

## Morphological Note of *Zygnema cruciatum* (Zygnemataceae, Chlorophyta) in Korea

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We described a freshwater filamentous zygnematacean species, *Zygnema cruciatum* (Vaucher) Agardh in Korea, based on light microscopy and scanning electron microscopy. *Zygnema cruciatum* is characterized by unbranched filaments of short cylindrical cells, two stellate chloroplasts per cell, a pyrenoid in each chloroplast. Cells are 32-39  $\mu\text{m}$  in width and 35-50  $\mu\text{m}$  in length. Conjugation is scalariform and female gametangia are cylindrical or slightly enlarged. Zygospores are yellow-brown, spherical or broadly ovoid, 35-44  $\mu\text{m}$  wide and 40-47  $\mu\text{m}$  long. Under SEM, wall of zygospore has pitted mesospore and pits are 1.4-1.8  $\mu\text{m}$  in diameter and 3-4  $\mu\text{m}$  apart from each other.

**Key Words:** conjugation, morphology, *Zygnema*, *Z. cruciatum*, Zygnemataceae

### INTRODUCTION

*Zygnema* (Agardh 1824) is an unbranched filamentous zygnematacean green algal genus that is widely distributed in various aquatic habitats. The slimy masses of the plant, surrounded by mucilaginous envelope, occur floating in small bodies of water. They are generally less slippery than the *Spirogyras* but more slippery than the *Mougeotias* (Transeau 1951; Bold and Wynne 1985). The genus is characterized by having short cylindrical cells, two stellate chloroplasts per cell, a prominent central pyrenoid in each chloroplast, nucleus between chloroplasts, non-flagellated amoeboid gametes, sexual reproduction by ladder-like or lateral conjugation, and zygospore formed either in a conjugation tube or in only one gametangium (Transeau 1934, 1951).

Approximately one hundred forty species have been described world-wide (Kützing 1843; Yamagishi 1965; Kadlubowska 1984; Johnson 2002; Novis 2004). Identification of a species within the genus was based mainly on morphological criteria such as vegetative cell size, details of sexual reproduction, zygospore size and shape, and color and ornamentation of the median spore wall (Randhawa 1959; Rundina 1998; Zarina *et al.* 2006). Their life cycle, however, is completed in a few weeks

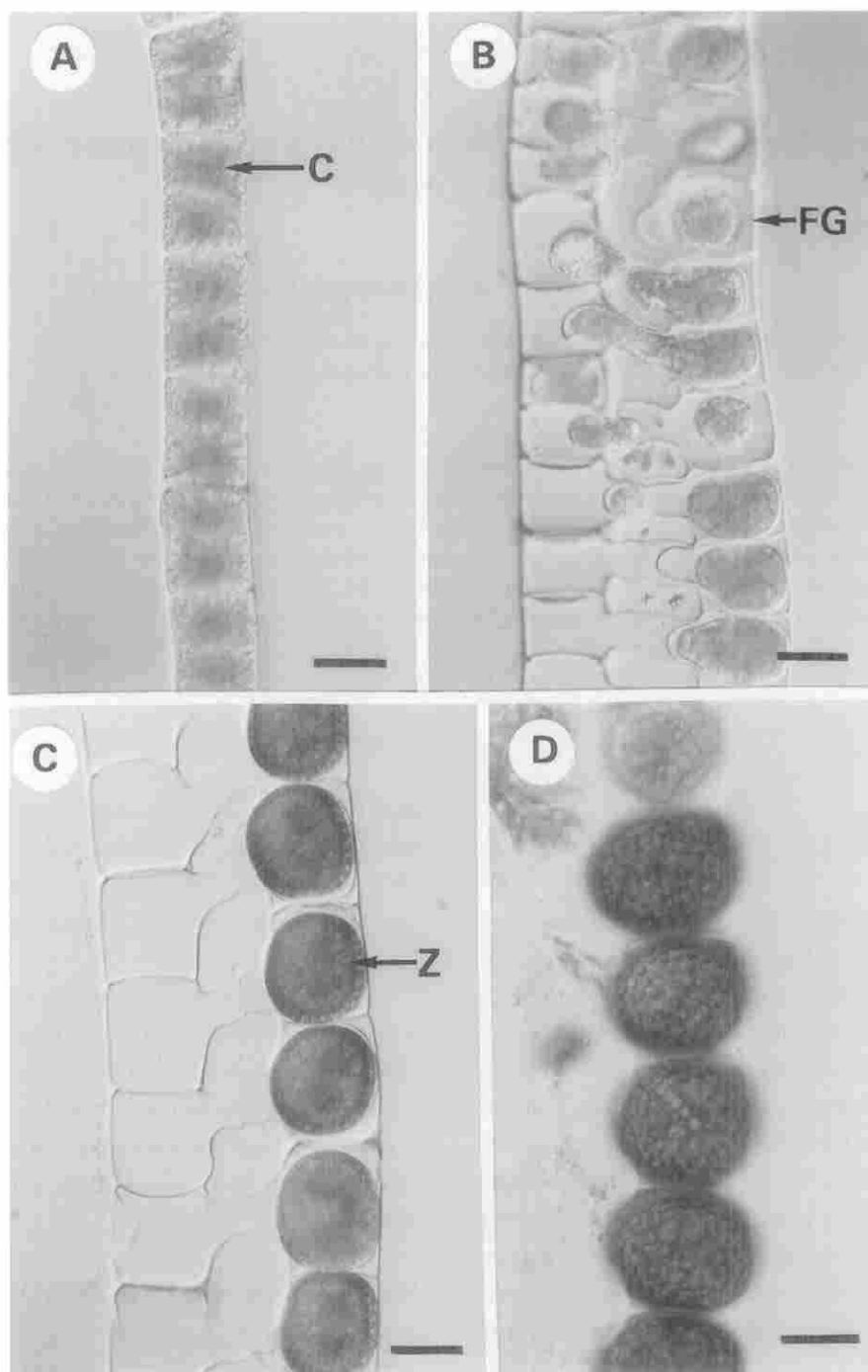
and reproductions are most abundant and more frequently found in temporary ponds and ditches (Transeau 1951). Therefore, it is difficult to identify the species due to the rarity of finding fertile materials in nature.

