
**OBSERVATIONS REGARDING PHYLOGENETIC POSITION OF DICHOTOMOSIPHONALES (BRYOPSIDOPHYCEAE)**

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**Abstract**

Akinetogenesis and gametogenesis were studied in *Dichotomosiphon tuberosus* from Hub River (Pakistan). Filaments were found to contain abundant amyloplasts, aikinetes were separate and arranged in chains with male gametes completely lacking flagella. Arguments are given in favour of keeping this monospecific genus in its own order, Dichotomosiphonales. The affinities of this order have been discussed with other siphonous algae. A diagrammatic scheme has been suggested to show the phylogenetic position of Dichotomosiphonales and to indicate its interrelationships with the other orders of Bryopsidophyceae.

**Introduction**

The oecocytic green algae were at the beginning grouped under Siphonales (Greville, 1830) and later put together under the order Siphonales (Oltmanns, 1904). *Dichotomosiphon* was initially described as *Voucheria tuberosus* A. Br. and placed under this order in the family Vaucheriacae (Ernst, 1902; Oltmanns, 1904; Virieux, 1911; Printz, 1927; West & Fritsch, 1927; Smith, 1933; Fritsch, 1935; Okamura, 1936). Later due to the removal of type genus *Voucheria* of this family from Chlorophyta and its placement in the Xanthophyceae (Chadefaud, 1945; Feldmann, 1946; Strain, 1948; Koch, 1951; Egerod, 1952; Parenfuss, 1955) *Dichotomosiphon* and *Pseudodichotomosiphon* were left with a disputed systematic position. For them a new family, Dichotomosiphonales was established within the Siphonales (Chadefaud, 1945; Feldmann, 1946). At this stage the order Siphonales appeared to the phycologists to constitute a heterogenous group from which several families were upgraded to the rank of orders (Feldmann, 1946, 1952, 1954; Fritsch, 1947; Chadefaud, 1954), as a result of that the order Dichotomosiphonales was erected (Chadefaud, 1954; Fe'dmann, 1954).

From the review of literature it becomes evident that the systematic position of *Dichotomosiphon* Ernst, 1902 within the siphonaceous green algae has not been completely settled. Certain workers continued to describe it within Vaucheriacae (Iyenger, 1951; Randhawa, 1952; Venkataraman, 1961). Some phycologists insisted to include the family Dichotomosiphonaceae under Siphonales (Fritsch, 1954; Smith, 1955; Chapman, 1962; Maeda et al., 1966; Prescott, 1969), some authors placed it under Caulerpales (Feldmann, 1946; Christensen, 1962; Morris, 1967), and some algal taxonomists included it under Bryopsidales (Fott, 1971; Wartenberg, 1972). Contrary to these views other systematicists were convinced to put it under its own order Dichotomosiphonales (Chadefaud, 1954; Feldmann, 1954; Siddiqui

*Dedicated to my teacher, Prof. Mohammed Nizamuddin (Univ. of Libya) on the occasion of his 50th birthday on 11-1-1975.*
& Nizamuddin, 1965; Bourrely, 1966; Kleining, 1969; Starmach, 1972; Chapman & Chapman, 1973). The present study was undertaken to justify phylogenetic position and to discuss systematic relations of Dichotomosiphonales with allied orders included under Bryopsidophyceae (Round, 1963, 1968; Wartenberg, 1972).

Materials and Methods

Specimens of Dichotomosiphon tuberosus (Braun) Ernst, 1902 was collected from the bank of Hub River near Bund Murad, Sind, Pakistan and were brought within a few hours to the laboratory. They were cultured in soil extract solution, obtained by mixing 100 gm of garden soil in 1 l of distilled water, heating and filtering. The pH value was adjusted to 7.5. Cultures were kept under fluorescent tube light of 1200 foot candles intensity at a temperature of 20±2°C. Aeration was brought about by a continuously working air pump, which kept the water circulating. Though the alga can not be safely differentiated from others with the naked eye (Faridi, 1970), identification was made after Faridi (1971).

