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A new planktonic diatom, *Craticula pseudocitrus* sp. nov., *Naviculales*, Bacillariophyceae found in Lake Kasumigaura, Japan

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Abstract A new planktonic diatom, *Craticula pseudocitrus* Tuji, Nakagawa, Sato et Yamaguchi, is described from Lake Kasumigaura. This taxon has been confused with *C. citrus*, but can be distinguished by density and pattern of striae, size of outline and form of central area. Phylogenetic tree analysis using *rbc*L and 18S rRNA supports the assignment of this taxon to genus *Craticula*. The seasonal pattern of this taxon is similar to total diatoms, as it appears in the spring and late autumn.

Key words: *Craticula citrus*, *Craticula pseudocitrus* sp. nov., Lake Kasumigaura, *Navicula citrus*.

Introduction

Most freshwater planktonic diatoms are classified as centric or araphid diatoms. Biraphid planktonic diatoms are very rare. Most of the appearance records of freshwater planktonic naviculoid diatoms may not be true plankton but rather tychoplankton. The phytoplankton of Lake Kasumigaura has been monitored by the National Institute for Environmental Studies (NIES) since 1996 (Takamura and Nakagawa, 2012). We found a small characteristic naviculoid diatom from the samples of the monitoring, and identified it as *Craticula citrus* (Krasske) Reichardt (Tuji and Nakagawa, pers. inf.). In this paper, we re-examined the identification, phylogeny and seasonal variation of this taxon.

Materials and Methods

1. *Sampling*

The sampling was done as a part of monthly comprehensive monitoring by NIES (Takamura and Nakagawa, 2012; National Institute for Environmental Studies, 2016). Water samples were taken from the surface to 2.0 m depth with an acrylic column sampler at Stations 3 (36°07.302′N, 140°22.652′E) and 9 (36°02.142′N, 140°24.222′E)

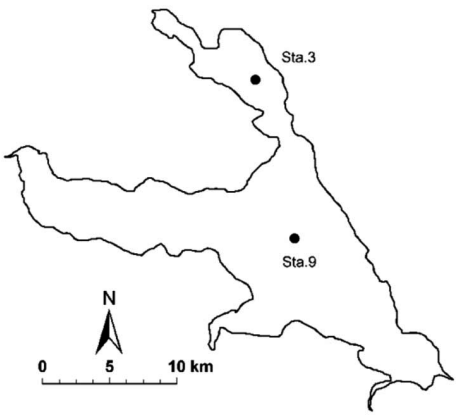


Fig. 1. A map of Lake Kasumigaura showing sampling sites.

(Fig. 1). The water sample (100 ml) for counting phytoplankton was fixed with Lugol's iodine solution (final concentrations; 0.2–0.4%), then placed in a sedimentation chamber and kept there for 24 hours. The number of each species was counted for each sample with an inverted microscope with 40× an objective lens.

2. *Establishment of a culture strain*

Surface water in the St. 9 was collected using 2 l of plastic bottle on May 13, 2015. Isolation of the diatom cell was conducted using a flow cytometer (EPICS ALTRA; Beckman Coulter, Germany) equipped with a cell sorter and an argon laser (excitation at a wavelength of 488 nm). The established culture strain was maintained using CSi medium (Kasai *et al.*, 2009) under a light intensity of 11–14 μmol photons/m^2/sec with a light cycle of 10 : 14 (light : darkness). This strain is available for the public via the Microbial culture collection of National Institute for Environmental Studies as strain number NIES-4015 (NIES, http://mcc.nies.go.jp/).

3. *Microscopic observations*

Planktonic samples without fixation were observed and taken photographs using a light microscopy (LM, BX41, Olympus, Japan) equipped with water immersion lenses and a camera (EOS kiss X7i, Canon, Japan). Planktonic samples and the strain were cleaned using concentrated nitric acid as described previously (Tuji and Tanimura, 2012). Cleaned samples were embedded using Zrax (Tuji and Tanimura, 2012). Light microscopy (LM) observation and imaging were done using a microscope (Axiophoto, Zeiss, Germany) equipped with a CCD camera (Infinity 4: Lumenera, Canada). A small part of the sample was filtered using a membrane filter (RTTP02500, Merck), placed on a SEM stub and dried at room temperature overnight. The stub was sputter-coated with platinum and examined with a scanning electron microscope (JEOL-6390LV, JEOL, Japan) equipped with lanthanum hexaboride cathode.

