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Robert G. Anderson

University of Missouri - Kansas City

Clarence B. Sinclair

University of Arkansas at Little Rock

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ADDITIONAL INFORMATION ON **BASICLADIA CRASSA**
HOFFMANN AND TILDEN

Robert G. Anderson and Clarence B. Sinclair

The University of Missouri at Kansas City and
Little Rock University, respectively

The alga **Basilcladia** was given generic status by Hoffman and Tilden (1930) following the work of Collins (1907) and Tiffany (1926). Hoffman and Tilden described the two species **B. crassa** and **B. chelonum**, the latter having been identified by Collins (1907) as **Chaetomorpha chelonum**. Tiffany (1926), as well as Hoffmann and Tilden (1930), described reproduction by means of biflagellated zoospores. The latter authors, as well as Smith (1950, p. 218), apparently were in doubt as to whether these biflagellated cells were zoospores; but, none the less, used the term "zoospores?". Leake (1938) saw biflagellated motile cells but was uncertain whether they were zoospores or gametes. She indicated the filaments would not grow in water culture. Leake (1946) later described aplanospore and zoospore germination within 3-4 days in hanging drop preparations. Kusunoki (1944) described and illustrated developing sporangia and the release of large and small zoospores through lateral papillae.

Hamilton (1948), using a series of line drawings, described the reproductive process as the release of biflagellated, spindle-shaped gametes from a parent-cell. These gametes were of the same approximate size as the motile cells described by Leake (1938). The gametes fused anteriorly to result in a spindle-shaped zygote with four (4) anterior flagella. If the gametes failed to fuse, they swam for 1-3 minutes, became spherical, lost their flagella, and degenerated in a few hours.

Ducker (1958) described **B. ramulosa** as a new species obtained from the back of an Australian fresh water turtle. She pointed out an increasing morphological complexity from **B. chelonum** → **B. crassa** → **B. ramulosa**, with **B. ramulosa** being more highly branched and filamentous than the other two species. Ducker did not, however, include in this series **B. sinensis** as described by Gardner (1937).

Up to this point **Basilcladia** has been described as growing only on the carapace of turtles. Proctor (1958) succeeded in culturing **Basilcladia** on an inorganic-soil extract agar medium. The organism also grew on sponge and carapace, and "spread by means of zoospores, and possibly gametes." These flagellated structures were seen to migrate to the walls of the glass container and to make loose attachment from which they were easily dislodged resulting in death of the alga. Proctor suggested that the physical structure rather than the chemical composition of the shells of turtles was important in limiting the distribution of the alga. He stated that pure cultures of the organism were needed for accurate information on possible nutritional requirements. Normandin

and Taft (1959) showed that *B. vivipara*, a new species, grew on the shell of the snail *Viviparus malleatus* Reeve. Explants of the alga from the snail grew well on culture media containing scrapings of turtle carapace. The reverse situation, i.e., *Basycladia* from turtles' backs, showed no appreciable growth in culture on shell scrapings of snail. The authors suggested the possibility of a minimum and specific growth substance existing in the snail shell material. They also stated that the cells releasing reproductive cells "should probably be considered as being sporangia."

In a motion picture study produced in 1964 by the Film Production Unit and the Department of Botany and Plant Pathology at Iowa State University, the release of the zoospores of *Basycladia* was well illustrated. (Liberation of zoospores in the alga *Basycladia*. The Ealing Corporation, Cambridge, Mass.)

It seems obvious to the writers that the true nature of the flagellated cells is unknown and for this reason these cells will be referred to in this work as reproductive cells.

This paper will present new evidence and illustrations of certain morphological features of *B. crassa*. This investigation was carried out at the University of Missouri at Columbia during the summers of 1962-1963 on material from Du Quoin, Ill., supplied by David Norton, a graduate student in the N. S. F. Summer Institute. Acknowledgement is extended to him for his valuable assistance.

The turtles upon which the alga was growing were identified as male specimens of the western painted turtle, *Chrysemys picta bellii* (Gray), by Dr. R. S. Campbell of the Zoology Department of the University of Missouri at Columbia. The turtles were collected at the fair-ground lake in Du Quoin, Ill., placed in a small darkened aerated container and transported to Columbia in the trunk of a car. The alga from the turtles was examined after being in darkness for periods of eight (8) to thirty-two (32) hours, and photographs were made. The pH of the water in which the turtles were maintained and in which the alga was examined ranged from 6.9-9.0. All photographs were made with a Spencer AO Microstar Microscope on Panatomic-X film.

The general vegetative morphology observed is the same as that previously described by Hoffmann and Tilden (1930), Hamilton (1948) and Smith (1950, p. 217). The alga has a rhizoidal system, modified as a holdfast, from which arise infrequently-branched multicellular filaments. The cells have a reticulate chloroplast and intercalary cell division was observed by the writers. Nowhere in the literature, however, is there mention of the cytokinetic process in the vegetative cells of *Basycladia*. Fig. 4 shows that wall formation proceeded centripetally. This process pinched the chloroplast and cytoplasm into two nearly equal portions. A few terminal portions of the filaments were observed, and the shape of the terminal and adjacent cells suggests terminal cell division.

