

Taxonomic studies on *Scenedesmus* in Japan 2. On *S. obtusus* MEYEN and *S. arcuatus* (LEMM.) LEMM.

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The morphology and taxonomy of *Scenedesmus obtusus* and *S. arcuatus* were studied. Their morphological features, especially diagnostic characters, were examined in culture. *S. obtusus*: mature coenobia flat or slightly curved; cells of 4-celled coenobia arranged in alternate series, cells of 8-celled coenobia arranged in alternate or various double series; mature cells ovate or elliptical, with nodular thickening at each pole. This species changed the cell shape and coenobial form with culture age, showing wide variations in cell arrangement. Therefore, those taxa distinguished from *S. obtusus* only by cell shape or cell arrangement should be considered to be synonym. *S. arcuatus*: coenobial form curved; cell shape reniform; cells of 4-celled coenobia arranged in cruciate series, cells of 8-celled coenobia arranged in double series. These characters were stable at the various conditions studied. This species is, therefore, distinct from *S. obtusus*.

Key Index Words: Chlorococcales; Chlorophyceae; coenobium; Scenedesmus; *S. obtusus*; *S. arcuatus*; taxonomy.

The present paper is the second of a series of taxonomic studies on *Scenedesmus* (Chlorococcales, Chlorophyceae) in Japan. It deals with morphology and taxonomy of *S. obtusus* MEYEN and *S. arcuatus* (LEMM.) LEMM., whose cells are more or less ovate in shape and arranged in double series. The circumscription of each species was clarified by examining morphological features and their variations, especially in cell arrangement, in culture.

The specimens used for this study were isolated from samples of waters collected from the habitats shown in Table 1. Each isolate was inoculated into a test tube containing medium AWB and cultured under the same conditions as those described in the previous paper (OOSHIMA 1981). For comparison, these cultures were transferred into seven different media (AWB, A, B, C, D10, D200 and F), and then were observed after two, four and eight weeks (OOSHIMA

1981).

Results and Discussion

Types of cell arrangement. Cell arrangements in 4- and 8-celled coenobia have been used as diagnostic criteria useful for distinguishing certain species and infraspecific taxa of *Scenedesmus*. From the results of the present study, however, this character proved to be stable in *S. arcuatus* but variable in *S. obtusus*.

Four-celled coenobia. Cells of *S. obtusus* were arranged in alternate series (Figs. 3-5) or cruciate series (Fig. 7) and rarely in the intermediate series between them (Fig. 8). Alternate coenobia were always dominant in all cultures of the 11 strains examined. With strain SC766, about 30% of 4-celled coenobia were cruciate in media B and D200. In the vigorous cultures of the other 10 strains, however, these cruciate coenobia were less

Table 1. Source of clonal cultures investigated.

Strain No.	Localities	Dates of collection	Cell No.
			colony
<i>Scenedesmus obtusus</i>			
SC474	Sanaruko-lake, Shizuoka	May 3, '76	8
SC485	Ashigaika-pond, Aichi	May 3, '76	8
SC487	Ashigaika-pond, Aichi	May 3, '76	8
SC493	Ashigaika-pond, Aichi	Aug. 21, '76	8
SC497	Ashigaika-pond, Aichi	Aug. 21, '76	4
SC536	Ashigaika-pond, Aichi	Aug. 21, '76	8
SC607	a small pond, Mito, Ibaraki	May 22, '77	4
SC635	Shishizukaike-pond, Ibaraki	Aug. 10, '78	8
SC707	a small pond, Ashino Park, Aomori	Oct. 28, '78	8
SC766	a moat around the castle, Okayama	Oct. 4, '79	4
SC920	a small pond, Takikawa, Hokkaido	Aug. 25, '83	8
<i>S. arcuatus</i>			
SC571	Shishizukaike-pond, Ibaraki	May 22, '77	8
SC591	Shishizukaike-pond, Ibaraki	May 22, '77	8
SC593	Shishizukaike-pond, Ibaraki	May 22, '77	8
SC610	a small pond, Mito, Ibaraki	May 22, '77	8
SC919	a small pond, Takikawa, Hokkaido	Aug. 25, '83	8
SC921	a small pond, Akabira, Hokkaido	Aug. 26, '83	8
SC922	a small pond, Akabira, Hokkaido	Aug. 26, '83	8

than 1%, at most 9%, in most media. When these cruciate coenobia were reisolated and cultured in fresh medium AWB, many 4-celled alternate coenobia were observed.

