesh sizes, stained for 45 min with a saturated solution of uranyl acetate, washed and then stained with lead citrate (Reynolds 1963) for 10-20 min. Sections were viewed with a Zeiss EM 10 electron microscope.

Cultured material from the North Alouette River was exposed to a higher light intensity, ca. 36 μEin·m⁻²·s⁻¹, for 4 wks. Germinated monospores were then removed from culture dishes with a micropipette and fine tweezers and then fixed for electron microscopy according to the
above techniques.

E. CHROMOSOMES

For the study of chromosomes, plants from both natural populations and cultured material were fixed in Carnoy's fluid I consisting of 3:1 absolute ethanol:glacial acetic acid (3:1; Sharma and Sharma 1965). In some species of red algae, cell division in culture occurs between the end of the light cycle and the beginning of the dark cycle (Cole pers. comm.). Therefore in an attempt to localise cell division, plants of Au. sp. were removed and fixed every 10 min, one hour before the dark cycle began and one hour after (plants were growing on a 12:12 L:D cycle). The alga was also maintained on a 24 h light cycle for one week and then fixed at one half hour intervals one hour before the lights were switched off in the incubator and one hour after. Filaments were stained with Wittmann's aceto-iron-haematoxylin-chloral hydrate solution (Wittmann 1965).

F. PRELIMINARY GROWTH STUDY AT DIFFERENT LIGHT INTENSITIES

In order to assess the growth of Au. sp. the calcofluor white fluorescent technique of Cole (1964) was used. Calcofluor White ST (4,4'-bis [4-anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-ylamino]-2,2'-stilbenedi-sulfonic acid) was discovered to bind to the cell wall of plants because of its ability to hydrogen bond with \( \beta-1,4 \) and \( \beta-1,3 \) polysaccharides (Haigler et al. 1980). Plants were exposed to 0.01% Calcofluor White ST (Cyanamid Can. Inc. Baie d'Urfe P.Q.) added to
culture medium for 24 and 48 h allowing the dye to bind with cell wall material. Each plant was then washed several times in fresh medium and set up under experimental conditions. Any new growth of the alga should not be labelled with the dye unless the cell wall already formed contributed to the production of new cell wall material.

Petri dishes containing labelled plants in normal culture medium were placed on a shaker in a growth chamber at 5°C on a 12:12 L:D cycle. Triplicate samples were set up at three different light intensities: light intensity A- full light 35 μEin·m⁻²·s⁻¹; light intensity B- under paper 6 μEin·m⁻²·s⁻¹; complete darkness. Portions of the plants were removed randomly at various intervals and observed using a Wild M20 fluorescence microscope (blue light). New plant growth was not labelled with the dye and did not fluoresce. The white secondary fluorescence induced by the dye was easy to distinguish from the red primary fluorescence of the chlorophyll within the chloroplasts.

New cells were counted, measured and photographed using Tri-X film at 4 min exposures, as outlined by Waaland and Waaland (1975). Ten different filaments were observed each time and 10-15 cells were measured.
<table>
<thead>
<tr>
<th>Collection</th>
<th>pH</th>
<th>Temp. °C</th>
<th>Au. sp.</th>
<th>Presence of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hairs</td>
</tr>
<tr>
<td>1978</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 May</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>13 July</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>15 Sept.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>29 Sept.</td>
<td>-</td>
<td>12.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1978</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Jan. +</td>
<td>5.4</td>
<td>1.5</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>15 Feb. +</td>
<td>5.7</td>
<td>2.5</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>20 Mar. +</td>
<td>5.5</td>
<td>5.2</td>
<td>++E</td>
<td>+</td>
</tr>
<tr>
<td>9 May</td>
<td>4.4</td>
<td>11.2</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>21 May</td>
<td>-</td>
<td>11.0</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>21 June +</td>
<td>7.1</td>
<td>9.4</td>
<td>++E</td>
<td>+</td>
</tr>
<tr>
<td>19 July</td>
<td>-</td>
<td>19.7</td>
<td>++E</td>
<td>+</td>
</tr>
<tr>
<td>15 Aug.</td>
<td>5.8</td>
<td>-</td>
<td>+G</td>
<td>-</td>
</tr>
<tr>
<td>1 Nov.</td>
<td>6.9</td>
<td>7.0</td>
<td>+G</td>
<td>+</td>
</tr>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Jan.</td>
<td>6.0</td>
<td>5.0</td>
<td>+G</td>
<td>-</td>
</tr>
<tr>
<td>25 Jan.</td>
<td>5.6</td>
<td>2.0</td>
<td>+G</td>
<td>+</td>
</tr>
<tr>
<td>22 April</td>
<td>6.2</td>
<td>9.0</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^{†}\)=prepared for EM; +, ++, +++=relative quantities; -=no data or structure absent; G=present in a germling state; E=algae heavily epiphytized.
TABLE II. Collections of *Audouinella* sp. from outside the North Alouette River study area (made in June 1978 by Dr. R. Sheath).

<table>
<thead>
<tr>
<th>Collection</th>
<th>Site Description</th>
<th>Temp. °C</th>
<th>River Velocity cm s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 June Satiam R. at Hwy 22, Oregon</td>
<td>epiphytic on moss at river bank, slow flowing</td>
<td>8.5</td>
<td>16.9</td>
</tr>
<tr>
<td>21 June Umpqua R. at Hwy 138, Oregon</td>
<td>epiphytic on <em>Lemanea</em> at river bank, partly shaded</td>
<td>12.5</td>
<td>90.5</td>
</tr>
<tr>
<td>21 June Rock Creek at 138, Oregon</td>
<td>epiphytic on <em>Lemanea</em> near bank on shoulders at water surface</td>
<td>16.5</td>
<td>54.5</td>
</tr>
<tr>
<td>*'23 June Neskoun Crk. at Hwy 101, Oregon</td>
<td>epiphytic on <em>Lemanea</em> in middle of river</td>
<td>11.0</td>
<td>120.4</td>
</tr>
<tr>
<td>*'24 June River a few miles north of Humptulips, Wash.</td>
<td>epiphytic on moss under tree overhang at river bank</td>
<td>12.8</td>
<td>90.5</td>
</tr>
<tr>
<td>*'25 June Lake Creek at Hwy 101, Wash.</td>
<td>epiphytic on <em>Lemanea</em> throughout river</td>
<td>16.0</td>
<td>36.8</td>
</tr>
<tr>
<td>13 July Kanaka Crk. B. C.</td>
<td>epilithic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1979

| 1 Sept. River 19 miles from Bamfield B. C. | epilithic in shaded area at river bank |          |                       |

*= fixed for EM; ' = grown in culture; 1 = average of two recordings.
TABLE III. Composition of enriched artificial Medium g·l⁻¹ (Sheath and Cole 1980)

<table>
<thead>
<tr>
<th>SALT</th>
<th>g·l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.028</td>
</tr>
<tr>
<td>KCl</td>
<td>0.007</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.07</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.015</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.16</td>
</tr>
</tbody>
</table>

+ 50 ml soil extract (Provasoli 1968)
+ 20 ml ES stock solution (Provasoli 1968)
+ 1 ml vitamin solution (Provasoli 1968)
G. OTHER EXPERIMENTS

Audouinella sp. was also grown in media of varying salinities using the same technique as outlined for the light intensity experiment. Artificial seawater and freshwater media were used and four different conditions were set up, 0.6%, 0.9%, 15.5%, 27.6% (Sheath and Cole 1980). After 28 days plants in all four experimental conditions were growing. However the plants in medium of 27.6% produced smaller cells and growth was slower. Due to contamination the experiment was discontinued and it is not included in the results.
RESULTS

A. FIELD COLLECTIONS OF AUDOUINELLA SP.

Material collected from the North Alouette River between May 1978 and April 1980 will be described according to the season in which it was obtained. The cell measurements referred to in this section are presented in Table IV.