Results and Discussion

I. Observations on Dichotomosiphon tuberosus.

Our material resembled in morphology, (fig. 1a), reproduction and development (fig. 1b,d) with the previous descriptions (Ernst, 1902; Sharma & Moghe, 1957; Islam, 1965; Siddiqui & Nizamuddin, 1965). The only new observation was the development of large, club-shaped, dark brown and terminal akinetes, full of starch grains, having a few chloroplasts and borne at the ends of special thin rhizoidal branches (fig. 1i). Kamat (1974) observed solitary rhizoidal akinetes in the specimens from Nagpur (India), but our material showed the akinetes occurring in chains. The akinetogenesis starts with the accumulation of cytoplasm at the tip of a rhizoidal branch, which behaves as akinete initial (fig. 1e). Later on bulging takes place (fig. 1f) and the filament becomes constricted in several segments (fig. 1g-i). The segments are rounded and finally separated by brown coloured partition walls. The cross partitioning takes place in a basipetal succession and the terminal most akinete enlarges attaining a length of 500—1500 μ and a diameter of 100—300 μ. It becomes tuberous and acquires a dark colour. Kamat (1974) noticed that akinetes were not separated from rest of the plant by a septum. On this basis our material differed from that of Kamat (1974), and probably there are several subspecies within D. tuberosus.

Antheridia were found to show a peculiarity (fig. 1b). During gametogenesis the proplastids contracts leaving the walls of antheridium, nuclear division is followed by the accumulation of cytoplasm, but the uninucleated pieces do not produce flagella as described by Ernst (1902). These rounded uninucleated but non-cililolated bodies (fig. 1c) escape by the gelatinization of the terminal part of an antheridium. This has repeatedly been observed and I initially considered it as cultural abnormality, but now it has been confirmed by the similar observations of Kamat (1974). The male gametes are therefore non-flagellated. Round (1971) disagrees with the occurrence of amyloplasts in Dichotomosiphon, but they were found in abundance in our specimens (fig. 1a,d).

II. Systematic position of Dichotomosiphonales.

Pseudeichotomosiphon was initially described as Vauceria constricta (Yamada, 1932), and was later established as a genus by Yamada (1934) on the
basis of erect growth, filaments showing frequent constrictions, in part dichotomous branching and thus a vegetative habit somewhat like that of *Dichotomosiphon*. It was thus placed with *Dichotomosiphon* in the same family, initially *Vaucheriaceae* (Yamada, 1934; Fritsch, 1935; Tseng, 1936) and later *Dichotomosiphonaceae* (Chadhé, 1945; Siddiqui & Nizamuddin, 1965; Maeda *et al.*, 1966). But this resemblance is highly superficial and it differs from *Dichotomosiphon* in not being associated with membrane thickenings at the constrictions, lacking starch, not being heteroplastidal and its plastids have thylacoids grouped by 3 (Hori & Ueda, 1967). Moreover it resembles *Vaucheria* in storing oil and in having the sex organs arranged along the sides of the filaments (Fritsch, 1935, 1954). Later on it was considered that *Pseudodichotomosiphon* can not be maintained (Luther, 1953; Fritsch, 1954; Perenfuss, 1962), and on the basis of above mentioned characters it is quite justified to put it back under *Vaucheria*. Therefore the inclusion of this pseudogenus in Dichotomosiphonales as suggested by Siddiqui & Nizamuddin (1965) may not be entertained.
Collins (1909) has described simply on vegetative characters another species, *Dichotomosiphon pusillus*, occurring in the marine environment and lacking sexual reproduction. It was later shown to be a species of *Boodleopsis*, and was renamed as *B. pusilla* by Taylor et al. (1953). This has been confirmed by Venkataraman (1961), Bourrelly (1966) and Taylor (1967), but Fott (1971) still considers it as a second species of *Dichotomosiphon*. In *B. pusilla* the thallus is in the form of a continuous turf or a mat, lower filaments are colourless, more or less buried in the mud and not sharply constricted, upper filaments often produce clubloid masses of short, unconfined and irregular branches, and pyriform to subspaticuliform sporangia like structures are sometimes present (Taylor, 1967). These characters show a resemblance with *Boodleopsis* rather than with *Dichotomosiphon*. Probably this may be a stage in the life-history of some member of the *Udotaceae*, therefore the consideration of Fott (1971) does not appear to be justified.