4. *Molecular analysis*

DNA was extracted from the cultured strain (NIES-4015) using the Chelex method (Walsh *et al.*, 1991). Polymerase chain reaction (PCR) and sequencing of *rbc*L and the 18S rRNA gene were then performed (Tuji *et al.*, 2014). Phylogenetic and molecular evolutionary analyses for the obtained sequences were conducted using the MEGA 7 computer program (Kumar *et al.*, 2016). Alignments were checked manually. Neighbor joining (NJ) and maximum likelihood (ML) trees were calculated using MEGA software with the best fits model (T92 + G + I model) by Bayesian information criterion scores (BIC), and the substitution nucleotide matrix parameters were calculated by the software. A tree using 1000 bootstrap replicates was generated. All positions containing gaps and missing data were eliminated.

Results and Discussion

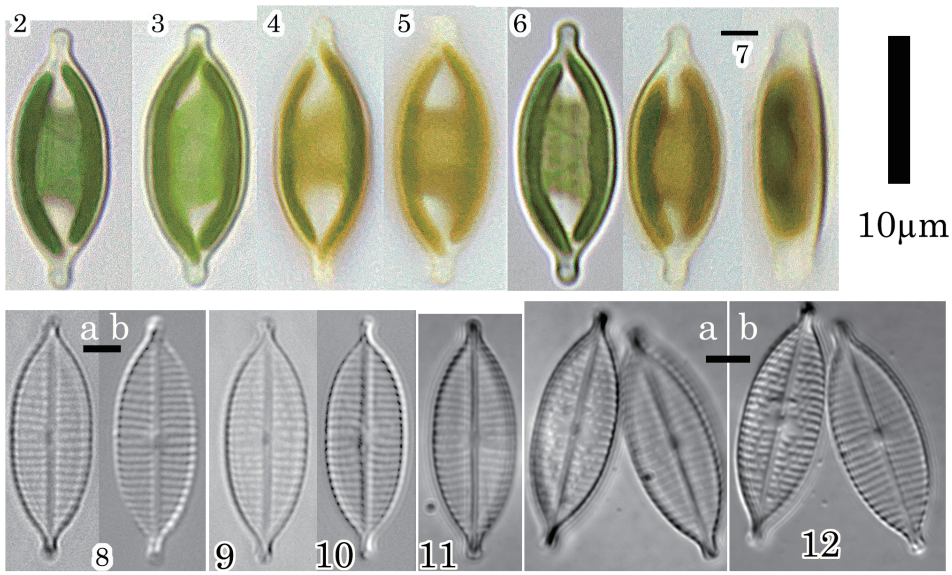
Morphological differences between Craticula citrus *and new species*

Morphological variation of shape and size of this taxon is very small in Lake Kasumigaura. One H-shaped chloroplast is observed in living cell (Figs. 2–7). The 'conspicuous silica granules' on the valve exterior described by Reichardt, as well as his finding that 'at the margins the bigger granules are elongated into rib-like structures' (Reichardt, 1997), were corroborated using SEM (Figs. 13–15). Other morphological characteristics found by LM (Figs. 8–12) and SEM (Figs. 13–18) also agreed with Reichardt's description of *Curaticula citrus* in (Reichardt, 1997). Though our observations of the size, length 16.0–17.5 μm, breadth 5.5–7.0 μm, and striae (19 per 10 μm), are more diminutive than the original description (length: 22 μm, breadth: 8 μm, 15 striae per 10 μm) in Krasske (1923), they agree with the descriptions of more recent works (Hofmann *et al.*, 2011; Burge and Bishop, 2015).

A photograph of the type material was presented by Lange-Bertalot (2001: plate 83, fig. 5). The size of this type individual (length: 19.5 μm,

Table 1. Length, breadth and density of striae (per 10 μm) of *Craticula citrus* in previous studies and *C. pseudocitrus* in this study

Taxon	Lengh	Breadth	Striae per 10 μm	Reference
Navicula citrus	22	8	15	Krasske (1923: 199)
Navicula citrus	19.5	7	16	Holotype in Lange-Bertalot (2001)
Navicula citrus	18–20	6.5	18–20	Hustedt (1961: 780)
Craticula citrus	18–22	6–7	18–20	Lange-Bertalot (2001: 111)
Craticula citrus	13–17	4.6–5.8	18–24	Burge & Biishop (2015)
Craticula pseudocitrus	16–17.5	5.5–7.0	19	This study



