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Description of an unrecorded diatom *Fragilaria saxoplanctonica* Lange-Bertalot & Ulrich (Bacillariophyceae) from Paldang Reservoir in Korea

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Diatoms are unicellular eukaryotic microalgae, and they are highly diversified in aquatic environments. We describe an unrecorded diatom species *Fragilaria saxoplanctonica* Lange-Bertalot & Ulrich (Bacillariophyceae) collected from Paldang Reservoir, Korea, on 4 April 2022. The valve was needle shaped and narrowly rectangular, its ends were rounded, and no spines were found on the outline of their valves. The valve was 67.2−70.2 μm in length and 1.4−2.9 μm in width. The ratio of width-to-length was 1 : 23.2−50.1. The pattern of striation was alternate or opposite, and the number of striae in 10 μm was 24−26. Molecular comparisons of the 18S rDNA and *rbc*L sequences showed that it belonged to the genus *Fragilaria*. These morphological and phylogenetic results confirmed that our species was *F. saxoplanctonica*, and it was the first record in Korea.

Keywords: 18S rRNA, *Fragilaria saxoplanctonica*, freshwater diatom, morphology, Paldang Reservoir, *rbc*L

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Introduction

Diatoms (Bacillariophyta) are eukaryotic, phototrophic, unicellular algae which are one of the major primary producers (Falkowski *et al*., 2004). The organisms occur globally in most aquatic environments, and their species numbers are estimated to 30,000−100,000 (Mann and Vanormelingen, 2013). Their high diversity, abundance, and environmental sensitivity contributed to their utilization as a biotic index for water quality assessment in Europe (Kolkwitz and Marsson, 1908). Until recently, they are one of the successful indicators for monitoring streams and rivers (Round, 1981; Stoermer and Smol, 1999; Chessman *et al*., 2007), and coincidentally, more precise identification and classification of diatoms are required for accurate water quality assessments. Traditionally, diatoms have been identified into two groups, centric diatoms and pennate diatoms, based on their morphology. Centric diatoms have radially symmetric valve, while pennate diatoms have polar symmetric valve (Round *et al*., 1990). The pennates are morphologically further subdivided into raphid and araphid (Round *et al*., 1990). Araphid pennates without a raphe on the valve surface have an unstable classification system, and the typical example is the genus *Fragilaria*.

The diatom genus *Fragilaria* (Fragilariaceae, Bacillariophyta) was originally described for species forming linear, flat, simple, fragile, and ribbon or zigzag-like colonies linking each other (Lyngbye, 1819). When only using a light microscopy (LM), fine structures of *Fragilaria* were not fully investigated, as a result, colony type became a significant character to differentiate *Fragilaria* from its morphological relatives. For example, morphologically and taxonomically closely related genus *Ulnaria* has undistinguishable valve outline, and thus it is distinguished with fan-shaped or rosette-like colonies (Ehrenberg, 1832; Hustedt, 1931). Meanwhile, further studies have discovered more colony types, such as loose aggregates (*F. tenera* (Smith) Lange-Bertalot, and *F. heatherae* Kahlert & Kelly), stellate (*F. lemanensis* (Druart, Lavigne & Robert) Van de Vijver, Ector & Straub), and none (*F. tenera* var. *nanana* (Lange-Bertalot) Lange-Bertalot & Ulrich, *F. spectra* Almeida, Morales & Wetzel, *F. gracilis* Østrup, and *F. perminuta* (Grunow) Lange-Bertalot) (Druart *et al*., 2007; Lange-Bertalot and Ulrich, 2014; Almeida *et al*., 2016; Kahlert *et al*., 2019). The reliance on colonies decreased, and with the introduction of scanning

electron microscopy (SEM), new microstructural criteria for the *Fragilaria*'s taxonomy were suggested and evaluated (Poulin *et al*., 1986). To date, morphological features such as valve length, width, ends and outline of the valve, central area, striae, and the presence of spine have been utilized to identify *Fragilaria*.

Until now, a total of 204 *Fragilaria* species are recorded in AlgaeBase (Guiry and Guiry, 2023). It accounts for the biggest part (45.8%) of the family Fragilariaceae (445 species), while the second and third genera only recorded 105 (23.6%; *Synedra* Ehrenberg) and 33 (7%; *Fragilariforma* Williams & Round). After the original description by Lyngbye in 1819, many new and unrecorded species have been reported globally until now.

