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Karyomorphological Studies on the Genus *Spirogyra* Link (Conjugales, Chlorophyta) from Korea

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Freshwater green algae are one of the important sources of bioenergy in the future. *Spirogyra* is a conjugating filamentous zygnematacean green algal genus that is widely distributed worldwide with more than 400 species. Despite its widespread occurrence throughout the world, cytological studies of the genus have been limited. We investigated karyological features and chromosome numbers for seven Korean *Spirogyra* species. Most of the species examined in the present study showed significant karyological features, inner organization of nucleolus, heavily stainable nucleolar substance and the diffuse-centric nature of chromosomes, typical of the Conjugales. Chromosome number ranged from n=12 in *S. varians* to n=38 in *S. africana*. Aberrant cytokinesis resulted in binucleate and tetranucleate cells, which sometimes provide cytological explanation for different morphology and ploidal changes in clonal culture of *Spirogyra* or even different cells within the same filament. The present chromosome data also substantiates the earlier held assumption that aneuploidy must have been the chief driving force for speciation and evolution of the genus *Spirogyra*.

Key words : aneuploidy, chromosome, cytotaxonomy, Conjugales, karyomorphology, *Spirogyra*

INTRODUCTION

Freshwater green alga *Spirogyra* (Conjugales, Chlorophyta) is one of the most economically important bioenergy source in the future (Sharif *et al.*, 2008). The silky masses of this alga are fast growing and often form bright-green floating or frothy masses in small bodies of water in the spring (Bold and Wynne, 1985). The genus is characterized by having cylindrical cells, nucleus suspended by a thread of cytoplasm, spiral-like chloroplast, non-flagellated amoeboid gametes, and sexual reproduction by conjugation (Transeau, 1951).

Spirogyra Link (1820) is widely distributed worldwide with more than 400 species (Randhawa, 1959; Kadlubowska, 1984; Devi and Panikkar, 1994). Species descriptions of the genus were mainly on morphological features such as filament width, chloroplast number, details of sexual reproduction, size and shape of zygospore and ornamentation of its wall. However, vegetative morphological variation in different ploidy levels was reported in clonal culture of *Spirogyra* (Hoshaw *et al.*, 1987) and similar variations in filament width and chloroplast number were found in nature (McCourt *et al.*, 1986). Floristic studies, with-

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Table 1. Collection site and chromosome numbers of *Spirogyra* populations in Korea (N: number of individuals examined for a given population).

Taxon	Collection site	Chromosome number (n=)
S. africana	Duksan stream, Yesan, Korea	n=38 (N=4)
	Chongchon stream, Chongchon, Korea	n=36 (N=4)
S. decimina	Maepo stream, Tanyang, Korea	n=24±1 (N=5)
	Chongchon stream, Chongchon, Korea	n=24±1 (N=10)
	Dukchon stream, Tanyang, Korea	n=22/23 (N=1)
S. dubia	Chonjeyeon waterfall, Cheju, Korea	n=19~25 (N=3)
	Chonjeyeon waterfall, Cheju, Korea	n=24±1 (N=10)
	Sasok stream, Chinchon, Korea	n=23 (N=3)
	Sosan pond, Sosan, Korea	n=23 (N=4)
S. gracilis	Chungju lake, Tanyang, Korea	n=24±1 (N=10)
S. koreana	Sasok stream, Chinchon, Korea	n=38 (N=12)
	Duchon stream, Hongchon, Korea	n=34/35 (N=2)
	Duchon stream, Hongchon, Korea	n=34 (N=5)
	Munbaik pond, Chinchon, Korea	n=36 (N=6)
S. varians	Maepo stream, Tanyang, Korea	n=ca. 12 (N=2)
S. variformis	Duksan stream, Yesan, Korea	n=ca. 35 (N=1)

out consideration of the effects of polyploidy and morphological variations in clonal culture and field-collected materials, have contributed to the diversity of the genus (McCourt and Hoshaw, 1990).