Figs. 2–12. *Craticula pseudocitrus* sp. nov., light microscopy (LM), bar = 10 μm. 2–7. Living cells showing H-shaped chloroplast (station 3, Lake Kasumigaura, Nov. 8, 2017). 8–12. Cleaned permanent slide, TNS-AL-TNS-AL-62279 (holotype slide), 8a, 9–11, 12a. Bright field microscopy (BF). 8b, 12b. Differential interference contrast microscopy (DIC).

breadth: 7 μm, 16 striae in 10 μm) showed dimensions slightly smaller than in the original description (Table 1).

Differences in the density of striae are important for distinguishing naviculoid diatoms. Furthermore, the size of the outline, pattern of the central area and striae, and form of protracted apices showed differences between the original description and type individual on the one hand and Lake Kasumigaura individuals on the other. Thus, we propose a new species for Lake Kasumigaura individuals.

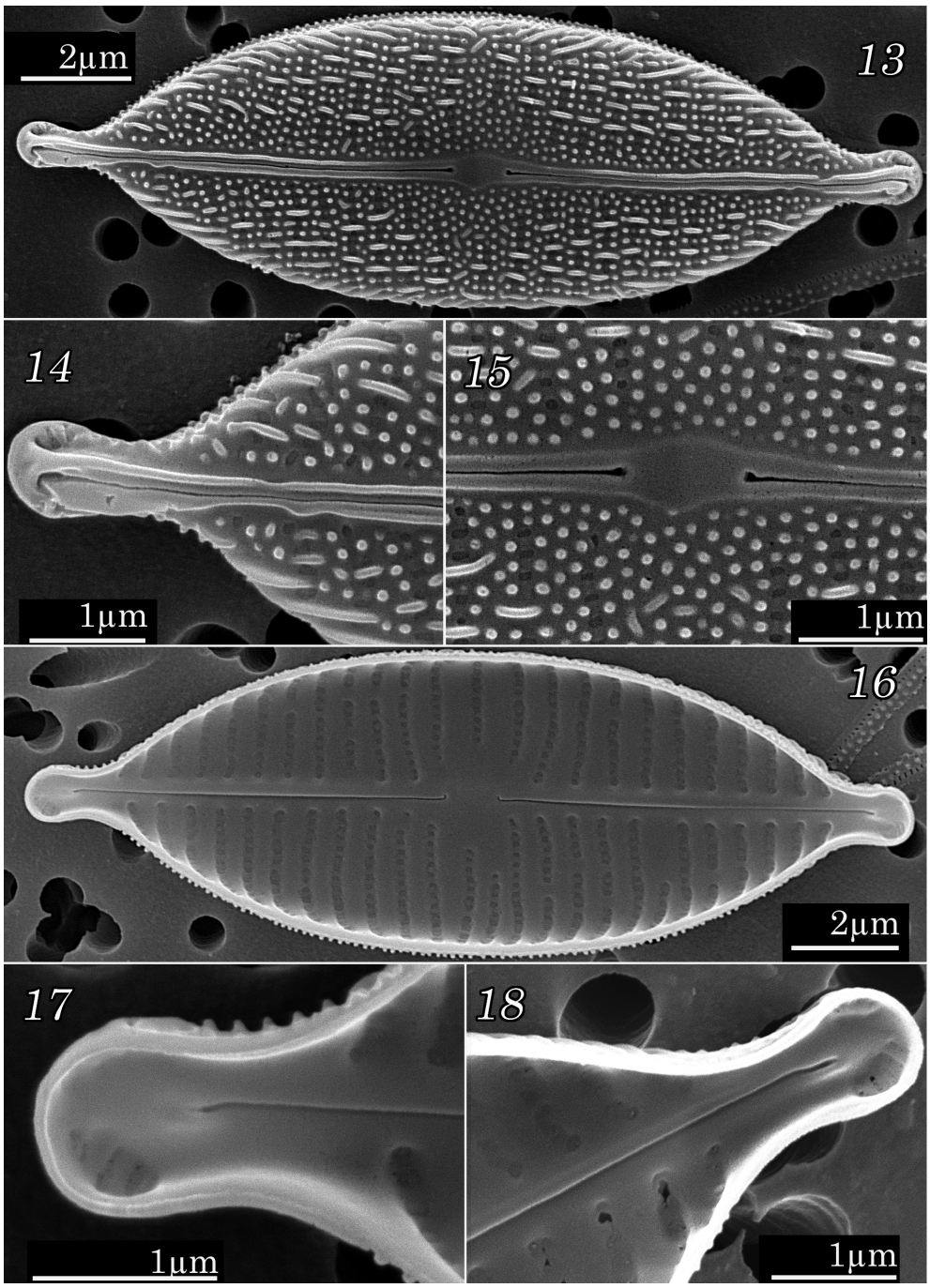
Craticula pseudocitrus Tuji, Nakagawa, Sato et Yamaguchi, sp. nov.

