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Report

Morphological characteristics *of Spirogyra neglecta* (Zygnemataceae) collected from spring water in the urban area of Nagoya City, Aichi, Japan

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Abstract

Morphological characteristics of *Spirogyra neglecta* (Hassall) Kützing collected from spring water in the urban area of Nagoya City on 8 August 2016 were described using microscopic photographs of the vegetative cells, conjugation processes, and zygospores. The vegetative cells with plane end walls were 54–74 μm wide, 79–377 μm long, as well as three chloroplasts making 1–2 turns (n = 171). The fertile cells had cylindrical shape. Both gametangia formed tubes, which were ladder-like during conjugation. The zygospores were ellipsoidal or ovoid, which were 49–69 μm wide and 67–127 μm long, with a smooth, thick yellow-brown median spore wall. (n = 156). Considering that *S. neglecta* closely resembles *S. ternata* Ripart in morphology, the description of these two species was compared using some monographs. The water temperature, pH, and electrical conductivity in the sampling site were 26.6°C, 7.0, and 19.3 mS m^{-1}, respectively. The concentration of the dissolved inorganic nitrogen was 4500 μgN L^{-1} (NO_3^--N contributed approximately 99 %) and PO_4^{3-}-P was only 1 μgP L^{-1}.

Key words: conjugation, *Spirogyra neglecta* (Hassall) Kützing, *Spirogyra ternata* Ripart, vegetative cell, zygospore

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Introduction

Spirogyra neglecta (Hassall) Kützing was described in Hassall (1842) as *Zygnema neglectum* and renamed in Kützing (1849). *S. neglecta* is commonly found in freshwater habitats worldwide, including Africa, Asia, Europe, and North America (Transeau, 1951; Kadlubowska, 1984; Devi and Panikkar, 1994; Johnson, 2011). However, this species is not listed in any representative monograph of the Japanese freshwater algal flora (Yamagishi, 1966; Yamagishi, 1977). In Japan, *S. neglecta* has only been reported in Hyogo Prefecture in the Kinki region (Sato, 2013), with no records in other regions. In this study, the morphology of the vegetative cells, conjugation, and zygospores of *S. neglecta* are reported using fresh samples collected from spring water in the urban area of Nagoya City in the Tokai region of Japan.

Ripart (1876) reported that *S. neglecta* morphologically resembles *S. ternata* and is difficult to classify. Transeau (1951), Devi and Panikkar (1994), and Johnson (2011) all have similar viewpoints. Only *S. ternata* is listed in the two above-mentioned Japanese representative monographs. Therefore, this study was conducted considering the differences in the morphological features of cells and zygospores of *S. neglecta* and *S. ternata*. The Japan Society for the Promotion of Science provided NOZAKI Kentaro with a grant-in-Aid for Scientific Research (C) to support this research (21K02911).

Methods

Samples of *S. neglecta* were collected from spring water in the schoolyard of Sugiyama Jogakuen Elementary School in the urban area of Nagoya City on 8 August 2016, located at