S. arcuatus sometimes produced 4-celled coenobia in more than 4-week-old cultures. These 4-celled coenobia were cruciate (Fig. 32), but rarely tetradesmoid (Fig. 31).

Both species, *S. obtusus* and *S. arcuatus*, never produced 4-celled linear coenobia which were common in some species of *Scenedesmus*.

Eight-celled coenobia. Figure 1 represents five types of cell arrangement in 8-celled coenobia found in *Scenedesmus*. As shown in Figure 2, 8-celled coenobia of Type II and III are further classified based on the variation in number of slits between the median cells which are in contact with each other near the inner poles but not in lateral contact. Type II-1 is double coenobium without slits, while Type II-5 is alternate coenobium with four slits. Similarly, Type III-1 is double

coenobium without slits and Type III-3 is coenobium with two slits.

Eleven strains of *S. obtusus* produced three of the five types, Type II, III and IV (Figs. 10-11, 17 and 19), but Type I and V were never found. All variations of Type II and III were observed in the vigorous cultures of these strains (Figs. 10-18).

With strains SC474 and SC920 which were established from 8-celled alternate coenobia of Type II-5 (Fig. 16), such alternate coenobia were over 80% in media AWB, B and D10, and 15-30% in media C, D200 and F. With the other 9 strains, however, the double coenobia of Type II-1 and III-1 (Figs. 10-11 and 17) were always dominant in all the media. The percentage of each type varied slightly among these strains.

When the alternate coenobia of each strain were reisolated and then cultured in fresh medium AWB, their mature cells produced not only alternate coenobia but also various

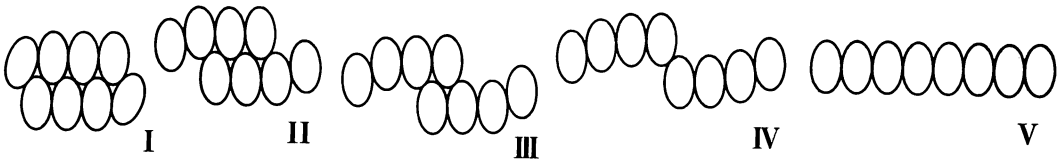


Fig. 1. Five types of cell arrangement in 8-celled coenobia found in *Scenedesmus*.

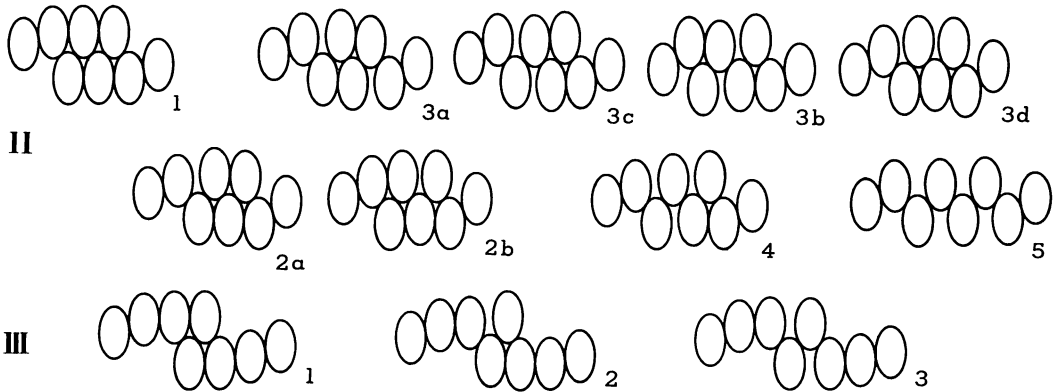


Fig. 2. Variations in cell arrangement of Type II and III.

double coenobia, Type II-1 to II-5, Type III-1 to III-3 and Type IV. Similarly, when the double coenobia of Type II-1 and III-1 were isolated and cultured in the same medium, their cells produced different kinds of coenobia including alternate coenobia of Type II-5.

The seven strains of *S. arcuatus* produced only 8-celled double coenobia of Type I, but never the other types. The double arrangement of cells in 8-celled coenobia proved to be one of the stable characteristics.

Some 8-celled coenobia in the vigorous cultures of *S. obtusus* and *S. arcuatus* were composed of cells irregularly arranged in double series (Figs. 20-23 and 34-35). When these irregular coenobia were transferred into fresh medium AWB, their cells produced many coenobia of which cells were regularly arranged in double series.