(Figs. 2–18)

Synonym: *Craticula citrus* sensu Reichardt (1997); *C. citrus* sensu Burge and Bishop (2015). non: *Navicula pseudocitrus* Manguin in Bourrelly and Manguin (1954).

Holotype: A slide TNS-AL-62279 in TNS (Department of Botany, National Museum of Nature and Science).

Isotype: A formalin-fixed specimen of following type strain, TNS-AL-62279m (Ak1279) in TNS (Department of Botany, National Museum of Nature and Science).



Figs. 13–18. Scanning electron microscopy (SEM) of *Craticula pseudocitrus* sp. nov. TNS-AL-62279 m (isotype material). 13–15. External views of frustules using scanning electron microscopy. 13. Whole frustule showing many conspicuous silica granules and bars. 14 Produced subcapitate apice. 15. Central area. 16–18. Internal views of frustules using scanning electron microscopy. 16. Whole frustule. 17, 18. Produced subcapitate apices of both side.

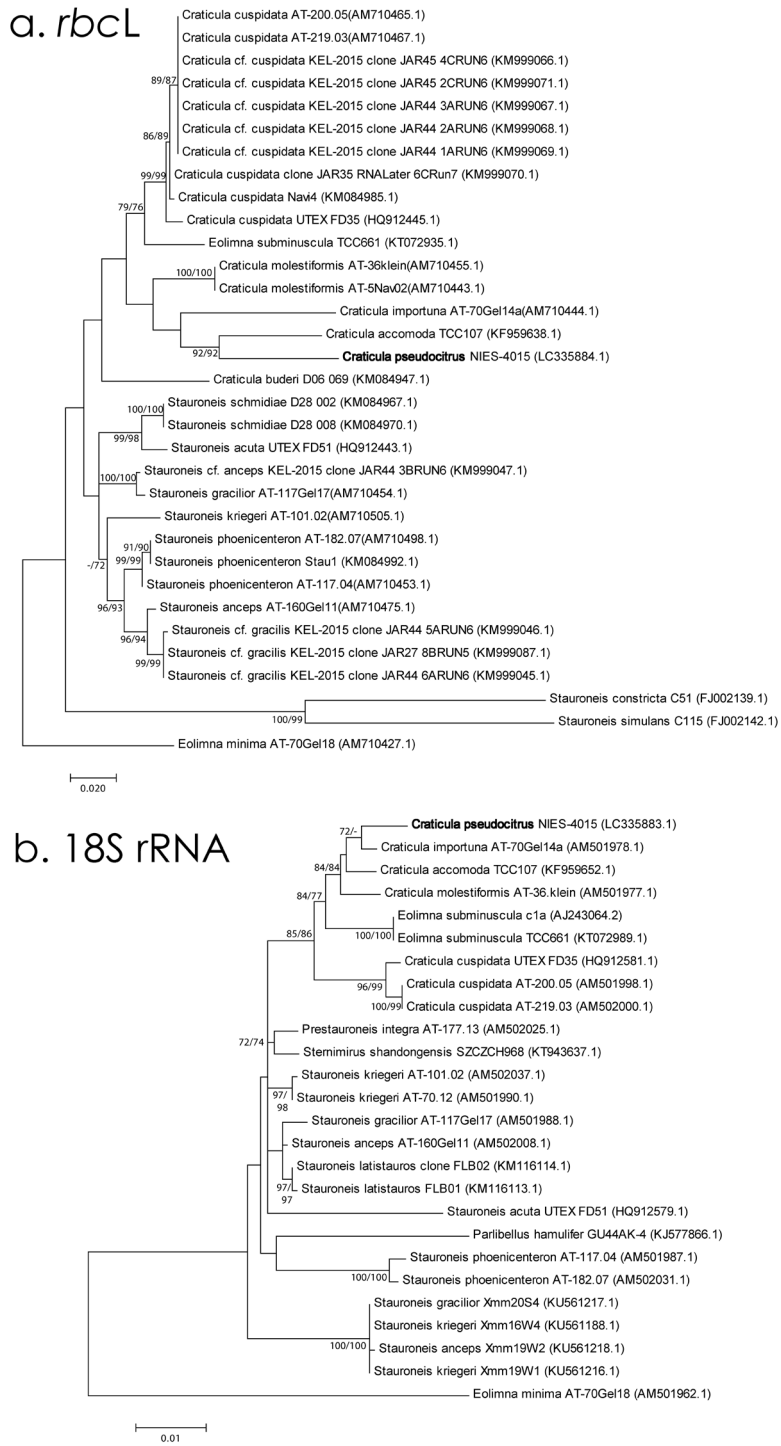


Fig. 19. Phylogenetic tree of *Craticula pseudocitrus* and related taxa determined by Maximum Likelihood (ML) method using *rbc*L (a) and 18S rRNA (b) genes. Accession numbers are followed by taxonomic names. Numbers at branches indicate NJ (Neighbor Joining)/ML bootstrap support values (only values higher than 70 are shown).

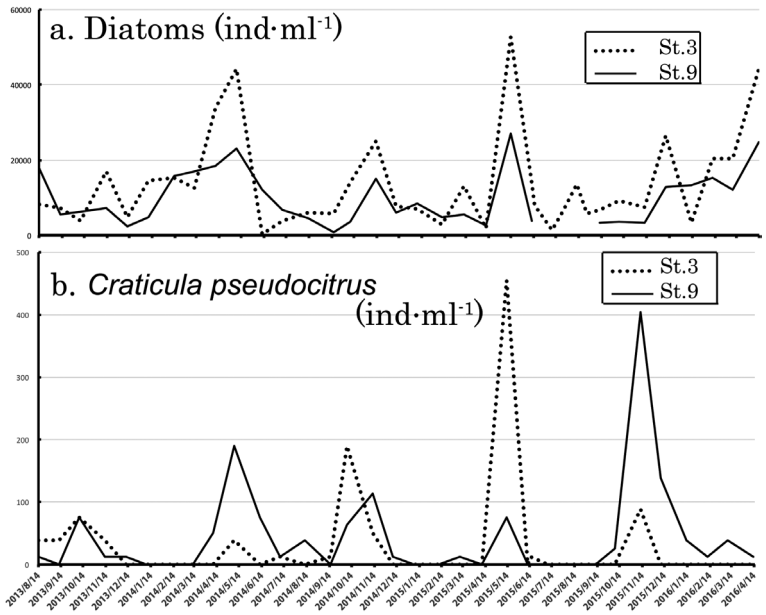


Fig. 20. Seasonal changes of the density (individuals ml^{-1}) of diatoms (a) and *Craticula pseudocitrus* (b).