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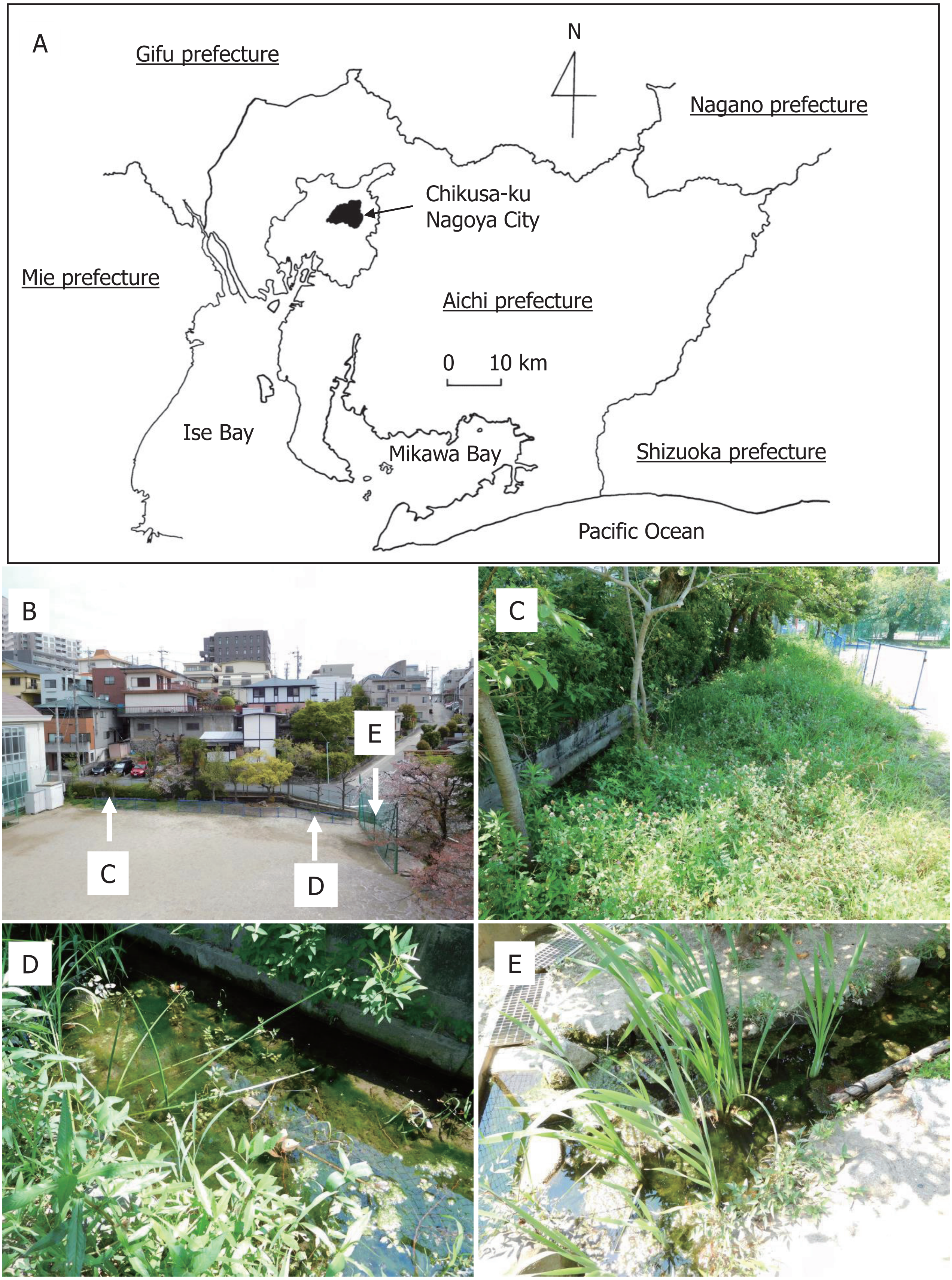

Figure 1. Map and photos of the sampling site. A: Location of Chikusa-ku, Nagoya City, Aichi Prefecture in Tokai region of Japan. B-E: Spring water located at the schoolyard of Sugiyama Jogakuen Elementary School in the urban area of Nagoya City.

