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Giffenia koreana sp. nov. (Bacillariophyta): A newly identified epipelic diatom in the tidal flat sediments of Suncheonman Bay, extant for over 1,400 Years

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Abstract

An unknown *Giffenia* taxon was sampled from the tidal flat sediment of Suncheonman Bay in the Republic of Korea on June 11, 2018. To identify the species, a detailed analysis using light and electron microscopy and a literature review were conducted. The important morphological characteristics—thin lip-shaped sternum, elongated alveoli, and eccentric raphe slit—were found in the species. These features are not present in previously described *Giffenia* taxa (*Giffenia cocconeiformis*). The new diatom species is characterized by (1) a slightly undulated valve face, (2) a lip-shaped sternum with a slightly rough surface, (3) numerous elongated alveoli on the valve face, and (4) an eccentric raphe slit. We propose *Giffenia koreana sp. nov.* as a new epipelic diatom taxon.

Key words: diatoms, *Giffenia*, novel species, wetland

Introduction

The genus *Giffenia* F.E. Round & Basson (1997) was established to accommodate taxa originally described in, or transferred to, the genus *Nitzschia* Hassall (1845) (Round & Basson 1997). *Giffenia* taxa exhibit oval or lanceolate frustules in valve view, rectangular frustules in girdle view, transapically undulated valve faces, slightly raised edges of the valves, a continuous raphe on the mantle side of each valve, two raphes located on each frustule, hooked raphe slits at the apices, an off-center indented sternum, nearly parallel striae consisting of multiseriate rows, externally elongated chambers formed by alveoli with multiseriate striae, and internally large foramina in the alveoli situated on the valve (Round & Basson 1997).

Giffenia cocconeiformis was first recorded in the slide collection of Cleve & Moller (1879), and Grunow (1880) later on described and recorded this species as *Nitzschia cocconeiformis* using samples from Bengal and the Gulf of Carpentaria. Hustedt illustrated the species in Schmidt's Atlas, Pl. 331/17, 18, from material collected at Dar es Salaam, and it has also been recorded by Cholnoky (1968) in South Africa. Kuntze (1898) recorded this species as *Homoeocladia cocconeiformis* (Grunow) Kuntze 1898. Round & Basson (1997) introduced the novel genus *Giffenia* from a re-investigation of *N. cocconeiformis* Grunow, using material deposited in the Natural History Museum (London) by Giffen. Currently, *Giffenia cocconeiformis* Round & Basson (1997) is the only known species in the genus *Giffenia* (Guiry & Guiry 2023).

Suncheonman Bay, one of the Republic of Korea's most representative wetlands, is a coastal wetland comprising 21.6 km² of tidal flats and 5.4 km² of reed fields. Suncheonman Bay was first registered on the Ramsar Convention