In Korea, research on diatoms has been conducted since the 1910s. According to the Database of the National Species List of Korea (DBNKo) (Park *et al*., 2020), the first study on *Fragilaria* was conducted by Chung and colleague in 1965, reporting four species, *F. capucina* Desmazières, *F. crotonensis* Kitton, *F. intermedia* (Grunow) Grunow, and *F. striatula* Lyngbye. Now, at least 20 species of *Fragilaria* have been discovered in Korean waters (Chung, 1968; Cho, 2000), and most species were researched from the Han River, one of the largest rivers in South Korea (Chung *et al*., 1965; Chung, 1968). Several *Fragilaria* species, including *F. crotonensis*, *F. radians* (Kützing) Williams & Round, and *F. rumpens* (Kützing) Carlson were investigated from Paldang Reservoir (Jung *et al*., 2014; Yun *et al*., 2014; Tan *et al*., 2020), and *F. rumpens* and *F. radians* were revealed as seasonal dominant species during summer in Paldang Reservoir (Yun *et al*., 2014). Although *Fragilaria* species have an importance to the hydroecology of Korea, descriptions about their micromorphology, species identification, and classification based on molecular data are still limited. Thus, additional morphological data and genetic information of *Fragilaria* are needed to distinguish and classify similar *Fragilaria* species observed in Korean waters.

In the present study, we isolated unique diatom *Fragilaria* cells in water samples collected from Paldang Reservoir, Korea, and characterized their morphological features and molecular gene sequences (18S rDNA and *rbc*L). We compared their characteristics to other diatoms, and then, identified it to be *F. saxoplanctonica*, which was previously not recorded in Korea. Hence, this study described the morphology and molecular taxonomy of the Korean *F. saxoplanctonica*.

Materials and Methods

Water sampling and sample culture

Paldang Reservoir is one of the major reservoirs and the largest drinking water source in South Korea. A water sample was collected on 13 April 2022 at shallow part of Paldang Reservoir (GPS code: 37°29′16″N 127°17′14″E; Fig. 1), and the sample was filtered with 200 μm mesh to remove zooplanktons. Water temperature, pH, and conductivity were measured with a YSI 566 Multi Probe System (YSI, Yellow Springs, OH).

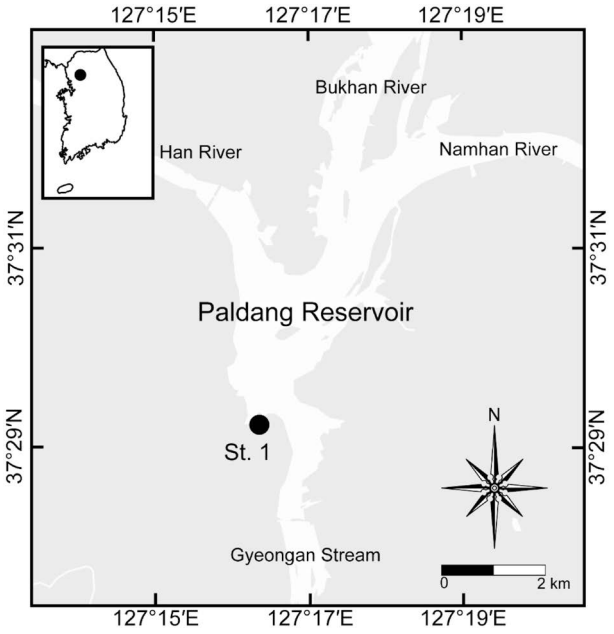

Fig. 1. A map of Paldang Reservoir in South Korea. A black circle (St.1) represents the sampling site.

In the laboratory, we spread the water sample on solid diatom medium (DM) that was prepared with 1% agar, cultured, and isolated single colonies. Next, a single diatom was further cultured in 50 mL plastic culture flasks. For DNA extraction, the strain was filtered with 10 μm pore sized membrane filter (Cat. No. TCTP04700, 47 mm diameter; Millipore, Billerica, MA), and stored at −20°C with 800 μL cetyltrimethylammonium bromide (CTAB) buffer.

