

A Taxonomic Study of the *Closterium calosporum* Complex (2)*

By

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3. Physiological studies

3.1 Vegetative reproduction at different temperature conditions

(a) Materials and methods

In order to disclose the optimum temperature for vegetative reproduction in each strain, one clone of each homothallic strain (R-12-smaller, R-12-larger, A-2, A-7, A-13, C-320, Ak, C-319, R-9-slender, C-318, N-134, N-143, N-147, and R-9-broader), a pair of mating clones of each heterothallic strain (M-10, R-5, and R-11), and a subclone (R-12-2-G1) of giant cells isolated from R-12-smaller were cultured for three weeks under seven different temperatures (5, 10, 15, . . . , 35°C). The pH of the medium was adjusted at 7.4. The culture conditions other than the temperature and pH were the same as the standard's. The growth rate of each strain was determined by measurements of the optical density at 675 nm of the culture with a spectrophotometer.

(b) Results

Figs. 15–16 show the growth rates of each strain. All the strains showed good growth under the three conditions, 15, 20 and 25°C. The temperature optima for them were at one of these temperatures. No significant difference in growth rate between plus and minus clones of M-10 was detected. The clone M-10 from Malaysia seemed to be adaptive to higher temperatures. R-12-smaller from Okinawa Pref. showed relatively good growth at 10°C and a similar trend in the growth rate was recognized in a subclone R-12-2-G1. The growth of R-12-larger, which originated from the same soil sample with R-12-smaller, was found to largely hindered at 10°C. R-12-smaller seemed to be adaptive to slightly lower temperatures than R-12-larger. A-2, A-7 and A-13 from neighbouring localities in Aichi Pref. showed good growth at 30°C. The clone A-7 showed a considerably high growth rate at 10°C. Though C-320 from Michigan did not grow well in all the conditions examined, it grew moderately at medium and lower temperatures. Ak from Hokkaido Pref. seemed to have relatively wide tolerance to temperature conditions. C-319 from Massachusetts grew only at medium temperatures and relatively good at lower ones. C-318 from Vermont grew well at medium temperatures. No significant difference in growth rate between minus and plus clones of R-5 from Okinawa Pref. was recognized, though the former

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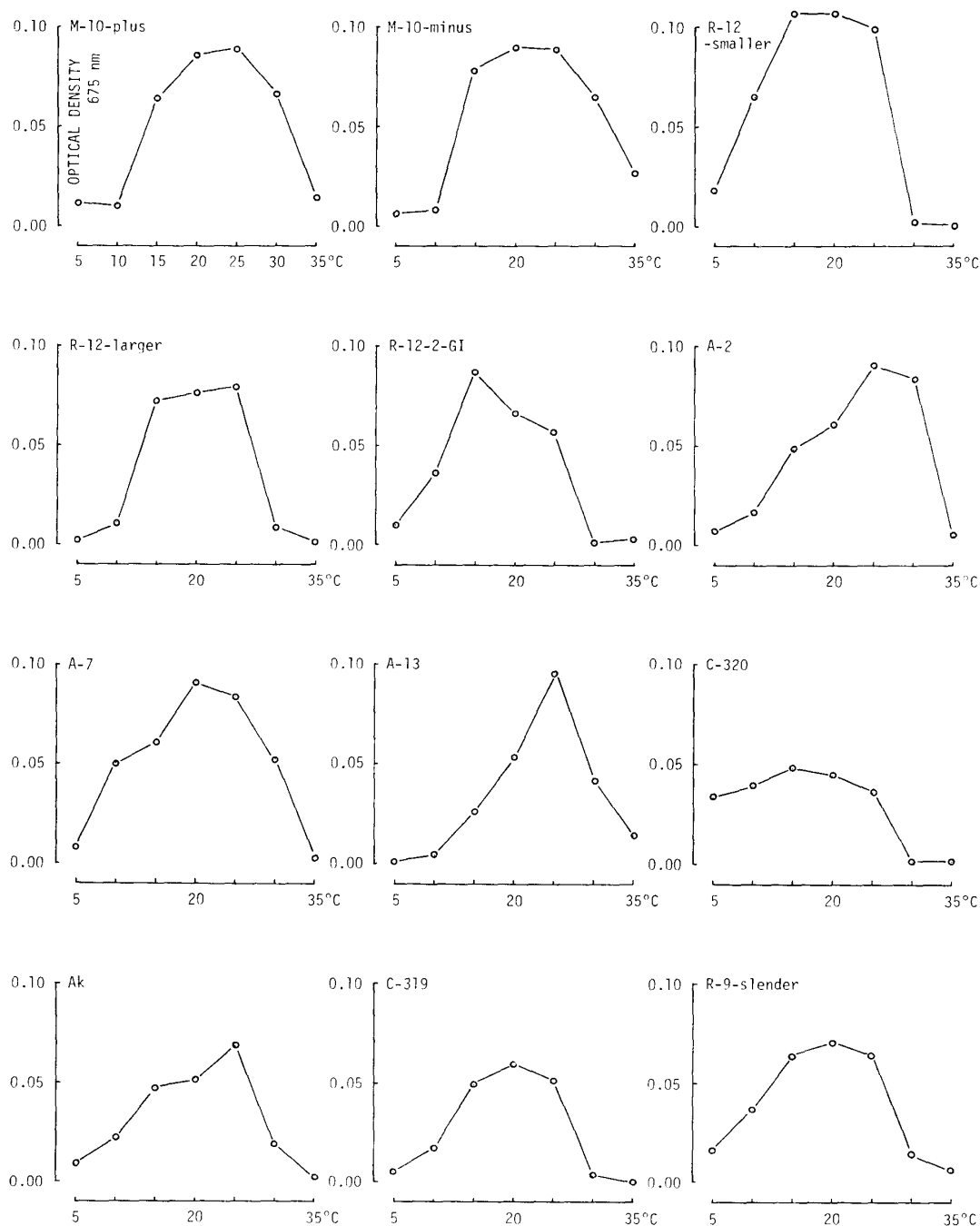


Fig. 15. The dependence of growth rates of ten strains on different temperatures.

showed relatively good growth at 10°C. Growth rates of plus and minus clones of R-11 from Okinawa Pref. were quite similar. In these clones, the good growth manifested at higher temperature conditions was remarkable. N-147, N-143 and N-134 from Himalayas showed relatively good growth at 10°C and minor growth at