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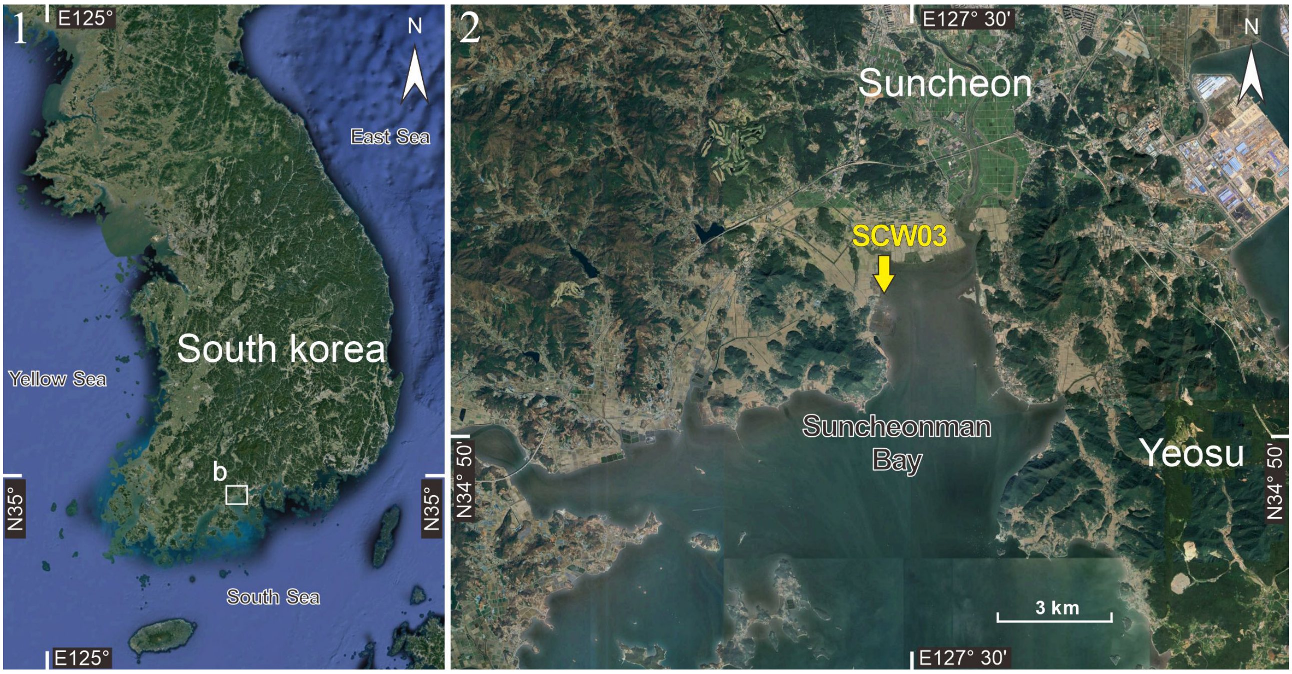
list in the Republic of Korea in January 2006 and was designated as a "wetland protected area" by the Ministry of Land, Transport and Maritime Affairs in December 2003 (Jang & Cheong 2010). In June 2008, Suncheonman Bay was designated as a national cultural property, "Myeongseung," number 41, and was included in the UNESCO World Heritage Tentative List in 2010 (Kim *et al*. 2013).

In our previous study, Park *et al*. (2021) investigated fossil diatoms and identified 87 diatom taxa, including six newly recorded diatom species in the Republic of Korea. These were sampled from a sedimentary core (SCW03) in Suncheonman Bay. In that study, an unknown *Giffenia* taxon, *Giffenia* sp., was found in the surface layer (0.1 m) of the sediment core, extending to a depth of 6.0 m at 0.5 m intervals, covering a duration of 1,400 years.

In a subsequent taxonomic study, we observed and identified the unknown *Giffenia* taxon reported by Park *et al*. (2021). The present study aims to describe this species as new to science using extensive light microscopy (LM) and scanning electron microscopy (SEM) observations, as well as comparisons with globally described *Giffenia* taxa.

Materials and methods

Coring was performed from the tidal flat sediment of Suncheonman Bay (34°52'10.1266" N, 127°29'27.5667" E) in the Republic of Korea using a peat core sampler (Peat Sampler, Eijkelkamp Soil & Water, Giesbeek, Netherlands, 52 mm in diameter) on June 11, 2018 (Figure 1, 2; Table 1). A sediment core (SCW03) with a total length of 6.0 m was drilled. The recovered core sediments, housed in a PVC pipe, were transported to the laboratory after being vacuum-packed in a plastic bag to prevent drying and oxidation. Once in the lab, the sediments in the PVC pipe were vertically bisected, and samples were taken for chronological and diatom analyses.



FIGURES 1, 2. Map of the study area. Fig. 1: A satellite map of the Republic of Korea. Fig. 2: Location of the study area, SCW03.

TABLE 1. Information of Sampling Sites.

Site	Address	Altitude(m)	Latitude (N)	Longitude (E)
SCW03	Haksan-ri, Byeollyang-myeon, Suncheon-si, Jeollanam-do, Republic of Korea	1.659	34°52'10.1266"	127°29'27.5667"

Age dating was conducted using shell fragments found in the SCW03 core and analyzed with an accelerator mass spectrometer (AMS) at the Korean Institute of Geoscience and Mineral Resources (KIGAM). The estimated ages were calibrated using the OxCal statistical analysis program (http://c14.arch.ox.ac.uk accessed on 20 May 2021).

