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Report

Zygospore formation of a *Spirogyra variformis* TRANSEAU (Zygnemataceae) collected from an irrigation canal of rice fields at Mikkabi, Hamamatsu, Japan

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Abstract

Zygospore formation of a *Spirogyra variformis* TRANSEAU collected from an irrigation canal of rice fields on 26 March 2014 was described using microscopic photographs of the vegetative and reproductive cells, and physical and chemical characteristics of their sampling site were shown in order to understand the habitable conditions of the *S. variformis*. Vegetative cell of the *S. variformis* was 40-50μm wide and 60-100 μm long with plane and end cell walls. One chloroplast was making 2-5 turns in each cell. Conjugation was scalariform-type, and tubes elongated both sides of the *S. variformis* filaments. Reproductive cells were mostly cylindrical, sometimes enlarged or inflated. Characteristics of fully-ripened zygospores were ellipsoidal, 40-50 μm in wide, 50-60 μm in long, smooth medium spore wall and brownish color. Water temperature at noon, electric conductivity and pH were 11.8°C, 26.2 mS m^{-1} and 6.5, respectively. Dissolved inorganic nitrogen (DIN) was 1.14 mg L^{-1} (NO_3^--N accounted for 97 %), and reactive phosphorous (PO_4^{3-}-P) was 0.004 mg L^{-1}.

Key words: conjugation, *Spirogyra variformis* TRANSEAU, springtime, zygospore

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Introduction

Filamentous green alga genus *Spirogyra* (Zygnemataceae) which often constructs a mat forming community in lake littoral, pond and slow-flowing streams, is a common genera found in freshwaters. Nearly 400 species of *Spirogyra* were described in monographs (Hoshaw and McCourt, 1988), and 90 species were found in Japan (Yamagishi, 1966, 1977). The formation process of conjugation and zygospores of *Spirogyra* is a suitable teaching material introducing sexual reproduction in biology learning (Nozaki, 2014, 2015a). *Spirogyra* species have been classified by the shape and color of conjugation and fully-ripened zygospores formed in sexual reproduction (Transeau, 1938; Yamagishi, 1966, 1977). Although conjugation and zygospore development of *Spirogyra* are a well-known phenomenon, little is understood about their detailed processes and mechanism, because of the difficulty in artificial reproducible induction of conjugation in laboratory experiments (Yamashita and Sasaki, 1979; Simons *et al*., 1984; Ikegaya *et al*., 2012; Zwirn *et al*., 2013; Nozaki, 2015b). Previous studies of *Spirogyra* classification have been almost entirely carried out using samples collected from natural environment, making the identification of the exact species very difficult. Consequently, ecological studies of *Spirogyra* were almost unidentified as a species (Graham *et al*., 1995; Berry and Lembi, 2000; Nozaki, 2001; Nozaki and Mitamura, 2002). In addition, only a few studies about taxonomic description have showed the physical and chemical parameters of the sampling site (*e.g*. Simons and van Beem, 1990; Nozaki, 2013). Thus, little is known about the abiotic habitable conditions of each species of *Spirogyra* from previous descriptions.

In the present report, zygospore formation of a *Spirogyra* collected from an irrigation canal of rice fields in springtime was described using microscopic photographs of the

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vegetative and reproductive cells, and physical and chemical characteristics of their sampling site were shown in order to understand the habitable conditions of the *Spirogyra*. This research was supported by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (No. 24501114) to Kentaro NOZAKI.

Methods

Spirogyra samples were collected from an irrigation canal of rice fields on 26 March 2014, located at latitude 34°46'48"N and longitude 137°32'23"E near the Ona Station of Tenryu-Hamanako Railway, Mikkabi, Hamamatsu City in the Tokai Region of Japan (Fig.1). Water temperature, pH (WAK-pH, Kyoritsurika Co.) and electric conductivity (CM21P, TOA-DDK Co.) were measured at 11:00 AM in the sampling site. *Spirogyra* and water samples were stored in a box with ice and were returned to the laboratory within 3 hours after sampling.

Fig. 1. Sampling site (A) and *Spirogyra* community (B) on 26 March 2014 (Ona, Mikkabi, Hamamatsu City).