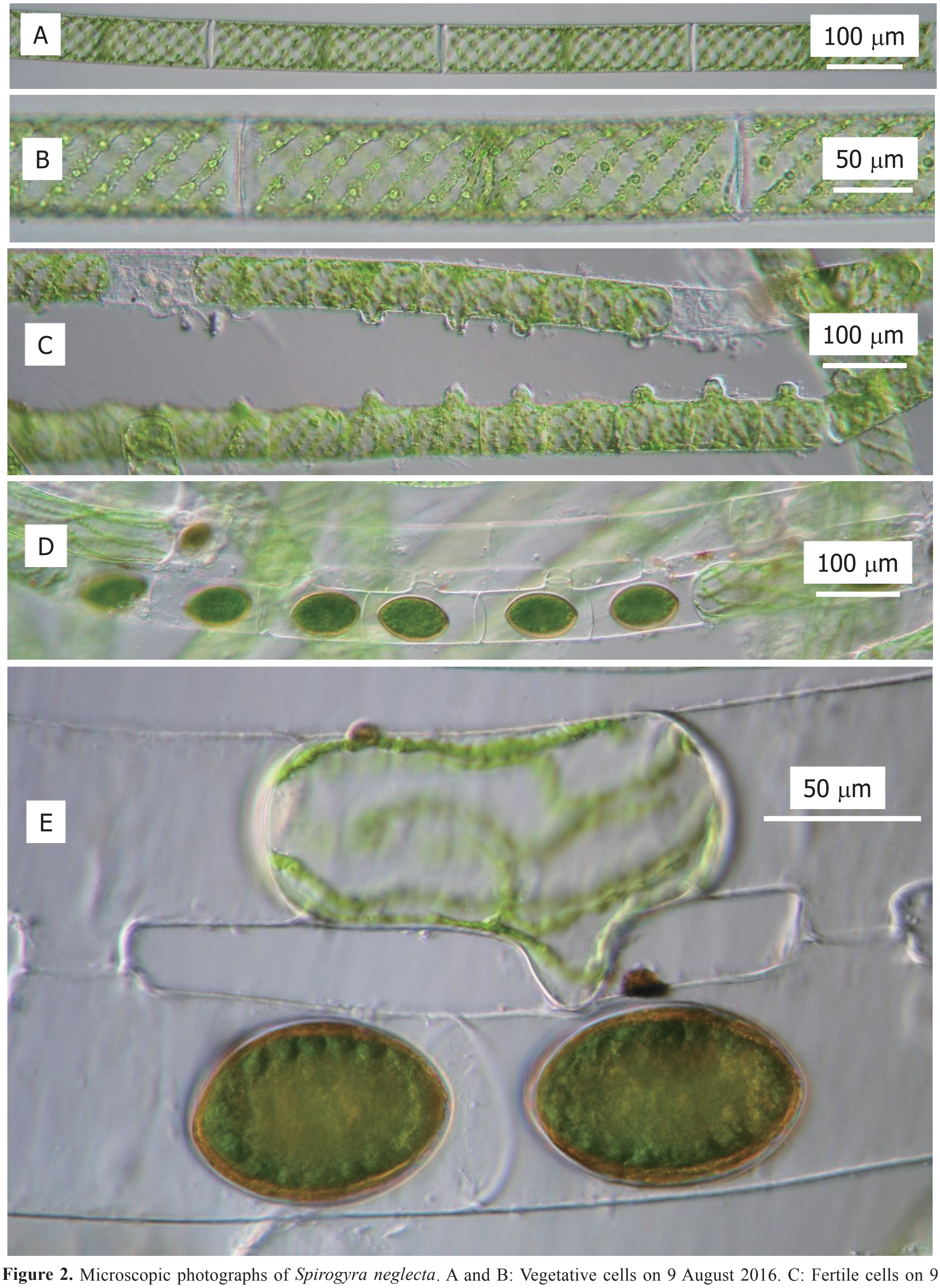

Figure 2. Microscopic photographs of *Spirogyra neglecta*. A and B: Vegetative cells on 9 August 2016. C: Fertile cells on 9 August 2016. D: Conjugation on 13 August 2016. E: Conjugation and zygospores on 20 August 2016.

