

## Taxonomy and Phylogeny of *Scenedesmus*

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The first taxa of the genus *Scenedesmus* were described in 1828 and studied for more than one hundred years only by light microscopy. The position, delimitation and subdivision of the genus has often been changed. The study of the cell wall structures in the transmission electron microscopy and finally DNA studies (G/C content, homologies, reassociation, sequence analyses) have been very important for progress in the taxonomy of the genus. The combined studies of light and electron microscopy and of DNA studies resulted in a better understanding of the relationship of taxa. Besides overinterpreted variability, polymorphism occurs with the switching of qualitatively different cell wall ultrastructures with the following generation. According to recent publications the genus is now divided into only two subgenera. Several taxa from the former family Chlorellaceae (now classes Trebouxiophyceae) were transferred to *Scenedesmus* and the genera *Tetrademus* and *Enallax* should become retransferred to *Scenedesmus*.

**Key Words:** *Chlorella*, *Dicloster*, DNA, electron microscopy, light microscopy, morphology, phylogeny, physiology, *Scenedesmus*, taxonomy, *Tetrademus*

### INTRODUCTION

The first species of the genus *Scenedesmus* were described by Turpin (1828) and placed by him in the diatoms, later by Ehrenberg (1834) in the Desmidiaceae, by Nägeli (1849) in the Chlorococcales, family Hydrodictyceae, and finally by Oltsmanns (1904) in the family Scenedesmaceae. The genus name was created by Meyen (1829). For this genus Hegewald and Silva (1988) enumerated about 800 taxa or combinations. The most important monographs are: Smith (1916), Chodat (1926), Kiriakov (1977), Komárek and Fott (1983) and Uherkovich *et al.* (1995) and for Korea: An (1989). Only Komárek and Fott (1983) and An (1989) considered also electron microscopy, all other monographs are exclusively based on light microscopy.

According to Komárek and Fott (1983) the family Scenedesmaceae has 6 subfamilies, the genus *Scenedesmus* belongs to the subfamily Scenedesmoideae. It includes 8 genera: *Pseudotetrademus* Hirose *et*

Akiyama, *Enallax* Pascher, *Tetrademus* G.M. Smith, *Didymocystis* Korschikov, *Dicloster* Jao, Wei *et* Hu, *Neodesmus* Hindák, *Scenedesmus* Meyen and *Westellopsis* Jao. Of these the genus *Pseudotetrademus* with its single species was transferred as a synonym to *Scenedesmus* (Hegewald *et al.* 1988) and the genus *Didymocystis* was transferred to the Oocystaceae (Hegewald 1988b). However, the genus *Pseudodidymocystis* Hegewald *et* Deason (Hegewald and Deason 1989), which belongs to Scenedesmaceae was split from *Didymocystis*.

According to Komárek and Fott (1983) the genus *Scenedesmus* is defined by flat coenobia of 2-32 cells, arranged in 1 or 2 rows, cells of different shape, but always elongate, with cell poles from acute to truncate/obtuse, cell wall smooth or with different sculptures, with or without spines, with 1 chloroplast and 1 pyrenoid. Reproduction by autospores, which are arranged within the mother cell into a new coenobium (Trainor 1996: autocoenobium).

Besides autospores, zoospores were first mentioned by Valz (1870) and later produced in culture (Trainor 1963, 1993, 1996), however, they could not be produced in any other laboratory. Trainor (1996) also reported the production and germination of zygotes. Since Valz (1879) we have only one doubtful record for zoospores in outdoor mass cultures (Lukavský 1991; Cepak 1993). Hence

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we conclude that, if sexuality occurs, it is negligible and reproduction is generally, in culture as in nature, by autospores. That means that all mutations occurring which do not significantly influence growth and competition will last and cannot be lost by genetic processes. So it is easy to understand that most of the occurring morphological mutations survive and hence that the genus has an enormous variability.

The genus *Scenedesmus* was divided into 2-6 subgenera or "groups" by Chodat (1926), Hegewald (1978), Hindák (1990), Kiriakov (1977), Komárek and Fott (1983), Lagerheim (1882) and Uherkovich (1966). We distinguish three groups, treated as subgenera in Hegewald (1978) and Kiriakov (1977), one group has acute cell poles (subgenus *Acutodesmus* Hegewald = subgenus *Fusodesmus* Kiriakov nom. inval.), another group has obtuse or truncate cell poles without spines (subgenus *Scenedesmus*) and the last group also has obtuse to truncate cell poles but the coenobia have spines and/or cell wall structures (subgenus *Desmodesmus* Chodat). We will discuss these subgenera separately.

#### The Subgenus *Acutodesmus* under the Light (LM) and Electron Microscope (EM)

The taxonomy of the subgenus *Acutodesmus* is mainly based on the cell form and cell arrangement, because only few other characteristics are available. An additional taxonomically useful characteristic was found by Krienitz (1987) in growth at high temperature (35°C). High temperature taxa were hitherto known from *Chlorella* Beijerinck (Kessler 1976) but for *Scenedesmus* only high-temperature strains were found (Hegewald 1984). By high temperature resistance Krienitz (1987) was able to separate e.g. *S. obtusiusculus* Chodat from *S. obliquus* (Turpin) Kützing, which otherwise could not be separated by Hegewald (1982). Both taxa are morphologically similar, *S. obtusiusculus* is usually single-celled and smaller than *S. obliquus*, which generally produces coenobia. However, many strains of *S. obliquus* can produce up to 100% single cells and these are smaller than coenobial cells (Hegewald 1982). The production of single cells is common in many *Scenedesmus* species, the coenobia of the species of the subgenus *Acutodesmus* generally disrupt into single cells before cell division. The release of single cells instead of coenobia from a mother cell depends on strain and environmental conditions. If growth conditions are good and the algae are growing fast (e.g. production of more than 8 daughter cells), they often produce only single cells. However, there are many

other factors of influence, e.g. suboptimal growth conditions or special accompanying bacteria. When Egan and Trainor (1989) postulate that low cell density is the unifying principle for unicell development in *Scenedesmus*, they are right because low cell density means good growth conditions (e.g. more light per cell) hence fast growth, however, the cell density itself is not a unifying principle for unicell development.