Turbidity of water samples was measured with the water analyzer (WA1, Nippon Denshoku Co.) using pre-filtered water. Water sample was transferred to a glass fiber filter (GF-75, Advantec Co.) in preparation for the analysis of water color and nutrient concentrations. Water color was also measured with a water analyzer (WA1, Nippon Denshoku Co.). Nutrient analyses were carried out on NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P concentrations, respectively. Nutrient analysis procedures followed the textbook of the Tokai Branch of the Japanese Society of Limnology (2014).

Spirogyra sample in this experiment were using dominant type (>90 % in cell numbers, unpublished data) in the sampling site (Fig. 2A). *Spirogyra* filaments were placed in a 10 cm glass Petri dish filled with filtered water in sampling site and kept in a growth chamber (MLR-351H, Sanyo Co.) at 15°C under 170 μmol m^{-2} s^{-1} (approximately 20000 lux) on a 12:12 hours of light and dark cycle. *Spirogyra* filaments were observed under an optical microscope (BX 51, Olympus Co.), and microscopic photographs of conjugation and zygospores were taken by digital camera (Camedia C-5060, Olympus Co.). Temperature of the growth chamber was set somewhat higher than that in the sampling site during the incubation period, because the increase of water temperature seemed to be a trigger inducing the conjugation of *Spirogyra* (Simons *et al*., 1984; Berry and Lembi, 2000; Nozaki, 2013; Nozaki, 2015b).

Results and Discussion

Zygospores formation of the *Spirogyra* filaments is illustrated in Figure 2A-E. Vegetative cells of the *Spirogyra* measured 40-50 μm wide and 60-100 μm long with plane end cell walls. One chloroplast was making 2-5 turns in each cell (Fig. 2A). Sexual reproduction of the *Spirogyra* was started by formation of papilla on cells opposite each other. After elongation of the papilla, 2 filaments aligned and conjugation tubes were formed between cells. Conjugation of the *Spirogyra* was scalariform-type (Yamagishi, 1999) and tubes elongated both sides of the filaments (Fig. 2B). Reproductive cells were mostly cylindrical, sometimes enlarged or inflated. Zygospores matured in approximately 20-30 days after sampling. Characteristics of fully-ripened zygospores were ellipsoidal, 40-50 μm in wide, 50-60 μm in long, smooth medium spore wall and brownish color (Fig. 2C-E). The *Spirogyra* in this report is identified to be *S. variformis* TRANSEAU according to previous studies