During the fall (September through November) Audouinella sp. collected in rock scrapings was in microscopic form only; the overall development was very poor. The river temperature averaged 9.9°C and pH 6.9 (Table I). Green filamentous algae (Oedogoniales, Ulotrichales; for details see Wehr 1979) were abundant in the river during the fall and in most cases the germlings were mixed with these green algal mats. Small germlings were found with erect filaments growing out in a runner-like fashion from a cushion of basal cells (Fig. 1, 2). Cells of the erect axis ranged in length from 12 to 38 μm and their diameter from 5 to 11 μm.

The basal system consisted of many interwining filaments (Figs. 3, 4). In general the cells of the basal system were smaller than those of the erect axes, with cell lengths ranging from 8 to 19 μm and cell diameters from 4 to 14 μm. Monosporangia did not develop but branches with/without hair cells developed even when germlings were only 9 cells long (Fig. 5). These hair cells varied in length from 16 to 74 μm and the diameter at the base of the cells was 2 μm.

In the winter (December through February) and the spring (March through April) growing macroscopic plants were abundant (Fig. 6). Winter water temperatures averaged 3.8°C and pH 5.7 (Table I). A current reading
TABLE IV. Cell measurements (μm, n=10-20)

<table>
<thead>
<tr>
<th></th>
<th>Plant Germlings  (North Alouette River)</th>
<th>Adult Plants (all collections)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>August through November &amp; Jan. 1980</td>
<td>May 1978 through to July 1979</td>
</tr>
<tr>
<td></td>
<td>erect axes</td>
<td>basal cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>range</td>
<td>12-38</td>
<td>8-19</td>
</tr>
<tr>
<td>mean</td>
<td>25.9</td>
<td>14.2</td>
</tr>
<tr>
<td>sd</td>
<td>12.83</td>
<td>5.08</td>
</tr>
<tr>
<td>diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>range</td>
<td>5-11</td>
<td>4-14</td>
</tr>
<tr>
<td>mean</td>
<td>8.07</td>
<td>11.16</td>
</tr>
<tr>
<td>sd</td>
<td>3.33</td>
<td>5.93</td>
</tr>
</tbody>
</table>
of 73 cm·s$^{-1}$ (average of three readings) was recorded for 15 February 1978 (Wehr 1979). During the winter of 1979, many healthy macroscopic plants were observed. The measurements of various cell parts and the appearance of the hair cells and monosporangia were similar to those found in the spring collections (Table IV). However, heavy flooding during late December 1979 and early January 1980 resulted in an influx of silt and sediment into the North Alouette River having adverse effects on the growth of the plants. In January and February 1980 only plant germlings were found and it was not until March that macroscopic plants were seen again. The germlings were similar in size to those collected during the fall. Filamentous green algae (Oedogoniales, Ulotrichales) and blue-green algae (Oscillatoriales) were intermixed on the rocks with the Au. sp.

In March and April small plants were mixed with blue-green (Chamaesiphonales, Oscillatoriales) and filamentous green algae (Klebsormidiales, Oedogoniales, Ulotrichales), while in May the population became dominant in the shaded areas and many "clean" (non-epiphytized) macroscopic plants were found (Fig. 6). Filamentous green algae were still present in the river but they were mainly concentrated in the sunny areas. In the spring water temperature averaged 6.7°C and pH ca. 5.3. A current reading taken on 18 May 1978 was 73 cm·s$^{-1}$ (average of three readings, Wehr, 1979).

During the winter (1979) and spring (1978-80), plants reached a maximum of 2 cm in height. They were easily recognizable as pink-grey clumps on the rock surfaces and they bore many branchlets terminating with hair cells and monosporangia (Figs. 6, 7).

In the summer months, June through August, most of the Audouinella collected was badly contaminated with the epiphytic blue-green alga Chamaesiphon (Fig. 60) and diatoms. Plants that were heavily epiphytized
had notably fewer hairs. In August, only germlings were observed in rock scrapings. The water temperature of the river, during the summer months averaged 14.5°C and pH ca. 6.5 (Table I); current 47 cm·s⁻¹ (Wehr 1979).

In summary, the winter and spring months appeared to be the best seasons for the growth of *Au.* sp. in the North Alouette River. Water temperatures were colder, water current faster and the competition for light and space in the shaded areas seemed to be reduced. Nutrient levels in the river were not measured. Competition for light and space is suggested based on seasonal observations of the area. Sexually reproductive plants were not found, even during periods of active growth.

It should also be noted, however, that the seasonality for *Au.* sp. appears to vary depending on the river and year in which the plant populations were studied. Following completion of this study, in the fall, 1980, macroscopic plants were found in the North Alouette River. The river temperature was 10.5°C and pH 6. These recordings are similar to the data obtained in 1978-79 (Table I). Collections made in Oregon and Washington (Table II) at the end of June 1978 contained "clean" macroscopic plants. River temperatures varied from 8.5 to 16.5°C and the alga was epiphytic on another freshwater red alga, *Lemanea* or submerged mosses (Table II). Plants were occasionally found in shaded areas but not always (Table II).

Macroscopic, non-epiphytized plants were also found in September in a river near Bamfield, B. C. (Table II). Large clumps grew near the river bank on rocks in a shaded area. *Audouinella* was the dominant alga in the area.
B. CYTOLOGICAL OBSERVATIONS OF FIELD AND CULTURED MATERIAL

Ultrastructurally Audouinella sp. had typical florideophycean features (Dodge 1973). The alga was characterized by a number of interesting morphological and ultrastructural features. The difficulty in obtaining good thin sections was due, in part, to the extremely thick cell wall (Fig. 10) which appeared to be fibrillar in nature (Fig. 56). Occasionally, a thin electron-dense outer cell wall layer was also observed (Fig. 57).

Each cell contained one chloroplast which varied significantly in its morphology. Occasionally it assumed a spiral form, but more often it was peripherally located in the cell with lobes extending towards the centre (Figs. 9-14). Thylakoids within the chloroplast were in a typical parallel arrangement with one or more encircling the others (Figs. 10, 12, 15, 16). In some electron micrographs, phycobilisomes were present on the surface of the thylakoid membranes (Figs. 15). Plastoglobuli varied in shape from spherical (Fig. 15) to disc-like structures (Fig. 11). A number of electron translucent DNA areas were evident in the chloroplast (Fig. 14).