Morphological observations

Colony and cell shape of the isolated diatom were observed with a light microscope (LM; Carl Zeiss Axioskop, Oberkochen, Germany) under 400× magnification. The ultrastructure of the cells was analyzed with SEM (MIRA-3, TESCAN, Brno, Czech Republic). The diatom from our study was identified and classified based on previous studies (Østrup, 1910; Krammer and Lange-Bertalot, 1991; Lange-Bertalot and Ulrich, 2014) and AlgaeBase (Guiry and Guiry, 2023).

DNA extraction and PCR

We extracted genomic DNA of the isolated *Fragilaria*

Table 1. A list of morphological characters of individual *Fragilaria* species.

Characters	*F. tenera* var. *nanana*[1]	*Fragilaria aquaplus*[1]	*Fragilaria gracilis*[2]	*Fragilaria saxoplanctonica*[1]	*Fragilaria saxoplanctonica*[3]
Valve length (μm)	50–70	22–45	63	40–170 (most frequently 50–140)	67.2–70.2
Valve width (μm)	2	1.5–2.4	3.6	1.5–2.5	1.4–2.9
Width-to-length ratio	1:25–35	1:9.2–30	1:17.5	1:16–113.3	1:23.2–50.1
Striae number in 10 μm	18.5–20	22–24	20	23–28	24–26
Valve outline	Needle shaped	Narrowly rectangular, needle shaped	Narrow and linear	Narrowly rectangular, needle shaped	Narrowly rectangular, needle shaped
Striation	Alternating	Opposite	Opposite	Alternating or opposite	Alternating or opposite
Spines	Pyramidal	Lacking	Lacking	Lacking	Lacking
Valve ends	Capitate	Subcapitate	Subcapitate	Never capitate or subcapitate, acutely rounded	Rounded

Data sources are indicated with superscripts: [1]Lange-Bertalot and Ulrich (2014), [2]Tuji (2007) and [3]this study.

sp. following the CTAB method (Richards *et al*., 1994). Polymerase chain reaction (PCR) was conducted to amplify the sequences of nuclear 18S rDNA and chloroplast *rbc*L with 18S primers (forward: 18F01, 5′-TAC CTG GTT GAT CCT GCC AGT AG-3′; reverse: 18R1780, 5′-GTT CAC CTA CGG AAA CCT TG-3′) and *rbc*L primers (forward: Dt-rbcL-F29, 5′-TCT GTA TCA GAA CGG ACT CG-3′; reverse: Dt-rbcL-R1010, 5′-AGG ATC ACC TTC TAA TTT ACC-3′). The composition of 20 μL PCR tubes were 11.8 μL sterile distilled water, 2 μL 10× Ex PCR buffer (TaKaRa, Shuzo, Kyoto, Japan), 2 μL dNTP mix (4 mM each), 1 μL each of two primers (10 pmol), 0.2 μL Ex Taq polymerase (2.5 U), and 2 μL of template. PCR was performed under the following conditions: (94°C for 3 min; 40 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; and 72°C for 10 min). DNA was purified with QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and Sanger sequencing (Bionics Co. Ltd, Seoul, South Korea) was performed. The ends of the sequences were trimmed and assembled with Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI).

Molecular identification and phylogenetic analysis

Fragilaria's 18S rDNA and *rbc*L sequences determined here were subjected to molecular identification using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI). In addition, we retrieved DNA sequences of 30 species (18S rDNA) and 32 species (*rbc*L) from the NCBI database and a previous result by Kahlert *et al*. (2019). The sequences were aligned using the MAFFT software (Katoh *et al*., 2019) and trimmed with Gblocks (Castresana, 2000). A maximum likelihood (ML) tree using the GTR + G + I model with 1,000 bootstrap replicates was constructed separately with the aligned 18S rDNA (987 bp) and *rbc*L (615 bp) in MEGA X (Kumar *et al*., 2018). The visualized phylogenetic tree was edited in Adobe Illustrator CC (Adobe Systems, San Jose, CA).

Results and Discussion

We analyzed and classified the species by morphological and molecular data. This is the first record of *F. saxoplanctonica* in Korea and provides morphological and taxonomic descriptions of this species.