Since Strasburger's (1875) first report for the process of cell division in *Spirogyra orthospira*, studies on the nuclear cytology (Godward, 1954, 1956; Godward and Newnham, 1965) and the cytotaxonomy of *Spirogyra* were carried out (Tatuno and Iiyama, 1971; Vedajanani and Sarma, 1978; Abhayavardhani and Sarma, 1983). However, despite its wide distribution throughout the world, chromosome numbers of the genus were determined for only forty-four identified species (Chaudhary and Agrawal, 1996). Chromosome numbers in *Spirogyra* ranged from n=2 in three Japanese species (Tatuno and Iiyama, 1971) to n=92±2 in *S. nitida* (Abhayavardhani and Sarma, 1983). Also, chromosomal features of the Conjugales and other algae have been reviewed by Sarma (1982, 1983).

To date, forty-nine species of the genus have been reported in Korea (Kim *et al.*, 2004). Most of the species were found in floristic studies (Chung, 1968; Chung and Kim, 1991). Although Kim *et al.* (2006) studied the genus in morphology and molecular phylogeny, species delimitation in the genus remains unclear. Karyological investigations on the seven Korean *Spirogyra* species, that formed zygospores in nature and cultured by unialgal culture, were conducted in the present study with a view to contributing to our understanding of morphological plasticity in clonal culture and in nature under changing environment conditions.

MATERIALS AND METHODS

A total of seven species, 16 populations, of *Spirogyra* were collected from various freshwater habitats in Korea during 1996~2006 (Table 1). The live cells were isolated under the dissecting microscope, washed thoroughly with sterile distilled water, and inoculated single filaments in the sterilized Woods Hole liquid medium (Nichols, 1973). Unialgal cultures were kept in 200 mL glass culture vessels at 20±1°C, on a 16 : 8-h light: dark photoperiod under 10~50 μmol m^{-2} sec^{-1} from cool-white fluorescent lamps (Pringsheim, 1967).

For cytological purposes, actively growing filaments of each isolate were sampled half hourly for 2 hrs after the onset of the dark phase. They were fixed in acetic acid-alcohol (1 : 3, v/v) fixative for 1 hr at room temperature. Double fixation, change of fixative after 1 hr of fixation, was done to completely remove the coloring matter from the filaments. Squash preparations were made following Godward's iron-alum acetocarmine technique (Godward, 1948, 1966). The preparations were examined, sealed with enamel and the suitable stages were photographed using light microscope

Table 2. Comparative morphological and karyological features of *Spirogyra* investigated in the present study.

Taxa	Cell width (μm)	Chloroplast number	Chromosome number (n=)	Chromosome size (μm)	Nucleolus size (μm)	Nucleus	
						Size (μm)	Position
S. africana	48~57	3~6	36, 38	0.7~3.2	4.7~19.0	3.1~47.5	central
S. decimina	28~36	2~4	22/23, 24±1	0.7~1.0	4.7~7.1	11.9~16.6	central
S. dubia	40~50	3~5	23, 24±1	0.7~2.7	4.7~5.9	11.3~18.0	near cell wall
S. gracilis	20~23	1	24±1	0.7~2.3	5.9~9.1	9.5~23.7	central
S. koreana	48~54	3~5	34/35, 36, 38	0.7~3.3	5.9~16.6	8.3~42.7	central
S. varians	24~39	1	ca. 12	–	7.1~11.9	14.2~23.7	central
S. variformis	46~54	1~3	ca. 35	1.0~2.0	3.0~6.0	7.3~12.7	central

(Olympus BH-2).

RESULTS

Chromosome number, comparative morphological and karyological features of *Spirogyra* examined in this study are shown in Table 2 and Figures 1~19.

1. Interphase Nucleus

The interphase nuclei are spherical and centrally located singly in the cell. They are characterized by the presence of a darkly stained, large, conspicuous, spherical body "the nucleolus" (Fig. 1). Occasionally, however, two nucleoli may also exist in a nucleus (Fig. 2). The nucleoli are generally characterized by well defined granular material commonly known as 'Geitler's nucleolar substance'. Chromocenters were not encountered either free in the nucleoplasm or in association with the nucleolus (Fig. 3).