The recent revision of the subgenus *Acutodesmus* by Holtmann (1994) gives more weight to the twisting of the plane of coenobia and of cells and to the manner of attachment of cells, as well as to the difference of the cell length of outer and inner cells within a coenobium. Based on this revision a key for the taxa is given as an appendix.

A genus morphologically very close to *Scenedesmus* subgenus *Acutodesmus* is *Diclostera*, described by Jao *et al.* (1976). The tropically/subtropically distributed taxon can be distinguished by the arrangement of 2 and 2 cells above each other and by 2 pyrenoids per cell. However, 2-celled coenobia with one pyrenoid per cell are often found in nature (Hegewald 1988a; Hortobágyi 1969 sub nom. *S. acuminatus* var. *elongatus* G.M. Smith, Schmidt *et al.* 1997) and these coenobia can hardly be distinguished from 2-celled *S. acuminatus* (Lagerheim) Chodat. Another related genus is *Tetradismus* with a bundle-like arrangement of cells, which is the only distinguishing characteristic from the subgenus *Acutodesmus*. The genus was transferred to *Scenedesmus* by Chodat (1913) and this was accepted by several authors, however, in Komárek and Fott (1983) the genus *Tetradismus* has been established again. One taxon, described as a variety of *Scenedesmus* (*Tetradismus*) *wisconsinensis* (G.M. Smith) Chodat, is var. *reginae* Holtmann (1994). This taxon has a cell arrangement as *Diclostera* but has only one pyrenoid and much shorter cells. Hence the placement of the taxon is doubtful.

To the subgenus *Acutodesmus* also belongs a species with rib-like structure: *Scenedesmus acutiformis* Schröder. This structure, which is best visible under the EM, is formed by the hemicellulosic cell wall layer, different from the structures in the other subgenera. Because of this structure the species was transferred to the genus *Enallax* by Hindák (1990). However, one or several weakly developed longitudinal ribs are usually found in other species of the subgenus *Acutodesmus*.

#### The Subgenus *Scenedesmus* under the LM and EM

The subgenus *Scenedesmus* includes all spineless

species with obtuse cell poles and without cell wall structures of the sporopolleninic cell wall. However, two taxa show granulations under the LM: *S. verrucosus* Roll and *S. parvus* (G.M. Smith) Bourrelly, but the granulation of the former species is a fluffy precipitation on the cell wall and not a cell wall structure. For the latter we do not know, but according to the irregular arrangement of the granulation we suspect, that it is also a precipitation.

The subgenus was revised by Hegewald *et al.* (1988), showing that the genera *Pseudotetradasmus* and *Raysiella* Edelstein et Prescott are not justified and the species of these genera are synonyms of species of *Scenedesmus* subgenus *Scenedesmus*. Also two subgenera became synonyms of the subgenus *Scenedesmus*: subgenus *Rhynchodesmus* Chodat with only one species, (*S. bacillaris* Gutwinskii = *S. producto-capitatus* Schmula) and *Clathrodesmus* Chodat with *S. raciborskii* Woloszyńska only (Hegewald 1978).

In the subgenus *Scenedesmus* I have been found some extraordinary cell arrangements: *Scenedesmus ellipticus* Corda has up to 32 cells arranged linearly in one coenobium and *S. arcuatus* (Lemmermann) Lemmermann has up to 16 cells arranged in three rows. This extraordinary arrangement led to the establishment of the above mentioned genus *Raysiella*, which was not placed in the subfamily Scenedesmoideae but in Tetrallantoideae because of a separation of cells. However, this separation was probably artificial, caused by the pressure of a cover glass.

### The Subgenus *Desmodesmus* under the LM and EM

The subgenus *Desmodesmus* has the highest number of taxa of all subgenera. It is characterized by 4 sporopolleninic layers as the outer cell wall layer (compared with three in the subgenera *Acutodesmus* and *Scenedesmus*). The fourth layer produces cell wall structures (Hegewald and Schnepf 1979) which are often also visible under the LM as granulation or ribs. Most species have short or long spines. The occurrence, arrangement and length of the spines has been the most important taxonomical characteristic for more than one hundred years and often still is today (Uherkovich *et al.* 1995). Bisalputra and Weier (1963) observed the cell wall ultrastructure under the EM for the first time and Pelicarić (1971) showed that different species have different structures. Finally Komárek and Ludvík (1971, 1972) used these structures for the taxonomy of the genus. These structures were later used as the most important differentiating criteria by Hegewald and co-workers (e.g.