Class Bacillariophyceae Haeckel, 1878: 95
Order Fragilariales P.C. Silva, 1962: 835
Family Fragilariaceae Kützing, 1844
Genus *Fragilaria* Lyngbye, 1819

Fragilaria saxoplanctonica Lange-Bertalot & Ulrich 2014 (Fig. 2)

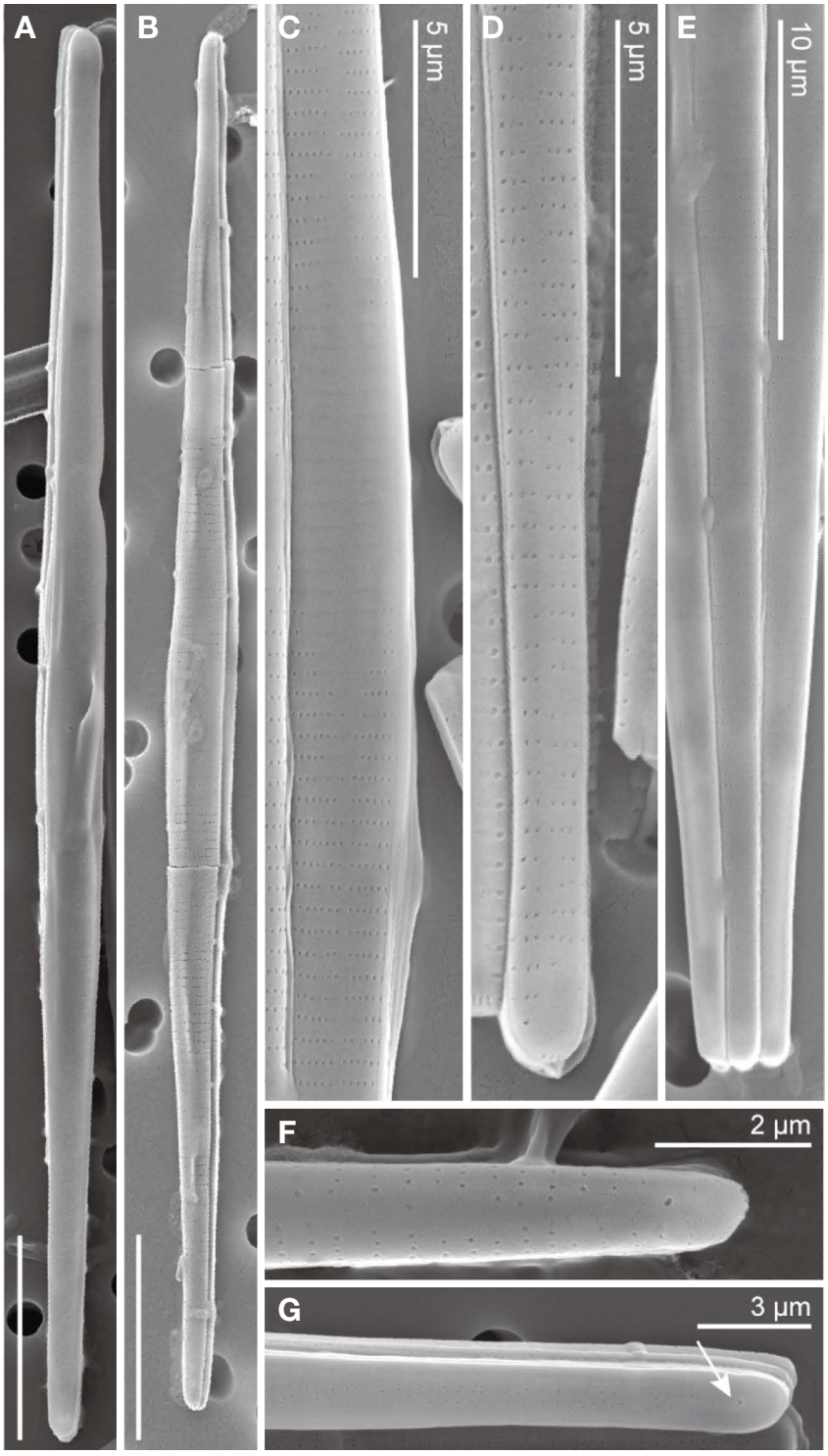

Fig. 2. SEM micrographs of the Korean *Fragilaria saxoplanctonica* isolated from Paldang Reservoir. A, B: valve in external view displaying the outline and striae. C: external view of central area with ghost striae. D: the striae patterns. E, F: external view of valve ends. G: girdle view. The arrow shows the external view of rimoportula. Scale bar = 10 μm in A and B.