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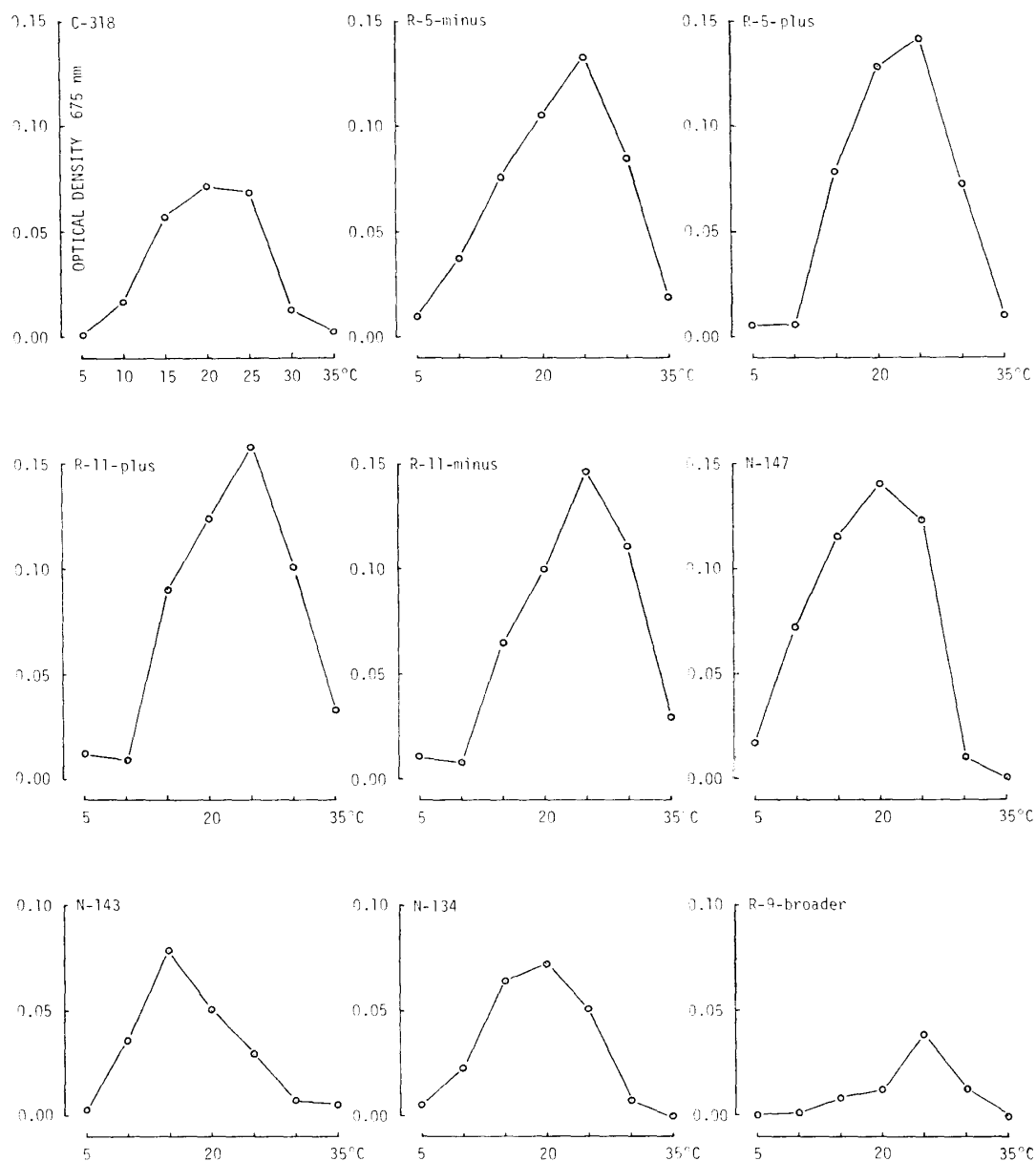


Fig. 16. The dependence of growth rates of seven strains on different temperatures.

30 C. R-9-broader from Okinawa Pref. did not grow well except at 25 C.

3.2 Sexual reproduction at different temperature and pH conditions

(a) Materials and methods

Seventeen strains were cultured under the 15 combinations of temperature (10, 20, 30°C) and pH (5, 6, 7, 8, 9) to estimate the relationships between sexual reproduction and different culture conditions. Intercrossing between R-5 and R-11 was also tried. Other culture conditions were the same with those described in 2.4.2-(a).

Individuals which accomplished the zygospore or parthenospore formation, and those which formed or were forming a conjugation papilla and were apparently at the conjugation processes, were counted, together with individuals in the vegetative phase, under a microscope after three weeks cultivation. The total number of individuals counted in each strain under respective culture conditions was about 200–500.

(b) Results

Tables 7–9 show the results of the examination. Since 20°C was generally suitable not only for vegetative reproduction but also for sexual reproduction in most of the strains examined, due references should be given to the results at 10°C and 30°C.

M-10 : The zygospore formation rates were very high at 20°C, pH 6–7. It is also notable that the zygospore formation rate at 30°C (pH 8) took the second order and the conjugation index* at this condition approached to that shown at 20°C (pH 6–7).

R-12-smaller : Zygospores and parthenospores were not formed at any conditions examined. The conjugation index was fairly high at 30°C (pH 5).

R-12-larger : Sexual activity is more or less higher at lower pH and lower temperature conditions but at 30°C (pH 5).

R-12-2-G1 : Sexual activity is higher at lower pH and lower temperature conditions.

A-2, A-7, A-13 : As for A-2, no sign of the sexual reproduction was observed at all the pH conditions in 10°C and at pH 7–8 in 20 and 30°C. A-7 and A-13 seemed to be similar to A-2 in the pattern of sexual reproduction induced under the diverse culture conditions.

C-320 : Zygospores and parthenospores were not formed at any conditions tested. The conjugation index was fairly high at 30°C (pH 5).

Ak : The zygospore formation rates shown at 10°C were comparable to those obtained at 20°C.

C-319 : Neither zygospore nor parthenospore were formed throughout the conditions examined except the case of 30°C and pH 5, in which one zygospore was observed.

R-9-slender : Zygospores and parthenospores were not formed at 30°C (pH 5–9) and at pH 8–9 in 10°C and 20°C.

C-318 : Considerably large values in the parthenospore formation rate and in the conjugation index at 10°C, pH 8 was notable.

R-5 : Zygospores and parthenospores were formed at almost all the conditions examined. The large value in the conjugation index at 10°C (pH 5) was notable.

R-11 : No sign of sexual reproduction could be observed at 10°C (pH 5–9). The values shown by the present strain in respective conditions were smaller than those obtained in R-5 without exception.

* cf. Table 9 and ICHIMURA, 1971

Table 7. Zygosporangium formation rate in each of the strains examined under 15 different culture conditions.

pH	5	6	7	8	9	R-12-smaller			R-12-larger			R-12-2-G1				
						5	6	7	5	6	7	5	6	7		
10°C																
	20	1.1	32.4	42.5	1.2	3.1			1.1	0.7	1.3	1.0	0.4	r	1.3	r
	30		1.3	4.0	14.6	2.5			1.0	1.0	0.5	0.5	0.6			r
			A-2													C-320
			A-7													
10																
20					8.3	4.3			0.4	0.4						
30					0.4	2.6			0.7	0.5	r					
			Ak													C-318
			C-319													
10	2.8	r	3.3	3.7	r				1.1	3.4	1.3					1.5
20		7.9	4.5	1.8	r				6.8	11.8	19.2		8.6	6.1	11.5	5.0
30																r
			R-5													
			R-11													
10	1.6	0.7	2.5	0.9	r											
20	6.8	7.8	21.9	6.0	0.7	5.8	3.1	5.8	0.8	r	0.7	0.8	0.6	r	4.4	2.9
30	0.7	1.1	3.6		r										22.4	1.6
			N-147													
			N-143													
			N-134													
10	r	r	r	r	2.3	17.4	17.3	11.9	3.7	46.3	51.7	67.6	59.6	11.2	r	1.4
20	r	r	r	r	25.1	10.3	r	0.5	2.1	50.2	41.4	37.8	36.6	34.7	2.2	1.4
30	r	r	r	r			r	3.0	3.0	4.4	8.2	2.7	2.0	5.0		
			R-9-broader													

Table 9. Conjugation index in each of the strains examined under 15 different culture conditions.