2. General Pattern of Nuclear Division

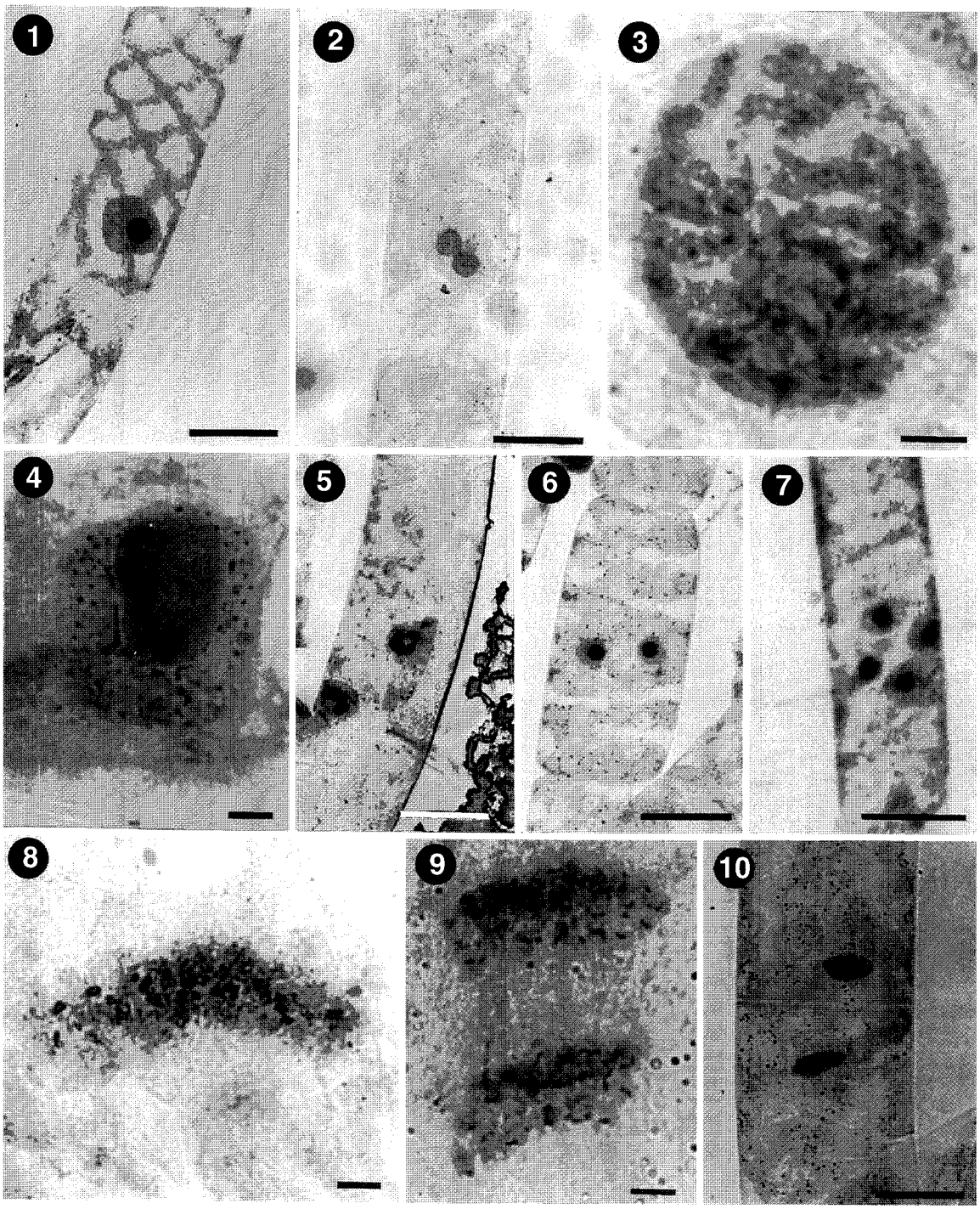
The interphase nuclei gradually enlarge to assume up to 3-fold increase in size at prophase (Fig. 4). The contents of the nuclei and nucleoli become more prominent with the advancement of prophase stage. At late prophase, the nucleoli appear granular in nature, with the dissolution of nuclear envelope, dissecting the nucleolar substance into few (3-6) globular masses (Fig. 5). The chromosomes at metaphase appear as condensed, small, discrete structures usually embedded in the heavily stained nucleolar substance (Fig. 8). They normally look doubled, representing the two sister chromatids lying parallel to each other from one end to the other. The chromosomes, thus, do not possess localized centromeres, and presumably are diffuse-centric in nature. Anaphase is normal and the chromatids segregate parallels (Fig. 9), clearly indicating the diffuse-centric nature of the chromosomes. The daughter groups of chromosomes also remain embedded in the stainable nucleolar substance. Due to this heavily stainable material embedding the chromosomes at meta- and anaphases, exact count of chromosomes often becomes difficult. The telophase nuclei are separated by the cell plate laid down in the center of the two daughter nuclei formed (Fig. 10).