With the aid of Hoffmann optics, large vacuolar regions were seen within each cell (Fig. 8). These vacuoles were also observed in electron micrographs and contained membrane material (Fig. 11). Electron micrographs also revealed other cell features. Floridean starch was accumulated in large amounts in the cell cytoplasm (Figs. 10, 13, 14). Golgi bodies were always associated with mitochondria (Figs. 17-19) and often a complex of several golgi bodies and mitochondria was observed (Fig. 17). Mitochondria had tubular somewhat irregular cristae and varied in shape from spherical (Figs. 17, 18) to elongate (Fig. 19). The nucleus (Fig. 19)
14) containing a prominent nucleolus (Fig. 58) was occasionally surrounded by endoplasmic reticulum (Fig. 59). In some electron micrographs, endoplasmic reticulum was present along the cell periphery adjacent to the plasma membrane (Fig. 20). As in most red algae, pit plugs were present between adjoining cells (Fig. 26). The pit plugs were dumb-bell in shape and bound on each end by a two layered plug cap (Figs. 53, 54). Convolutions of dilated endoplasmic reticulum surrounded the plug area (Fig. 55).

Large lipid-like bodies were very common in natural and culture collections. Light microscopy revealed prominent vesicular bodies in living (Fig. 22) and haematoxylin stained material (Figs. 23-26). These bodies were shown to be lipids, using the stain Sudan IV (Jensen 1962). In most of the material observed the lipid bodies appeared to aggregate at the pit plugs (Fig. 26) and sometimes seemed in a divided state (Fig. 25). Electron micrographs revealed small and large dense lipid bodies between the starch granules in the cytoplasm (Figs. 10, 21).

Monosporangia and hair cells were produced terminally and laterally on branches and branchlet cells (Figs. 27, 44). Monosporangia were of common occurrence (Figs. 30-33), but occasionally bisporangia were observed (Fig. 28). Monosporangia ranged in size between 11 and 19 \( \mu m \) in length and 8 and 11 \( \mu m \) in diameter with no difference between field and cultured material (Table V). Regeneration of the monosporangia was common, development occurring laterally in a dichotomous-like pattern (Figs. 30-32, 41-43). The cell subtending the empty monosporangium elongated into the empty sporangium, the nucleus divided and a new monosporangium developed (Fig. 30). Several successive monosporangia developed (Fig. 42) and as many as six old monosporangial wall layers were present
Often after several regenerations a new branch cell extended into the empty monosporangium which in turn divided to produce a new monosporangium above it (Figs. 29, 31, 32).

Monospores were released through an opening at the top of the monosporangium (Figs. 23, 33, 41) and often a piece of wall layer remained in the centre of the empty sporangium (Figs. 31, 32). Prior to release the monospores became granular in appearance and when released they assumed a spherical shape (Fig. 34). After release of the monospore the pit plug separating the monosporangium from its subtending cell remained intact (Fig. 43).

In culture, monospores never settled to the bottom of the petri dish but germinated whilst attached to the free floating mother plant. Prior to initiation of a germ tube, the spore became oval in shape (Figs. 35, 36) and monopolar germination occurred (Fig. 37). A rhizoidal-type of filament was formed (Figs. 38, 39) and the cytoplasm moved into the developing filament before a cross-wall was formed, leaving an empty spore behind (Fig. 40). The new filament contained an abundance of floridean starch and chloroplasts. New filaments were pale pink in colour and became easily contaminated with bacteria in culture. Attempts to trigger monospore release in culture were unsuccessful.

The thin-walled hair cells were colourless (Figs. 27, 44) and varied in length from 19 to 243 μm. The absence of chlorophyll in the hair cells was demonstrated by fluorescence microscopy (Figs. 91, 92). A cross-wall between a hair cell and its subtending cell was always observed and it was concluded that it developed early in the production of a hair cell. As the hair cell grew outward the cytoplasm became concentrated at the tip of the cell (Fig. 45). A pit plug occurred between each hair cell and its
subtending cell (Fig. 46).

The fine structure of the hair cells was characterized by the presence of a nucleus and lack of chloroplasts or any proplastid type of organelle (Fig. 50). However, many mitochondria and associated golgi bodies were present (Figs. 47-52). The golgi bodies appeared to be very active in the production of vesicles (Figs. 48, 49, 51, 52) and in many of the cells a substantial amount of endoplasmic reticulum was present (Fig. 49). A few of the golgi vesicles contained an electron-opaque substance which has not been identified chemically (Fig. 52, arrow).

Cultured material displayed some morphological variations not observed in plants from the field. Often some of the filaments became rhizoidal (Fig. 62), with lengths ranging between 38 and 46 \( \mu \text{m} \) (± 3.8) and diameters between 6 and 7 \( \mu \text{m} \) (± 0.5). Cells of the erect axes often formed chains of short bulbous cells (Fig. 61) with sizes ranging from 19 to 27 \( \mu \text{m} \) in length (± 3.7) and 8 to 9 \( \mu \text{m} \) in diameter (± 0.6). In general, the cells of the erect axes did not reach the maximum length of those collected in the field. Irregularly-shaped tip cells occasionally developed (Fig. 63). New apical cells commonly grow by pushing through dead cells (Fig. 64-66) and remnants of the dead cells were still evident even after the new cells had developed completely (Figs. 64, 65).

C. CHROMOSOME COUNTS AND CELL DIVISION

The aceto-iron-haematoxylin-chloral hydrate solution readily stained the chromatin in the nuclei. However, chromosome counts were difficult to obtain because cell division was not synchronous and it was limited to the growing tips, developing lateral branches and monosporangia. When
distinguishable, chromosomes were very small and not evenly spread. Condensed chromosomes were found occasionally at the tip of hair cells. There were four chromosomes, two very small ones ca. 0.3 μm and two larger ones ca. 0.4 μm. During late prophase eight chromatids were visible (Fig. 67).

Several other morphological features of cell division were noted. Interphase nuclei in monosporangial branch cells differed in appearance from those in cells of the main axes. The nuclei consisted mainly of densely staining chromatin surrounded by a small amount of nucleoplasm and in some 3 to 4 distinct masses of chromatin could be counted (Fig. 68). Interphase nuclei in cells of the main axes were not always visible but when evident (usually in the tip cells) they contained a distinct nucleolus and chromatin surrounded by a clear area of nucleoplasm (Figs. 69, 70). Prior to division, the apical cell of the main axes often elongated leaving the nucleus in a central "bulge" (Figs. 69, 71). During late interphase, chromatin began to condense in association with the nuclear envelope (Figs. 71, 72). At metaphase the chromatin formed dark staining disc-shaped metaphase plates in several orientations. The plates were either parallel, perpendicular (Fig. 73) or oblique to the longitudinal axis of the cell. Metaphase plates were also found in hair cells (Fig. 74). Chromatids separated as two masses and moved towards the poles in horse-shoe configurations (Figs. 75, 76). Cytokinesis occurred after the two new nuclei were in the interphase condition (Figs. 77, 78). Occasionally, two interphase nuclei were observed in the cells subtending monosporangia (Fig. 79).
D. PRELIMINARY STUDIES ON THE GROWTH OF AU. SP. AT DIFFERENT LIGHT INTENSITIES

Growth in culture was studied using calcofluor dye as an indicator. In general, plant cross-walls stained darker with the dye than the remainder of the cell (Fig. 80). New developing tip cells of the main axes and branches were easily distinguishable (Figs. 81, 82, 85, 86) and cell elongation was also noted (Figs. 83, 84). Elongation occurred by the production of new cell wall material only. If the original (labelled) cell wall had contributed to the area of elongation the labelled part would have become diffused through the entire cell wall. This did not occur (Figs. 83, 84). New cells tended to be lighter in colour not only when observed with blue light (Fig. 86, 88, 90), but also when seen with regular white light (Figs. 85, 87, 89). The monosporangial wall exposed to the dye did not absorb it well so that it was not possible to differentiate the monosporangia which developed later (Figs. 91, 92).