pH	5	6	7	8	9	R-12-smaller			R-12-larger			R-12-2-GI					
						5	6	7	5	6	7	5	6	7			
M-10																	
10°C					r	r	r	r	15.3	12.5	16.3	3.4	2.3	17.7	8.6	0.9	r
20	10.7	68.2	61.7	2.2	7.8	2.0	1.0	r	9.9	7.3	8.9	3.6	3.6	12.5	r	0.8	r
30		1.8	27.3	58.4	14.3	10.0	r	r	20.6	5.6	5.1	1.5	3.9	11.6	r	r	2.0
A-2																	
A-7																	
10						r	r	r									r
20	14.5	33.5	29.6			27.6	14.6	11.7	3.6	7.5							3.8
30	3.6	33.4	56.9			65.6	71.9	38.7	30.5	35.3	18.8						12.8
C-319																	
Ak																	
10	2.8	r	3.8	6.4	r				4.7	11.9	10.4	r	r	24.6	19.0	47.1	34.1
20		8.9	6.3	8.2	r				17.9	39.7	72.4	11.0	r				r
30	2.3	r	r	r	4.6				7.9	r	r						r
R-5																	
10	38.5	11.0	8.3	6.6	4.9				5.0	r	3.8			8.1	5.6	4.2	2.6
20	39.9	35.6	50.0	20.7	8.6	26.8	12.4	15.6	7.9	1.9	4.1	7.9	r	42.5	44.9	69.9	48.6
30	13.2	11.1	13.0	8.5	7.0	3.9	r	4.6	0.9	r				1.2	r	r	1.9
N-147																	
N-143																	
10	r	r	r	r	4.6	21.6	22.0	14.3	19.6	56.8	60.1	79.6	72.3	22.4	r	2.3	16.6
20	r	r	r		36.0	15.8	r	10.5	15.2	66.1	54.6	48.8	61.5	70.7	1.0	2.0	36.4
30	3.1	r				r	r	3.0	3.0	6.6	13.3	7.4	8.0	8.6	r		
N-134																	
R-9-broader																	
R-5-plus × R-11-minus																	
R-5-minus × R-11-plus																	
R-9-slender																	
C-318																	
C-320																	

The rate was calculated by regarding as the induced type all the cells which showed a sign of sexual reproduction ranging from conjugation papilla formation to partheno- or zygospore formation.

R-5 × R-11 : The zygospore and parthenospore formation rates and the conjugation index observed in the combination of R-5-minus and R-11-plus were similar to those detected in the intra-crossing of R-11, while the values obtained in the reverse combination were closer to those in the intra-crossing of R-5.

N-147, N-143, N-134 : These three strains seemed to have a similar trend, in that considerable numbers of cells showing a sign of sexual reproduction were formed at 10°C.

R-9-broader : No sign of sexual reproduction could be found at the conditions of 30°C, though a negligible rate of the parthenospore formation was obtained at pH 5 in this temperature regime.

3.3 Fertility of zygospores

(a) Materials and methods

Zygospores obtained in intercrosses between R-5 and R-11 were used. The zygospores, which were formed at 20°C, pH 5–8, were isolated from the cultures with micropipettes and inoculated one by one in a small cell (Lab-Tek's chamber slides for tissue culture) containing about 0.5 ml of CA. The isolates were cultured at a room temperature of 25–35°C for about three weeks. The culture conditions other than the above-mentioned were the same with those described in 2.1-(a). The observations were carried out by aid of an inverted microscope.

(b) Results

Table 10 shows the results of the examination to test the fertility of the zygospores which were formed by the intercrosses between a plus clone of R-5 and a minus clone of R-11, and between the reverse combination. Though the zygospore formation rates obtained in crosses between R-5-plus and R-11-minus were larger than those between R-5-minus and R-11-plus at all the pH conditions examined, the fertility of the zygospores formed in the former combination was inferior to those formed in the latter. While there was slight correlation between the zygospore formation rates and the rates of vigorous zygospores in the case of the combination, R-5-minus and

Table 10. Fertility of zygospores formed by intercrossing between R-5 and R-11 under different pH conditions.

Clone	pH conditions for zygospore formation	Zygospore formation rate %	Number of zygospores isolated	Withered zygospores after germination		Zygospores producing offspring	
				no.	%	no.	%
R-5-3 (+)	5	4.4	15	4	27	0	0
	6	2.9	15	10	67	1	6
R-11-6 (–)	7	22.4	16	11	69	0	0
	8	1.6	16	5	31	1	6
R-5-2 (–)	5	0.7	16	3	19	10	62
	6	0.8	16	3	19	11	68
R-11-5 (+)	7	0.6	7	2	29	2	29
	8	rare	8	2	25	2	25

Table 11. Coefficient of variation in width and distance between apices of the *Closterium* population in natural habitat.

Organism	No. of individual observed	CV (%)		Reference
		Width	Distance	
<i>C. attenuatum</i>	86	4.3	5.9	NISHIHAMA (1972)
<i>C. calosporum</i> v. <i>brasiliense</i>	81	7.2	9.3	
<i>C. cynthia</i> v. <i>jenneri</i>	60	6.4	7.7	
<i>C. diana</i>	56	4.0	5.3	
<i>C. diana</i> v. <i>pseudodiana</i>	94	7.0	6.8	
<i>C. intermedium</i>	92	7.4	—	
<i>C. intermedium</i> v. <i>hibernicum</i>	85	4.0	—	
<i>C. juncidum</i>	98	9.8	—	
<i>C. rostratum</i>	57	4.3	7.4	
<i>C. setaseum</i>	62	6.6	8.4	
<i>C. venus</i> v. <i>incurvum</i>	50	11.0	9.0	
<i>C. calosporum</i> v. <i>maius</i>	50	2.7	5.9	WATANABE (1974)
<i>C. cynthia</i>	50	10.7	8.8	
<i>C. malinvernianiforme</i>	50	4.7	—	
<i>C. malinvernianiforme</i>	33	8.9	9.4	
<i>C. parvulum</i>	50	3.3	6.0	
<i>C. rostratum</i>	50	3.9	7.1	
<i>C. cynthia</i>	25	4.2	9.4	WATANABE et al. (1979)
<i>C. intermedium</i>	50	2.2	11.6	
<i>C. navicula</i>	25	4.0	6.9	
<i>C. parvulum</i>	25	2.8	8.8	
<i>C. rostratum</i> v. <i>onchosporum</i> f. <i>papuanum</i>	25	1.6	8.2	

R-11-plus, such correlation could not be recognized in the case of the reverse combination. These results seem to suggest that the optimum condition for zygospore formation is not always highly suitable for yielding vigorous offspring.

4. Discussion and conclusion

The propriety of representing a population by one or a few clones was investigated by the two experiments (2.2.1, 2.2.2), and the results to sustain that this view is justified at least in the three strains examined. The extents of variations in natural populations of *Closterium* shown in Table 11 may also validate the above conclusion. Coefficients of variations (CV) of the width were 1.6–11.0% (a mean value=5.5%) in the natural populations, while they were 1.4–5.7% in the 49 clones cultured under the standard condition. This shows that phenotypic and also genotypic variations in natural populations are so small in many cases that the above conclusion is supported. The width of cells can be used as a reliable character when the materials obtained from the field are compared with those cultured in the laboratory. The CVs of the distance between apices were 5.3–11.6% (a mean value=7.9%) in natural populations, while they were 3.4–10.1% in the 49 clones cultured under the standard condition.

This shows that the distance is also a reliable character, though the reliability is less than in the case of the width. The phenotypical variations caused by the environmental flux may be small so far as the fluctuations do not exceed a crucial limit. Thus the culture condition employed as the standard should be designed to be located within the limits. Regarding the materials so far examined in the present study, the standard culture condition seem to satisfy this requirement. Naturally, there has been a question that artificial culture for a long time would alter the natural attributes of an alga. However, so far the three strains from the United States are concerned, no significant differences were detected between the measurements on the size of vegetative cells by the present author and those by Dr. COOK (1963) in spite of the cultivation for more than ten years. But an activity in sexual reproduction in two of these strains seems to have been lost or weakened during the cultivation. The strains from Malaysia are sharply contrasted with those from Nepal in the temperature requirements for growth and induction of sexual reproduction, and those different properties have been maintained for five years that were covered by my experiments.