5. *Scenedesmus obtusus* MEYEN Nova Acta Phys.-Med. Acad. Caes. Leop.-Car. **14**: 775. pl. 43. figs. 30-31. 1829.

Synonyms: *Scenedesmus alternans* REINSCH, Abh. Senckenb. Naturf. Ges. **6**: 135. pl. 20. fig. D-5. 1866.

S. bijugatus (TURP.) KUETZ. var. *alternans* (REINSCH) HANS. Prodrum Algenfl. Böhmen 114-115. 1886.

S. bijugatus var. *disciformis* CHOD. Mat. Fl. Crypt. Suisse **1**(3): 213. fig. 87. 1902.

S. bijugatus f. *irregularis* WILLE, Ost. Bot. Zeits. **53**: 92. fig. 4. 1903.

S. bijuga (TURP.) LAG. var. *alternans* (REINSCH) BERGE, Ark. Bot. **6**(1): 57. 1906.

S. bijuga var. *alternans* f. *parvus* G.M. SMITH, Trans. Wisc. Acad. Sc. Arts Lett. **18**: 448. pl. 30. figs. 106-108. 1916.

S. bijuga var. *irregularis* (WILLE) G.M. SMITH, l.c. **18**: 448. pl. 28. figs. 59-62. 1916.

S. bijuga var. *alternans* f. *irregularis* G.M. SMITH, Trans. Amer. Microsc. Soc. **45**: 189. pl. 16. figs. 24-27. 1926.

S. ovalternus CHOD. Zeits. Hydrol. **3**: 164. fig. 51. 1926.

S. ovalternus var. *graevenitzii* (BERN.) CHOD. l.c. **3**: 165-166. fig. 52. 1926.

S. platydiscus (G.M. SMITH) CHOD. var. *alternans* (REINSCH) CHOD. l.c. **3**: 176. fig. 69. 1926.

S. graevenitzii (BERN.) MARG. P. Inst.

Biol. Apl. 22: 91. figs. 14d, i. 1956.

S. ovalternus var. *irregularis* KISS, In UHERK. *Scenedesmus*-Arten Ung. 48. fig. 137. 1966.

S. bijugatus var. *alternans* f. *parvus* (G.M. SMITH) PHIL. Chlorococcales 256. figs 164h, j. 1967.

S. bijugatus var. *graevenitzii* (BERN.) PHIL. l.c. 254. figs. 164a, b. 1967.

Steiniella graevenitzii BERN. Prot. Desm. 189-190. figs. 463-466. 1908.