Under different light intensities, plants grew significantly faster (probability level p<0.05) at the higher light intensity (A) than at the lower light intensity (B), (Fig. 93). The diameters of new tip cells and the cells immediately below the tip ranged between 8 and 9 μm at both light intensities. However, cell lengths for both these cells were significantly larger at the higher light intensity (A), ranging from 20 to 65 μm. At the lower light intensity cell lengths ranged from 15 to 50 μm (Figs. 94, 95). The cell lengths for the higher light intensity were comparable to the cell sizes recorded for field collected material (Table IV).

Samples of Au. sp. labelled with calcofluor dye and then placed in the dark showed no new growth after 3 months. The plants were still alive
and reinitiated their growth when returned to normal culture conditions.

E. SPECIES DESCRIPTION

The following description of Audouinella sp. was derived from the present study:

Uniseriate filaments in tufts, reaching a maximum of 2 cm in height; plants composed of an erect and prostrate system; the basal system made up of many branched intertwining filaments; the erect system wall developed and much branched; cells of main axis cylindrical, lengths range from 27-65 μm; diameters range from 9-19 μm; cell lengths increase under higher light intensities; branch cell lengths 38-50 μm, diameters 8-11 μm; chloroplasts one per cell, spiral shaped but more often irregularly band shaped, parietal in the cell, thylakoids single in a parallel arrangement with one to three peripheral thylakoids, pyrenoids absent; nucleus one per cell with a prominent nucleolus, chromosomes n=4, two ca. 0.4 μm, two 0.3 μm; many long and short lateral branches along the main axis, denser towards the base; short lateral branches often terminating in hair cells and/or monosporangia; monosporangia lengths 11-19 μm, diameters 8-11 μm; hair cells mainly on lateral branches, occasionally terminal on the main axis, lengths 16-243 μm, diameters 2-6 μm tapering towards the tip; sexual reproduction unknown; plants attached to rocks, moss or other freshwater red algae in fastrunning creeks and streams mostly in shaded areas; abundant in the winter and spring; germlings common in rock scrapings during the summer and fall.
DISCUSSION

A. TAXONOMY

The *Audouinella* sp. in the present investigation was originally identified as *Au. violacea* (Stein and Borden 1979), although it should have been assigned the older epithet *Au. hermanni* (Israelson 1942). In order to confirm the identification, the species description derived from this study was compared to descriptions of 24 freshwater species in the Acrochaetiaceae (Appendix Table I). Nine of the species listed: *Chantransia beardslei* Wolle; *C. hercynica* Kütz.; *C. compacta* Ralfs; *C. dalmatica* Kütz.; *C. serpens* Israelson; *C. subtilis* Mobius; *C. leibleinii* Kütz.; *C. ramellosa* Kütz.; and *Balbiania meiospora* Skuja have descriptions with so few characteristics that they cannot be distinguished from *Audouinella* observed in this study. Seven of the freshwater species have been reported to have more than one chloroplast per cell: *C. cylindrica* Jao; *C. tenella* Skuja; *C. eugenea* Skuja; *Au. glomerata* Jao; *Au. sinensis* Jao; *Acrochaetium godwardense* Patel; and *Rhodochorton venezuelensis* D'LaCoste and Ganesan. However the number of chloroplasts per cell can easily be mistaken in *Au.* species. A single lobed chloroplast viewed through the light microscope can appear to be more than one. Therefore I suspect that most of the numbers provided for the above species are questionable with the exception of those for *R. venezuelensis* which appear satisfactory (D'LaCoste and Ganesan 1972).

Based on vegetative characteristics alone, *Au.* sp. could be any one of the above asexual species as well as the remaining seven asexual species which have been described in more detail: *Au. lanosa* Jao; *C. macrospora*
Wood; C. pygmaea Kütz.; Au. chalybea Bory; Ac. indica Raikwar; Ac. sarmaii Kahn, and Ac. amahatanum Kumano. However, most of these species do not appear to be unique and require further study.

Sexual reproduction has been reported in two freshwater species: Audouinella violacea [= C. boweri Murray and Barton (Drew 1936)] and Rhodochorton investiens (Lenorm.) Swale and Belcher [= Balbiania investiens (Lenorm) Sirodot]. In concluding their study Swale and Belcher (1963) remarked that "it appears that there is no fundamental difference between Balbiania and members of the Rhodochorton-Acrochaetium complex, such as Rhodochorton violaceum (Kütz.) Drew (= Au. violacea). In agreement with Drew (1928) they used the generic name Rhodochorton and named their plant R. investiens. I believe that this plant should have been synonymized with R. violaceum (for complete descriptions see Appendix Table I).

Sexual reproduction was not observed in the Au. sp. studied here and until it is found the alga should not be called Au. hermanni (= Au. violacea).

In this study Au. sp. was not compared to the many type descriptions of the marine members of the Acrochaetiaeae. Using descriptions provided in current literature (Woelkerling 1971; Dixon and Irvine 1977) five marine species were found to be similar in only a few aspects. These include: Au. efflorescens (J. Ag.) Papenfuss, Au. pectinatum (Kylin) Hamel, Au. thuretti (Bornet) Woelkerling, Au. corymbifera (Thuret) Dixon and Au. purpurea (Lightf.) Woelkerling. All the species listed have both erect and basal systems; the first four species reproduce asexually by monospores and the cells contain one parietal chloroplast, varying in shape from irregular and band-like to spiral and ribbon-like (note: Au. efflorescens can have more than one chloroplast). These species are strictly marine in habitat except for Au. purpurea which is reported as
growing terrestrially and in intertidal regions where freshwater runoff is predominant (Rosenvinge 1900; Knaggs 1967b).

B. MORPHOLOGICAL PLASTICITY

The current study showed that the number of chloroplasts per cell and the general habit of the plant are the only stable vegetative characteristics of Au. sp. There was always one chloroplast per cell and both erect and basal systems were present even in the germling state. The remainder of the morphological features generally used in taxonomic descriptions were variable and were not included in the preceding discussion. These features were: the length and diameter of hair cells, cells of the main axes and branch cells; chloroplast type which varied from spiral in the apical cells to more band-shaped in the older cells; and the basal system composed of many intertwining filaments which varied depending on the size of the "clumps" that were collected and whether or not they were teased apart. These features could be used to differentiate species only if allowances are made for morphological plasticity.

All the studies on morphological plasticity in the Acrochaetiaceae have been done exclusively on marine members. West (1968, 1971) noted in Au. pectinatum and Acrochaetium proskaueri that during ontogeny there was much variability in chloroplast morphology, origin and frequency of sporangia, branches and hair cells. He concluded that these characteristics were unreliable as taxonomic criteria. While studying temperature effects on various species of Audouinella Garbary (1979b) also found that branching was variable. In addition he reported that sporangial size, other cell dimensions, and the thickness of the cell wall were extremely
variable.

Stegenga and various co-workers (Stegenga and Borsje 1976, 1977; Stegenga and Vroman 1976; Stegenga and Mulder 1979) in their studies of the life histories of Ac. polyblastum (Rosenv.) Borg., Ac. hallandicum (Kylin) Hamel, Ac. dasyae Collins, Ac. densum (Drew) Papenfuss and Au. microscopica (Nag.) Woelkerling found that branch frequency and pattern, presence of hair cells, morphology of the basal system, number of erect axes and the number of pyrenoids were variable. But, unlike West (1968, 1971) and Garbary (1979b), they noted that chloroplast type, cell diameter and to some extent the monosporangial dimensions were stable characteristics. The general plant structure, type of spore germination and carposporophyte were also considered stable characteristics.