As stated earlier, the eight subgroups, which were recognized by morphological characters, were found to have been differentiated in some physiological characters such as growth rate at different temperature conditions, zygospore formation rate at different combinations of temperature and pH, etc. Fig. 17 shows the monthly mean temperature and the precipitation at the meteorological stations nearest to the localities of the 17 strains. The strains from Malaysia that were originally growing under high temperatures showed high growth rates and high degrees of induction of sexual reproduction when cultured under high temperatures, and the strains from Nepal which inhabited in the areas with low temperatures were found to be successful under the experimental conditions with low temperatures. Malaysia and Nepal are similar in having relatively small annual variations of temperatures. In order to advance our understanding on the differentiation of physiological attributes of the algae concerned, data must be accumulated on (1) diurnal temperature variations on the natural habitats and (2) the experimental cultures using an equipment which can adjust "day conditions" and "night conditions". As for the strains from the habitats located in middle latitudes, further accumulation of the data on water conditions seems to be important.

From the results of crossing of plus and minus clones of R-11 and different crosses between R-11 and R-5, it was estimated that the plus clone of R-11 has quite weak potential of sexual reproduction (ICHIMURA & WATANABE, 1974). This view should be questioned, however, considering the fact that the zygospores produced by crossing between plus clone of R-11 and minus clone of R-5, though small in number, were good in both the germination rate and the subsequent development. These results, together with the fact that the conditions such as temperatures and pH have a great influence on sexual activity of the algae, suggest that some important methodological problems still remain to be dissolved, when experimental approaches based on the so-called biological species concept are applied to these plants.

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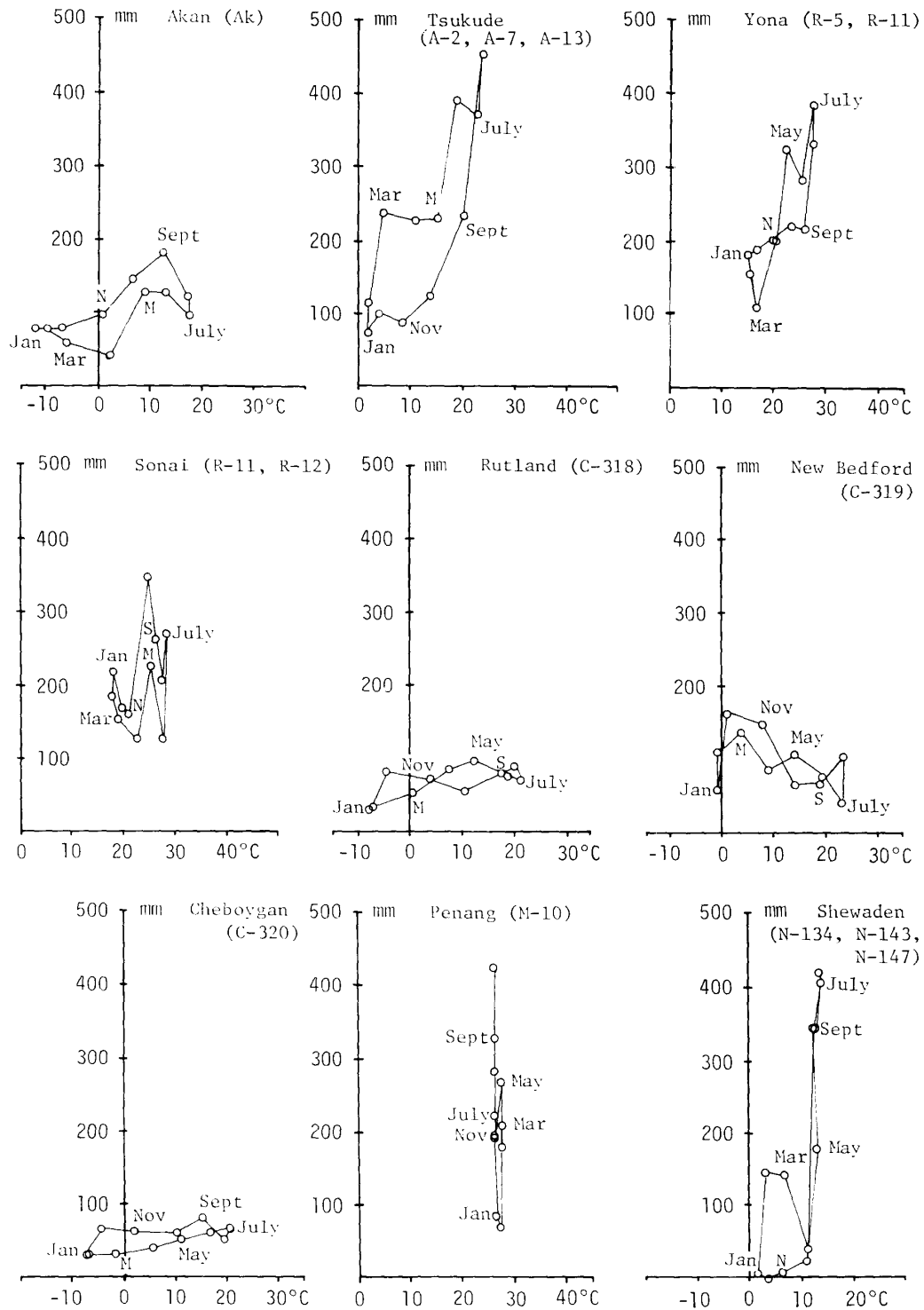


Fig. 17. Monthly mean temperature and precipitation at the meteorological stations nearest to the localities of the 17 strains.

Spontaneous occurrence of giant cells in desmids is widely recognized by physiological workers (STARR, 1958; KING, 1960; BRANDHAM, 1965; KASPRIK, 1973; ICHIMURA & WATANABE, M. M., 1976; NISHIHAMA, 1977), and it is generally attributed to a change of ploidy levels caused by chromosome doubling. It seems probable that this generally accepted view holds true in the present materials, though the chromosome studies still have not been carried out. The resemblance in cell shape among the strains of Group I suggests that similar phenomena might be involved as an important part of the differentiation of this group. Triple zygospore formation in desmids is also known for long (ARCHER, 1865; WEST, W., 1892; WEST & WEST, 1897; STARR, 1954; BRANDHAM, 1965). Recently, LING & TYLER (1972 a, b) studied in detail the process of the triple zygospore formation and germination in *Pleurotaenium*. They stated "The one successful germination produced a cell significantly larger than normal, suggesting that the offspring is hyperhaploid (BRANDHAM, 1965)". A similar situation can be assumed in the triple zygospores of M-10 (Pl. I, fig. 9) and N-134 (Pl. VIII, figs. 2-3), and also in the zygospores formed by crossing between R-12-smaller and -larger (Pl. II, fig. 7).

Since different traits of vegetative cells among the 17 strains were recognized by culturing under the same experimental conditions, the differences may be regarded as caused by genotypical differences. It may be clear from the results described above that *C. calosporum* sensu KRIEGER (1935) and KOSSINSKAJA (1960) is genotypically not homogeneous but represents an aggregate which consists of morphologically and physiologically differentiated elements. A taxonomic revision of this aggregate, which is widely distributed from the Arctic regions to the tropics, is apparently necessary.

It goes without saying that a possible scope of applying an experimental method to a taxonomic problem is greatly restricted, and the results obtained in the present work should be combined with the information that has been accumulated in the floristic work. A marked advance through the accomplishment of the work along this line can not be expected in case of such common algae as *C. calosporum* s. lat., however, because satisfactory descriptions for such algae are often neglected in the floristic manuals. Further, the genetic relationships or evolutionary backgrounds among the eight subgroups detected in the present examination are almost completely unknown. In spite of these shortages, a formal taxonomic revision for the algae concerned is here given, since it will be useful to communicate the results described above and would make the investigations conducted in the future more meaningful.