Information on Basycladia crassa

The rhizoidal system of a single filament was highly interwoven with those of other filaments. A complete individual rhizoidal system was difficult to observe for the above reason. In a few cases chloroplasts were observed in the rhizoidal outgrowths, but photographs showing these chloroplasts could not be obtained. Walls of cells near the rhizoidal system were thicker than cell walls in the upper portions of the filaments, and these walls were lamellated. Lamellated cell walls were not as apparent on the vegetative and parent-cells as on cells in the basal regions.

Reproduction is initiated by the transformation of a vegetative cell into a parent-cell. These parent-cells develop one or more papillae at various loci through which the motile cells may eventually escape. Papillae develop simultaneously on numerous adjacent cells. A papilla begins development as a blunted angular protrusion but soon becomes somewhat dome-shaped. Wall thickness of the papilla and that of the reproductive cell from which it is produced are approximately the same throughout. The terminal portion of the dome-shaped projection completed its development 12-15 minutes after slide preparation and gelatinized preparatory to reproductive cell release. That more than one papilla per cell may develop was observed in a few cases, and cells with two (2) open papillae were observed. The parent-cell prior to eruption was highly granular, making it difficult to recognize individual reproductive cells, and there were no apparent swimming movements.

The most commonly observed method of release of reproductive cells was the forceful eruption of flagellated motile cells. The earliest released cells were surrounded by a gelatinous membrane which soon disintegrated; latter members from the same parent-cell escaped individually by swimming movements and were compressed while moving through the cell aperture (Figs. 1, 2). Measurements of the cell aperture of an individual parent-cell in enlarged photographs showed an increase in diameter during this escape; however, the length and width of the parent-cell remained essentially the same. Thus it would seem that the wall material surrounding the aperture is not of the same consistency as the adjacent wall material. Figs. 1 and 2 show part of the release sequence. The above release sequence was photographed after the alga had been subjected to a twenty-seven (27) hour dark period. Similar release sequences were observed after an eight (8) hour dark period and after dark periods ranging through thirty hours.

The motile reproductive cells are biflagellated, spherical to slightly ellipsoidal, with a somewhat crescent-shaped red eyespot and appear to contain many irregularly shaped parietal chloroplasts arranged posteriorly to give a cuplike form. The flagella are located at the anterior end which is devoid of chloroplasts (Fig. 6). The red eyespot was observed at different locations in the cell on both sides of the equatorial plane, but not at the extreme anterior or posterior ends. These motile cells vary in diameter from 10-20 μ . Hamilton (1948) stated that the reproductive cells escaped with flagella trailing, but after escaping, swam anterior end first. This observation was confirmed by the writers

(Fig. 2); however, cells were also observed leaving the parent-cell flagellar end foremost (Fig. 1).

The writers also observed, after a thirty-two (32) hour dark period, a rather unusual release of reproductive cells. This consisted of the release, through the pore of the papillae, of various sized masses of flagellated cells which adhered to each other without subsequent disjunction. These did not exit forcefully, but were released in a slow, outpouring manner, with each mass being constricted as it passed through the pore (Fig. 3). Each cellular mass was surrounded by a gelatinous material. The most striking characteristic of this entire group of cells was the absence of flagellar activity, although flagella were definitely present. It has been suggested to the writers that the release of these cellular masses was the result of a physiological imbalance induced by the unusual environmental conditions. Actively swimming cells were rarely seen after a thirty-two (32) hour dark period.

Some reproductive cells, which were never released, were also observed in parent-cells which had either closed or open papillae.

In the summer of 1963, quadriflagellated reproductive cells with red eyespots were seen on several occasions and were photographed (Fig. 5a, b). This is the first recorded evidence of quadriflagellated reproductive cells in *Basilcladia*. The release sequence for these cells was similar to that of the biflagellated cell release.

DISCUSSION

Many writers — Leake (1948), Hoffmann and Tilden (1930), and Smith (1950) — indicated that the biflagellated reproductive cells which escaped from the parent cell of *Basilcladia* were zoospores. However, Hamilton (1948) recorded the fusing of these same types of structures and stated that they were gametes. Hamilton also stated that the organism was homothallic. Smith (1950, page 214) stated that *Cladophora* reproduced asexually by means of quadriflagellate zoospores. Fritsch (1956) stated that certain species of *Cladophora* and *Rhizoclonium* produce biflagellated zoospores. Prescott (1962) stated that biflagellated zoospores are common, especially in *Cladophora*. These organisms, along with *Basilcladia*, all belong to the Order Cladophorales (Smith, 1950; Fritsch, 1956; & Prescott, 1962). Hamilton (1948) did not report the presence of zoospores in *Basilcladia* in his studies. At no time during our investigations did we see fusion of any reproductive cells although two (2) to several cells were observed adhering in masses. The presence of both biflagellated and quadriflagellated reproductive cells without subsequent fusion only further serves to indicate the lack of understanding of the nature and function of these cells. The aplanospores described by Leake (1946), and the captive cells seen by Hamilton (1948) may very well be different time stages of the same type structure. We did not observe and nowhere in the literature is mention made of the presence of akinetes.