Reference: Lange-Bertalot & Ulrich 2014, p. 30, fig. 13: 1–9, 14: 1–8.

Description: The frustules of the diatom shaped needle and narrowly rectangular. Each cell existed solely, and no colony formation was observed. The external valve showed no linking or marginal spines, and the ends of the

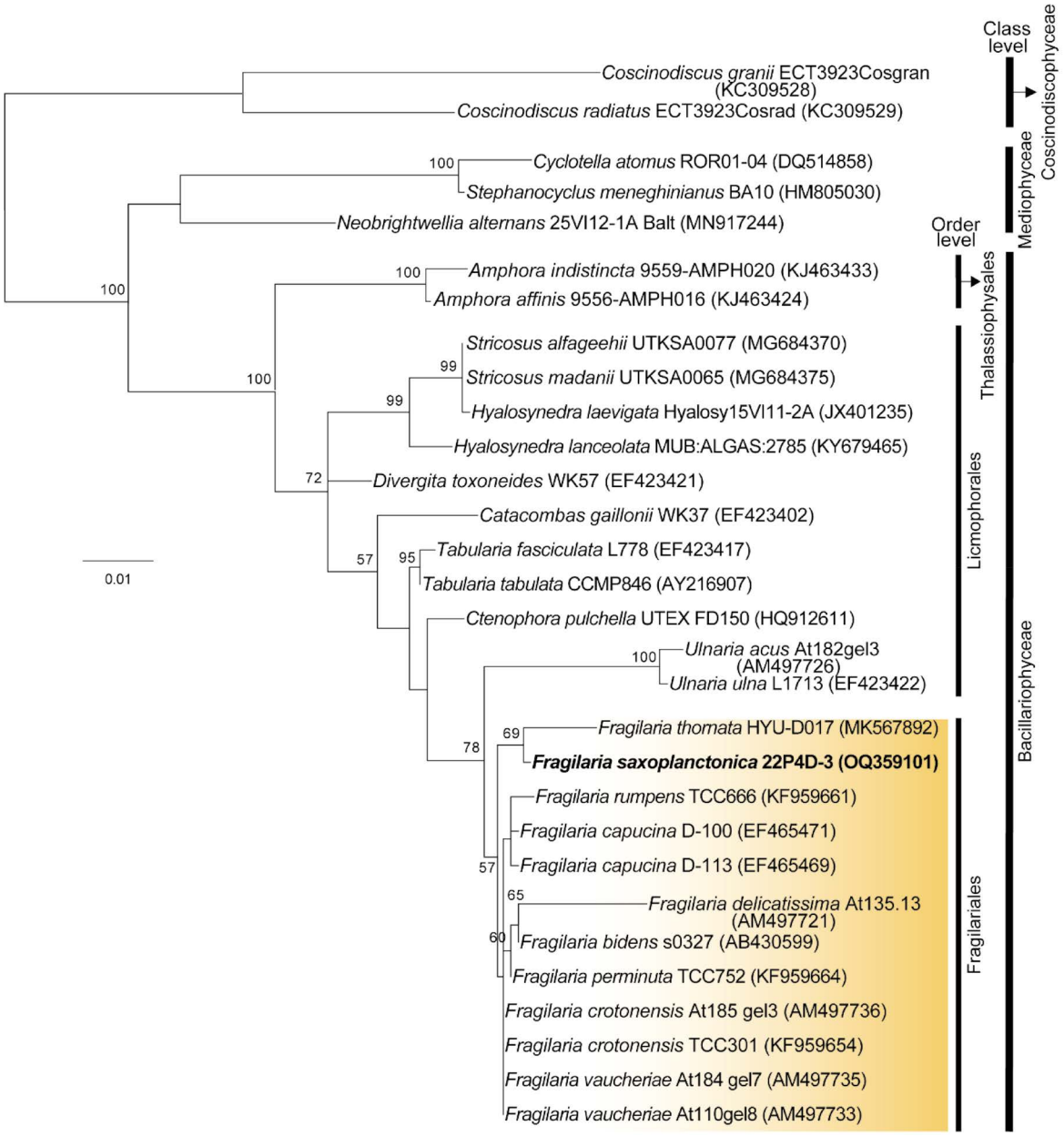

Fig. 3. A phylogenetic tree of diatoms using the maximum likelihood (ML) method based on 18S rRNA sequences. The genus *Fragilaria* is highlighted in brown color, and our species is specified in bold. Coscinodiscophyceae was used as an outgroup.