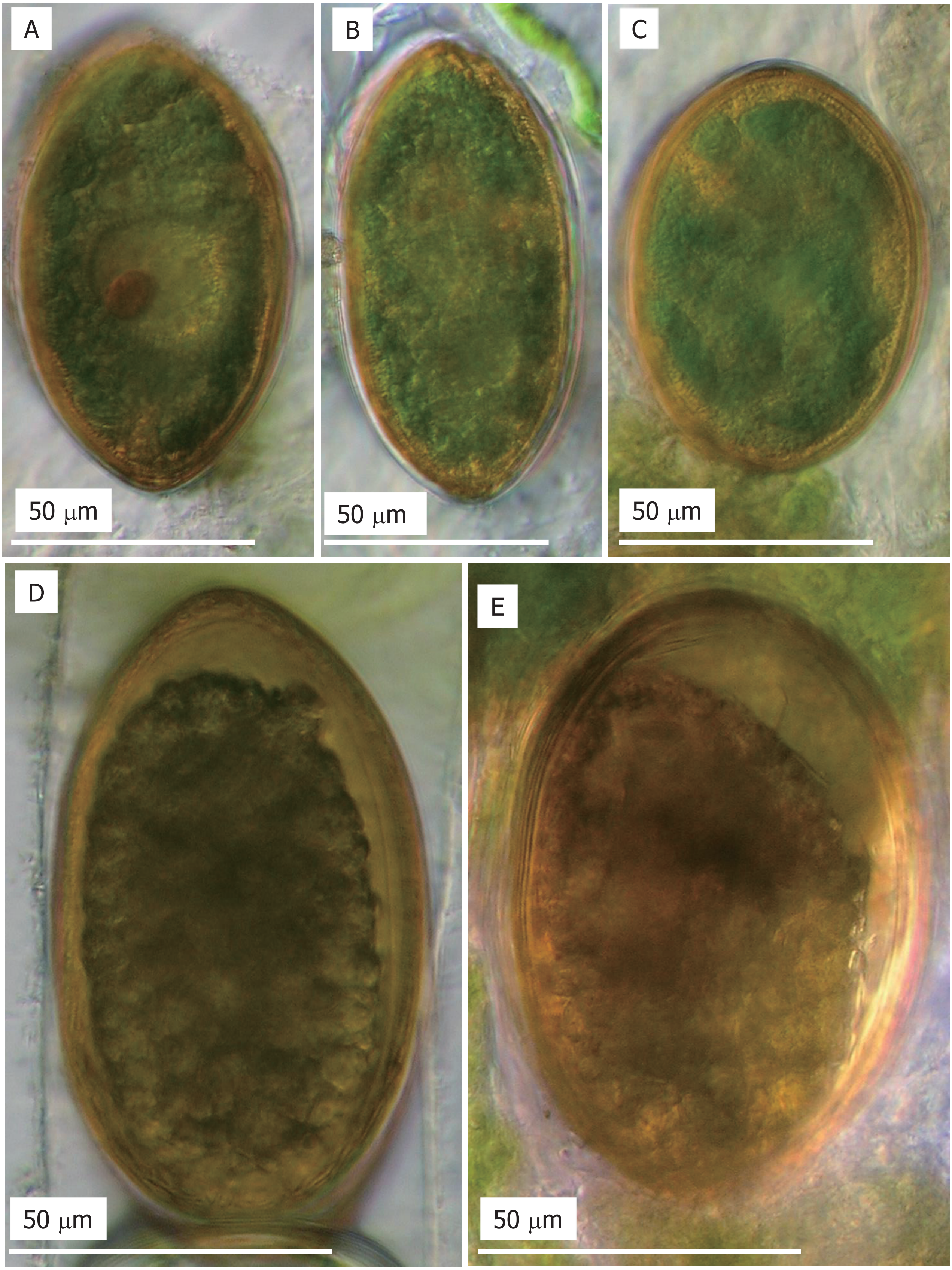

Figure 3. Microscopic photographs of zygospores of *Spirogyra neglecta.* A and B on 14 September 2016. C on 22 September 2016. D on 16 October 2016. E on 31 August 2016.

latitude 35°03'53"N and longitude 136°57'04"E (Figures 1A–1C). The *S. neglecta* community resembled a mat, and the fragments of the community were floating (Figures 1D and 1E). The water temperature, pH (WAK-pH, KYORITASU-RIKA Co.), and electrical conductivity (CM21P, TOA-DKK) were measured at 10:40 AM at the sampling site. The *S. neglecta* and water samples were stored in a box with ice and were returned to the laboratory within 1 h after sampling.

Filaments of *S. neglecta* were gently washed in distilled water and placed in a 10-cm glass Petri dish filled with filtered water in the sampling site and kept in a growth chamber (MLR-351H, SANYO Co.) at 25 ℃ under 170 μmol photon m^{-2} s^{-1} (approximately 20000 lux) on a 13:11 h light and dark cycle. The filaments of *S. neglecta* were observed under an optical microscope (BX51, OLYMPUS Co.) and microscopic photographs were taken using a digital camera (DP27, OLYMPUS Co.). The width and length of cells and zygospores in fresh samples were measured using software (cellSens Standard, OLYMPUS Co.). Specimens were donated to the Department of Botany, National Museum of Nature and Science (TNS-AL61281ma-md in TNS).

The turbidity of the water sample was measured using a water analyzer (WA1, NIPPON DENSHOKU Co.) and raw water. The water sample was filtered to a glass fibre filter (GF-75, ADVANTEC Co.) to analyze water colour and nutrient concentrations. The water colour was also measured using a water analyzer (WA1, NIPPON DENSHOKU Co.). A spectrophotometer (U-100, HITACHI Co.) was used to perform nutrient analyses on NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P concentrations using. The nutrient analysis procedures followed those of Matsumoto and Nozaki (2014).