3. Cytokinesis

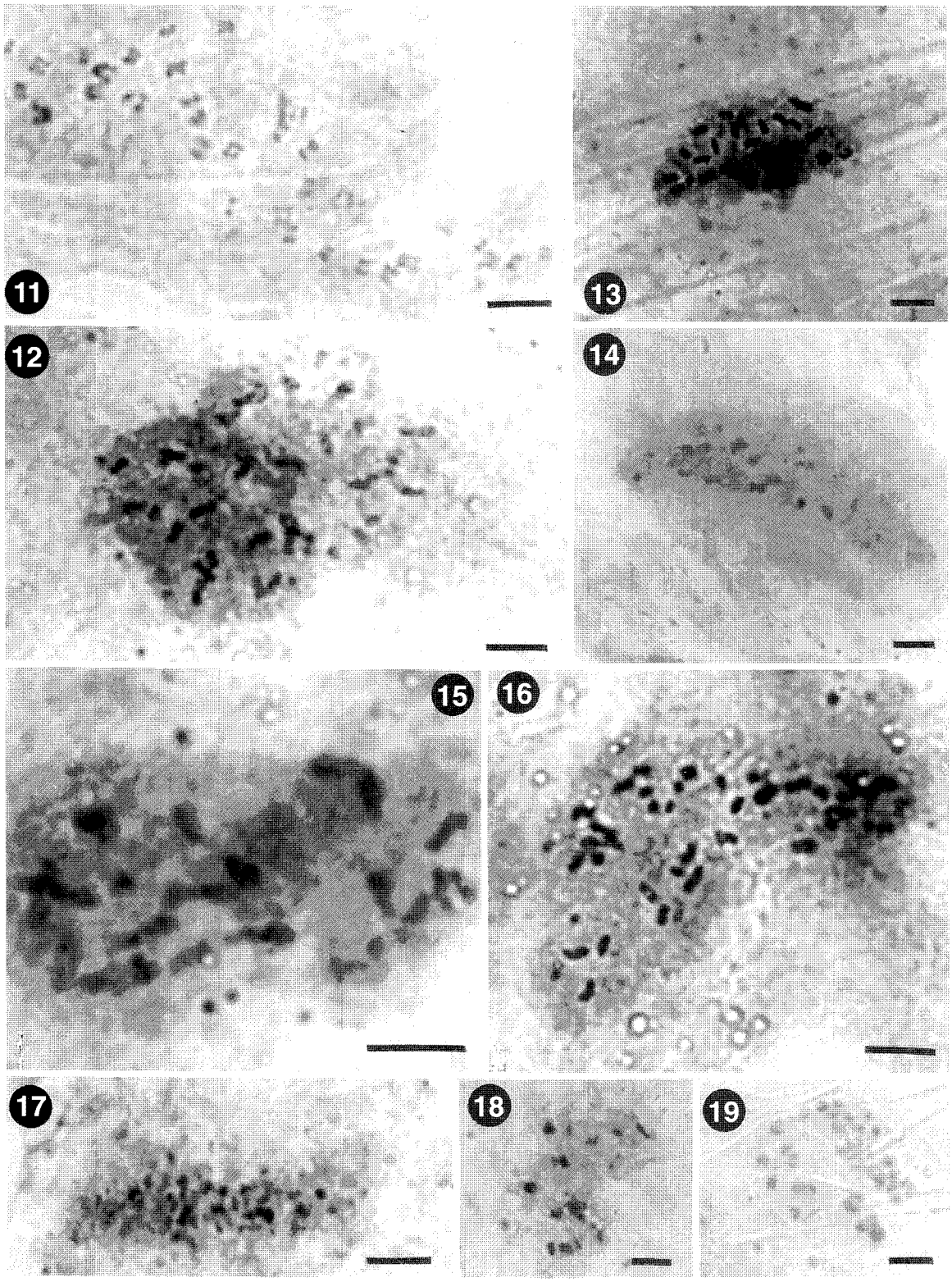
The karyokinesis is followed by cytokinesis, separating the two daughter nuclei by transverse cell plate formation across the barrel-shaped cell. Rarely, however, abnormal separation in some species of *Spirogyra* was also encountered. Consequently, of the two adjacent daughter cells while one cell becomes binucleate (Fig. 6), the other cell becomes anucleate. The latter does not survive long and is eliminated from the filament after degeneration. The binucleate cell with fused or unfused nuclei divides normally in the subsequent cell cycles. Interestingly, a tetranucleate condition (Fig. 7) was also noticed, indicating further failure of cytokinesis during the subsequent cell division.