According to the features of apices of vegetative cells, the 17 strains were arranged into two groups at the beginning of the present paper. COOK (1963) stated in a note on *C. diana* var. *minus* "The apices are variable within all clones ranging from acutely rounded to clearly truncate" and stated also in a note on *C. calosporum* "However, if the practice of separating species according to the nature of the apices were to be consistently followed, *C. calosporum* could easily be separated into at least two species with vegetative cells corresponding to *C. parvulum* and *C. diana*". The results of the present examinations show that the shape of the apices is more or less

C. spinosporum HODGETTS (1925) : According to the original description, this species is characterized by the obliquely truncated apex and the zygosporangium with conical spines. At the same time, Hodgetts described the variety *minus* for the algae having a shorter cell. This is the first report for the algae which have an obliquely truncated apex and form a zygosporangium with spines. KRIEGER (1935) treated this species as a synonym of *C. calosporum* var. *maius*, and var. *minus* as a synonym of the typical variety of *C. calosporum*. However, *C. spinosporum* may be quite different from *C. calosporum* because they apparently differ in the shape of apex. Most of the algae which have been reported under the name of *C. calosporum* var. *calosporum* may be more rightly referred to *C. spinosporum*. The misunderstanding regarding the conception of the two species began from SKUJA (1928) who reported algae with the "abgestutzt-abgerundet" apex under the name of *C. calosporum* and its form *major*. Since the typical variety and var. *minus* of *C. spinosporum* are not different in the features of cell apex, except a slight discordance in distance, the present author here regards them as belonging to the same taxon.

C. calosporum var. *calosporum* f. *erectum* PRESCOTT (1975) : This form seems to have been proposed on the basis of its relatively longer cell, its obliquely truncated apex and its spiny zygosporangium. However, this alga is apparently closer to *C. spinosporum* and seems to be situated at an intermediate position between the typical variety and var. *minus* of *C. spinosporum*. In my opinion, this form should be reduced to a synonym of *C. spinosporum*.

4.2 Taxonomic treatment of the strains examined

The 17 strains examined can be classified into two groups, Calosporum-group and Spinosporum-group, each of which is composed of four subgroups. The three subgroups of the Calosporum-group are assigned to three varieties of *C. calosporum* and the remaining one is regarded to be an independent species, and the four subgroups of the Spinosporum-group are arranged in four varieties of *C. spinosporum*. The subgroup with the smallest cell (C-318) of the Calosporum-group from the United States is identified with *C. calosporum* var. *calosporum* in accord with COOK (1963). The second subgroup (R-5 and R-11) from Okinawa Pref. is identified with *C. calosporum* var. *galiciense*. The third (N-147, N-143, and N-134) from Nepal and the fourth (R-9-broader) from Okinawa Pref. do not correspond to any taxon described so far, and they are newly named here respectively. The same is true of the subgroup which is the smallest in cell size (M-10) from Malaysia, the second subgroup (R-12-smaller and -larger) from Okinawa, and the fourth one with the largest cell size (C-320, Ak, C-319 and R-9-slender) from Hokkaido, Okinawa and the United States in the Spinosporum-group.

The measurements of vegetative cells represent the results of the standard culture. When a taxon includes more than two clones, the maximum and minimum values in description means those obtained from all the clones measured. As for the details of the measurements, Appendixes at the end of the text should be referred. The strains

examined are characterized in common by the following morphological features: vegetative cells lunate, without girdleband, gradually attenuated toward the apices, having almost same curvature at the outer margin, with endopore and a smooth wall; zygospores nearly globe-shaped, with spine or warts.

Abbreviations W=width of vegetative cells
 D=distance between apices
 D/W=distance width ratio
 C=curvature at the outer margin
 Z=dimensions of zygospores without spines
 S=length of spines

C. calosporum WITTROCK var. *calosporum* (Pl. IV, figs. 8–9, Pl. V, figs. 1–9, Pl. IX, fig. 5)

WITTROCK 1869, p. 23, fig. 11. — COOK 1963, p. 12, fig. 9. — RINO 1971, p. 14, pl. 2, figs. 3–5.

Cells acutely rounded at the apex; inner margin nearly straight in the median portion. Hinge-like separation type. W: 10.0–11.5 μm , D: 72–91 μm , D/W: 6.9–8.5, C: 137–164°, Z: 22.5–28.5 \times 22.0–25.0 μm , S: 0.3–3.0 μm .

Strain and locality: C-318 (Vermont, U S A)

COOK (1963) had identified this strain with *C. calosporum* var. *calosporum*. The present alga agrees with WITTROCK's one in width, distance between apices, and curvature, but differs from the latter in the number of pyrenoids and the size of zygospore. Measurements of the curvature of WITTROCK's alga were made based on his original figures.

C. calosporum var. *galiciense* GUTWIŃSKI (Pl. VI, figs. 1–9, Pl. IX, fig. 4)

GUTWIŃSKI 1896, p. 41, pl. 6, fig. 21.

Apices slightly broader than those of var. *calosporum* and showing a faint tendency to truncate; inner margin nearly straight or faintly concave in the median portion. Hinge-like separation type. W: 11.5–15.5 μm , D: 72–120 μm , D/W: 5.4–9.6, C: 92–135°, Z: 24.5–32.0 \times 23.0–29.0 μm , S: 1.5–5.0 μm .

Strains and localities: R-5 (Okinawa Isl., Okinawa Pref.), R-11 (Iriomote Isl., Okinawa Pref.)

This alga is so similar to *C. parvulum* NÄG. in its vegetative stage that it is difficult to distinguish them in the vegetative stage. So it is possible that this alga was sometimes reported as *C. parvulum*.

C. calosporum var. *himalayense* M. WATANABE var. nov. (Pl. VII, figs. 1–9, Pl. VIII, figs. 1–3, Pl. IX, figs. 1–3)

Cellulae 80–128 μm distantes inter apices, 13.5–16.5 μm latae, 5.3–8.5 plo distantis quam latibus; margines exterior aequaliter curvati, 81–123° arcus; margines interior fere linearis ad media; apices oblique rotundati.

Semicellulae ad conjugationes non omino disjunctae; zygosporae sphaericae vel sub-sphaericae, 25.0–34.0 \times 22.5–32.5 μm latae; processus conici, 0.5–3.5 μm longi.

Strains and localities : N-134 (Shewaden, Nepal), N-143 and N-147 (Suke, Nepal)

FÖRSTER (1965) reported an alga as *C. calosporum* from the upland, 4200 m alt., in Khumbu, Nepal Himalayas. KUSEL-FETZMAN (1969) also reported an alga under the name of *C. calosporum* from the upland, 4920 m, in Khumbu. HIRANO (1964) reported an alga as *C. calosporum* from Nuristan, 2500 m alt., in Afghanistan. Full descriptions of the algae were not given in these reports, but the size and shape of vegetative cells were indicated. Considering the described features, the algae mentioned above are possibly identical with the present variety. Since the habitats of these algae as well as those of the three strains examined in the present study are located at high altitudes, where annual mean temperatures may be less than 10°C, they must be adapted to considerably low temperatures for a certain period of a year. *C. calosporum* var. *himalayense* is estimated to be restricted to the alpine regions with a cold climate. Geographical distribution of this alga at the highlands of inner Asia is a problem of some interest.

C. selenastrum M. WATANABE sp. nov. (Pl. VIII, figs. 4–9, Pl. IX, fig. 6)

Cellulae 142–202 μm distantes inter apices, 24.0–28.5 μm latae, 6.1–8.1 plo distantis quam latibus; margines exterior aequaliter curvati, 112–148° arcus; margines interior concavi, vel fere linearis ad media; apices oblique rotundati, poro in latere dorsali; membrana laevis, sine cingulis; pyrenoidea 2–6 (plerumque 3–5) unaquaque in plastide.