Results and Discussion

Figures 2A–2E show microscopic photographs of vegetative cells and conjugations. The vegetative cells were 54–74 μm wide and 79–377 μm long with plane end cell walls. In each cell, three chloroplasts made 1–2 turns (Figure 2A and 2B). The conjugation was ladder-like with tubes formed by both gametangia (Figures 2C–2E). The fertile cells had a cylindrical shape, which did not inflate. Chloroplast numbers and gametangia morphology are same as in original description of *S. neglecta* (Hassall, 1842). Microscopic photographs of various shapes of zygospores are shown in Figure 3A–3E. Well-ripened zygospores were ellipsoidal or ovoid, 49–69 μm wide and 67–127 μm long, with a smooth and thick yellow-brown median spore wall. The suture line could not be observed. Table 1 shows the morphological characteristics of *S. neglecta* and *S. ternata* based on previous studies. However, the original description studies of both species provide insufficient information for classification and lack an illustration plate. Thus, *S. neglecta* refers to morphological information from Transeau (1951) to Schagerl and Zwirn (2015), whereas *S. ternata* refers from Yamagishi (1966) to Johnson (2011).

The morphology of the gametangia and the orientation of zygospores in the filament show differences between *S. neglecta* and *S. ternata* in some monographs. Although Transeau (1951) and Johnson (2011) reported that fertilized gametangia of *S. neglecta* is usually inflated, with zygospores developing at right angles to the long axis of the filament, the zygospores were not so orientated in their illustration plates. On the microscopic photograph of the conjugation of *S. neglecta* in Schagerl and Zwirn (2015), zygospores were orientated in right angles to the filament. On the other hand, Ripart (1876), Kadlubowska (1984), Yamagishi (1966), and Yamagishi (1977) reported the same morphological characteristics in *S. ternata*. On the microscopic photograph of the conjugation of *S. ternata* in Yamagishi (1987), zygospores in shorter cells were orientated in right angles to the filament. Moreover, the illustration of *S. ternata* in Johnson (2011) showed zygospores orientated in right angles to the filament, which is contrary to the description. This result indicates possible mistakes in illustrations. Devi and Panikkar (1994) reported that the sterile cells of *S. neglecta* inflated, which is described as the only difference in *S. ternata*. However, the sterile cells in this study did not inflate (Figures 2D and 2E). Therefore, the morphology of the gametangia and the orientation of the zygospores are insufficient for distinguishing between *S. neglecta* and *S. ternata*.

Because the form of conjugation and the shape of the zygospores of *S. neglecta* are very similar to those of *S. ternata* (Table 1), the width and length of the vegetative cells and zygospores are examined as classification characteristics in this study. The relationship between the width and length of the vegetative cells is shown in Figure 4. The solid line quadrate represents the minimum and maximum values of *S. neglecta*, and the dotted line quadrate represents the *S. ternata* (Table 1). Approximately 77% of all measured values (n = 171) were within the range of *S. neglecta*. The relationship between width and length of the zygospores

Table 1. Morphological characteristics of *Spirogyra neglecta* and *S. ternata* based on previous studies. *n.d.* indicates no description.