4. Chromosome numbers

The chromosomes numbers for the seven species, *S. africana* (n=36, 38, Figs. 11-12), *S. decimina* (n=23, 24±1, Figs. 13-14), *S. dubia* (n=23, Fig. 15), *S. koreana* (n=36, Fig. 16), *S. gracilis* (n=24 ±1, Fig. 17), *S. varians* (n=ca. 12, Fig. 18), *S. variformis* (n=ca. 35, Fig. 19) were determined in



Figs. 1-10. Nuclear division of *Spirogyra* in Korea. 1. Unsquashed interphase nucleus showing single, darkly-stained, spherical nucleolus located inside the nucleus; 2. Two nucleoli in a nucleus; 3. Interphase nucleolus showing inner organization after squashing; 4. Squashed early prophase nucleus showing enlarged nucleolus and the differentiated heterochromatic segments in the nucleoplasm; 5. Disruption of nucleoi into few darkly stained blobs; 6. Occasional existence of binucleate condition of cell; 7. Tetranucleate condition of the cell; 8. Metaphase showing diffuse- or polycentric chromosomes embedded in the heavily stained nucleolar substance; 9. Anaphase showing daughter groups of chromosomes embedded in the stainable nucleolar material; 10. Telophase showing cytokinesis between the two daughter nuclei formed. Scale bars: 1, 5 and 6=50 μm; 2, 7 and 10=25 μm; 3, 4, 8 and 9=5 μm.



Figs. 11-19. Somatic chromosomes of *Spirogyra* in Korea. 11-12. *Spirogyra africana* (n=36, 38); 13-14. *S. decimina* (n=23, 24±1); 15. *S. dubia* (n=23); 16. *S. koreana* (n=36); 17. *S. gracilis* (n=24±1); 18. *S. varians* (n=ca 12); 19. *S. variformis* (n=ca. 35). Scale bars: 5 μm.

the present study. Out of the seven species, *S. africana* (n=36/38), *S. decimina* (n=23/24±1), *S. gracilis* (n=24±1) and *S. varians* (n=ca. 12) are reported for the first time. Morphologically distinct three species, *S. decimina*, *S. dubia* and *S. gracilis*, exhibited the same chromosome number of n=24±1, and two species with similar vegetative morphology, *S. dubia* and *S. koreana*, are characterized by n=23/24±1 and n=34/35, 36, 38 chromosomes, respectively (Table 2).