To date, ten species of *Zygnema* have been recorded in Korea by floristic studies (Yi 1980; Chung 1993). However, morphology and taxonomy of the genus from Korea are barely known. We described a freshwater green alga, *Zygnema cruciatum* in Korea. Specimens were examined by light microscopy and scanning electron microscopy.

### MATERIALS AND METHODS

Specimens of *Zygnema cruciatum* were collected from the Miho stream, Cheongju, Korea, during June 2007. Some specimens were preserved in 5% formaldehyde-water and deposited at the herbarium of the Chungbuk National University (CBNU). Live cells were isolated by micro forceps and the isolates were grown in Woods Hole liquid medium (Nichols 1973; Simons *et al.* 1984). The unialgal cultures were maintained at  $20 \pm 1^\circ\text{C}$  on a 16:8h LD photoperiod under  $10\text{-}50 \mu\text{mol m}^{-2} \text{sec}^{-1}$  from cool-white fluorescence lamps (Pringsheim 1967). The morphology was examined with a light microscope (Nikon Optiphot, Nikon) and scanning electron microscope (JSM-7000F, Jeol). For SEM, zygospores were fixed

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**Fig. 1.** *Zygnema cruciatum* (Vaucher) Agardh. (A) Vegetative filament with two stellate chloroplasts in each cell. (B) Ladder-like conjugation showing the fusion of amoeboid gametes and the formation of zygospores. (C) Mature fruiting filament with yellow-brown and spherical zygospores in cylindrical female gametangia. (D) Zygospore wall showing pitted type of ornamentation. Abbreviations: C, chloroplast; FG, female gametangium; Z, zygospore (Scale bars: A, B and C = 30  $\mu$ m; D = 20  $\mu$ m).

in 2% glutaraldehyde in culture medium for 1 h, collected on 2.5  $\mu$ m pore size filters, and rinsed in distilled water. Then, the zygospores were post fixed in 1% osmium tetroxide for 1 h, dehydrated through an alcohol series (50%, 60%, 70%, 80%, 90%, and 100% ethanol), and critical point dried. Dried zygospores were mounted on stubs, coated with gold and observed under a SEM

(Marchant 1973; Hull *et al.* 1985).