Kleining (1969) has mentioned one more but unnamed fresh water species of *Dichotomosiphon* with a greater filament diameter. Faridi (1975) has observed that the specimens of *D. tuberosus* occurring in Mall Mandi, Peshawar (Pakistan) differ from the type in minor details, the filaments being about 10 µm smaller and the oogonia being oval instead of globose (fig. 1d). It is quite possible that the new species of Kleining (1969) is simply an ecotype or a subspecies, because it also contains exactly the same carotenoids as the type species. Therefore, till now *D. tuberosus* is the sole representative of *Dichotomosiphonacea* Chaheada ex Smith, 1950. Chaheada (1945) and Feldman (1946) both used a vernacular name, *Dichotomosiphon*, which is in violation of the International Code of Botanical Nomenclature. G.M. Smith (Fresh-water algae of the United States, ed. 2, 1950, p. 281) was the first to use the correct termination,—aceae. Following are the salient features on the basis of which this family differentiates itself from the other families of Bryopsidophyceae.

1. *D. tuberosus* is a cosmopolitan fresh water alga occurring in shallow water of a few meter depth or in subaerial habitat around springs, but the vast majority of siphonous greens (except Protosiphonaceae and a few Cladophoraceae) inhabit marine environment, more especially in the warmer regions of the world.

2. The cell wall of *D. tuberosus* is completely devoid of cellulose and contains about 91—92% D-xylose and 8—9% D-glucose, all of the sugar residues are of B configuration and are connected by 1,3 linkages (Maeda et al., 1966; whereas cellulose and xylan are present in the cell wall of Bryopsidaceae, cellulose, xylan and mannan in Derbesiaceae and mannan in Codiaceae (Frei & Preston, 1964). Cellulose I constitutes the cell walls of Desmidaceae, Shiponocladiaceae and Valoniaceae (Kregtz, 1962; Parker & Leeper, 1969) and cellulose II that of Acrosph. 0haceae (Morris, 1967).

3. The filaments of *D. tuberosus* are heteroplastidial, containing spindle shaped chloroplasts and lens shaped leucoplasts (fig. 1a,d); while Bryopsidophyceae are generally homoplastidial except Cauerpales (Chaheada, 1954), the only plastid present being chloroplast.

4. The chloroplasts of *D. tuberosus* are completely devoid of pyrenoids, though they are present in practically all the other families of siphonaceous green algae except Bryopsis muscosa (Famintzin 1912) and Cauerpalesae (Smith, 1955).
5. Against other coenocytic green algae the thylacoids present in the chloroplasts of *D. tuberosus* are not electronoptically present (Hori & Ueda, 1967).

6. *D. tuberosus* is the only siphonous alga which contains the ester siphonine, without the carotoid siphonoxanthine indicating its complete esterification, whereas Caulerpales, Codiales and Derbesiales contain both of them, in Valoniales only siphonoxanthine is present and in the other orders both of them are absent (Kleining, 1969).

7. In Bryopsidophyceae starch is synthesized and generally deposited on the chloroplasts except Caulerpales and Udoteaceae (Chapman, 1962), but in *D. tuberosus* it is synthesized by the amylogetic leucoplasts and deposited between the chloroplasts (fig. 1a,d).

8. The reproductive organs in *D. tuberosus* are borne at the ends of terminal forking branches of the main filaments (fig. 1b,d,i), while in other coenocytic green algae except Udoteaceae (Nizamuddin, 1969) they are usually lateral.

9. In *D. tuberosus* asexual reproduction occurs by large tuberous akinetes borne in catenate series (fig. ii) and zoospores are absent, though akinetes do not occur in the other siphonous greens except *Bryopsis muscosa*, where irregular akinete-like bodies may develop from rhizoids (Fott, 1933), and zoospores are the normal asexual means of reproduction in most of the families like Acrosiphonaceae, Cladophoraceae, Derbesiaceae, Protosiphonaceae, Siphonochladaeae and Valonaceae.

10. The sexual reproduction of *D. tuberosus* is of an advanced oogamous type, whereas practically all the other families of Bryopsidophyceae exhibit either isogamy or anisogamy.