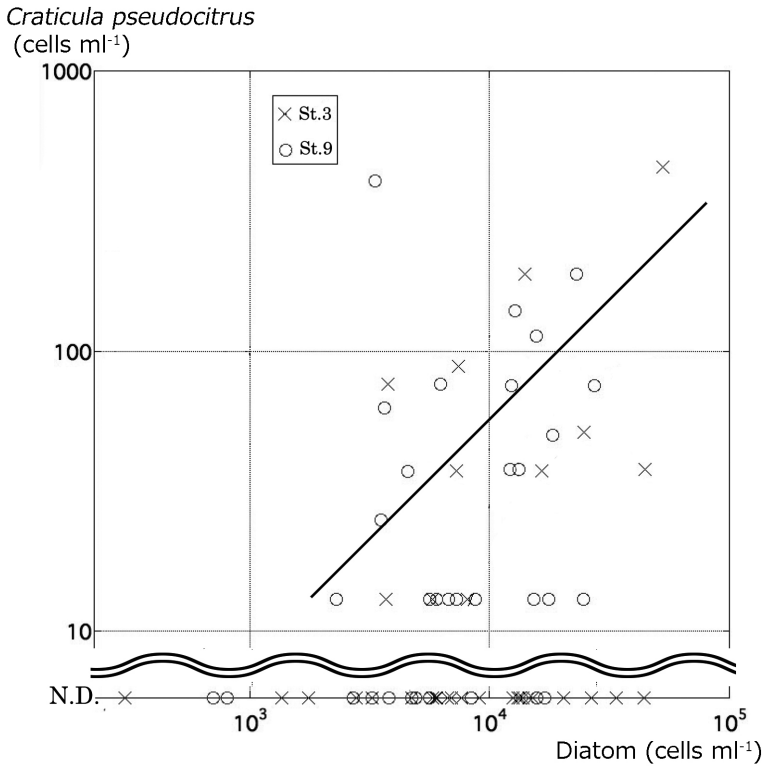


Fig. 21. The relationship between the density (individuals mL^{-1}) of diatoms and *Craticula pseudocitrus*.

Type strain: NIES-4105 maintained in the NIES collection, National Institute for Environmental Studies (NIES).

Type locality: Lake Kasumigaura, Ibaraki, Japan.

Valves are elliptical-lanceolate with protracted, rostrate apices. The axial area is straight and narrow; the central area is absent or small. Length 16.0–17.5 μm, breadth 5.5–7.0 μm. Striae are almost parallel throughout, 19 striae per 10 μm. Several striae are slightly curved around the central area, and sometimes becomes short. The density of these striae are coarser than other striae. The striae at the ends are slightly convergent. Many conspicuous silica granules and bars exist on the outside of valves. Striae are interrupted by these silica bars. The following characteristics are different from *Craticula citrus*, which it otherwise resembles. Several striae of *C. pseudocitrus* at the central area has courser than others, but the density of striae are almost some for *C. citrus* (Lange-Bertalot 2001: plate 83, fig. 5). The form of the central area is asymmetrical, butterfly-shaped in *C. citrus*, but *C. pseudocitrus* has a small or invisible central area. The produced apices are wide and parallel in *C. citrus*, but narrow and swollen in *C. pseudocitrus*. The thickened longitudinal elements, which are formed by the aligned frets in *Craticula cuspidata* (Mann and Stickle, 1991), is not exist. However, rib-like structures found in this taxon, should be comparable with it.

Phylogeny of C. pseudocitrus

Phylogenetic trees of *C. pseudocitrus* and related taxa using rbcL and 18S rRNA are shown in Fig. 19. *C. pseudocitrus* occupies the same clade as *C. accomoda* (Hust.) Mann *et al.*, *C. importuna* (Hustedt) K. Bruder et Hinz, *C. molestiformis* (Hust.) Mayama and *C. subminuscula* (Manguin) Wetzel et Ector (= *Eolimna subminuscula* (Manguin) Molser *et al.*); all are smaller members of the genus *Craticula*. A larger *Craticula* member, *C. cuspidata*, and the clade of small *Craticula* members, form a monophyletic group. Genus *Stauroneis* is a sister group for the *Craticula* clade. These results support the assignment of *C. pseudocitrus* to genus *Craticula*.

Seasonal pattern of C. pseudocitrus *in Lake Kasumigaura and its ecology*

The seasonal patterns of diatoms and *C. pseudocitrus* are presented in Fig. 20. The two patterns are almost identical, and show a high abundance in spring and the autumn. A positive correlation between densities of diatoms and *C. pseudocitrus* was found (Fig. 21).

The optimum pH for *C. citrus* was reported about 8–9, and the concentration of sodium is about 40 mg/l in North America (Burge and Bishop, 2015). Arguably, then, *C. citrus* sensu Burge and Bishop (2015) should not be *C. citrus*, but close to *C. pseudocitrus*. The pH in Lake Kasumigaura is about 8–10, and the concentration of sodium is about 20–50 mg/L, adapted to the optimum pH and concentration of sodium at North America.

The ecology of genus *Craticula* has been reported as 'Epipelic in freshwater bodies' (Lange-Bertalot, 2001). *C. pseudocitrus* is observed in planktonic samples in Lake Kasumigaura. Though it can be a tychoplankton, we have not seen many individuals of this taxon from epipelic samples in Lake Kasumigaura, and we found only this taxon for small naviculoid diatoms among the planktonic samples from Lake Kasumigaura.

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