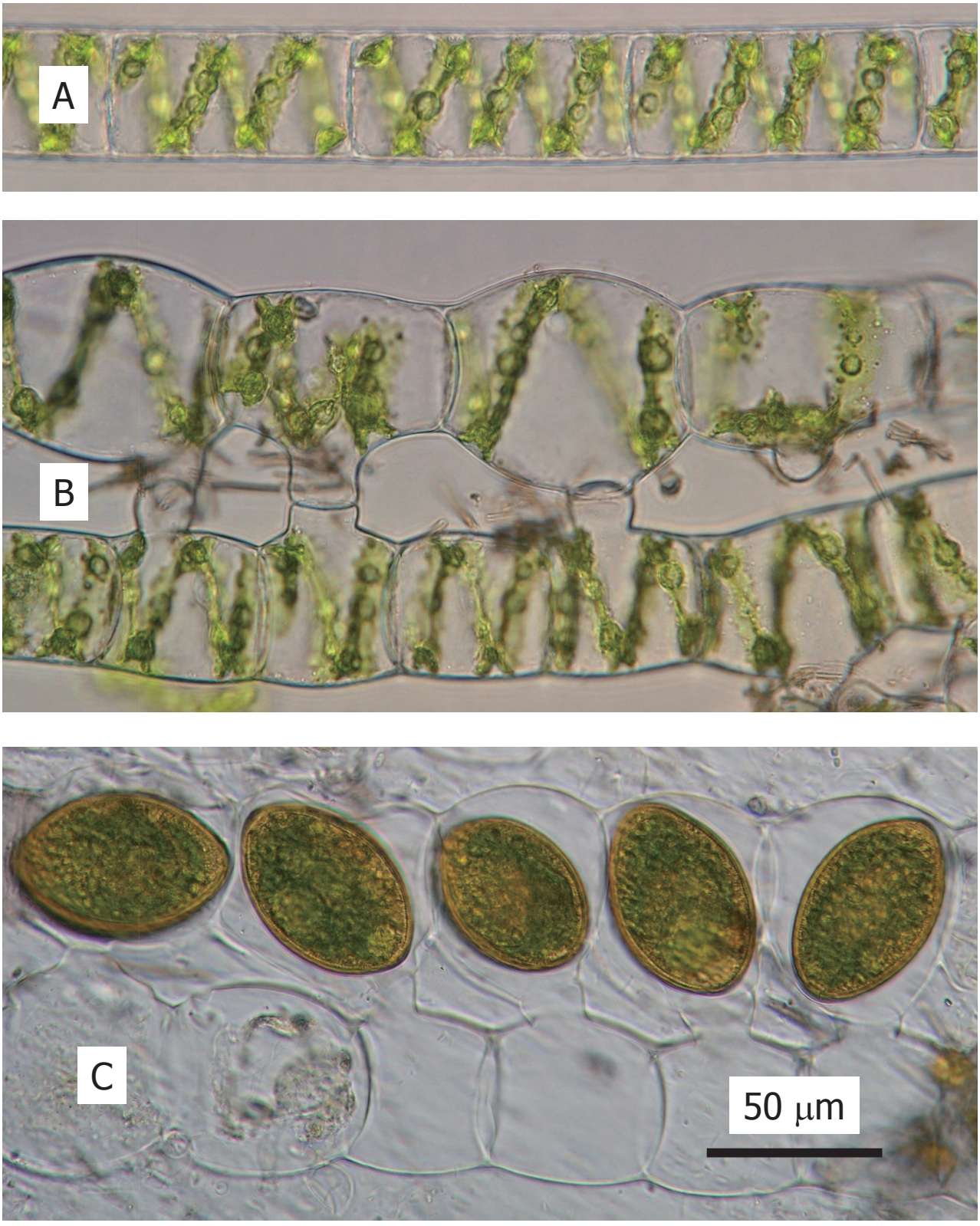
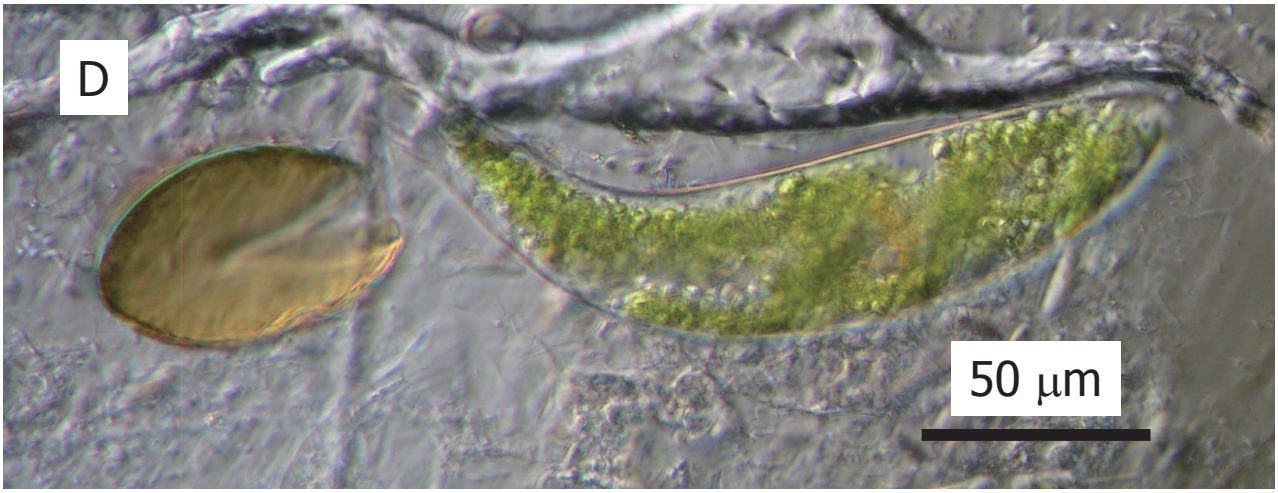


Fig. 2. Microscopic photographs of zygospore formation of *Spirogyra variformis* TRANSEAU. A. Vegetative cells on 26 March,B. Reproductive cells during conjugation on 27 March, C. Zygospores on 11 April, D. Crushed zygospore on 19 March, E. Well-ripened zygospores on 26 April.