11. Male as well as female gametes of *D. tuberosus* are non-ciliated (fig. 1c,d), while all the other siphonous green algae produce isocontous and usually biflagellate gametes.

12. In *D. tuberosus* fertilization occurs within the oogonium, but other families of coenocytic greens are generally characterized by the gametic copulation taking place outside the thallus except Sphaeropleales, which is still controversial to be placed under Bryopsidophyceae (Prescott, 1969; Fort, 1971; Chapman & Chapman, 1973).

13. In Bryopsidophyceae in general the zygote develops immediately after fertilization into a new plant, while in *D. tuberosus* it after developing a thin envelope undergoes a period of rest within the oogonium, which does not become detached from the parent filament prior to germination.

14. A peculiarity is exhibited by the germinating oospore of *D. tuberosus*. The germ tube elongates, produces a dichotomy and immediately forms a rhizoidal branch very close to its basal part.

15. Another peculiarity observed in the plastids of *D. tuberosus* is the occurrence of striated tubules and a characteristic association between the golgi bodies and endoplasmic reticulum but the mitochondria are not involved in this association as in the case of *Vaucheria* (Moestrup & Hoffman, 1973).
On the basis of above mentioned characteristics it appears quite justified to place Dichotomosiphonaceae under its own order Dichotomosiphonales Chadeeaud, 1954. Both Chadeeaud (1954) and Feldmann (1954) independently erected this order in the same publication and page priority does not count. It seems rather difficult to decide who should be credited with the name. It is suggested to choose Chadeeaud since he was the first person (1941) to point out that Dichotomosiphon should be removed from the Siphonales.

III. Affinities of Dichotomosiphonales with other siphonous algae.

*D. tuberosus* represents the most advanced type in Bryopsidophyceae. Apart from the above mentioned differences Dichotomosiphonales also shows resemblances with the other orders of coenocytic green algae. The affinities with different orders are described separately to indicate their phylogenetic relationships. Most closely related order is taken first and then the others in a decreasing order of kinship. Dichotomosiphonales resembles with Caulerpaceae (Caulerpaceae and Udoteaceae) in the following characters.

1. Main thallus is attached by downward growing colourless rhizoidal branches.

2. Filaments are siphonous, sometimes dichotomous and with subradichotomous constrictions.

3. The skeletal substance of the cell wall is β-1, 3-linked xylan, which is microfibrillar and the chains are helically coiled (Frei & Preston, 1964; Haleem, 1972). Though *Dichotomosiphon* and *Caulerpa* xylans have respective microcrystal widths of 22 and 24 Å (Parker & Leeper, 1969), the microcrystals appear as component part of microfibrils. Cellulose is completely absent.

4. The protoplasm is heteroplastidial containing chloroplasts as well as leucoplasts.

5. Apart from the principal carotencids the ester siphonein is also present, though contrary to *D. tuberosus* Caulerpaceae also contain siphonaxanthin (Kleining, 1969).

6. Chloroplasts completely lack pyrenoids, while the sub-microscopic plan of structure of the chloroplasts is not similar in the two orders (Hori & Ueda, 1967).

7. Starch is synthesized solely by special amylogenic leucoplasts, chloroplasts being entirely free from starch.

8. Zoospores are absent, the common mode of asexual reproduction in *D. tuberosus* is by means of akinetes (fig. 1j), whereas fragmentation is the usual way of vegetative multiplication in *Caulerpa*.

9. The gametangia are terminal, though they are lateral in *Caulerpa*, which may also exhibit holocarpy (Ernst, 1931).

10. The oogonia or macrogametangia become bright yellow at maturity.
Dichotomosiphonales and Codiales (Bryopsidaceae and Codiaceae) resemble each other in the following characters.

1. Mode of branching of the main thallus is dichotomous, though *Bryopsis* exhibits bi—or tripinnate fronds.

2. Filaments comprising the vegetative thallus are devoid of true cross walls, but there are ingrowths of wall material forming septa, *Bryopsis* lacks septation but bears distinct constrictions at the basal point of pinnae.