For diatom analysis in the SCW03 core, 1.0 g of sediment was sampled at 0.1 m depth (surface of the core) and continued to a depth of 6.0 m at 0.5 m intervals. The sediment samples were dried at 60 ºC for 24 h, boiled with 10 mL of 30% hydrogen peroxide (H_2O_2), and then rinsed with distilled water to remove organic matter. Cleaned diatom samples were mounted using Mountmedia (Wako, Japan) to create permanent slides for analysis via light microscope (LM; Eclipse Ni, Nikon, Tokyo, Japan). Microphotographs were captured using a digital camera (DS-Ri2, Nikon, Tokyo, Japan) connected to the LM. Some cleaned samples were filtered using 2.0 µm polycarbonate membrane filters (Nuclepore, Whatman, Maidstone, UK), and these membranes were affixed to aluminum stubs. The samples on the stubs were coated with gold-palladium for ultra-structure analysis in diatoms using a field emission scanning electron microscope (FE-SEM; MIRA 3, TESCAN, Brno-Kohoutovice, Czech Republic). Morphological analyses of diatoms were performed using ImageJ v1.32 software (NIS-Elements BR4.50.00, Nikon, Tokyo, Japan). Taxonomical nomenclature and identification were based on recent references.

Results

Division: Bacillariophyta
Class: Bacillariophyceae Haeckel 1878
Subclass: Bacillariophycidae D.G. Mann 1990
Order: Bacillariales Hendey 1937
Family: Giffeniaceae Round & Basson 1997
Genus: *Giffenia* Round & Basson 1997

Giffenia koreana S.D. Lee & M. Park *sp. nov.* (Figs 3–23)

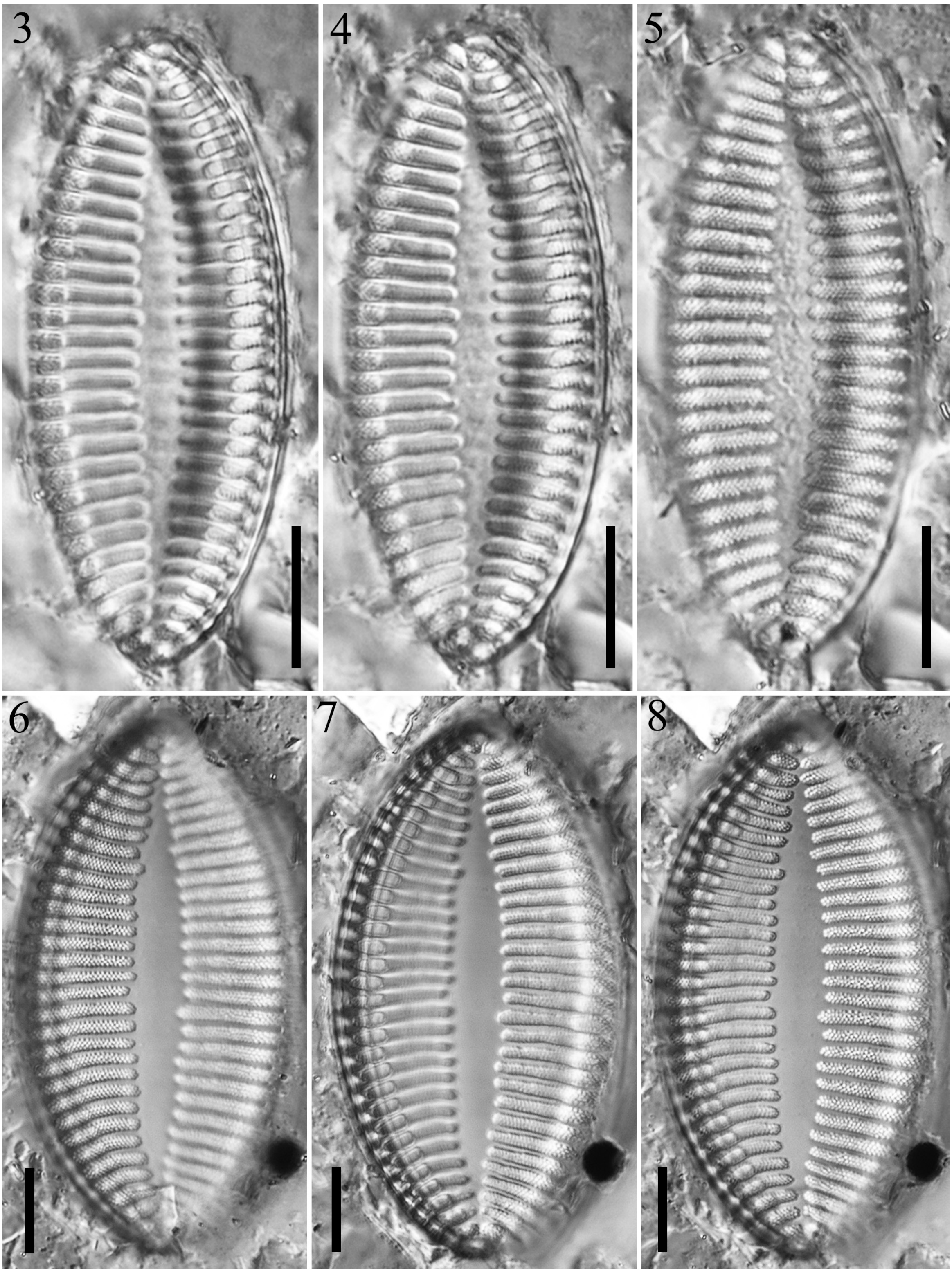
Light Microscopy Observations (Figs 3–8)