DISCUSSION

This is the first report on chromosome number and cytological study for seven species of *Spirogyra* from Korea. The structure and behavior of the nucleus during mitosis of *Spirogyra* examined in the present study showed the distinct karyological features of the filamentous Conjugales (Godward, 1954, 1956; Vedajanani and Sarma, 1978; Abhayavardhani and Sarma, 1983). The stained interphase nuclei are characterized by single, big, intensely stained, prominent nucleoli situated in the center of the nuclei. Inner organization of the nucleoli, when squashed, can be easily visualized (Fig. 3). On advancement of nuclear division, the nucleoli appear more granular and their contents get more aggregated into few bigger blobs (Fig. 5), ultimately dispersing into the nuclear area after dissolution of the nuclear envelope. This heavily stained nucleolar substance called 'Geitler's nucleolar substance' suspends chromosomes into its mass and segregates to daughter nuclei in association with the chromosomes maintaining its persistence throughout the nuclear cycle.

The chromosomes of the genus are unusual compared to those of other algae because many species possess minute dot-like chromosomes, although large rod-shaped chromosomes are found in some species of *Spirogyra* (Sarma, 1983; Hoshaw and McCourt, 1988). Nevertheless, sister chromatids can be often seen lying parallel to each other without any point of attachment (Fig. 11), comparable to centromeres of other typical eukaryotic algae. Chromosomes of most species are polycentric or diffuse centromeres, except in a few *Spirogyra* species (Godward, 1954). The parallel disjunction of chromosomes during anaphase, absence of any lagging chromosome fragments at equatorial plate in the irradiated material, and the establishment through colchicines application of definite spindle mechanism operating for genome separation clearly indicate the diffuse-centric nature of chromosomes.

The number of chromosomes ascertained in the present study for the seven Korean *Spirogyra* species ranges from n=12 in *S. varians* to n=38 in *S. africana* (Table 2), in contrast to the lowest chromosome number n=2 (Tatuno and Iiyama, 1971; Abhayavardhani and Sarma, 1983) and the highest n=92±2 (Abhayavardhani and Sarma, 1983). In the present study, morphologically distinct three taxa, *S. decimina*, *S. dubia* and *S. gracilis*, exhibited the same chromosome number of n=24±1 and this chromosome number was reported in two Indian species by Abhayavardhani and Sarma (1983). Similarly, *S. varians* gave a chromosome count of n=ca. 12 in this study and this number was reported in three species of the genus from India, *S. azygospora*, *S. paraxoxa*, and *S. variformis* by Abhayavardhani and Sarma (1983). However, *S. varians* is characterized by 24~39 μm broad filaments with 1 chloroplast, whereas the above three species are characterized by 1~5 chloroplasts per cell and the width of filaments ranged from 45~90 μm. Contrastingly, two species with similar vegetative morphology, *S. dubia* and *S. koreana*, were characterized by n=23/24± 1 and n=34/35, 36, 38 chromosomes, respectively (Table 2). These results indicate that there is no clear correlation between the filament width or chloroplast number and chromosome numbers, as in previous studies (Vedajanani and Sarma, 1978; Abhayavardhani and Sarma, 1983). In addition, these results indirectly indicate their divergence due to chromosomal rearrangements or gene level changes.

The perusal of chromosome numerical data, present and the previous counts, clearly indicate that aneuploidy must have played a decisive role in the speciation and evolution of the genus *Spirogyra* (Sarma, 1983; Chaudhary and Agrawal, 1996). Though not common as in desmids (Sarma, 1983; Chaudhary and Agrawal, 1996), chromosomal races also exist in *Spirogyra* species including the present forms (Table 2). The present karyological data suggest that the variations in the chromosomes umber, mostly aneuploid numbers, are primarily due to "agmatoploidy" rather than nondisjunction of chromosomes operating in typical eukaryotic algae. The process of aberrant cytokinesis resulting in binucleate and anucleate daughter cells, encountered in certain instances, seem

to be an additional mode of chromosome numerical variability within the filament and population. While the anucleate cell degenerates in due course, the binucleate cell divides normally to produce daughter cells with two nuclei each. Thus, the present results provide cytological evidence for the existence of different ploidal status within the clone and for the existence of polyploidy species complex in *Spirogyra* (Hoshaw *et al.*, 1987).

Based on the present chromosome data, though limited, it may be concluded that the different taxa of *Spirogyra* are often characterized by their chromosome number, and hence the latter may be considered an important attribute in the taxonomy of the genus.

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