Vegetative cell Width µm	Vegetative cell Length µm	Vegetative cell Chloroplast Number, turn	Fertile cell Conjugation	Fertile cell Gametangia	Zygospore Width µm	Zygospore Length µm	Zygospore Shape	Zygospore Median wall, Colour	Zygospore Orientation	Reference
Spirogyra neglecta (Hassall) Kützing 1849										
n.d.	*n.d.*	3, <6 turns	*n.d.*	no inflation	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	Hassall (1842) p.37
47	*n.d.*	3, *n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	globular	*n.d.*	*n.d.*	Kützing (1849) p.441
55-67	100-300	3, 1-2.5 turns	scalariform and lateral	enlarged, inflated	54-64	75-100	ovoid	thick and smooth, yellow	right angles to the filament	Transeau (1951) p.175, p.298-299 Plate27 Figures 14-15
57-67	*n.d.*	2-4, *n.d.*	ladder-like and lateral	cylindric	54-68	70-110	ovoid	thick, smooth with a suture line, yellow-brown	not so orientated	Kadlubowska (1984) p.304, p.303 Figure 464
49-60	115-215	3, 2-4 turns	scalariform	cylindric, sterile cells inflated	49-57	69-83	ellipsoid	smooth, yellow	not so orientated	Devi and Panikkar (1994) p.50-51, p.108 Figures 171-172
55-67	100-300	3, 1-2.5 turns	ladder-like and lateral	inflated	54-64	75-100	ovoid	thick, smooth with a suture line, yellow-brown	right angles to long axis of filament	Johnson (2011) p.157, p.594 Plate 148F
58-68	*n.d.*	2-3, *n.d.*	scalariform	enlarged	56-68	68-80	ellipsoid	smooth	right angles to the filament	Schagerl and Zwirn (2015) p.78, p.75 Figure 3f
54-74	79-377	3, 1-2 turns	ladder-like	cylindric	49-69	67-127	ellipsoid, ovoid	thick and smooth, yellow-brown	not so orientated	This study
Spirogyra ternata Ripart 1876										
70	*n.d.*	3, 0.5 turn	*n.d.*	inflated	77	n.d	ellipsoid	*n.d.*, brown	oblique to the filament	Ripart (1876) p.162-163
(55-) 60-85	(48-) 85-330	3-4, 1-2 turns	scalariform	cylindric, inflated	50-72	94-104	ellipsoid, ovoid	smooth, yellow	oblique and right angles to the filament	Yamagishi (1966) p.97, Plate 6 Figures 4-6; Yamagishi (1977) p.451, p.450 Plate 156 Figure 3
54-70	*n.d.*	3-4, *n.d.*	ladder-like	inflated	55-70	66-125	ellipsoid	thick and smooth, yellow brown	oblique and right angles to the filament	Kadlubowska (1984) p.315, p.316 Figure 490
49-66	66-149	3, 1-2 turns	scalariform	cylindric	42-53	59-73	ellipsoid	smooth, yellow	not so orientated	Devi and Panikkar (1994) p.71, p.122 Figures 284-285
54-70	*n.d.*	3-4, *n.d.*	ladder-like	inflated	55-70	66-125	ellipsoid	thick and smooth, yellow brown	not so orientated	Johnson (2011) p.601, p.594 Plate 148G

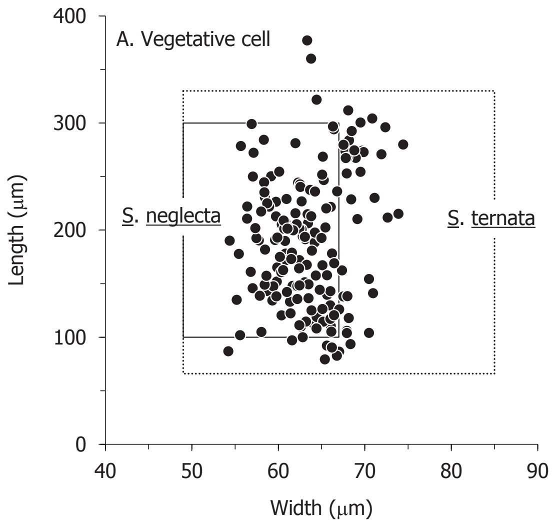

Figure 4. Relationship between the width and length of the vegetative cells of *Spirogyra neglecta* (n=171). The solid line quadrate represents the ranges of minimum and maximum values of *S. neglecta* based on previous studies and the dotted line quadrate represents the *S. ternata.*

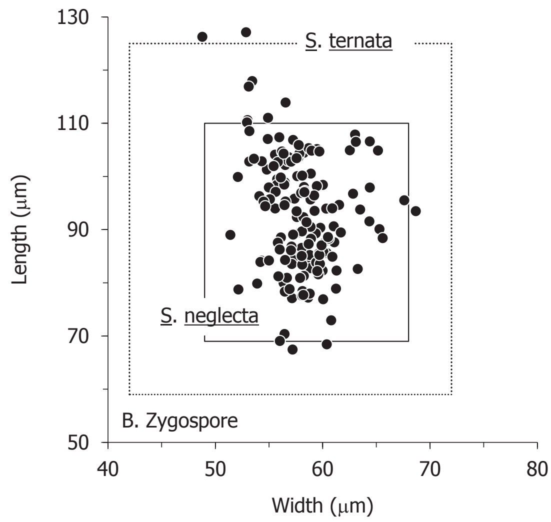

Figure 5. Relationship between the width and length of the zygospores of *Spirogyra neglecta* (n=156). The solid line quadrate represents the ranges of minimum and maximum values of *S. neglecta* based on previous studies and the dotted line quadrate represents the *S. ternata*.

is shown in Figure 5. Approximately 94% of all measured values (n = 156) were within the range of *S. neglecta*. Although the range of width and length of the vegetative cells and zygospores of *S. neglecta* reported in previous studies are smaller than those of *S. ternata*, 80%–90% of the values obtained in this study site are within the range of *S. neglecta*. Therefore, the *Spirogyra* collected in this study was appropriately identified as *S. neglecta*. Considering that Transeau (1951) did not describe *S. ternata* and Yamagishi (1966) did not describe *S. neglecta*, the results of this study also suggest that these two species are synonymous.