The stability of the general plant structure in Au. sp. agrees with the reports by Stegenga and co-workers. Those features which West and Garbary considered variable were also variable in Au. sp. (note: the thickness of the cell wall was not measured in Au. sp.).

C. SEASONALITY

In general Au. sp. was most abundant in the winter and spring. Drew (1935) also noted that R. violaceum was abundant in the winter and spring. Dillard (1966) found in North Carolina, that Au. violacea was dominant only when water temperatures were less than 15°C. The North Alouette River rarely exceeded 15°C and the disappearance of Au. sp. in macroscopic form did not appear to be related to the temperature.

The seasonality of Au. sp. appeared to be very specific to the river or stream and the year in which it was collected. Wehr (1979) hypothe-
sized that the change in species composition in the Alouette River watershed could possibly be due, in part, to periodic disturbances, such as nutrient pulses and floods. He also suggested that the distribution of red algae was shade limited. A better understanding of the seasonality of Au. sp. can be achieved by a long term ecological study.

D. CYTOLOGY

In the current study of Au. sp. the fine structure was constant throughout the year and in all the samples that were processed for electron microscopy. Several cytological features of interest were observed. Large lipid bodies were evident in many of the cells in field and cultured material, often located close to the pit plug. Pearlmutter and Cole (1980) reported the same structures in a clone of R. purpureum. Using cytochemical tests they showed that these bodies were lipids and that they were formed initially in the cell cytoplasm. They also noted that the lipids aggregated at the pit plugs and were in close association with the endoplasmic reticulum.

Pueschel (1977) found large lipid bodies surrounded by an apparent reticulum of bifurcating membranes in the outer cortex of Palmaria palmata (L.) O. Kuntze. These lipid bodies were similar to the "corps en cerise" located in the cortical cells of several Laurencia spp. (Bodard 1968; Feldmann and Feldmann 1950). A membrane surrounding the lipid bodies in Au. sp. was not observed. Young et al. (1980) showed that the "corps en cerise" in Laurencia snyderae Dawson contained the brominated natural product β-synderol, a halogenated sesquiterpenoid. The lipid bodies of Au. sp. in light micrographs look similar to those in
the trichoblasts and cortical cells of _L. snyderae_ but further work on their contents is merited. The function of the lipid bodies in _Au._ spp. is unknown.

According to some electron micrographs of single sections of _Au._ sp. it may appear that there was more than one chloroplast per cell. However it was clearly seen as a single unit in light microscope observations. The chloroplast was spiral to irregular band-shaped and lacked pyrenoids. Hara and Chihara (1973) claimed that all freshwater red algae contained chloroplasts of the _Polysiphonia_ or _Batrachospermum_ types, lacking pyrenoids. These types infer that there were many discoid parietal chloroplasts per cell; a claim which cannot be applied to all freshwater red algae (e.g. _Au._ sp., _Au._ hermanni, _Chroodactylon ramosum_ (Thwaites) Hansg.) (Sheath and Hymes 1980).

Pit plugs of _Au._ sp. were dumb-bell shaped and bound by a two layered plug cap. All members of the Acrochaetiaceae that have been observed ultrastructurally contained pit plugs similar to these (Lee 1971; Pueschel and Cole 1981 pers. comm.). This type of pit plug is similar to the _Nemalion_-type described by Feldmann et al. (1977). Dilated endoplasmic reticulum in the area of the pit plug was common in _Au._ sp. and other species of red algae (e.g., Feldmann et al. 1977; Aghajanian and Hommersand 1978).

E. HAIR CELLS

The most interesting feature observed in _Au._ sp. was the unicellular hair cell. The ultrastructure of these cells has not been described before. Hair cells appear to be totally lacking in members of the
Bangiophyceae, while in the Florideophyceae they have never been detected in members of the Gigartinaceae and Phyllophoraceae (Gigartinales) or in the Delesseriaceae, Dasyaceae and Rhodomelaceae (Ceramiales) (Dixon 1973).

In his survey of the Florideophyceae, Rosenvinge (1911) noted that unicellular hair cells were the most common. These hair cells were usually terminal on branches or cells of the main axes. They developed as outgrowths of peripheral cells, contained no chloroplasts (except for *Plumaria elegans* (Bonnem.) Schm.) and were separated by a transverse wall as a small cell, early in development. He also observed that the young hair cells contained a nucleus and were filled with dense protoplasm. As the cell lengthened it became vacuolate, forcing the nucleus to the tip surrounded by a thin layer of protoplasm. West (1971) noted the same type of development of unicellular hair cells in *Ac. proskaueri*. However, he also found short lateral projections along the axis of some of the hair cells and he did not detect any chloroplasts or a nucleus within the cells.

Rosenvinge (1911), West (1971) and Akatsuka (1978 Gelidiaceae) reported that hair cells did not always develop but usually occurred when growth was active. Hair cells were short-lived; they developed early and died long before plant growth stopped (Rosenvinge 1911). Dixon (1973) commented that in the northern hemisphere red algal hair cells were abundant in the spring and early summer and absent in the winter. He suggested that this might be related to periods of active growth.

In *Au.* sp. hair cells were common all year on large healthy plants as well as germlings. Development of the hair cells followed the same pattern described by Rosenvinge (1911). The nucleus of the hair cell was easily discernable in haematoxylin stained material and often the
chromatin appeared to be in a condensed state at the tip of the cells. Occasionally metaphase plates were observed but not any other stages of mitosis. In the hair cells of *Callithamnion byssoides* Arnott ex Harv. in Hook (Ceramiales) the nucleus was observed to divide in two (Rosenvinge 1911). It is difficult to explain why nuclear division occurs in the cells.

The function of the hair cells is still unknown. In general it is believed that they absorb nutrients for the alga (Rosenvinge 1911, Dixon 1973). West (1971) showed that an increase in the number of hair cells in *Ac. proskaueri* was controlled by an increase in light intensity. This could perhaps be due to the increase in active growth of the alga. Van den Hoek and Flinterman (1968) found that hair cells in the brown alga, *Sphacelaria furcigera* Kütz. developed when the cultures were depleted of nutrients. Kylin (1917) stated that the hair cells of *Dumontia filiformis* Grev. grew in cultures that did not contain nitrates but did not develop in cultures to which nitrates were added. It is possible that the fine structure of *Au*. sp. hair cells supports the hypothesis that these cells serve an absorptive function. The cell wall is extremely thin in contrast to vegetative cells and the surface area of the cells is great. They contain mitochondria, endoplasmic reticulum, a large nucleus, golgi bodies and many vesicles. All of these cellular inclusions are instruments which could allow absorption to occur. Further studies are still required to determine the function of these cells.
F. MONOSPORE RELEASE AND REGENERATION

In an extensive study of Ac. virgatulum (Harv.) J. Ag. monospores were shown to be released in three different ways; i) an apical slit or tear in the monosporangium wall; ii) an apical rupture; and/or, iii) a laterally placed dehiscence (Boney 1967). Monospore and filament regeneration in old monosporangia appeared to be common. In Au. sp. monospores were released through an opening in the apical wall of the monosporangium. Regeneration of monosporangia, filaments and occasionally hair cells through the monosporangia was observed. Regeneration of damaged apical cells also occurred.