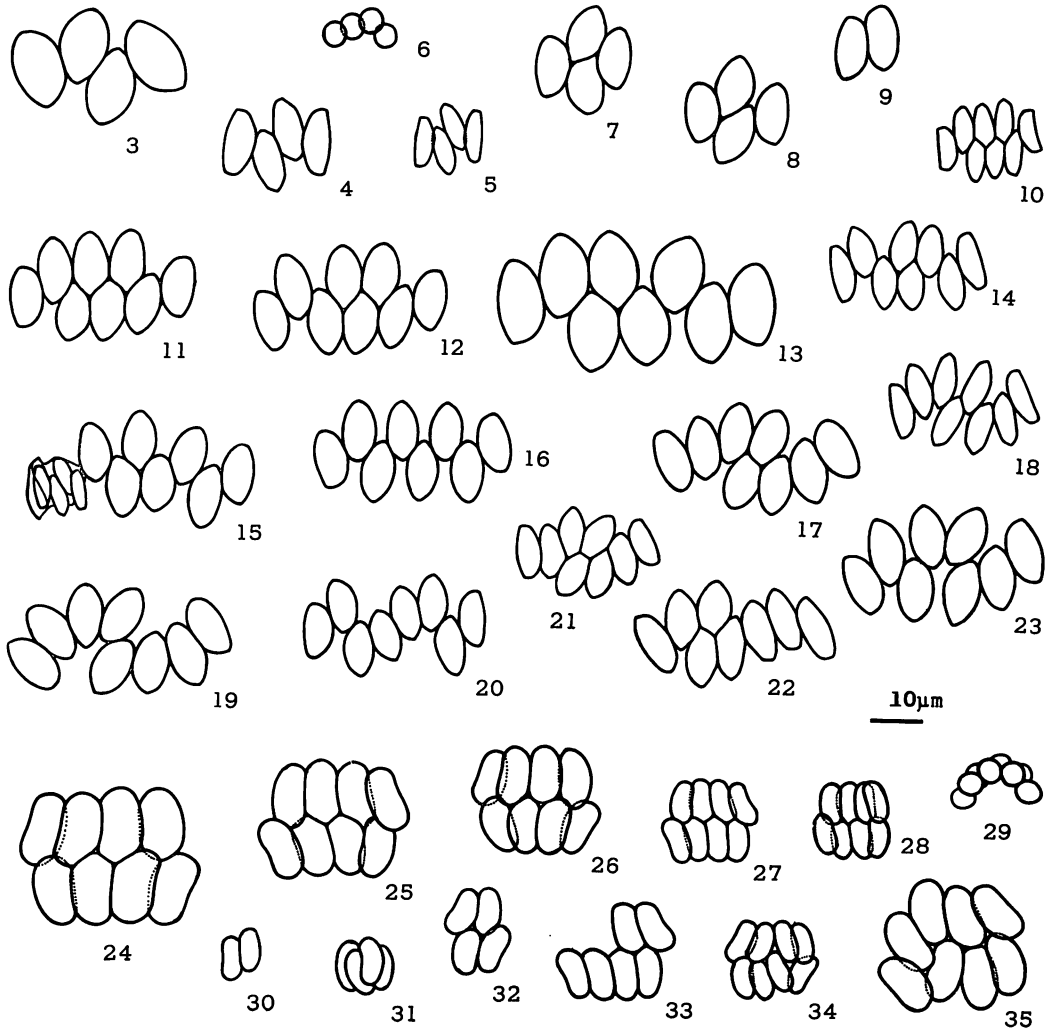
Scenedesmus ecornis (RALFS) CHOD. var. *disciformis* CHOD. l.c. 3: 172-173. fig. 161. 1926. (pro parte)

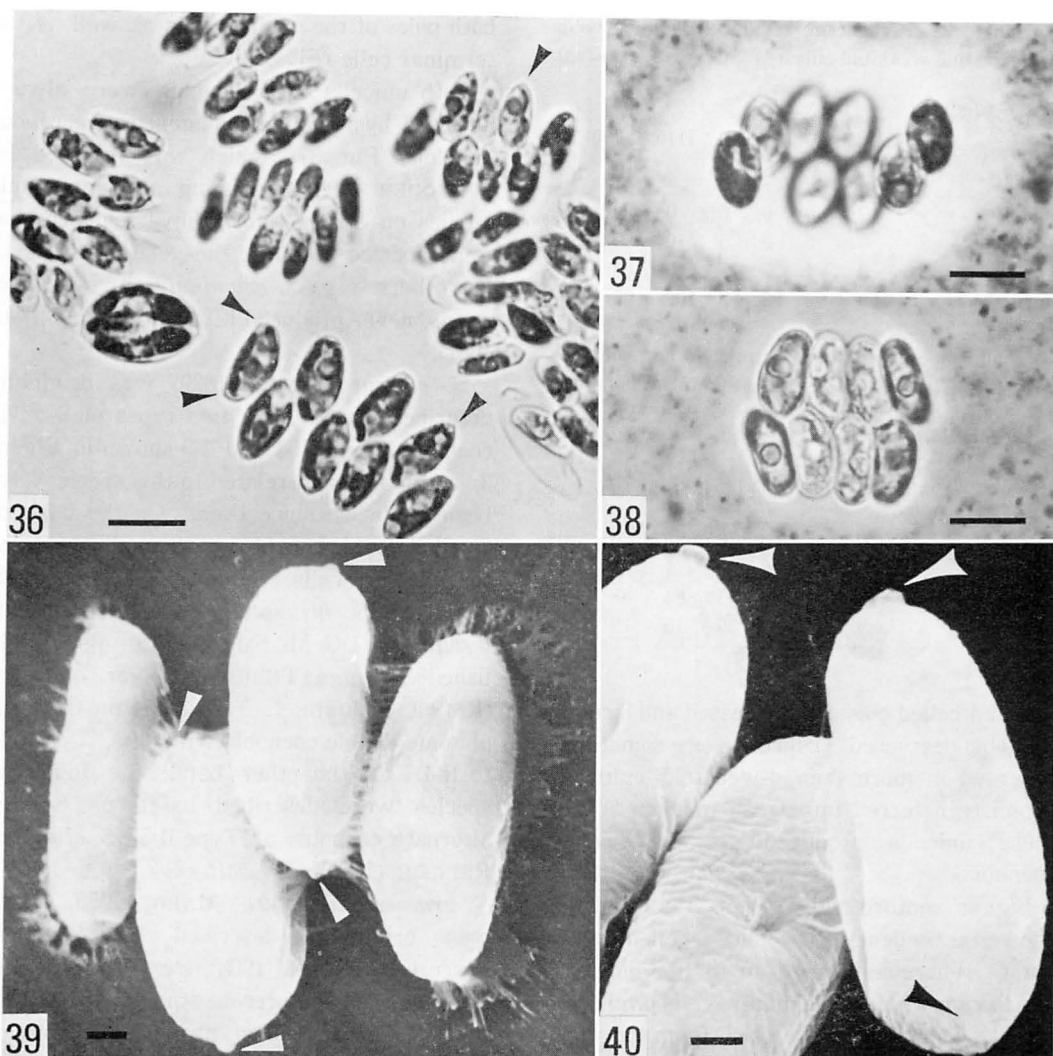
Coenobia of 2-4-8 (generally 4 and 8) cells, enclosed by a hyaline, homogeneous, gelati-