valves were rounded. The valve length was 67.2–70.2 μm, width was 1.4–2.9 μm, and the ratio of width-to-length was 1 : 23.2–50.1. Striae were alternate or opposite, composed of 2–4 areolae near the apices and 5–7 areolae in the middle except the central area with ghost striae. The number of striae in 10 μm was 24–26. In addition, one rimoportula per valve existed.

Taxonomic remarks: According to the original description, the similar species of *F. saxoplanctonica* was *F. tenera* var. *nanana*, which was once called *F. nanana* Lange-Bertalot. SEM analysis, however, revealed differences such as low density of striae (18.5–20 at 10 μm), pyramidal spines, and the capitate ends (Table 1). Two species, *F. aquaplus* Lange-Bergalot & Ulrich and *F. gracilis* showed more similar characteristics such as narrow, linear, and needle shaped valve view, no spines, and opposite striations (Table 1). Despite of relative similarities, *F. aquaplus* has shorter valve length (22–45 μm), and *F. gracilis* has wider valve width (3.6 μm) and lower striae density (20 in 10 μm). In addition, both species have subcapitate

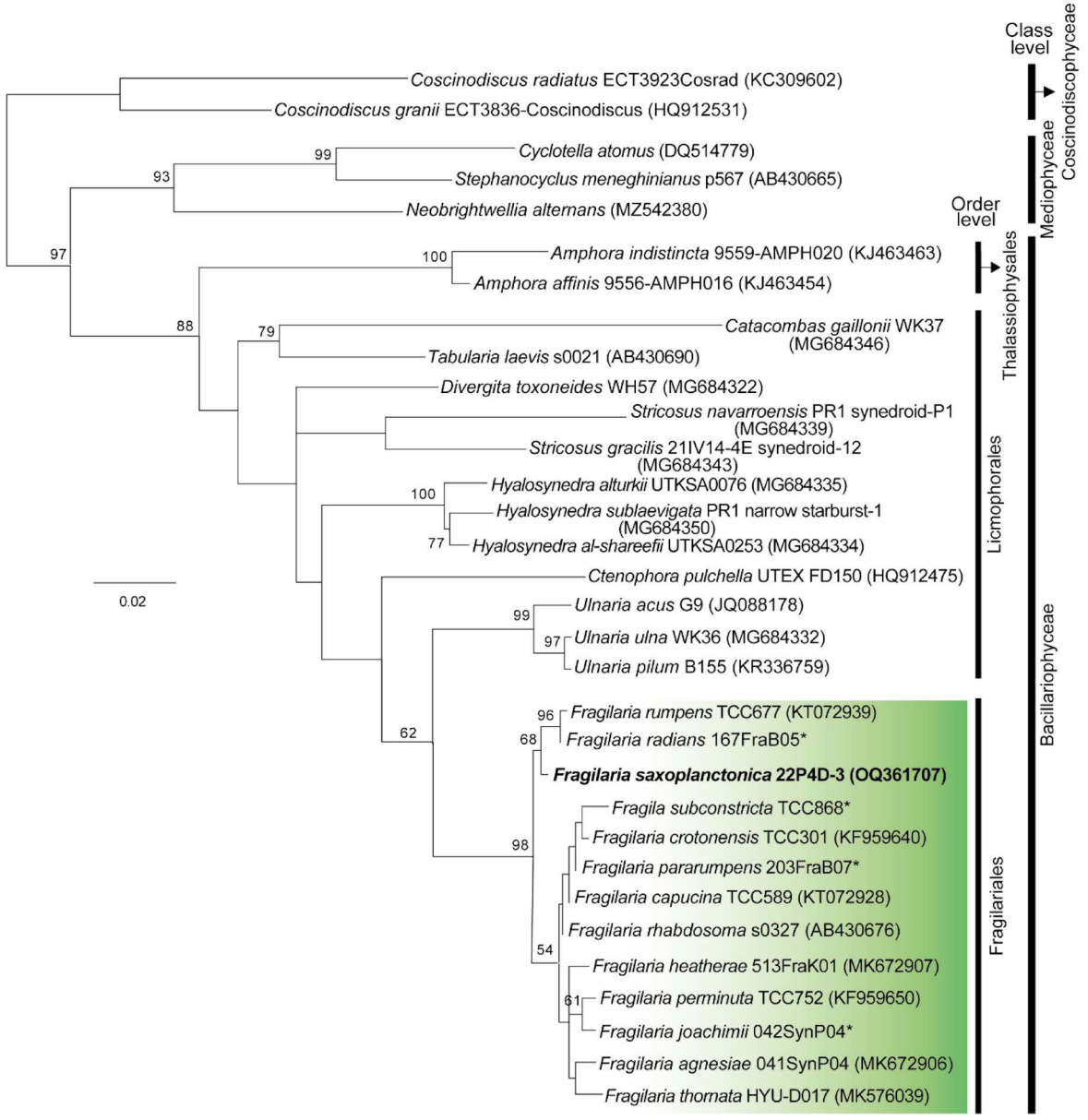

Fig. 4. A phylogenetic tree of *Fragilaria* using the maximum likelihood (ML) method based on chloroplast *rbc*L sequences. The genus *Fragilaria* is highlighted in green color, and our species is specified in bold. Strains marked with asterisk (*) are taken from Kahlert *et al.* (2019). Coscinodiscophyceae members were used as an outgroup.

ends. All characteristics of the cells in this study were consistent with *F. saxoplanctonica* isolated from a lake in Saxony, Germany (Lange-Bertalot and Ulrich, 2014).

Ecology: This species was collected from freshwater. Temperature at sampling was 14.8°C, pH was 9.3.

Site of collection: Paldang Reservoir, Gyeonggi-do, Korea (GPS code: 37°29′16″N 127°17′14″E).

Date of collection: April 13, 2022.

Gene sequences: partial 18S rRNA (GenBank No. OQ359101), and partial *rbc*L (GenBank No. OQ361707) gene sequences.