G. CHROMOSOMES

During late prophase, eight chromatids (four larger ca. 0.4 μm and four smaller ca. 0.3 μm) were counted in Audouinella cells. These chromatids were significantly smaller than those reported by Magne (1964) in his survey of the Rhodophyta. He noted that most chromosomes ranged from 2-4-(5) μm in size. Three different sizes of chromosomes were found in the spermatia of 15 species of Porphyra from the west coast of North America (Mumford and Cole 1977). The shorter chromosomes ranged in size from 0.5 to 1.0 μm and are comparable to those of Au. sp. The size and shape of the chromosomes in Au. sp. resemble the minute chromosomes in mosses (Ramsey 1966, 1974).

Chromosome numbers in the Acrochaetaceae have not been studied extensively, probably because cell division is difficult to synchronize and the chromosomes are so small. Magne (1964) reported elongate
chromosomes in *Au. floridula*, each cell containing ca. 20 chromosomes (n or 2n). Woelkerling (1970) found chromosome numbers of n=5 and 2n=10 in cells of *Ac. botryocarpum*. West (1970b) counted between 12 and 14 chromosomes in the prophase nuclei of intercalary vegetative cells of *R. concrescens*. In *Au. sp.* 8 chromatids were counted in late prophase stage and it is likely that West (1970b) was also observing chromatids. Hence, his count was probably n=6 or 7.

In the red algae the majority of the Florideophyceae have high chromosome numbers. Dixon (1966) recorded three ranges of counts: 30-32; 59-65; and 87-98. Ten species of algae in the Florideophyceae have chromosome counts of 4 or 5, one species in the Acrochaetiaeae, three species in the Gelidiaceae, five species in the Gigartinales and one species in the Chaetangiaceae (Dixon 1966; Moore 1973, 1977). In the Bangiophyceae chromosome counts range between 2 and 5, also similar to *Au. sp.* (e.g., in Mumford and Cole 1977; Yabu and Tokida 1963; Kito 1978).

It has been observed in the red algae that the aggregation of chromosomes into clumps along the metaphase plate is common, making it impossible to determine numbers at this stage (Dixon 1966; Mumford and Cole 1977). Dixon also stated that during anaphase and telophase the movement of chromosomes to the poles is very rapid and the two sets of chromosomes separate as two masses. The chromosomes of *Au. sp.* also clumped at the metaphase plate and moved to the poles as two masses. The anaphase condition was easily detectable because the chromosomes were associated in a characteristic horse-shoe type of configuration. This has also been noted in *Bangia* spp. (Cole pers. comm.).
H. GROWTH USING CALCOFLUOR DYE

Calcofluor white dye has been used as an nontoxic label in studies of algal growth (Cole 1964; Waaland and Waaland 1975). It has also been used as a vital stain to localise cellulose and chitin (Haigler et al. 1980). Cole (1964) showed that actively growing tips of the gametophytes of some members of the Laminariales (Phaeophyta) fluoresced brightly when labelled with dye but the reproductive structures in general absorbed the dye weakly. In *Aud.* sp. the monosporangia also absorbed the dye weakly, making it impossible to tell if new growth had occurred. Waaland and Waaland (1975) showed that elongation in five species of red algae was confined to the narrow bands at each end of the lateral walls of growing intercalary cells. In contrast, all growth of *Aud.* sp. was apical and elongation was confined to apical cells only. Elongation occurred by the production of new cell wall material at the growing tip.

I. CONCLUSION

According to the present study several features of the freshwater Acrochaetaceae merit further investigation. Hair cells are particularly abundant on *Au.* sp. It would be of value to establish the function of these cells and to correlate this with the ultrastructure. A determination of the function and frequency of lipid bodies in the Acrochaetaceae would be of interest; an analysis of the contents would be requisite. In addition, the taxonomy of the freshwater members of this family requires amendments with close observations of morphological plasticity.
LITERATURE CITED


Dillard, G. E. 1966. The seasonal periodicity of *Batrachospermum macrosporum* Mont. and *Audouinella violacea* (Kütz.) Ham. in Turkey Creek, Moore County, North Carolina. *The Journal of the Elisha*


Murray, G. and Barton, E. S. 1891. On the structure and systematic position of Chantransia: with a description of a new species.


Swale, E. M. F. and Belcher, J. H. 1963. Morphological observations on wild and cultured material of Rhodochorton investiens (Lenormand)


Wolle, F. 1887. Fresh-water Algae of the United States; Desmids of the United States, Bethlehem, PA.
FIGURE LEGENDS: All figures are of Audouinella sp. from the North Alouette River unless otherwise specified. The micrographs are from cultured material unless otherwise stated. The following abbreviations will be used; 3:1 W = alga fixed in Carnoy's 3:1 alcohol:acetic acid and stained with Wittman's aceto-iron-haematoxylin-chlorohydrate solution; Hoffmann = Hoffmann modulation contrast system; Met. = filaments fixed in methacrylate.
Fig. 1. Light micrograph of plant germlings; collected 15 August 1979. X 400.

Figs. 2-4. Met. stained with PAS/AN.

Fig. 2. Note erect and basal cells. X 500.

Fig. 3. Detail of a cross-section of basal cells. Note different shapes of cells. X 4,200.

Fig. 4. Section showing the filamentous condition of the basal cells (arrow). X 2,300.

Fig. 5. New branch developing following nuclear division. Filament fixed and stained in 3:1W. X 3,000.
Fig. 6. Light micrograph showing the erect axes containing many hair cells and monosporangia on the tips of branches; collected 21 May 1979. X 300.

Figs. 7-9. Hoffmann.

Fig. 7. Detail of the erect axes showing various stages of development of the monosporangia (arrows). X 4,000.

Fig. 8. Detail of the erect axes. Note vacuolate condition of the cells (arrows); collected 11 January 1979. X 1,500.

Fig. 9. Light micrograph of the cell chloroplast (arrows). The chloroplast is peripheral and somewhat lobed; collected 11 January 1979. X 3,200.
Figs. 10-12. The fine structure of *Au.* sp.; cross sections of cells.

**Fig. 10.** Cell with peripheral chloroplast (C), thick cell wall (CW), floridean starch (S) deposited in the centre of the cell and electron dense lipid deposits (arrow); collected and fixed 9 May 1979. X 14,400.

**Fig. 11.** Peripheral chloroplast (C) containing plastoglobuli (arrow) and note the vacuolate (V) area within the cell; collected and fixed 9 May 1979. X 17,200.

**Fig. 12.** Mitochondria (M) and golgi bodies (double arrow) evident with chloroplast containing three peripheral thylakoids (arrow) encircling the remaining thylakoids; collected 23 June 1978 from Neskoun Creek, OR. X 13,100.
Figs. 13-16. Plant was collected and fixed 9 May 1979.

Fig. 13. Section of filament showing the cell wall, peripherally lobed chloroplast and floridean starch deposits (S). X 6,000.

Fig. 14. Cell cross-section: note the nucleus (N), floridean starch (S), and chloroplast (C) containing electron translucent DNA areas (arrows). X 14,000.

Fig. 15. Detail of chloroplast showing plastoglobuli (electron dense), one peripheral thylakoid and phycobilisomes (arrows) on the surface of the thylakoids. X 53,600.