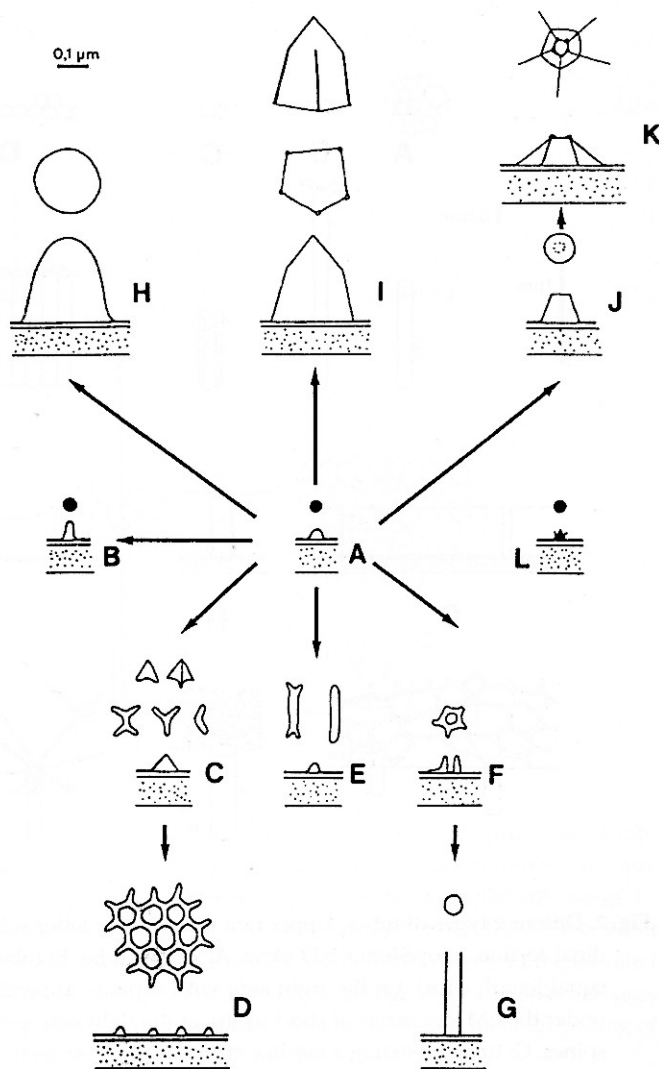


Fig. 1. Warts and related cell wall structures of *Scenedesmus* subgenus *Desmodesmus*. All figures are illustrated in cross section (lower figures) and in top view (upper figures). The inner hemicellulosic cell wall layer is dotted. In the outer sporopolleninic layer three inner layers and an outer structure-producing layer are distinguishable. A: simple round wart, B: elongate wart, C: (2-)-3-4 armed warts, D: net-like structure composed from 3-4 armed warts, E: elongated rod-like structures, usually found between two cells, F: short tube-like structure composed of armed warts, G: long elongate tube, H: large round warts, I: large round warts with stiffenings, J: large truncate warts, K: as J but with wing-like stiffenings, L: compact warts.

Hegewald *et al.* 1990; Hegewald and Schnepf 1991). There are three groups of structures: warts and related structures (Fig. 1), tubes (Fig. 2) and rosettes (Fig. 3). All the structures of Figs 1-3 vary and if strongly developed are visible under the LM, e.g. large warts (as granulation) or bundles of tubes (as teeth and spines) or rows of tubes (as ribs) as well as large rosettes (as prop or teeth-