Semicellulae ad conjugationes non omnino disjunctae; zygosporae sphaericae vel sub-sphaericae, 42.5–47.5 : 37.5–45.0 μm latae; processus conici, 2.5–7.0 μm longi.

Strain and locality : R-9-broader (Okinawa Isl., Okinawa Pref.)

This species resembles *C. parvulum* var. *maius* WEST in vegetative morphology but is distinguished from the latter by its zygospore covered with conical spines. There is a possibility that this alga has been reported under the name of *C. parvulum* var. *maius* by previous authors.

C. spinosporum HODGETTS var. *spinosporum* (Pl. II, figs. 8–9, Pl. III, figs. 1–9, Pl. X, figs. 6–8)

HODGETTS 1925, p. 72, fig. 7A–B.

SYN.: *C. spinosporum* var. *minus* HODGETTS 1925, p. 74, fig. 7C–D.

C. calosporum var. *calosporum* f. *erectum* PRESCOTT 1977, p. 39, pl. 36, figs. 11, 14.

Cells remarkably truncated at the apex; inner margin concave or straight at the median portion. Complete separation type. W: 10.5–13.5 μm , D: 65–125 μm , D/W: 6.0–9.7, C: 77–113°, Z: 24.5–33.0 × 22.0–29.0 μm , S: 0.5–3.5 μm .

Strains and locality : A-2, A-7, A-13 (Tsukude Vil., Aichi Pref.)

A-7 and A-13 seem to correspond to the typical form of var. *spinosporum*, while A-2 may be closer to var. *minus* sensu HODGETTS (1925).

C. spinosporum var. *malaysiense* M. WATANABE var. nov. (Pl. I, figs. 4–9, Pl. X, fig. 5)

Cellulae 77–105 μm distantes inter apices, 9.0–11.0 μm latae, 8.0–10.7 plo distantis quam

latibus; margines exterior aequaliter curvati, 90–119° arcus; margines interior concavi; apices oblique truncati.

Semicellulae ad conjugationes omnino disjunctae; zygosporae sphaericae vel subsphaericae, 25.0–29.0 × 24.0–27.5 μm latae; processus conici, 2.0–3.0 μm longi.

Strain and locality : M-10 (Penang Isl., Malaysia)

C. spinosporum var. *ryukyuense* M. WATANABE var. nov. (Pl. II, figs. 1–7, Pl. X, figs. 1–4)

Cellulae 48–82 μm distantes inter apices, 9.5–14.5 μm latae, 4.4–6.1 plo distantium quam latum; margines exterior aequaliter curvati, 103–140° arcus; margines interior fere linearis vel tumidi ad media; apices oblique truncati.

Semicellulae ad conjugationes omnino disjunctae; zygosporae sphaericae vel subsphaericae, 21.5–34.5 × 20.0–28.0 μm latae; processus conici, 0.5–3.0 μm longi.

Strains and locality: R-12-smaller and R-12-larger (Iriomote Isl., Okinawa Pref.)

C. spinosporum var. *crassum* M. WATANABE var. nov. (Pl. IV, figs. 1–7, Pl. XI, figs. 1–4)

Cellulae 100–182 μm distantes inter apices, 12.5–19.0 μm latae, 6.9–11.1 plo distantis quam latibus; margines exterior aequaliter curvati, 67–123° arcus; margines interior concavi, vel tumidi ad media; apices oblique truncati.

Semicellulae ad conjugationes omnino vel non omnino disjunctae; zygosporae sphaericae vel subsphaericae, 31.5–41.5 × 29.0–37.5 μm latae; processus conici, 2.5–5.0 μm longi.

Strains and localities : Ak (Akan, Hokkaido Pref.), C-319 (Massachusetts, USA), C-320 (Michigan, USA), R-9-slender (Okinawa Isl., Okinawa Pref.)

As previously stated, there are some differences in quantitative characters between C-319 and C-320. C-319 also differs from the other three strains in its slightly tumid inner margin at the median portion. Thus there remains a certain problem in regarding the four strains as a single group. Many of the algae which have been reported as *C. calosporum* var. *maius* from many parts of the world seem to be attributable to the present variety because they have been reported as having a truncated apex. Reexamination of these algae is needed.

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6. Appendixes

Appendix 1. Variation in vegetative characters among clones of the Calosporum-group.

Clone	Width (μm)				Distance (μm)				Ratio				Curvature (deg.)							
	Min	Max	Mean	SD	CV(%)	Min	Max	Mean	SD	CV(%)	Min	Max	Mean	SD	CV(%)	Min	Max	Mean	SD	CV(%)
C-318	10.0	11.5	10.5	0.3	3.0	72	91	81.5	4.6	5.6	6.9	8.5	7.8	0.4	5.4	137	164	148.9	7.4	5.0
R-5-3	12.0	13.5	12.7	0.3	2.7	82	110	93.6	5.9	6.4	6.5	8.5	7.4	0.5	6.5	101	128	115.4	6.4	5.5
R-5-5	12.5	14.0	13.0	0.3	2.5	77	102	90.5	5.7	6.3	6.3	7.5	7.0	0.4	5.1	108	129	117.4	5.7	4.9
R-5-6	11.5	14.0	13.0	0.4	3.4	80	118	93.8	7.1	7.6	6.5	7.8	7.2	0.4	5.7	106	122	116.3	5.0	4.3
R-5-25	11.5	13.5	12.0	0.5	4.1	78	97	87.6	3.8	4.4	6.3	7.9	7.3	0.4	5.3	106	133	122.4	7.4	6.1
R-5-28	12.0	15.5	12.3	0.7	5.4	82	103	92.3	4.7	5.1	6.4	8.5	7.2	0.5	6.7	100	131	118.8	7.3	6.2
R-5-2	11.5	15.0	12.8	0.7	5.7	75	120	93.3	8.4	9.0	5.8	8.9	7.3	0.7	9.3	108	124	116.1	4.6	4.0
R-5-24	11.5	13.5	12.6	0.5	3.7	79	103	92.1	5.3	5.8	6.5	8.2	7.4	0.7	9.1	92	135	116.5	10.4	8.9
R-5-26	12.5	14.5	13.7	0.5	3.4	72	101	84.7	5.4	6.3	5.4	7.6	6.3	0.4	6.8	104	133	117.7	7.4	6.3
R-5-27	11.5	14.5	12.6	0.5	4.2	74	105	88.4	7.0	7.9	6.0	7.8	6.9	0.5	7.4	102	135	118.8	9.0	7.6
R-5-30	12.0	14.0	12.5	0.5	3.8	80	114	98.7	6.6	6.7	7.0	9.6	8.0	0.7	8.4	100	117	108.1	4.4	4.1
R-11-5	12.0	14.0	12.7	0.5	4.1	72	96	84.0	5.6	6.6	5.7	7.3	6.6	0.4	6.0	110	129	117.1	5.7	4.9
R-11-6	11.5	14.5	13.3	0.7	4.9	82	105	92.6	5.3	5.7	6.4	7.8	7.0	0.3	4.8	103	128	112.8	7.7	6.8
R-11-7	12.5	14.5	13.6	0.4	2.7	85	105	94.4	4.8	5.0	6.3	7.5	6.9	0.3	4.4	93	120	112.5	6.2	5.5
R-11-9	13.0	15.0	14.0	0.5	3.4	80	104	91.7	5.3	5.7	6.0	7.3	6.6	0.4	5.4	103	125	114.6	5.0	4.4
R-11-10	13.0	15.0	14.0	0.5	3.3	74	103	88.7	6.4	7.3	5.4	7.2	6.4	0.4	6.8	108	127	117.3	4.8	4.1
N-147-4	14.5	16.0	15.2	0.4	2.4	80	112	99.5	6.6	6.6	5.3	7.4	6.5	0.5	7.2	87	120	104.7	9.0	8.6
N-147-5	13.5	16.5	14.5	0.9	6.1	83	108	95.0	7.7	8.1	6.1	7.1	6.5	0.3	4.6	96	114	105.6	5.8	5.5
N-147-6	13.5	16.5	15.6	0.5	3.3	87	112	99.0	5.4	5.5	5.9	6.7	6.3	0.2	3.6	96	123	106.7	6.3	5.9
N-143-10	15.0	16.5	15.6	0.4	2.4	90	115	103.0	6.1	5.9	6.0	7.3	6.6	0.3	5.2	98	120	107.0	6.2	5.8
N-134-3	14.5	16.0	15.1	0.3	1.8	100	123	114.2	4.5	3.9	7.1	8.0	7.6	0.3	3.8	86	108	94.6	5.9	6.3
N-134-4	14.5	15.5	15.0	0.3	1.7	100	118	111.3	3.8	3.4	7.0	8.0	7.4	0.2	3.3	82	107	95.0	6.0	6.3
N-134-5	14.5	15.5	14.9	0.2	1.6	97	128	112.2	6.4	5.7	6.9	8.5	7.6	0.4	5.4	81	106	94.6	6.9	7.2
R-9-10	25.0	27.5	26.0	0.5	1.9	142	195	178.6	10.0	5.6	6.2	7.5	6.9	0.3	4.9	112	141	130.8	5.0	3.8
R-9-11	24.0	27.0	25.3	0.6	2.4	148	194	168.5	9.4	5.6	6.1	7.5	6.7	0.3	4.6	118	148	136.9	11.4	8.3
R-9-40	24.5	28.5	26.3	1.1	4.1	160	202	179.9	10.2	5.6	6.3	8.1	7.0	0.4	6.0	120	139	131.2	4.9	3.7
R-9-42	24.0	26.5	25.3	0.5	1.9	156	202	177.6	9.3	5.2	6.6	8.0	6.9	0.3	4.3	121	145	130.8	6.2	4.7