Fig. 16. Detail of the chloroplast (C) with one single peripheral thylakoid (arrow). X 35,000.
Figs. 17-18. Detail of mitochondria (M) and associated golgi bodies (G); ER = endoplasmic reticulum; collected 23 June 1978 from Neskoun Creek, OR. Fig. 17 X 31,800; Fig. 18 X 39,400.

Fig. 19. Elongated mitochondria (M) and the associated golgi body (G); collected 9 May 1979. X 27,600.

Fig. 20. Detail of the cell showing the association of the endoplasmic reticulum (ER) with the plasma membrane: note the electron translucent DNA area (double arrow) within the chloroplast (C). X 30,900.

Fig. 21. Portion of a cell with electron-dense lipid deposit in the cell cytoplasm (arrow). X 24,000.
Figs. 22-26. Light micrographs showing the deposit of large lipid bodies (LB) within the cells of the erect axes.

Fig. 22. Note the vesicular appearance of the lipid bodies (LB), Hoffmann, collected 11 January 1979. X 3,400.

Figs. 23-26. Sections fixed and stained 3:1 W.

Fig. 23. Lipid bodies (LB) deposited in many cells of the filaments Hoffmann; collected 1 September 1979 near Bamfield, B. C. X 3,300.

Fig. 24. The lipid bodies are prominent within the cells (arrows). X 1,700.

Fig. 25. Lipid body possibly in a divided state (arrow). The lipid bodies appear to be concentrated near the pit plugs. X 2,600.

Fig. 26. In this micrograph the lipid bodies are somewhat granular in appearance (clear arrows) and tend to be concentrated at the pit plugs (P). Hoffmann. X 2,600.
Figs. 27-31. Light micrographs of monosporangia and bisporangia.

Fig. 27. Branch containing monosporangia (M) and a hair cell (H), phase contrast, collected 21 May 1979. X 1,200.

Fig. 28. Bisporangium (arrow) adjacent to monosporangium (M); collected 15 February 1979. X 4,000.

Fig. 29-31. Cells fixed and stained in 3:1 W.

Fig. 29. Third regeneration of a monosporangium: note the old cell wall layers left behind (arrows). X 4,600.

Fig. 30. Regenerating monosporangium (arrow): note the wall layers remaining in the monosporangium and the cell below it. X 4,000.

Fig. 31. Note the wall layers remaining in the cell below the empty monosporangium (clear arrow) and the piece of cell wall in the centre of an empty monosporangium (solid arrow). X 3,700.
Fig. 32. Light micrograph showing empty monosporangia, a newly developed monospore (M) and the six remaining wall layers of the cell below it, 3:1 W. X 3,600.

Fig. 33. Opening at the tip of the monosporangium where the monospores escape (arrow), collected 21 May 1979. X 1,700.

Figs. 34-40. Germination of the monospores.

Fig. 34. Monospore just after release from a monosporangium. The cell is granular in appearance and spherical, collected 25 January 1980. X 2,500.

Fig. 35-38. Cells fixed and stained in 3:1 W.

Fig. 35,36. Monospores assuming an oval shape upon germination. Fig. 35 X 3,700; Fig. 36 X 3,400.

Fig. 37. A rhizoidal-type of filament emerges. X 3,900.

Fig. 38. Cytoplasm (arrows) moves from the spore into the germ tube. X 3,200.

Figs. 39,40. The empty monospore (arrow) cut off from the elongated germ tube by a cell wall. Fig. 39 X 350; Fig. 40 X 3,000.
Fig. 41. Fine structure of branch cells terminating in monosporangia: note blue-green alga growing epiphytically within an old monosporangial cell wall (arrow); collected 18 May 1978. X 1,800.

Figs. 42, 43. Empty monosporangia; note the many wall layers (arrows Fig. 42) and the remaining pit plugs (arrow Fig. 43); Fig. 42 collected 18 May 1978. X 9,600; Fig. 43 collected 9 May 1979. X 17,700.
Figs. 44-46. Light micrographs of hair cells.

Fig. 44. Hair cells (H) of various lengths; phase contrast; collected 21 May 1979. X 400.

Fig. 45. Detail of a hair cell showing concentration of the cell cytoplasm at the tip of the hair cell (arrow); phase contrast; collected 21 May 1979. X 1,700.

Fig. 46. Hair cell fixed and stained 3:1 W to show the nucleus (N). The pit plug can also be seen. X 4,000.
Fig. 47. Fine structure of a developing hair cell. Note the presence of mitochondria (M) and golgi bodies (G) and the lack of chloroplasts; collected 9 May 1979. X 18,200.

Fig. 48. Longitudinal section of hair cell: note the mitochondria (M) and small vesicles (VS) within the cell; collected 9 May 1979. X 7,000.
Figs. 49-52. Cross-sections of the fine structure of hair cells. Samples were collected 9 May 1979.

Fig. 49. Note the presence of an elaborate golgi body system (G), endoplasmic reticulum (ER) and mitochondria (M). X 34,100.

Fig. 50. Section showing a prominent nucleus in the cell centre. X 30,400.

Fig. 51. Detail showing the golgi body (G) and the many vesicles produced. Note that none of the vesicles coalesce with the plasma membrane; M = mitochondria; N = nucleus. X 47,300.

Fig. 52. Detail of golgi body (G). Some of the vesicles appear to contain an electron-dense substance (small arrow); M = mitochondria. X 56,300.
Figs. 53, 54. Fine structure of the pit plug and its two-layered plug cap (arrows); Fig. 53 collected 23 June 1978 Neskoun Creek. X 39,500; Fig. 54 collected 21 May 1979. X 64,900 respectively.

Fig. 55. Section showing dilated endoplasmic reticulum (ER) in the region of the pit plug; collected 9 May 1979. X 40,000.

Fig. 56. Micrograph showing the fibrillar nature of the cell wall (CW); collected 18 May 1978. X 31,000.

Fig. 57. Electron-dense wall material (arrow) found occasionally at the periphery of the cell wall; collected 9 May 1979. X 24,000.
Fig. 58. Longitudinal section of the cell nucleus (N) containing a prominent nucleolus (NU); collected 23 June 1978, Neskoun Creek, OR. X 39,200.

Fig. 59. Micrograph showing the endoplasmic reticulum (arrows) associated with the nuclear membrane; collected 9 May 1979. X 21,900.

Fig. 60. The fine structure of a blue-green alga (Chamaesiphon) (BG) epiphytic on Aud. sp. (AUD); collected 9 May 1979. X 9,600.
Figs. 61-63. Observations of unusual features in cultured material.

Fig. 61. Small bulbous cells developing on the erect axes. X 3,600.

Fig. 62. One cell of a rhizoidal-like filament developing on an erect axis; Hoffmann 3:1 W. X 3,400.

Fig. 63. Irregular development of the tip cells: note the abnormal cross wall (arrow); 3:1 W. X 3,100.

Figs. 64-66. Regeneration of the new cells developing through dead cells. All sections were fixed and stained in 3:1 W.

Figs. 64,65. Note the remains of the dead cell alongside the periphery of the new cells formed (arrows). X 3,200 and X 3,600 respectively.

Fig. 66. New developing cell pushing through a dead tip cell (arrow). X 3,300.
Fig. 67-72. All samples were fixed and stained in 3:1 W.

Fig. 67. Eight chromatids (Cr) in a cell subtending a branch during prophase. Note the four larger chromatids and the four smaller ones. X 4,700.