Schagerl and Zwirn (2015) reported that many *Spirogyra* species morphologically identified could have been described too narrowly, based on 16 *Spirogyra* strains collected from Central Europa that were compared with species descriptions provided in previous monographs. Therefore, *S. neglecta* and *S. ternata* seem to be also difficult to distinguish morphologically. In recent years, studies that collate *Spirogyra* morphology and its genetic information have been published in the United States (Stancheva *et al*., 2013) and Japan (Takano *et al*., 2019). To advance the taxonomic study of *Spirogyra*, the re-examination of the descriptions of previous monographs using clear microscopic photographs of cells, conjugations and zygospores will be required as in Ferrer and Caceres (2017).

The abiotic habitable conditions of the study site are described in the following section. The physical and

Table 2. Physical and chemical parameters at the sampling site on 8 August 2016.

Sampling time	10:40
Water temperature	26.6℃
pH	7.0
Electrical conductivity	19.3 mS m^{-1}
Turbidity	1.0 mg L^{-1}
Water Colour	4.7 mg L^{-1}
Dissolved oxygen	12.1 mgO_2 L^{-1}
NH_4^+-N	29 μgN L^{-1}
NO_2^--N	30 μgN L^{-1}
NO_3^--N	4450 μgN L^{-1}
PO_4^{3-}-P	1 μgP L^{-1}
SiO_2	10 $mgSiO_2$ L^{-1}

chemical parameters of the water sample collected from the sampling site are shown in Table 2. The water temperature, pH, and electrical conductivity are 26.6 ℃, 7.0, and 19.3 mS m^{-1}, respectively. The water was clear and slightly brownish, as shown in the turbidity and colour values. The saturation value calculated from the water temperature and dissolved oxygen concentration (12.1 mgO_2 L^{-1}) was 150 % supersaturated. Therefore, this result shows that the photosynthesis activities of *S. neglecta* and other photosynthetic organisms were high at the time of the sampling. The concentration of dissolved inorganic nitrogen (NH_4^+-N + NO_2^--N + NO_3^--N) was 4500 μgN L^{-1} (NO_3^--N contributed approximately 99 %), and PO_4^{3-}-P was only 1 μgP L^{-1}. Nozaki and Matsumoto (2022) reported similar results for water chemistry at this sampling site, with an annual mean concentration±SD of dissolved inorganic nitrogen being 5200±450 μgN L^{-1} and PO_4^{3-}-P being only 3±3 μgP L^{-1}.

The results show that *S. neglecta* could form zygospores during the summer. Simmons and van Beem (1990) reported that *Spirogyra* species with filaments wider than 50 µm, such as *S. neglecta* produced zygospores during summer in the Netherlands. However, the factors and mechanisms affecting the reproductive stage during summer of *S. neglecta* are still unknown clear and further research is required.

References

Devi, K. U. and M. V. N. Panikkar (1994) Species of the Genus *Spirogyra* from Kerala, India. Bibliotheca Phycologica Band 97, J. Cramer, Berlin.

Ferrer, N. C. and E. J. Caceres (2017) Vegetative and reproductive morphology of five *Spirogyra* species (Zygnematales, Charophyta) in Argentina. *Algological Studies*, **153**: 17-34.

Hassall, A. H. (1842) Observations on the genera *Zygnema, Tyndaridea*, and *Mougeotia* with descriptions of new species. *Annals and Magazine of Natural History, Series 1*, **10**: 34-47.

Johnson, L. R. (2011) Phylum Chlorophyta. Family Zygnemataceae. In *The freshwater algal flora of the British Isles*. An identification guide to freshwater and terrestrial algae. Second edition, John, D.M., Whitton, B.A. and A. J. Brook (eds.): 576-608. Cambridge University Press, Cambridge.

Kadlubowska, J. Z. (1984) 10. Spirogyra Link 1820. In *Chlorophyta Part 8*, *Conjugatophyceae I: Zygnemales Freshwater Flora of Central Europe*, Ettl, H., Gerloff, J., Heynig, H. and D. Mollenhauer, D. (eds.): 258-494. Gustav Fischer, Stuttgart (*in German*).