Molecular affiliation by 18S rRNA and *rbc*L: 18S rDNA (1,645 bp) and *rbc*L (978 bp) sequences of *F. saxoplanctonica* were determined by using the Korean isolate. There were no recorded sequences of *F. saxoplanctonica* in GenBank database. That is, the 18S rDNA and *rbc*L sequences of *F. saxoplanctonica* were sequenced for the first time. The 18S rDNA sequence of our species was nearly identical (99.82% similarity) with *F. tenera* var. *nanana* (AM497739), and *rbc*L sequence was 98.06% similarity to *F. heatherae* (MK672907). Due to the lack of 18S rDNA from *F. heatherae* and *rbc*L from *F. tenera* var.

nanana, the relationship between the three species could not be compared.

Our 18S rDNA and *rbc*L ML trees both include three classes (Coscinodiscophyceae, Mediophyceae, and Bacillariophyceae), and within Bacillariophyceae, there are three orders: Thalassiophysales, Licmophorales, and *Fragilaria*les (Figs. 3 & 4). Both trees show that *Fragilaria* members cluster together, and the genus is closely related to genus *Ulnaria*, forming a separate clade from *Fragilaria*. Within the *Fragilaria* clade, *F. thornata* Tan & Kim forms a sister relationships with *F. saxoplanctonica* in the 18S rDNA ML tree (Fig. 3), and with *F. rumpens* and *F. radians* in the *rbc*L ML tree (Fig. 4). Although both trees share many common species, it showed considerable differences within the *Fragilaria* clade at the species level. These results show that the sequences from our isolated diatom clearly belong to the genus *Fragilaria*.

In conclusion, morphological and molecular characteristics of freshwater diatoms isolated from Paldang Reservoir were analyzed and identified as *F. saxoplanctonica*. This species has the same morphological key characteristics compared to the known *F. saxoplanctonica*, and its 18S rDNA and *rbc*L sequences were clustered within the clade of *Fragilaria*. These results confirmed that our species was *F. saxoplanctonica*, and it was the first record in Korea.

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