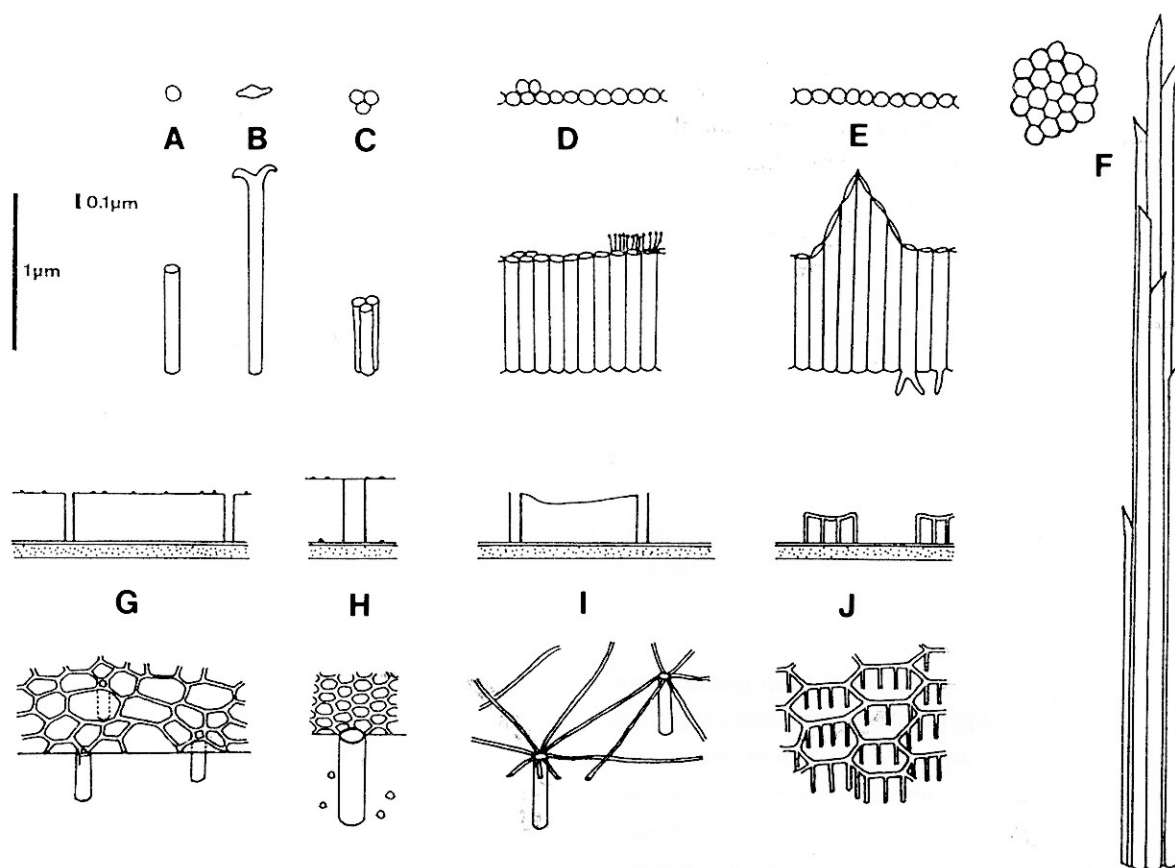


Fig. 2. Different types of tubes. Upper two rows: below letter side view, above letter top view. Lower two rows: below letter longitudinal section, above letter 3-D view. A: simple tube, B: tube with forked top, C: bundles of tubes, D: linearly arranged tubes of equal length (ribs), on the right side with capitate appendages, E: as D, but tubes of different length (can be misinterpreted under the LM as a series of short teeth), at the right side with basal rod-like appendages, F: bundle of tubes of different length = spines, G: tubes elevating a net-like structure, in cross section angular, H: as G, but tubes are round in cross section, I: tubes connected by string-like structures, J: tubes carrying rod-like structures which are connected to a net-like structure.

like structures). Nonvisibility of e.g. ribs under the LM does not mean that the studied specimens do not have ribs, but they may be reduced and only detectable under the EM. However, in the past high taxonomic importance was especially attached to the ribs and many taxa are based on the occurrence and development of ribs (e.g. many taxa described by Hortobágyi 1960). We cannot give here a detailed discussion of these structures, but only some information which may be necessary for the discussion of the relationships. All the warts in Fig. 1 are foldings of the fourth sporopolleninic layer, only the type of Fig. 1L is different, being compact and not hollow structures. These are found only in *Scenedesmus lefevrii* Deflandre and related taxa. The most common type of warts is shown in Fig. 1A, it is a low round simple wart, found especially in the section *Armati*, e.g. in *Scenedesmus armatus* (Chodat) Chodat. The armed warts (Fig. 1C) and the rod-like structures (Fig. 1E) are also

typical of this section and the latter are especially found between cells. The simple but elongated warts (Fig. 1B) are only found in *S. costato-granulatus* var. *elegans* (Hortobágyi) Hegewald et Krienitz. Only in this variety and the species itself are the large warts found (Fig. 1H+I), which occur as single warts or aggregated as ribs. Another type of large warts (Fig. 1J+K) is found in *S. serratus* (Corda) Bohlin and the related *S. lunatus* (W. et G.S. West) Chodat. The tubes of Fig. 2 rarely occur as single elements (Fig. 2A) and only in *S. komarekii* Hegewald as bundles, scattered all over the surface (Fig. 2C). Most common is the aggregation to ribs, found especially in the section *Armati*, however, the capitate appendages of Fig. 2D occur only in three species which are taxonomically not related (*S. abundans* (Kirchner) Chodat, *S. lunatus*, *S. pleiomorphus* Hindák). Tubes as the fourth cell wall layer elevating structure are found in subsection *Desmodesmus*, e.g. *S. communis* Hegewald and in subsec-