Appendix 2. Variation in vegetative characters among clones of the *Spinosporum*-group.

Clone	Width (μm)						Distance (μm)						Ratio						Curvature (deg.)								
	Min	Max	Mean	SD	CV(%)		Min	Max	Mean	SD	CV(%)		Min	Max	Mean	SD	CV(%)	Min	Max	Mean	SD	CV(%)	Min	Max	Mean	SD	CV(%)
M-10-1	9.0	11.0	9.7	0.4	3.9	78	98	86.5	5.2	6.0	8.0	9.5	8.9	0.4	4.1	90	119	106.8	7.9	7.4							
M-10-4	9.0	10.5	9.6	0.3	2.9	77	105	87.7	5.7	6.5	8.2	10.7	9.2	0.6	6.3	94	111	102.5	4.3	4.2							
R-12-2	9.5	11.5	10.4	0.6	5.3	48	65	57.1	3.4	5.9	4.7	6.1	5.3	0.4	6.8	108	139	119.1	6.2	5.2							
R-12-2-G1	12.5	15.5	14.6	0.7	5.0	62	94	80.7	6.5	8.0	4.9	6.0	5.5	0.3	4.6	126	156	137.3	7.1	5.1							
R-12-3	13.0	14.5	13.6	0.4	2.6	60	82	69.6	5.6	8.1	4.4	6.1	5.2	0.4	8.0	110	140	119.7	7.9	6.6							
R-12-6	12.5	14.0	12.9	0.4	2.8	60	81	68.4	5.2	7.5	4.6	6.0	5.3	0.3	6.4	103	124	112.3	6.1	5.4							
A- 2-22	11.0	12.5	11.5	0.5	4.1	65	90	75.7	5.8	7.7	6.0	7.8	6.6	0.5	7.6	91	113	104.9	5.5	5.2							
A- 7- 3	12.0	13.5	12.4	0.3	2.4	87	113	100.0	7.1	7.1	7.1	9.0	8.0	0.5	6.2	77	101	90.3	5.3	5.9							
A- 7- 4	11.5	13.5	12.5	0.4	3.4	84	115	95.5	6.2	6.5	7.0	8.7	7.7	0.3	4.4	85	102	93.7	4.8	5.1							
A- 7- 5	11.5	13.5	12.5	0.4	3.0	92	122	107.0	7.3	6.9	7.2	9.4	8.4	0.5	6.4	78	100	88.5	5.7	6.5							
A-13- 3	11.5	13.5	12.2	0.5	3.7	80	122	98.1	9.0	9.2	7.1	9.5	8.1	0.6	7.1	84	100	91.0	4.0	4.4							
A-13- 4	11.5	13.5	12.5	0.4	3.4	83	113	98.6	8.3	8.5	6.7	8.7	7.9	0.5	6.5	80	103	91.5	5.8	6.4							
A-13- 5	10.5	13.5	12.4	0.5	4.2	78	125	98.8	10.0	10.1	6.8	9.7	8.1	0.7	8.6	82	97	90.1	4.0	4.5							
C-320	13.5	15.0	14.0	0.3	2.2	114	140	128.1	6.3	4.9	8.3	10.1	9.2	0.5	5.4	67	95	81.8	7.4	9.1							
Ak-46	12.5	15.5	14.7	0.4	3.0	100	160	131.0	9.6	7.3	7.8	10.5	8.8	0.5	5.9	99	120	109.2	5.5	5.0							
C-319	15.5	16.5	16.0	0.3	1.8	112	138	121.3	5.2	4.3	6.9	8.5	7.6	0.3	4.6	98	123	109.5	5.9	5.4							
R-9- 5	16.5	18.5	17.3	0.4	2.1	128	168	148.9	8.5	5.7	7.5	9.4	8.5	0.5	5.7	83	104	94.9	5.5	5.8							
R-9- 6	17.0	19.0	17.7	0.3	1.9	128	182	164.2	10.4	6.4	7.3	10.0	9.2	0.6	6.5	83	116	97.8	8.5	8.6							
R-9-12	16.0	18.0	17.4	0.4	2.4	145	173	154.8	7.8	5.0	7.9	9.4	8.9	0.4	4.5	84	111	95.1	5.7	6.0							
R-9-13	14.5	17.0	16.0	0.7	4.2	118	170	141.6	11.2	7.9	7.7	11.1	9.0	0.9	9.8	89	116	101.5	6.7	6.6							
R-9-22	17.0	17.5	17.1	0.2	1.4	125	166	145.2	10.4	7.2	7.4	9.5	8.5	0.6	7.3	85	113	96.4	7.3	7.6							

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Explanation of Plates IX-XI.

Plate IX

Figs. 1-3. *Closterium calosporum* v. *himalayense* (1: N-134, 2: N-147, 3: N-143), fig. 4 *C. calosporum* v. *galiciense* (R-5), fig. 5 *C. calosporum* v. *calosporum* (C-318), fig. 6 *C. selenastrum* (R-9-broader).