## RESULTS AND DISCUSSION

### *Zygnema cruciatum* (Vaucher) Agardh 1824

Cleve 1868. p. 29, pl. IX, figs 1-3; Transeau 1915. p. 21; Transeau 1951. p. 36, pl. I, fig. 9; Randhawa 1959. p. 243,

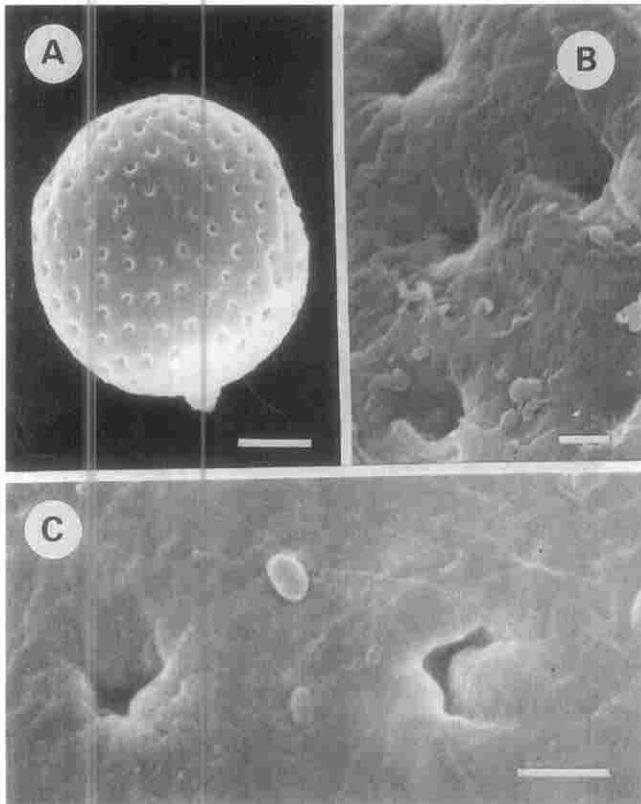


Fig. 2. Zygospore of *Zygnema cruciatum*, photographed with the scanning electron microscope. (A) Pitted type of the median spore wall. (B) Detail of mesospore pits. (C) Enlarged view of two pits (Scale bars: A = 10  $\mu\text{m}$ ; B and C = 1  $\mu\text{m}$ ).

fig. 197; Kadlubowska 1984. p. 180, fig. 254; Rudina 1998. p. 75, pl. 27, figs 5-6; Johnson 2002. p. 506, pl. 127, fig. G.

**Basionym:** *Conjugata cruciata* Vaucher 1803

**Synonym:** *Tyndaridea cruciata* (Vaucher) Hassall (1842)

**World distribution:** British Isles, India, Japan, New Zealand, Russia, Sweden, United States of America

**Specimens examined:** Miho stream, Cheongju, Korea (Jun. 09, 2007).

**Description:** Plants are unbranched filaments of short cylindrical cells with plane end wall (Fig. 1A). The cells have two stellate chloroplasts and each chloroplast with a conspicuous central pyrenoid. Vegetative cells are 32-39  $\mu\text{m}$  in width and 35-50  $\mu\text{m}$  in length. Sexual reproduction is scalariform conjugation (Fig. 1B). Zygospores are formed in only female gametangia that remain cylindrical or slightly enlarged on the conjugating side (Fig. 1C). Zygospores are yellow-brown, mostly spherical or broadly ovoid, 35-44  $\mu\text{m}$  wide, and 40-47  $\mu\text{m}$  long. Mature spore wall has pitted type of ornamentation (Fig. 1D). Under SEM, wall of zygospore has pitted mesospore (Fig. 2A) and pits are 1.4-1.8  $\mu\text{m}$  in diameter and 3-4  $\mu\text{m}$  apart from each other (Figs 2B and 2C).

*Conjugata cruciata* Vaucher (1803) was transferred to the genus *Zygnema cruciatum* by Agardh in 1824 based on the vegetative cell shape and spore shape. Cleve (1868) described additional characteristics such as vegetative cell width, shape and size of spore, brown and ornamented median spore wall. Transeau (1915, 1951) also observed the occurrence of both zygospores and aplanospores. Our specimens correspond well with the previous descriptions in those morphological characteristics, except for the fact that the aplanospores described previously were not observed in this study. Distinct features of this species are the more than 35  $\mu\text{m}$  wide vegetative cell width, cylindrical female gametangia, spherical zygospores, pitted median spore wall, and 1.4-1.8  $\mu\text{m}$  in pits diameter. This species is not common in the freshwater habitats in Korea.

The species can be compared with *Z. cylindrosporum* Czurda and *Z. stellinum* (Vaucher) Agardh (cf. Transeau 1951; Randhawa 1959). It resembles *Z. cylindrosporum* and *Z. stellinum* in width of the filament, in scalariform conjugation and zygospores in only one of the gametangia, in the color and ornamentation of the mesopore. *Z. cylindrosporum* has cylindrical female gametangia and cylindric-ovoid zygospores, and *Z. stellinum* contains enlarged female gametangia, ovoid zygospores and bigger diameter of pits.

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