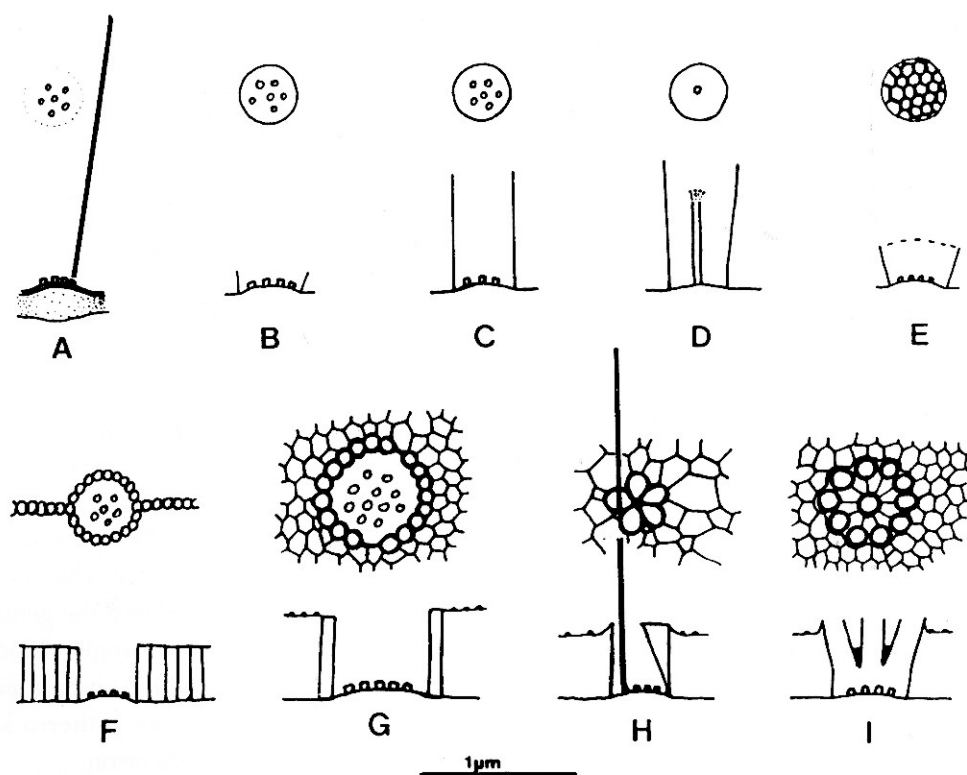


Fig. 3. Different types of rosettes ("apical openings"). A: basic type without surrounding structures. Basal bristle producing knob-like structures and one bristle illustrated. Under the rosette the hemicellulosic layer is thicker but electron microscopically less dense (the layer is not illustrated in B-I), B: as A but surrounded by a simple wall, which sporadically has radial stiffenings, C: chimney-like rosette, D: as C but instead of bristle-producing knob-like structures a tube with capitate appendages, E: as B but covered with a net-like structure, F: rosette surrounded by tubes and included in a rib, G: opening-like rosette in the elevated fourth sporopolleninic layer, H: as G but covered by tubes which are open to the center of the rosette, hence bristles can pass through (one bristle illustrated), I: as H but composed of a larger number of tubes arranged in 1 (or 2) central tubes and a ring of outer tubes.

tion *Denticulati* Hegewald or as structure elevating elements in *S. intermedius* Chodat (Fig. 2J). Also the different types of rosettes of Fig. 3 are typical of related taxa (e.g. subsections). However, they show some variability within the subsection *Desmodesmus* as also the elevating tubes, which can be round in cross section or 3-, 4- or 5-cornered and the net-like structures, which can be regular or can have different mesh sizes. For species of the subsection *Desmodesmus* an example is given in Fig. 4 of the combination of LM and EM characteristics for distinguishing taxa. Often only the combination of characteristics allows taxa to be recognized which otherwise are identifiable with difficulty or not at all under the LM or EM.

For more than one hundred years there have been many discussions and publications about variability or polymorphism in *Scenedesmus*, but besides cell or coenobium shape these consider only differences in spine number and length or other quantitative characteristics

(e.g. Trainor 1964, 1993). However, we have to accept this as a normal variability of taxa and it should not be overinterpreted. Besides this quantitative variability in *Scenedesmus* we also have "true" polymorphism, that means the occurrence of qualitatively different structures, as described first for "*Chlorella*" *fusca* Shihira et Krauss var. *fusca* = *Scenedesmus abundans* (Fott et al. 1975; Hegewald 1989; Hegewald and Schnepf 1991), but which was also found in other taxa: *Scenedesmus kissii* Hortobágyi (Hegewald 1989), *S. komarekii* (Hegewald 1989), *S. multififormis* Hegewald et Hindák (Hegewald et al. 1990), *S. spinosus* Chodat (Hegewald et al. 1990). In *S. abundans* the common cell arrangement in nature and in well growing cultures is 2- or 4-celled coenobia with a spine on each corner of the coenobium and one in the middle of the outer cells. Under the EM the cell wall has no structures beside chimney-like rosettes. With depletion of nutrients the following generation is single-celled with very short and delicate spines and a cell wall struc-

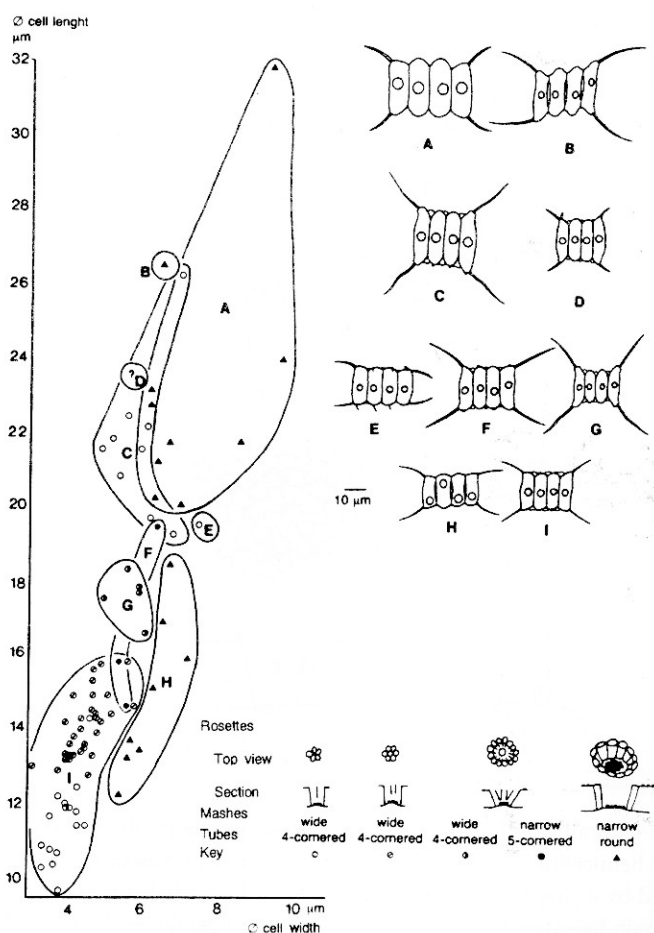


Fig. 4. Average cell sizes to average cell widths, habitus and electron microscopical characteristics of taxa of the subgenus *Desmodesmus* series *Desmodesmus*. The different types of rosettes, net/like structures and tubes are characterized by a sign ("Key") A: *Scenedesmus maximus* (W. et G.S. West) Chodat, B: *S. tropicus* Crow, C: *S. protuberans* Fritsch et Rich, D: *S. curvaticornis* Proschkino-Lavrenko, E: *S. spec.*, F: *S. spec.*, G: *S. spec.*, H: *S. perforatus* Lemmermann, I: *S. communis* Hegewald.

ture of long-armed warts and rosettes of the *S. armatus* type. With further depletion of nutrients the following generation is spineless but with well-developed ribs with capitate appendages and a net-like cell wall structure elevated by tubes. They can be single-celled or produce 2- or 4-celled spineless coenobia. After the addition of nutrition the next generation will again develop the spiny coenobia as mentioned first. In general all observed polymorphism in *Scenedesmus* is influenced by the nutrition and long-spined coenobia are developed at good nutrition supply, while spineless cells or coenobia with strong ribs are developed at low nutrition concentration.