Fig. 68. Monosporangial branch cells containing nuclei with partially condensed chromatin (arrows). In some of the cells it is possible to count 3-4 condensed masses (clear arrow). X 3,000.

Fig. 69. An interphase nucleus (N) in a central 'bulge' of a tip cell: note nucleoplasm around the large densely staining nucleolus. X 3,300.

Fig. 70. An interphase nucleus (N) of a tip cell: note the nucleus is not in a central 'bulge' but a clear zone of nucleoplasm can be seen around the nucleolus. X 3,300.

Figs. 71,72. Pre-prophase tip cells: chromatin beginning to condense along the nuclear membrane (arrow). X 2,600 and X 2,900 respectively.
Figs. 73-79. All cells were fixed and stained in 3:1 W.

Fig. 73. A metaphase plate perpendicular to the longitudinal axis of the cell (arrow). X 3,200.

Fig. 74. A metaphase plate parallel to the longitudinal axis of a hair cell (arrow). X 7,700.

Fig. 75, 76. Anaphase; chromosomes are in a horse-shoe type of configuration (arrows). Fig. 75 X 3,300; Fig. 76 X 3,000.

Fig. 77, 78. Telophase; daughter nuclei separating to the poles: note commencement of cytokinesis. Fig. 77 X 3,200; Fig. 78 X 7,300.

Fig. 79. A cell subtending a monosporangium containing two nuclei (arrows). X 4,800.
Figs. 80-92. All figures are of cultured material that has been labelled with calcofluor dye, washed and allowed to grow in fresh culture medium. Material in Figs. 80, 82, 84, 86, 88, 90 and 92 was photographed while exposed to blue light. Material in Figs. 81, 83, 85, 87, 89 and 91 was photographed with regular white light.

Fig. 80. Development of new cells 12 days after labelling with dye. Note the fluorescing lipid bodies. X 1,000.

Figs. 81, 82. Development of new tip cells 2 days after labelling with dye. X 1,200.

Figs. 83, 84. New tip cells 7 days after labelling with dye. Note that the tip cell elongated after it was labelled. X 600.
Figs. 85, 86. Newly developed branch cells 7 days after labelling with dye. X 650.

Figs. 87, 88. Thirteen days after labelling with dye; development of new cells of the main axis and branches. X 650.
Figs. 89,90. Thirteen days after labelling with dye. Note development of short bulbous cells. X 300.

Figs. 91,92. Seven days after labelling with dye. The monosporangia did not absorb the dye well. Note that the hair cell did not fluoresce. X 800.
Figs. 93-95.  
KEY: bar = standard deviation

Light A [●]  = 35 \( \mu \text{Ein} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)
Light B [□]  = 6 \( \mu \text{Ein} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)

Fig. 93.  
Growth of Au. sp. at different light intensities; number of new cells·day\(^{-1}\) (10 apices counted).
Fig. 94. Growth of Au. sp. at different light intensities; length (μm) of new apical cells during growth.

Fig. 95. Growth of Au. sp. at different light intensities; length (μm) of the cell below the apex during growth.
APPENDIX

Key: + structure present; - no information provided; L = length; W = diameter; all cell sizes are in \( \mu m \)
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<thead>
<tr>
<th><strong>27 descriptions of freshwater members of the Acrochaetiaceae.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alga</strong></td>
</tr>
<tr>
<td>*<em>Chlamydomonas</em> <strong>hetero-</strong></td>
</tr>
<tr>
<td><strong>C. convoluta</strong> Kutz. 1845.</td>
</tr>
<tr>
<td><strong>C. cylindrica</strong> Jao 1941.</td>
</tr>
<tr>
<td><strong>C. dalmatitica</strong> Gütz. 1845.</td>
</tr>
<tr>
<td><strong>C. goreana</strong> Skuja 1941.</td>
</tr>
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<td><strong>C. goreana</strong> Skuja 1941.</td>
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<td><strong>C. goreana</strong> Skuja 1941.</td>
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<tr>
<td><strong>A. minutissima</strong> Skuja 1847.</td>
</tr>
<tr>
<td><strong>A. ramosa</strong> Jao 1940.</td>
</tr>
<tr>
<td><strong>A. serrata</strong> Jao 1942.</td>
</tr>
<tr>
<td>*<em>Acrochaetium</em> <strong>unicastrum</strong> Kurz 1978.</td>
</tr>
<tr>
<td>Taxa</td>
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<tr>
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<tr>
<td>F. microspora Wood 1897</td>
</tr>
<tr>
<td>F. spongiosa Kütz. 1894</td>
</tr>
<tr>
<td>C. serena Kütz. 1862</td>
</tr>
<tr>
<td>C. subulata Mobius 1894</td>
</tr>
<tr>
<td>C. tenella Skuja 1934</td>
</tr>
<tr>
<td>A. indica Kähn 1887</td>
</tr>
<tr>
<td>R. tenuissima Kähn 1937</td>
</tr>
<tr>
<td>Genus</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td><em>Chondria</em></td>
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<tr>
<td><em>Batrachia minuta</em></td>
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</tbody>
</table>

**ii) Sexual species**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Habitat</th>
<th>Basal System</th>
<th>Erect System</th>
<th>Height of Plant [cm]</th>
<th>Chlorelll-type and Color</th>
<th>Pyrenoids</th>
<th>Hair Cells</th>
<th>Sexual Reproduction</th>
<th>Nowozyzangium and Monospores</th>
<th>Tetrasporangium and Tetraspores</th>
<th>Main Axis Cells</th>
<th>Branch Cells</th>
<th>Habitat and References Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chondria</em></td>
<td><em>atenuata</em></td>
<td>branching, somewhat second-order</td>
<td>brown</td>
<td>brown</td>
<td>1.8</td>
<td>bright purple or red</td>
<td>absent</td>
<td>terete plastid</td>
<td>L=9.6; W=2.4</td>
<td>L=13; W=2-3; W=15-5-20</td>
<td></td>
<td>attached to rocks or other aquatic plants in Belgium and Germany; Kutz. (1860); in streams with <em>Batrachia</em> in Europe; Starmach (1972); Nott (1973); Desvaux (1809).</td>
<td></td>
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<tr>
<td><em>C. virgata</em></td>
<td>*Rz}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>L=4.0; W=5</td>
<td>L=10-18; V=6</td>
<td></td>
<td>from river, turbulent areas, shallow water; Rz. (1965); Drew (1925).</td>
<td></td>
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</tr>
<tr>
<td><em>C. brevicaulis</em></td>
<td><em>Murphy and Barton</em></td>
<td>1899</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>L=17.0; W=8.5, widest</td>
<td>-</td>
<td>attached to leaves; Murray and Barton (1899); Murphy and Barton (1899).</td>
<td></td>
<td></td>
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<tr>
<td>Algae</td>
<td>Sexual Species</td>
<td>Habit</td>
<td>Basal System</td>
<td>Erect System</td>
<td>Height of Plant (mm)</td>
<td>Chloroplast-type and Color</td>
<td>Pyrenoids</td>
<td>Hair Cells</td>
<td>Sexual Reproduction</td>
<td>Meiosporangium and Tetraspores</td>
<td>Tetrasporangium and Tetraspores</td>
<td>Meio-Ars Cells</td>
<td>Branch Cells</td>
<td>Habitat and References Used</td>
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