nous envelope, 4- or 8-celled mature coenobia flat or slightly curved, young coenobia always curved; cells of 4-celled coenobia arranged in alternate series, rarely in cruciate series, cells of 8-celled coenobia arranged in alternate or various double series; mature cells ovate or elliptical, up to 16.5 μm long, up to 9.5 μm wide, young cells slightly angular and slender, 8-9 μm long, 3.5-4 μm wide; each pole of cells with a single nodular thickening; cell wall smooth, without teeth or spines; unicells ovate, similar in shape and size to cells composing a coenobium.

Type locality: Potsdam, Germany.

Geographical distribution: Europe, America, Africa, India, Java, Burma, China, Siberia





Figs. 3-23, 36-37 and 39-40. *S. obtusus*. 3-8. 4-celled coenobia; 6. vertical view of young coenobium; 9. 2-celled coenobium; 10-16. 8-celled coenobia of Type II; 15. mature coenobium with three slits, of which a terminal cell is releasing a 4-celled daughter coenobium; 17-18. 8-celled coenobia of Type III; 19. 8-celled coenobium of Type IV; 20-23. irregular coenobia of 8 cells; 36. mature cell forming a 8-celled daughter coenobium and some 8-celled coenobia showing nodular thickenings at outer poles (arrows); 37. 8-celled coenobium of Type III-1 enclosed by a thick hyaline gelatinous envelope; 39. 4-celled alternate coenobium showing papillae (arrows); 40. part of 4-celled coenobium showing papillae (arrows). Figs. 24-35 and 38. *S. arcuatus*. 24-29. 8-celled coenobia; 29. vertical view of young coenobium; 30. 2-celled coenobium; 31-32. 4-celled coenobia; 33. broken coenobium with six cells; 34-35. irregular coenobia of 8 cells; 38. 8-celled coenobium enclosed by a gelatinous envelope. Scales: 39-40. 1 μ m; the others. 10 μ m.

and Japan.

Specimens examined: SC474, SC485, SC487, SC493, SC497, SC536, SC607, SC635, SC707, SC766 and SC920 (Tab. 1).

Table 2 and 3 show the percentage of

unicells and coenobia recorded in the 2-week-old cultures of strains SC766 and SC920. The percentage of coenobia, especially 4- and 8-celled coenobia, varied among the strains examined. As all the cultures became old,

Table 2. Percentage of unicells and coenobia observed in 2-week-old cultures (SC766 : *S. obtusus*).

Type of coenobium	Medium						
	AWB	A	B	C	D10	D200	F
unicell	0%	0%	0%	0%	0%	0%	0%
2-celled	0	0	0	5	0	2	0
4-celled	0	4	7	7	0	3	7
8-celled	100	96	93	88	100	95	93

Table 3. Percentage of unicells and coenobia observed in 2-week-old cultures (SC920 : *S. obtusus*).