In the subgenus *Desmodesmus* single cell production is

common in the culture of strains but is often also found in nature. Many single-celled specimens are described as species of the genus *Lagerheimia* (incl. *Chodatella*), as e.g. *L. balatonica* (Scherffel in Kol) Hindák. However, *Scenedesmus* (Scenedesmaceae) and *Lagerheimia* (Oocystaceae) are easy to distinguish because of the cell wall, which is hemicellulosic and sporopolleninic in *Scenedesmus* (Fig. 1A) and has cellulosic fibers in *Lagerheimia*. Also the release of autospores is different: by rupture of the cell wall in *Scenedesmus* and by dissolution in *Lagerheimia*. Some strains of *Scenedesmus* are only known with single cells as e.g. *Chlorella fusca* var. *fusca* UTEX 343 (= *Scenedesmus abundans*) and *Chlorella ultrasquamata* Fott et Cléménçon (= *Scenedesmus verrucosus* Roll). Hanagata *et al.* (1996) found single-celled *Scenedesmus komarekii* on bark. This little known species was also found in soil. Hence the genus *Scenedesmus* is not only planktonic. In a single-celled form it can be found in soil and aerophilic on bark and it is certainly much more common than hitherto known, but was misidentified as a *Chlorella* taxon.

### Physiological and DNA Studies

For the genus *Scenedesmus* Hellmann and Kessler (1974) and Kessler (1980) tried to find some taxonomically useful differences in physiology or % GC (Guanine + Cytosine) content of DNA. Many differences found were only strain characteristics or their validity for a species could not be verified, if only one strain per species was studied. Although these authors found differences in % GC between species of the subgenus *Desmodesmus* they could not do so for the subgenus *Acutodesmus*. All their studied strains of this subgenus finally belong to two taxa only: *Scenedesmus obliquus* and *S. acutiformis*. The latter, which was as mentioned before transferred to *Enallax*, fits well in % GC with the subgenus *Acutodesmus*, hence should be retransferred to *Scenedesmus*.

If we summarize all data on % GC of DNA for the genus *Scenedesmus* (Table 1) we can distinguish the subgenus *Scenedesmus* which has the lowest values of the genus (50-52% GC) from the subgenus *Acutodesmus* (55-58 % GC), but this subgenus cannot be distinguished by the % GC content from the subgenus *Desmodesmus*. This subgenus has a very wide range of values (51-69 % GC). Although the measurements of the different authors are rather similar, the values for *S. intermedius* differ. This may reflect a high % GC variability in that species, which also shows an extremely variable morphology (Hegewald, An, Schnepf and Tsarenko, in press). The

**Table 1.** The DNA base composition (Mol % G+C) of *Scenedesmus* strains. P + H = Paschma and Hegewald (1986); K = Kessler *et al.* (1997); Hea = HUSS *et al.* (1989)

	P + H	Kea	Hea
<i>Scenedesmus acutiformis</i>	-	55	-
<i>Scenedesmus (Tetradesmus) wisconsinensis</i>	56	-	-
<i>Chlorella fusca</i> var. <i>rubescens</i> (= <i>S. rubescens</i> )	-	57	57
<i>Scenedesmus obliquus</i>	57	57-58	55
<i>Scenedesmus obtusiusculus</i>	-	57-58	-
<b>Subgenus Acutodesmus: 55-58</b>			
<i>Scenedesmus obtusus</i>	50	52	-
<i>Scenedesmus bacillaris</i>	-	52	-
<i>Chlorella fusca</i> var. <i>vacuolata</i> (= <i>S. vacuolata</i> )	-	50-52	52
<b>Subgenus Scenedesmus: 50-52</b>			
<i>Scenedesmus insignis</i>	51	-	-
<i>Scenedesmus denticulatus</i>	51-52	52	-
<i>Scenedesmus denticulatus</i> var. <i>linearis</i>	53	-	-
<i>Scenedesmus communis</i>	54-55(56)	52-55	-
<i>Scenedesmus protuberans</i>	52-54	-	-
<i>Scenedesmus maximus</i>	54-55	-	-
<i>Scenedesmus perforatus</i>	55	-	-
<i>Scenedesmus tropicus</i>	55	-	-
<i>Scenedesmus armatus</i> et varieties	53	55-57	56
<i>Scenedesmus komarekii</i>	-	54	-
<i>Scenedesmus spinosus</i>	54	-	-
<i>Scenedesmus abundans</i>	54	53-55	-
<i>Chlorella fusca</i> var. <i>fusca</i> (= <i>S. abundans</i> )	-	55	56
<i>Scenedesmus cuneatus</i> (= <i>S. aldavei</i> )	56	-	-
<i>Scenedesmus intermedius</i>	58	62	-
<i>Scenedesmus serratus</i> (= <i>S. pseudodenticulatus</i> )	58	-	-
<i>Scenedesmus subspicatus</i>	59	-	-
<i>Scenedesmus grahneisii</i>	59	-	-
<i>Scenedesmus bicellularis</i>	-	61	-
<i>Scenedesmus costato-granulatus</i>	67-68	67	-
<i>Scenedesmus lunatus</i>	69	-	-
<b>Subgenus Desmodesmus: 51-69</b>			