Cells are solitary. Valves have an oval to lanceolate outline, measuring 67.7–80.6 µm in length and 37.5–40.6 µm in width. The valve face is slightly undulated. Around the apical axis, one side is slightly concave while the other side is slightly convex. Valves are symmetric along the apical plane. The sternum appears straight on the side without a raphe, and curved on the side with a raphe. The thin lip-shaped sternum is located nearly at the valve's center and has a slightly rough surface. Numerous alveoli, situated at a density of 7 per 10 µm, are found on the valve face. The alveoli are almost straight near the center of the valve and become slightly curved closer to the apex.

Scanning Electron Microscopy Observations (Figs 9–23)

Valves are oval to lanceolate in valve view (Figs 9, 10, 12, 15). In girdle view, cells appear rectangular (Figs 9, 11, 15). The mantle is more or less vertical. The valve face is transapically slightly undulated (Figs 9, 15). Around the apical axis, the valve surface is slightly concave on one side and slightly convex on the other (Figs 9, 15, 17). The edges of the valve are slightly rounded (Figs 9, 13, 15). The lip-shaped sternum is located on the central valve face, which has a slightly rough surface and numerous irregular fine pores (Figs 9, 10, 15, arrow). On one side (raphe-less side), the sternum is straight, and on the other side (raphe side), it is slightly curved outward (Figs 9, 10, 13). The internal valve face features slightly circular openings for the alveoli (Fig. 12). These internal alveolar openings are located through slightly large circular foramina (7 per 10 µm) situated at the junction between the valve face and the mantle (Fig. 12).