Type of coenobium	Medium						
	AWB	A	B	C	D10	D200	F
unicell	0%	—	0%	0%	0%	0%	0%
2-celled	0	—	2	0	1	0	1
4-celled	91	—	56	65	84	88	65
8-celled	9	—	42	35	15	12	34

*A : dead

2- and 4-celled coenobia increased and 8-celled coenobia decreased. Unicells were sometimes observed in more than 4-week-old cultures. When transferred into fresh medium AWB, these unicells produced 4- and 8-celled coenobia.

Bigger mature cells (Figs. 3, 13 and 36) showed a tendency to produce 8-celled coenobium. These cells were up to 16.5 μm long and 9.5 μm wide, while mature cells producing 4-celled coenobium were 11-13.5 μm long and 6.5 μm wide (Figs. 7-8, 11-12, 15-17, 19 and 23). With all the strains examined, cells produced in medium B were more or less stumper than those in the other media.

Observations of 4- and 8-celled coenobia by light microscopy showed that nodular thickenings were situated at outer poles of the median cells and near both poles of the terminal cells (Fig. 36). Although the nodular thickenings were visible in cells of strains SC485, SC487 and SC920, it was sometimes difficult to find them in cells, especially mature ones, of the other 8 strains. When some coenobia of strain SC920 were treated with hypochlorous acid and examined using scanning electron microscopy, these nodular thickenings appeared as papillae on

both poles of the median cells as well as the terminal cells (Figs. 39-40).

Both unicells and coenobia were always enclosed by a hyaline homogeneous gelatinous envelope (Fig. 37), which was reported by SCHRÖDER (1902). During the taxonomic studies on *Scenedesmus*, I have revealed that some species produced the gelatinous sheath but others, e.g. *S. acuminatus* and *S. javanensis*, never produced it in culture (OOSHIMA 1981).

S. obtusus MEYEN (1829) was originally described based upon two types of 8-celled coenobia, Type II-1 and II-5 shown in Figure 2, and many taxa related to this species have been published since then. On the basis of 8-celled double coenobia without slits between the median cells (Type II-1), WILLE (1903) described *S. bijugatus* (TURP.) KUETZ. f. *irregularis*. G.M. SMITH (1926) also established *S. bijuga* (TURP.) LAG. var. *alternans* (REINSCH) BORGE f. *irregularis* on the basis of some double coenobia with slits, Type II-2 to II-4. On the other hand, the following species were described based on 8-celled alternate coenobia of Type II-5: *S. alternans* REINSCH (1866), *S. ovalternus* CHOD. (1926), *S. graevenitzii* (BERN.) MARG. (1956) which was originally described as *Steiniella graevenitzii* BERN. (1908), etc. Nevertheless, the results of the present study indicate that these taxa mentioned above are synonyms of *S. obtusus*.

6. *Scenedesmus arcuatus* (LEMM.) LEMM. Forschungsbr. Plön. 7: 112. pl. 1. figs. 2-4. 1899.

Basionym; *Scenedesmus bijugatus* (TURP.) KUETZ. var. *arcuatus* LEMM. Bot. Cent. 76: 150. 1898.

Synonyms: *S. bijugatus* f. *arcuatus* (LEMM.) W. et G.S. WEST, Trans. R. Irish Acad. 33 (B2): 105. pl. 10. figs. 12-14. 1906.

S. arcuatus var. *platydisca* G.M. SMITH, Trans. Wisc. Acad. Sc. Arts Lett. 18: 451. figs. 101-105. 1916.

S. platydiscus (G.M. SMITH) CHOD. Zeits. Hydrol. 3: 175-176. fig. 68. 1926.

S. alternans REINSCH var. *arcuatus* (LEMM.) FOTT et KOMÁR. Preslia 32: 126-127. pl. 8.

fig. 10. 1960.

S. alternans var. *platydiscus* (G.M. SMITH) FOTT et KOMÁR. *l.c.* 32: 126. *pl.* 8. *fig.* 6. 1960.

S. disciformis (CHOD.) FOTT et KOMÁR. *l.c.* 32: 129. *pl.* 8. *fig.* 3. 1960. (pro parte)

Coenobia of 2-4-8 (generally 8) cells, enclosed by a hyaline, homogeneous, gelatinous envelope, 4- or 8-celled coenobia always curved, mature coenobia sometimes slightly curved; cells of 4-celled coenobia arranged in cruciate series, cells of 8-celled coenobia arranged in double series, with small interstices among the cells; cells reniform, sometimes elliptical, with broadly rounded ends, inner poles of the median cells more or less angular by mutual compression, outer wall of the terminal cells concave; mature cells up to 16.5 μm long, up to 9 μm wide, young cells slender, 6.5-7 μm long, 3 μm wide; cell wall smooth, without teeth, spines or granulations; unicells reniform, sometimes elliptical, similar in shape and size to cells composing a coenobium.