3. Cell wall is composed of 3-1, 3-linked xylan, though contrary to *D. tuberosus* cellulose is also present in *Bryopsis* and mannan in *Codium* (Frei & Preston, 1964).

4. In addition to the principal carotenoids ester siphoncin is also present, but contrary to *D. tuberosus* Codiales also possess siphonaxanthin and some of them lack lutein and lutein epoxide (Kleining, 1969).

5. General plan of sub-microscopic structure of the chloroplasts is more or less the same (Hori & Ueda, 1967), they lack pyrenoids which are sometimes present in *Bryopsis*.

6. Akinetes or akinete-like bodies are produced except *Codium* and zoosporangia are completely absent.

7. Holocarpy is absent and there are distinct gametangia, which are different in morphology from the vegetative thallus.

Due to these resemblances some phycologists still consider to place Bryopsidaceae, Caulerpaeae, Codiaceae, Dichotomosiphonaceae and Udoteaceae together within the same order (Morris, 1967; Fott, 1971; Wartenberg, 1972). Though the differences among them are strong enough to warrant this consideration and would make the single order polyphyletic and heterogeneous. Fott (1971) has gone further to include even Derbesiaceae within the same order. Apart from the remarkable differences it shows affinities with the above mentioned families in morphology, cell wall constituents, plastid structure, carotenoids and reproduction indicating a common ancestry. Derbesiales resembles Dichotomosiphonales in the following characters.

1. *Derbesia* and *Dichotomosiphon* possess dichotomously branched, tubular and coenocytic thalli, though the first one sometimes shows monopodial branching due to an unequal elongation of the dichotomies.

2. The filaments show transverse septa at the base of the older branches or membrane thickenings at each constriction, which is absent in *Derbesia*.

3. Xylan takes part in the constitution of the cell wall, though contrary to *D. tuberosus* the cell walls of *Derbesia* also contain cellulose and mannan.

4. Protoplasm includes numerous small spindle shaped chloroplasts without pyrenoids, but chloroplasts of *Derbesia* may contain 2—3 pyrenoids.

5. Together with principal carotenoids siphoncin is also present, although against *D. tuberosus* siphonaxanthin is also present in *Derbesia*.
Dasycladales, Siphonocladales and Valoniales are purely marine found in tropical as well as sub-tropical seas and Acrosiphonales. Cladophorales and Sphaeropleales are found in fresh, brakish and marine waters. They exhibit remarkable differences with Dichotomosiphonales, resemblance lies only in the following few points.

1. Thalli are multinucleate and coenocytic.

2. Crystalline skeletal materials of the cell walls are polysaccharides containing β-D glucose, either partly or completely (Parker & Leeper, 1969), microcrystals appear as component parts of microfibrills.

3. Principal carotenoids present are α and β-carotenes, lutein, lutein epoxide, violaxanthin (—anthaxanthin-zeaxanthin see Hager, 1967) and neoxanthin, although contrary to D. tuberosus siphonein is lacking.

4. Gametes are uninucleate, though against these orders those of D. tuberosus are without flagella (fig. 1c,d).

Apart from prominent differences Dichotomosiphonales also resembles with Vaucheriales in the following characters.

1. Plant body is filamentous, coenocytic, branched and without true partition walls.

2. Thallus increases in length by elongation of the terminal portion.

3. Apart from densely green and vegetative portions there are certain colourless rhizoidal branches.

4. Cytoplasm contains numerous nuclei and lanceolate or discoid chloroplasts or chromatophores generally lacking pyrenoids.

5. Asexual organs of reproduction are club shaped and multinucleate.

6. Sexual reproduction is of a well defined and specialized oogamous type.

7. Male gametes are small and uninucleate and are liberated by an apical rupture of the antheridal wall.

8. Oogonia are spherical and oval and produce a single large non-ciliated ovum, an oogonium produces a small beak-like opening at the apex just before fertilization.