Kützing, F. T. (1849) *Species algarum*: 441. F. A. Brockhaus, Lipsiae (*in Latin*).

Matsumoto Y. and K. Nozaki (2014) 3. *Mizu No Kagaku Bunseki* (Analysis of water chemistry). In *Midika Na Mizu No Kankyo Kagaku, Jisshu Sokutei Hen* (Manual on methods for environmental studies of inland waters): 81-124, Asakura Publishing, Tokyo (*in Japanese*).

Nozaki, K. and Y. Matsumoto (2022) Relationship between water chemistry and human impact on adjacent springs in urban area: Possibility as a teaching material of natural science education in Japanese elementary school. *Wetland Research*, **12**: 43-72 (*in Japanese with English abstract*).

Ripart, M. (1876) Notes sur quelques espèces rares ou nouvelles de la flore cryptogamique du centre de la France. *Bulletin de la Société Botanique de France*, **23**: 158-168 (*in French*).

Sato, H. (2013) A list of freshwater algae in Hyogo Prefecture. *Human and Nature,* **24**: 63-83 (*in Japanese*).

Schagerl, M. and M. Zwirn (2015) A brief introduction to the morphological species concept of *Spirogyra* and emanating problems. *Algological Studies*, **148**: 67-86.

Simons, J. and A. P. van Beem (1990) *Spirogyra* species and accompanying algae from pools and ditches in The Netherlands. *Aquatic Botany*, **37**: 247-269.

Stancheva, R., Hall, J. D., McCourt, R. M. and R. G. Sheath (2013) Identity and phylogenetic placement of *Spirogyra* species (Zygnematophyceae, Charophyta) from California streams and elsewhere. *Journal of Phycology*, **49:** 588-607.

Takano, T., Higuchi, S., Ikegaya, H., Matsuzaki, R., Kawachi, M., Takahashi, F. and H. Nozaki (2019) Identification of 13 *Spirogyra* species (Zygnemataceae) by traits of sexual reproduction induced under laboratory culture conditions. *Scientific Reports*, **9**: 7458.

Transeau, E. N. (1951) The Zygnemataceae: 175, 298-299. The Ohio State University Press, Columbus.

Yamagishi, T. (1966) Studies on the Genus *Spirogyra* Collected in Japan. *Science Report of the Tokyo Kyoiku Daigaku, Section Biology*, **12**: 73-105.

Yamagishi, T. (1977) Family Zygnemataceae. In *Illustrations of the Japanese freshwater algae*, Hirose, H. and T. Yamagishi (eds.): 416-461. Uchida Roukakuho Publishing, Tokyo (*in Japanese*).

Yamagishi, T. (1987) *Spirogyra ternata* Ripart var. *ternata.* *Photomicrographs of the Fresh-water Algae*, **7**: 66. Uchida Roukakuho Publishing, Tokyo.

(Guest Editor: Dr. TUJI Akihiro, Department of Botany, National Museum of Nature and Science)

摘　要

愛知県名古屋市の都市部の湧水から採集されたアオミドロ *Spirogyra neglecta*（接合藻綱）の形態的特徴

野崎健太郎

2016年8月8日に名古屋市の都市部に位置する湧水から採取されたアオミドロ属 *Spirogyra neglecta*（Hassall）Kützing

の形態的特徴を，栄養細胞，接合過程および接合胞子の顕微鏡写真を用いて記載した．栄養細胞は隔膜が平板状，葉緑体は3本で1～2回転，細胞幅54～74 μm，長さ79～377 μm（n = 171）であった．生殖細胞は円筒形，接合は梯子状で接合管は両端から伸びていた．接合胞子は楕円形あるいは卵形で，幅49～69 μm，長さ67～127 μm，胞子膜中層は平滑で厚く黄褐色であった（n = 156）．*S. neglecta* は *S. ternata* Ripart と酷似しているため，いくつかの先行研究を用いて，これら2種に関する記述を比較した．調査地の水温は 26.6℃，pH は 7.0，電気伝導度は19.3 mS m^{-1}であった。溶存無機態窒素濃度は4500 μgN L^{-1}で硝酸態窒素が約 99 % を占め，リン酸態リンはわずか1 μgP L^{-1}であった．

キーワード：接合，*Spirogyra neglecta*（Hassall）Kützing，*Spirogyra ternata* Ripart，生殖細胞，接合胞子

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