Type locality: Leipzig, Germany.

Geographical distribution: Europe, America, Africa, India, Java, Burma, Australia, China, Siberia and Japan.

Specimens examined: SC571, SC591, SC593, SC610, SC919, SC921 and SC922 (Tab. 1).

Table 4 shows the percentage of coenobial types recorded in 2-week-old cultures of strain SC593. Each coenobium in 2-week-old cultures of the seven strains examined had eight cells. Two- and four-celled coenobia came into being in 4-week-old cultures. In much older cultures, unicells and colonies with five to seven cells (Fig. 33) were often

Table 4. Percentage of unicells and coenobia observed in 2-week-old cultures (SC593: *S. arcuatus*).

Type of coenobium	Medium						
	AWB	A	B	C	D10	D200	F
unicell	0%	—	0%	0%	0%	0%	0%
2-celled	0	—	0	0	0	0	0
4-celled	0	—	0	0	0	0	0
8-celled	100	—	100	100	100	100	100

*A: dead

observed, and they seemed to be produced by breaking up of 8-celled coenobia. When transferred into fresh medium AWB, both unicells and broken coenobia with five to seven cells produced 8-celled coenobia.

Cells of each colony were usually alike in shape and size. Although they increased in size with age, their characteristic reniform shape did not change.

All coenobia were always enclosed by a hyaline homogeneous gelatinous sheath (Fig. 38). The gelatinous sheath was thinner than that of *S. obtusus*.

S. arcuatus (LEMM.) LEMM. (1899) was originally described as *S. bijugatus* (TURP.) KUETZ. var. *arcuatus* LEMM. (1898) based on 8- and 16-celled coenobia. The dimensions of mature cells recorded for the 7 strains agreed closely with those of cells in the original description. During the present investigation, however, 16-celled coenobia were never found in all the cultures.

G.M. SMITH (1916) reported *S. arcuatus* var. *platydiscus* with five drawings (figs. 101-105 in his plate), describing only the flat form of 4- and 8-celled coenobia. Some taxonomists have recognized this taxon: PRESCOTT (1951), UHERKOVICH (1966), HEGEWALD *et al.* (1978), *etc.* However, one of the 8-celled coenobia in SMITH'S drawings (fig. 103) was apparently curved and three of them (figs. 101, 104 and 105) also seemed to be slightly curved. His 4-celled coenobium (fig. 102) was quite similar to Figure 32 of the present paper. Accordingly, this variety can hardly be recognized as an independent taxon.

FOTT and KOMÁREK (1960) adopted *S. alternans* REINSCH (1866) as the correct name and proposed two new combinations, *S. alternans* var. *arcuatus* (LEMM.) and *S. alternans* var. *platydiscus* (G.M. SMITH). Nevertheless, these two varieties are quite different from *S. alternans* var. *alternans* in shape and arrangement of cells. According to the results obtained in the present study, I propose that *S. arcuatus* (LEMM.) LEMM. should be recognized as a distinct species and that *S. alternans* should be regarded as

synonymous with *S. obtusus* MEYEN as described above.

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大島海一：日本産セネデスムス属の分類学的研究 2. *Scenedesmus obtusus* と *S. arcuatus* について

日本産セネデスムス属2種の形態と分類について報告する。*Scenedesmus obtusus* では、成熟するにつれて、定数群体全体の形は湾曲から平板状へ、細胞の形は多少角ばった細長い長円形から卵形あるいは長円形へと変化した。また、定数群体内における細胞の配列パターンも様々なタイプが出現した。その各タイプの相対的出現頻度は培養株ごとに差異があり、同一株でも培地によって違いが認められた。細胞の形の変化と細胞配列の可塑的変異が、本種のシノニムを多数記載させてきた主な原因と思われる。一方、*S. arcuatus* では対照的な結果が得られた。細胞は腎臓形で、定数群体全体の形は常に湾曲し、細胞の配列パターンは4細胞群体で十文字配列、8細胞群体で二重配列のみであった。これらの安定した形質から、本種は *S. obtusus* と容易に識別できる独立した分類群と考えられる。(252 藤沢市亀井野 1866 日本大学農獣医学部生物学研究室)