9. Fertilization occurs within the oogonium, the zygote develops a thick oospore wall and under goes a period of rest.

These resemblances are not due to a common ancestry but indicate a very marked parallelism. The list of differences among them would be at least two times larger.
IV. Phylogenetic interrelationships of * Dichotomosiphonales. *

In general there have been two views regarding the origin of Chlorophyta. One view held that Palmellaceae is the most primitive group (Chodat, 1897), whereas the other considered Chlamydomonadae as the most primitive family (Klebs, 1893; Blackman, 1900). The later view appears to be more convincing due to the universal appearance of flagellated cells, vegetative or reproductive in a vast majority of Chlorophyta. It is generally agreed that Chlorococcales and Tetrasporales have been evolved directly from Volvocales, but difference of opinion lies regarding the origin of Ulotrichales. According to one they have been derived from Tetrasporales (Blackman, 1900; Smith, 1933, 1955; Prescott, 1969) and according to the other directly from Volvocales (Fritsch, 1935; Nizamuddin, 1964), but there are more evidences in favour of the first view, where Tetrasporales might have given rise to Ulotrichales through forms like *Chaetopeltis* . What appears to be more logical in the main sequence of evolution is the transformation of a motile unicell into non-motile one and then the development of an attached unicel, which would later easily develop into a simple filament like *Ulothrix*. Therefore it looks more convincing that Chlorococcales has given rise to Ulotrichales through forms like *Chlorosphaera* (fig. 2).

![Phylogenetic diagram](image)

Fig. 2. A suggested phylogenetic interrelationship among Bryopsidophyceae. 1, Hemisiphoniidae. 2, Cystosiphoniidae. C. Eusiphoniidae.
There have been diverse views regarding the origin of siphonaceous green algae. Some phycologists think that Sphaeropleales and Cladophorales have been evolved from Ulotrichales and the others from coenocytic genera (Fritsch, 1935; Iyengar, 1951; Smith, 1955), others consider that all of them have been evolved independently from coenocytic Chlorococcales (Papenfuss, 1951; Egerod, 1952; Prescott, 1969). Fott (1971) is of the opinion that siphonocladaeous algae have been derived from Ulotrichales and truly siphonaceous ones from Chlorococcales, but Chapman & Chapman (1973) discard the origin of siphonaceous algae from Chlorococcales and trace a gradual evolution from a simple Ulotrichaceous ancestor. They do not appear to be convinced by the two lines of evolutionary development proposed by Hori & Ueda (1967) on the basis of plastid ultrastructure. Nizamuddin (1964) suggested a single line of successive evolution starting from Ulotrichales and terminating in Siphonales, cladophoraceous algae being intermediate. It has long been stated that Siphonales is not a valid ordinal name, not being based on a legitimate family (Silva, 1962); but it is really astonishing that this name is still in use (Prescott, 1969; Denfer et al, 1971; Starmach, 1972; Chapman & Chapman, 1973).

After considering the morphology, cytology, reproduction, life-cycle and the recent concepts regarding carotenoids, cell wall structure and the ultrastructure of the plastids it seems most probable that there should have been two phylogenetic lines among Bryopsidophyceae (fig. 2). Codiales, Caulerpales and Dichotomosiphonales appear to have been evolved directly from Chlorococcales through Protosiphonales. The origin of these diploic orders from haploic Protosiphonales probably took place through haplo-diploic forms like Derbesiales, in which the haploid generation resembles Protosiphonales and diploid generation the other orders of Eusiphonidae (3). Apart from life-history and general morphology Derbesiales also occupies an intermediate position between Protosiphonales and Eusiphonidae in the type of carotenoids and cell wall constituents. The other phylogenetic line passes through Cladophorales, which has partly been evolved from Ulotrichales through forms like Lola and partly from Chlorococcales through forms like Charactium. Dasycladales, Siphonocladales and Valoniales were probably evolved by the gradual suppression of haploid and elaboration of diploid generations of haplo-diploic Cladophorales through forms like Acrosiphonales. Sphaeropleales arose independently from Ulotrichales. Cystosiphonidae (2) appears, therefore, to have been evolved from Hemiisiphonidae (1).

The suggested scheme (fig. 2) shows the phylogenetic position of Dichotomosiphonales and its interrelationships with the other orders of Bryopsidophyceae. This is in accordance with the affinities of Dichotomosiphonales with other siphonaceous green algae as discussed above.

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