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The Need for Laboratory Culture of Algae as a Means to Accurate Classification

By R. L. HULBARY

We are still confronted with a situation in which many genera of algae have species that cannot be identified without knowledge of stages in the life cycle which do not lend themselves to preservation as dried herbarium specimens. Corollary to this situation and arising from it is the difficulty in establishing a soundly based nomenclatural type. Such familiar genera as *Chlorococcum*, *Characium*, *Spirogyra*, *Stigeoclonium*, *Trachelomonas*, *Chlamydomonas*, *Haematococcus* must be present in more than one stage of the life cycle in order to be classified. Many Chlorophyta, such as *Stigeoclonium* and *Cladophora*, or Cyanophyta, such as *Calothrix* and *Stigeonema*, do not have suitable keys even when sufficient stages are found together in natural habitats. These probably have more species named than actually exist in nature due to the erroneous descriptions of two forms which are actually stages in the life cycle of one species. Our position may be illustrated as similar to that of the Paleobotanist who must describe form genera for stem fossils, seed fossils, and root fossils separately because he finds them separate and cannot be sure, for example, which root fossil belongs with which seed. While the Paleobotanist must await the long chance of finding the seed or sporangial parts attached to the stem and roots for clarification of the problem, Phycologists may, in most instances, cultivate the form in question and obtain specimen material suitable for identification. Through study of ontogeny under culture conditions, criteria useful in the preparation of effective keys to species may be revealed.

As with nearly every other group of organisms, in algae the reproductive stages must be present in order to effect identification. Sometimes this means vegetative reproductive features, such as, sporangia, zoospores, aplanospores or akinetes. In other cases gametes, gametangia and zygospores must be present. It is frequently necessary to have both asexual and sexual reproductive structures as in *Chlamydomonas* and some genera of Desmidiaceae. Pure cultures, unialgal cultures and, in many instances, easily maintained mixed cultures viable for a few days or weeks in window-sill aquaria are required for accurate classification.

In groups such as Volvocales dried herbarium specimens are impossible. Preserved specimens are possible and should be maintained. Accurate vouchers of these forms are, however, most satisfactory only as unialgal agar or liquid cultures. Adequate vouchers of the unicellular species of the group can only be maintained in pure or unialgal culture.

Though Cyanophyta lend themselves to drying and re-soaking for examination probably better than any other group, monographs of various genera needed so seriously will never be adequate and certainly not complete until variation of species of the difficult genera under culture and field conditions have been elucidated, recorded and compared. Many species of Blue-Green Algae in the manuals are certainly minor physiological variants of other species. If this latter statement is incorrect, only observation under cultivation will establish its incorrectness. If progress is to be made in the classification of algae, monography can no longer be divorced completely from culture techniques.

The efficacy of culture procedures in the determination of difficult groups of algae is borne out beautifully in the recent work of Starr (1953a, 1953b) on coccoid unicellular Chlorophyta. Isolation on simple agar and liquid media of unicellular forms appearing identical at the beginning of the experiment resulted in development of zoospores, autospores, and, in some instances, gametes, permitting the allocation of the several forms to six distinct genera (*Chlorococcum*, *Radiosphaera*, *Dictiochloris*, *Bracteococcus*, *Spongiochloris*, *Trebouxia*). It was found that by culturing these coccoid forms new structural and cytological criteria were uncovered making key construction easier and classification more nearly natural (phylogenetic). The shape and position of the plastid; the presence or absence of a pyrenoid and such zoospore characteristics as presence or absence of walls and equal or slightly unequal flagellar length were criteria permitting delineation. These distinctive features would have been of no value in dried herbarium material and they do not preserve easily or adequately except in pure or unialgal cultures.

A chaetophoraceous alga collected from stones in the filters of the sewage plant at Iowa City in February, 1950, seemed to belong to the genus *Stigeoclonium*. It had a prostrate irregularly branched filamentous thallus with a few erect tapering filamentous branches. In preliminary aquarium cultures of this alga, unbranched filaments attached with a ulothrixoid holdfast cell and a terminal cell tapering acutely to a sharp point began to appear

(Chang 1952). Later unialgal cultures initiated with single cells and carried through several sexual and asexual generations proved that both types of filamentous plant body occurred. This polymorphism was verified by repeated collections from the sewage plant at frequent regular intervals (one week in summer and two weeks in winter). Because of one phase in its life cycle we have identified this alga as *Stigeoclonium subsecundum* Kuetzing. If it were collected in midsummer and keyed out on the basis of its unbranched filament phase started from quadriflagellate macrozoospores, it would not fit in any *Stigeoclonium* key published thus far. In contrast, it might in this phase key to *Ulothrix*, *Uronema*, *Hormidium*, or *Stichococcus*. If collected in fall, winter, or early spring, the branching heterotrichous phase of the alga could be identified with some certainty as *Stigeoclonium*. Both phases occur together in spring, summer and fall. Unless they are cultured through a life cycle they would appear definitely as two distinct species belonging to genera in two different families, as our system of classification stands today.

Such examples of polymorphism in the life cycle of other species of *Stigeoclonium* have been reported in the past by Livingston 1900, Juller 1937, Godward 1942, and Reynolds 1950. In spite of this, even our most modern keys and, therefore, classifications, are based on the traditional concept of the type method, apparently taking no cognizance of these life cycle studies.

Obviously something should be done to alleviate the present chaotic situation. Silva and Starr, 1953, suggest that since in many taxa of algae no single method of documenting a proposed taxon appears adequate to foster nomenclatural precision and stability, the international rules, at least where they apply to algae should be modified to allow the use of other methods. They recommend that the nomenclatural type in unicellular algae should be "(1) original figures and/or description, (2) preserved portion of type culture (or mixed natural population), and (3) living type culture." This admirable suggestion should be extended to include all algae where difficulties of preservation and polymorphism in the life cycle prevail. The proposal would require the establishment of type culture collections. Such collections exist now at Cambridge, England (E. G. Pringsheim), Charles University, Prague, Paris (Bourrelly), and the University of Indiana, Bloomington, Indiana, (R. C. Starr) with prospects for several other special collections in various parts of the world.

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