Plate X

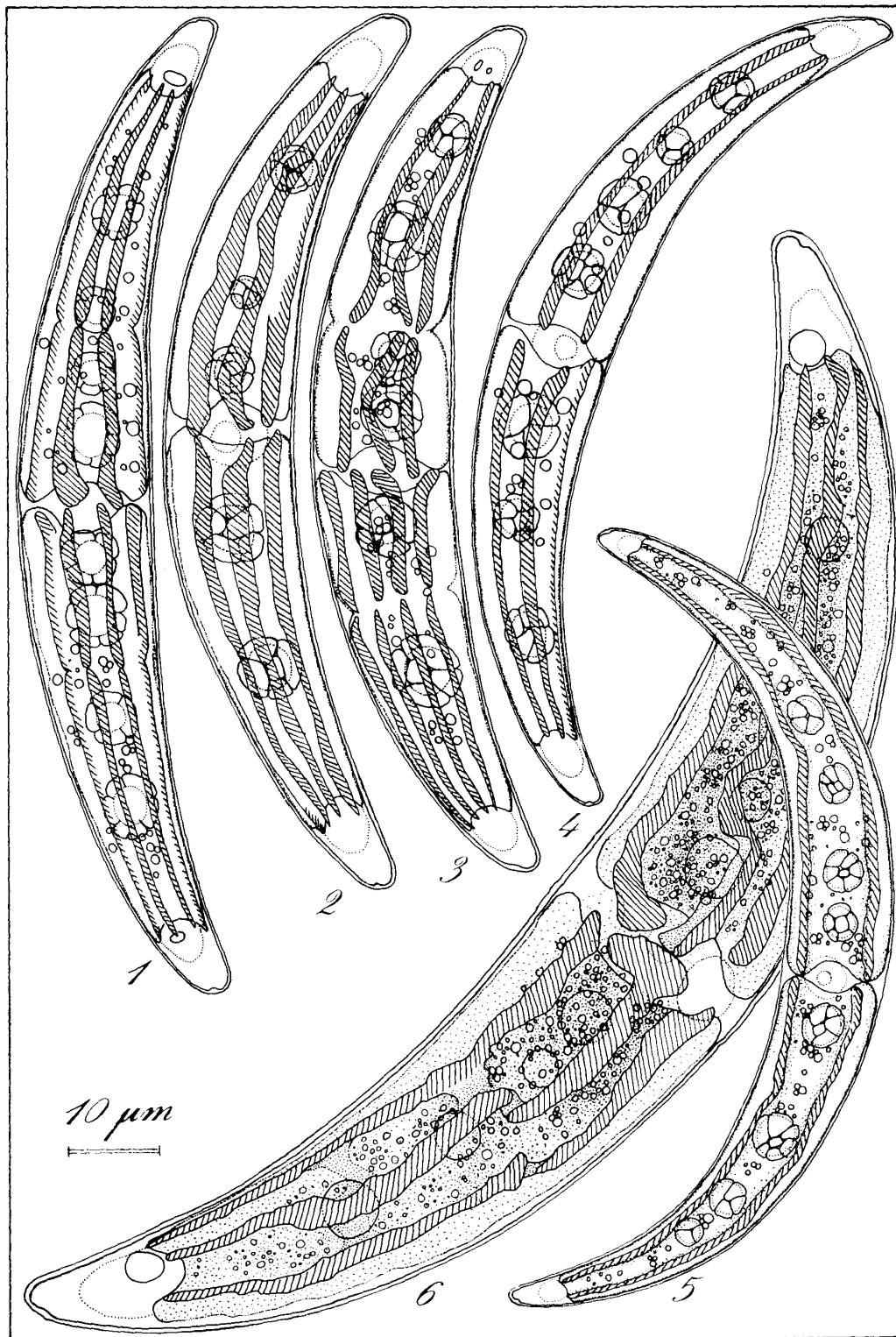
Figs. 1-4. *C. spinosporum* v. *ryukyuense* (1-2: R-12-smaller, 3-4: R-12-larger), fig. 5 *C. spinosporum* v. *malaysiense* (M-10), figs. 6-8 *C. spinosporum* v. *spinosporum* (6: A-2, 7: A-7, 8: A-13).

Plate XI

Figs. 1-4. *C. spinosporum* v. *crassum* (1: Ak, 2: R-9-slender, 3: C-320, 4: C-319).

Plate IX

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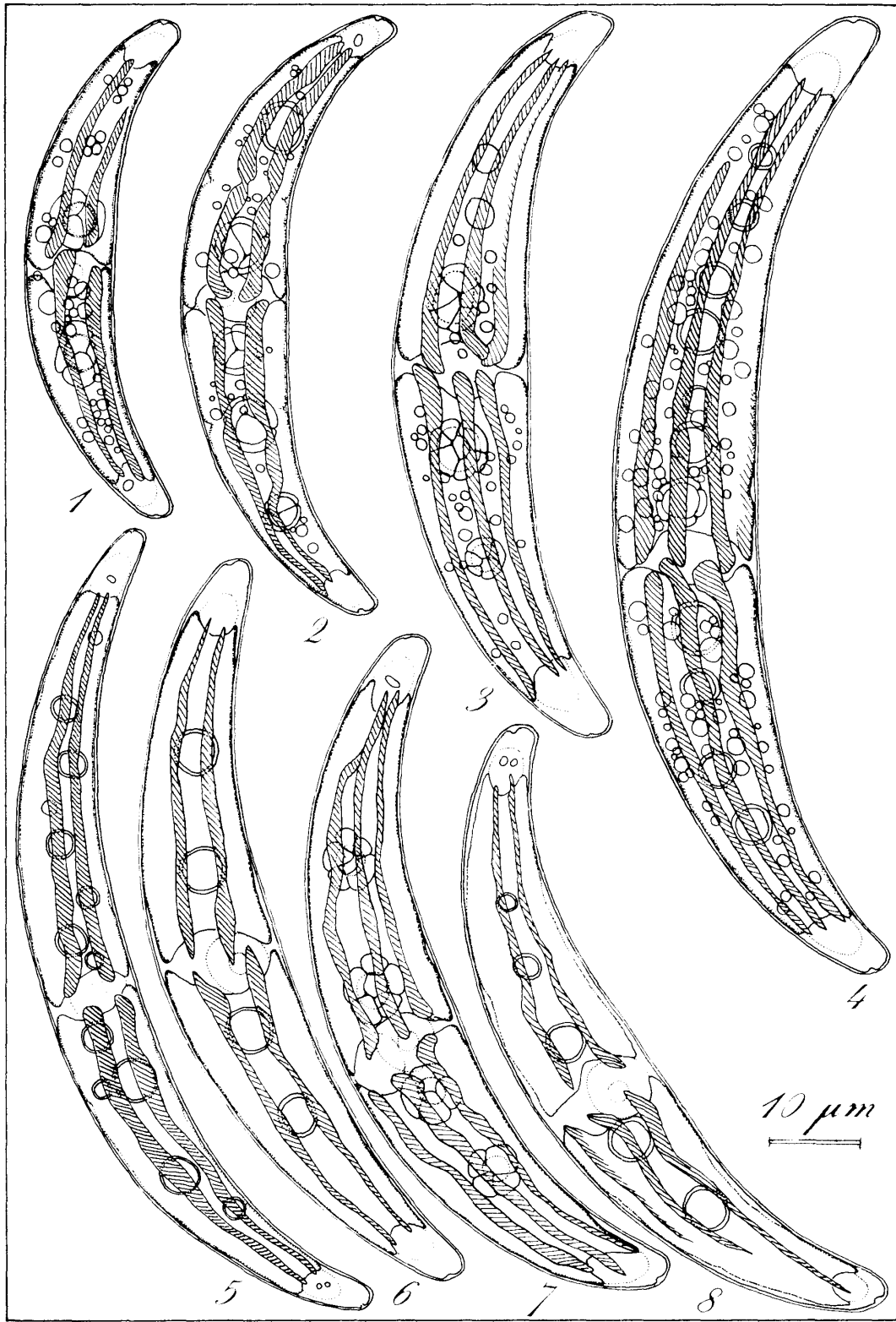


Plate XI

WATANABE: A Taxonomic Study of the *Closterium calosporum*