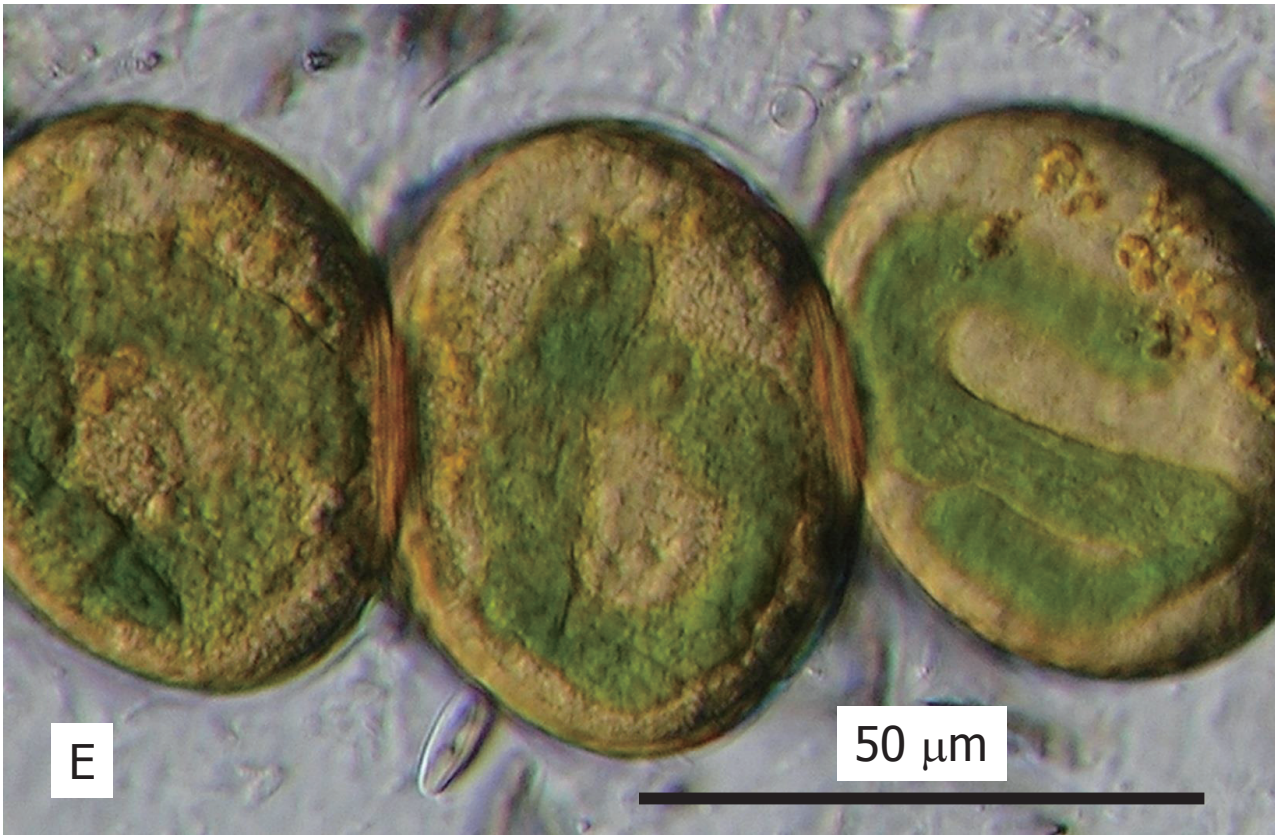


Fig. 2. Continued.

(Transeau, 1938; Yamagishi, 1966; Yamagishi, 1977, Kim *et al*., 2004; Nozaki, 2013). Morphological characteristics of *S. variformis* are shown in Table 1.