The factors responsible for the forceful and/or extrusive release of reproductive cells are unknown. Actively swimming reproductive cells which remained within the parent-cell eventually escaped by autonomous movements. Although there was some apparent synchronization of release of biflagellated cells from groups of three or four parent-cells, there was no observable acropetal or basipetal sequence of release. It is probable that this release of reproductive cells is the result of light stimulus (Hamilton, 1948). The time of actual reproductive cell formation (prior to, or after, light stimulus) within the parent cell is not known. The authors have shown that papillae formation occurs during the first 15 minutes of the light period.

SUMMARY

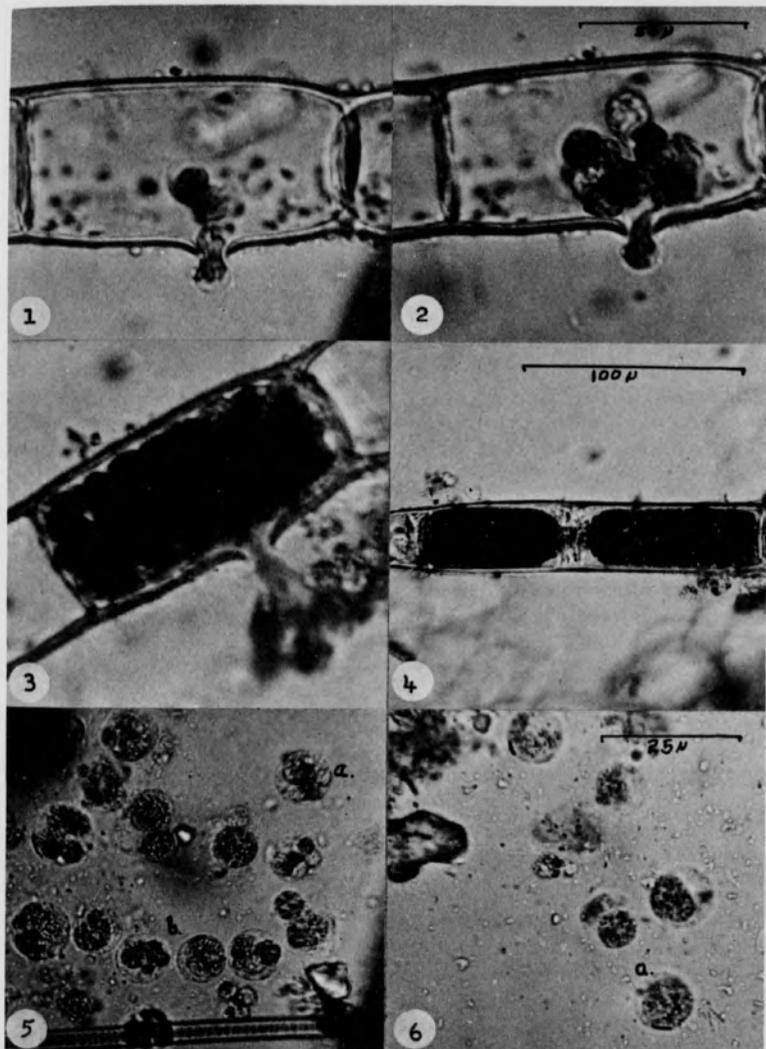
Observations and photographs of *Basicladia crassa* are presented. The vegetative cells have a reticulate chloroplast and divide by constrictive cytokinesis; cell division is intercalary and also appears to be terminal. Rhizoidal outgrowths from the basal cell may or may not have chloroplasts. The reproductive parent-cells were formed from vegetative cells and were seen to release both biflagellated and quadriflagellated motile cells. Quadriflagellated reproductive cells are reported for the first time for this organism. The flagellated cells were released through pores of rapidly formed papillae by means of forceful expulsion and by autonomous swimming movements, and an extrusive, slowly escaping movement. Some reproductive cells were never released from the parent-cells.

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Figs. 1-6. -Figs. 1-2. Two cells in normal release sequence. -Fig. 1. Anterior end foremost. X43 obj. -Fig. 2. Posterior end foremost. X43 obj. -Fig. 3. Extrusive release of clumps of reproductive cells. X43 obj. -Fig. 4. Centripetal wall formation dividing reticulate chloroplast and cell into two (2) unequal parts. X20 obj. -Fig. 5. Quadriflagellated reproductive cells at a and b. X90 obj. -Fig. 6. Biflagellated reproductive cells with crescent shaped red eyespot at a. X90 obj.