spineless taxa (*S. lunatus*, *S. costato-granulatus* Skuja, *S. bicellularis* Chodat, *S. grahneisii* (Heynig) Fott) show the highest values of % GC but may have no closer relationship. Kessler *et al.* (1997) placed *Chlorella fusca* var. *vacuolata* Shihira et Krauss in the subgenus *Acutodesmus*, however, the taxon has no spindle-like acute cells, hence has to be placed in the subgenus *Scenedesmus*. Here it also fits well according to % GC content.

The polyamine pattern, which is important for the taxonomy of bacteria also proved to be useful for the algal taxonomy. While Hegewald and Kneifel (1981) found taxonomically useful differences of polyamine composition in the family Scenedesmaceae, Kneifel and Hegewald (1980) could not find any differences in the genus *Scenedesmus*, and could not separate any subgenera or the genus *Tetradesmus*. However, when Hegewald

and Kneifel (1982) studied the polyamine composition of the genus *Chlorella* (Chlorellaceae), they found that *Chlorella fusca* var. *fusca*, var. *vacuolata* and var. *rubescens* Kessler *et al.* as well as *C. zofingiensis* Dönzing and *C. homosphaera* Skuja had the same polyamine composition as *Scenedesmus*. At that time it was already known that *C. fusca* var. *fusca* is a *Scenedesmus* (Hegewald 1982), however, the other varieties of *Chlorella fusca* were not transferred to *Scenedesmus*. Kalina and Punčochárová (1987) split the genus *Chlorella* and retransferred *Chlorella fusca* var. *rubescens* to *Halochlorella* Dangeard. However, their figures clearly show single-celled *Scenedesmus obliquus*/*S. obtusiusculus* Chodat with its typical unstructured bristles. Also the DNA reassociation (Huss *et al.* 1989) and the % GC content of DNA (Kessler *et al.* 1997) is similar in *Chlorella fusca* var. *rubescens* and *Scenedesmus obliquus*

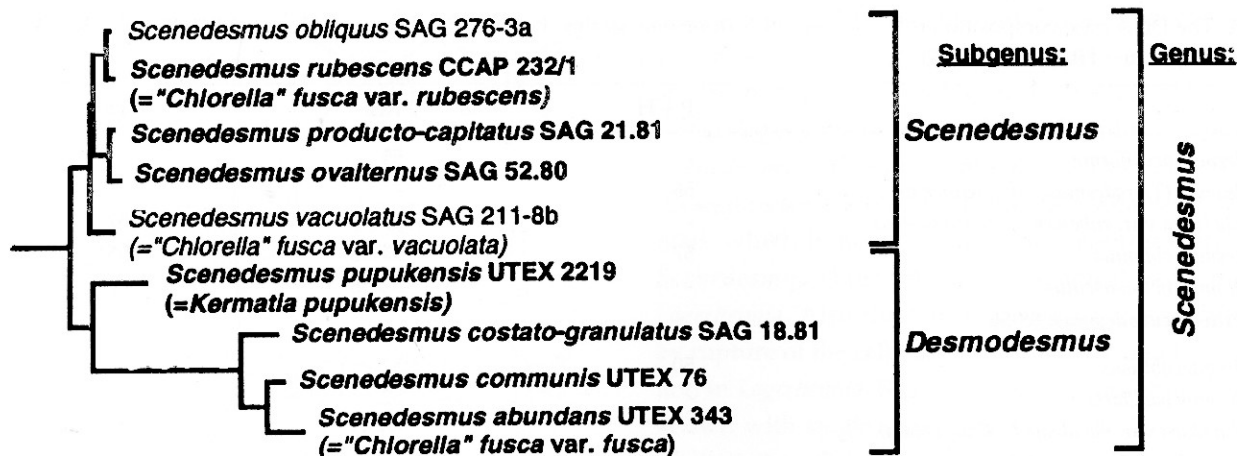


Fig. 5. Phylogenetic relationship of some *Scenedesmus* species based on 18S rRNA sequences. Extracted from Kessler *et al.* (1977, Fig. 2).

(incl. *S. obtusiusculus*). Finally Kessler *et al.* (1997) transferred *Chlorella fusca* var. *rubescens* to *Scenedesmus*, however, as an independent species: *Scenedesmus rubescens* (Dangeard) Kessler *et al.* It should rather be treated as a synonym of *S. obliquus* or possibly *S. obtusiusculus*. Most valuable for phylogenetic relationship is the sequencing of rRNA. According to 18S sequence analysis Kessler *et al.* (1997, see Fig. 5) could again demonstrate the close relationship of *S. obliquus* and "*Chlorella*" *fusca* var. *rubescens* (both fit into the former subgenus *Acutodesmus*) and *S. bacillaris* (= *S. producto-capitatus*) with *S. obtusiusculus* (= *S. ovalternus* Chodat) (both fit into the former subgenus *Scenedesmus*), while "*Chlorella*" *fusca* var. *vacuolata* branches earlier, cannot be placed in one of the former subgenera *Scenedesmus* or *Acutodesmus*. The species of the subgenus *Desmodesmus* (*Scenedesmus costato-granulatus*, *S. communis*, "*Chlorella*" *fusca* var. *fusca*) are branching separately from these five taxa. Hence the authors conclude that only two subgenera can be accepted.