Physical and chemical parameters of the water sample collected from sampling site are shown in Table 2. Nozaki (2013) described formation processes of conjugation and zygospores of *S. variformis* collected from a Japanese lowland marsh in early spring. At that time, water temperature, electric conductivity and pH at 13:17 PM were 9.3°C, 25.9 mS m^{-1} and 6.5. NO_3^--N and PO_4^{3-}-P concentrations were 1383 and 4.5 μg L^{-1}. These environmental parameters were very similar to those of this study site. Thus, the possible abiotic habitable conditions of *S. variformis* are about 10°C in water temperature, 25 mS m^{-1} in electric conductivity and 1 mg L^{-1} in NO_3^--N concentration.

Results of this study suggest that *S. variformis* might be

Table 1. Morphological characteristics of *Spirogyra variformis* Transeau according to previous studies.

Refernce	Collecting site	Vegetative cells		Chloroplast	Zygospores	
		width (μm)	length (μm)		wide (μm)	long (μm)
Transeau (1938)	Cape Town, Africa	43-53	(70-)108-140(-200)	1	45-54	58-90
Yamagishi (1966)	Kanagawa, Japan	43-50	60-140	1	45-54	58-90
Yamagishi (1977)	Kanagawa, Japan	43-50	60-140	1	45-54	58-90
Kim *et al*. (2004)	Deoksan,Yesan-gun, Chungcheongnam-do, Korea	46-54	197-695	1-3	42-49	62-74
Nozaki (2013)	Fukui, Tsuruga, Japan	40-50	60-100	1	40-50	50-60

propagating in spring and forming zygospores in late spring to early summer. Seasonal periodicity of the reproductive stage of the *Spirogyra* species varied with the filament width shown in previous studies. Berry and Lembi (2000) found that unidentified *Spirogyra* species with 45 μm width and one or two chloroplasts such as *S. variformis* dominated from March to April in a shallow artificial lake, in Columbus, Indiana of the United States. Large numbers of zygospores were observed to be produced during crash of the *Spirogyra* community. On the other hand, Simmons and van Beem (1990) reported that *Spirogyra* species having filaments wider than 50 μm produced zygospores in summer in The Netherlands. However, the mechanism of filament width affecting seasonal varieties in the reproductive stage is still not clear and further research is required.

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Table 2. Physical and chemical parameters at sampling site on 26 March 2014.

Sampling time	11:00
Water temperature (℃)	11.8
Electric conductivity (mS m^{-1})	26.2
pH	6.5
Turbidity (degree)	0.3
Color (degree)	4.3
NH_4^+-N (μgN L^{-1})	30.1
NO_2^--N (μgN L^{-1})	4.9
NO_3^--N (μgN L^{-1})	1101
Dissolved inorganic nitrogen (mgN L^{-1})	1.1
PO_4^{3-}-P (μgP L^{-1})	3.7

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摘　要

静岡県浜松市三ヶ日町の水田用水路から採集された アオミドロ属 *Spirogyra variformis* TRANSEAU の接合胞子形成

野崎健太郎

2014年3月26日に静岡県浜松市三ヶ日町の水田用水路から採集されたアオミドロ属 *Spirogyra variformis* TRANSEAU の接合胞子形成を顕微鏡写真と採集地点の水環境とともに記述した。細胞は，幅40～50 μm，長さ60～100 μm で，細胞間の隔膜は平板状であった。葉緑体は1本で，2～5回転していた。接合体は，並び合った2本の糸状体の両方から接合管が伸び，梯子状に形成された。接合体は大部分が円筒状であったが，時には拡張していた。熟した接合胞子は，楕円形で幅40～50 μm，長さ50～60 μm，胞子中層膜は黄褐色で平滑であった。南中時の水温は11.8℃，電気伝導度は26.2 mS m^{-1}，pH は6.5 であった。溶存無機態窒素濃度は1.14 mg L^{-1}で，およそ97% は硝酸態窒素であった。リン酸態リン濃度は，0.004 mg L^{-1} であった。

キーワード：接合体，*Spirogyra variformis* TRANSEAU，春季，接合胞子

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