According to an as yet unpublished pilot study of sequencing the ITS-2 region of DNA (An, Friedl and Hegewald, manuscript) we can confirm this conclusion. They also found that *Tetradismus* as well as *Enallax* could not be separated from *Scenedesmus* and that the genus *Diclostera* is far from the genus *Scenedesmus* and the above discussed variety *Scenedesmus wisconsinensis* var. *reginae* is correctly placed.

#### Fossil Records

Batton and Lister (1988a) found fossil *Scenedesmus acuminatus* and Batton and Lister (1988b) described a new taxon, *S. bifidus* Batton et Lister, which is related to *S. acuminatus*. Further taxa are found in formations about

70 to 100 million years old, besides *S. obliquus* also two new species from the former subgenus *Acutodesmus*: *S. tschudy* Fleming and *S. hanley* Fleming (Fleming 1989). Two specimens with obtuse cell poles are also found, but these have no spines or cell wall structures, and hence belong to the subgenus *Scenedesmus*. So the subgenus *Desmodesmus* seems to be the phylogenetically youngest subgenus. According to Kessler *et al.* (1997) the strain of *Chlorella minutissima* described as *Kermatia pupukensis* by Kalina and Punčochárová (1987) has to be placed in *Scenedesmus* subgenus *Desmodesmus*. However, it has no cell wall structures, hence should belong to the subgenus *Scenedesmus*. It could be a primitive ancestor of the subgenus *Desmodesmus*. It is interesting that this "relict" is known only from New Zealand. The spiny species (*S. communis*, *S. abundans*) branch in Fig. 5 very late, indicating that they are the phylogenetically youngest taxa.

Often the question is raised of whether the *Scenedesmus* species are distributed world wide. Apparently many species are indeed distributed world wide e.g. *S. obliquus*, *S. communis*, *S. armatus*. However, if considered in detail many *Scenedesmus* species have local races, as could be shown—especially for the phylogenetically youngest subgenus *Desmodesmus*—for e.g. *S. intermedius* (Hegewald, An, Schnepf, and Tsarenko Schnepf, in press) and for *S. serratus* (An, Hegewald and Tsarenko, manuscript) and both species are shown to have races e.g. in New Zealand.

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- 19a Cells in one row arranged linearly: many species of the subgenus *Desmodesmus*, if short teeth or ribs weakly developed or overlooked (e.g. *S. bicellularis* Chodat, *S. costato-granulatus* var. *elegans* (Hortobágyi) Hegewald et Krienitz)
- 19b Cells in two rows ..... (subgenus *Scenedesmus*)
- 20a Cells are arranged alternately ..... *S. parvus* (G.M. Smith) Bourrelly
- 20b Cells are arranged in two rows, outer cells between these two rows ..... *S. verrucosus* Roll
- 21a Coenobium curved ..... 22
- 21b Coenobium flat ..... 25
- 22a Small warts on each cell pole, 4-celled coenobia linear, central gap between cells ..... *S. raciborskii* Woloszyńska
- 22b Cells without polar warts, 4-celled coenobia not linear ..... 23
- 23a Cells alternately arranged, touching subpolarly ..... *S. curvatus* Bohlin
- 23b Cells are not alternately arranged, touching with lateral walls ..... 24
- 24a Cells in two rows, cells elongate ( $\pm 2.2 : 1$ ), curved, cell wall smooth  
..... *S. arcuatus* Lemmermann var. *arcuatus*
- 24b Cells in two rows, but the outer cells between these two rows, cells ovate ( $\pm 1.5 : 1$ ), not curved, cell wall smooth or verrucous ..... *S. verrucosus* Roll
- 25a Cells in one row, linearly to weakly alternating ..... 26
- 25b Cells in two rows or strongly alternating ..... 28
- 26a Cell shape cylindrical, cell walls parallel, cells arranged linearly ..... 23
- 26b Cell shape elongate oval or slightly curved, with swollen truncate polar area, cells arranged linearly or weakly alternating ..... *S. bacillaris* Gutwinski (= *S. producto-capitatus* Schula)
- 27a Cells touching without any gaps ..... *S. ellipticus* Corda
- 27b Cells connected subpolarly, hence with central gap ..... *S. ellipticus* var. *clathratus* (Biswas) Hegewald
- 28a Cell poles swollen, cells arranged strongly alternating ..... *S. indicus* Philipose
- 28b Cell poles not swollen ..... 29
- 29a Cell arranged alternately, coenobia with four cells, cells  $< 9 \mu\text{m}$  ..... *S. parvus* (G.M. Smith) Bourrelly
- 29b Cells in two rows, if alternating  $> 7 \mu\text{m}$ , coenobia with four or eight cells ..... 30
- 30a Cells kidney-shaped, connected with each other in two rows without gaps  
..... *S. arcuatus* var. *platydiscus* G.M. Smith
- 30b Cells ovate or elongate ovate ..... 31
- 31a Cells are elongately ovate, in two rows, alternately or if touching with the whole cell length the cell rows are shifted for one and a half cell width, average cell length  $> 8.5 \mu\text{m}$ , cell wall smooth ..... *S. obtusus* Meyen
- 31b Cells are ovate, arranged in two rows, but one outer cell arranged between the two cell rows on both sides, cell rows shifted for half cell width, medium cell length  $< 8.5 \mu\text{m}$ , cell wall smooth or with granules  
..... *S. verrucosus* Roll