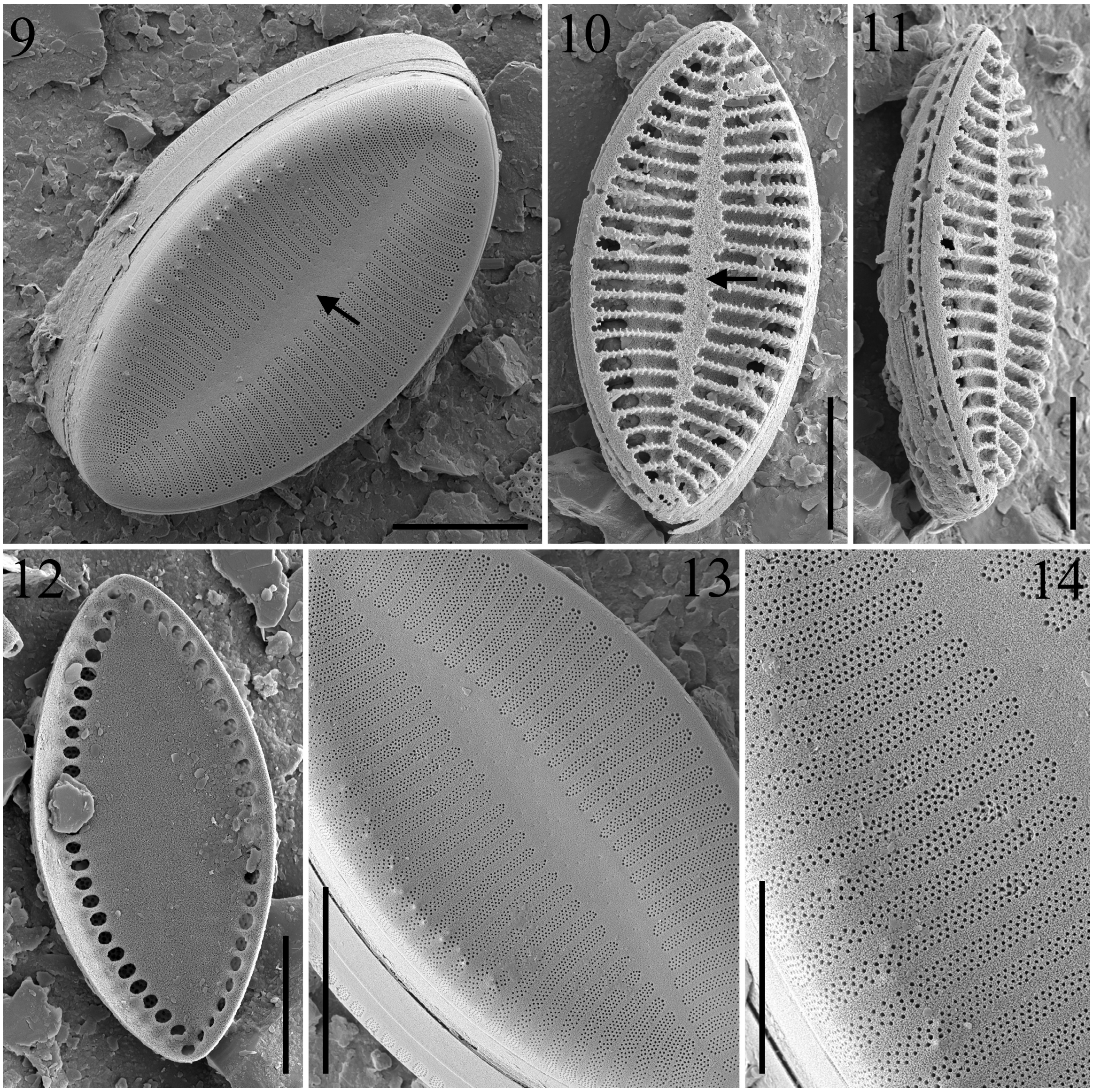
An elongated transapical chamber, or alveolus, is located on the outer valve surface (Figs 13, 14). The alveoli are positioned at a density of 7 per 10 µm on the valve surface. The shape of the alveolus is asymmetric relative to the apical axis and consists of conspicuously multiseriate striae (4–5 striae per alveolus). The alveoli are almost straight closer to the center of the valve (Fig. 16, arrow) and slightly curved nearer to the apex (Figs 18, 19, arrow). The raphe is positioned on one side of each valve, along the mantle (Figs 11, 15, 16). The two raphes are located on opposite sides of the two valves. On the valve face at the raphe side, rows of striae cease at the valve face's ends (Figs 15, 16), but on the other side (raphe-less side), rows of striae continue downward onto the mantle (Figs 14, 22). On the mantle at the raphe-less side, the number of pores increases and their size decreases, leading to irregularly increased pores density within the striae. A hyaline area appears between the raphe slit and the alveoli on the valve (Figs 16, 18, 19). The rows of striae are located at a density of 11 per 10 µm on the valve face. The striae are almost parallel to the transapical axis, except at the apices, where they curve slightly closer to the valve apex. The size of pores near the raphe slit on the valve are larger (Figs 16, 18, 19, arrowheads) than those elsewhere on the valve face. The raphe ends are downturned, resembling either a J-shape (Figs 18, 19) or a square root symbol shape (√) at the valve apex (Figs 17, 22). On the mantle at the raphe side, nail-shaped clusters of areaole are positioned below the raphe slit (7 per 10 µm) (Fig. 23, arrows). The girdle consists of three cupulae, first valvocopulae is wide (Figs 22, 23).



FIGURES 3–8. LM images of the undulating external valve view of *Giffenia koreana sp. nov.* on the holotype (Figs 3–5) and isotype (Figs 6–8). Fig. 3, 4: The valve face focused on the slightly convex side. Fig. 5: The valve face focused on the slightly concave side, with an uneven central sternum positioned along the apical axis. Figs 6–8: The valve face observed on various focuses. Scale bar: 10 µm.

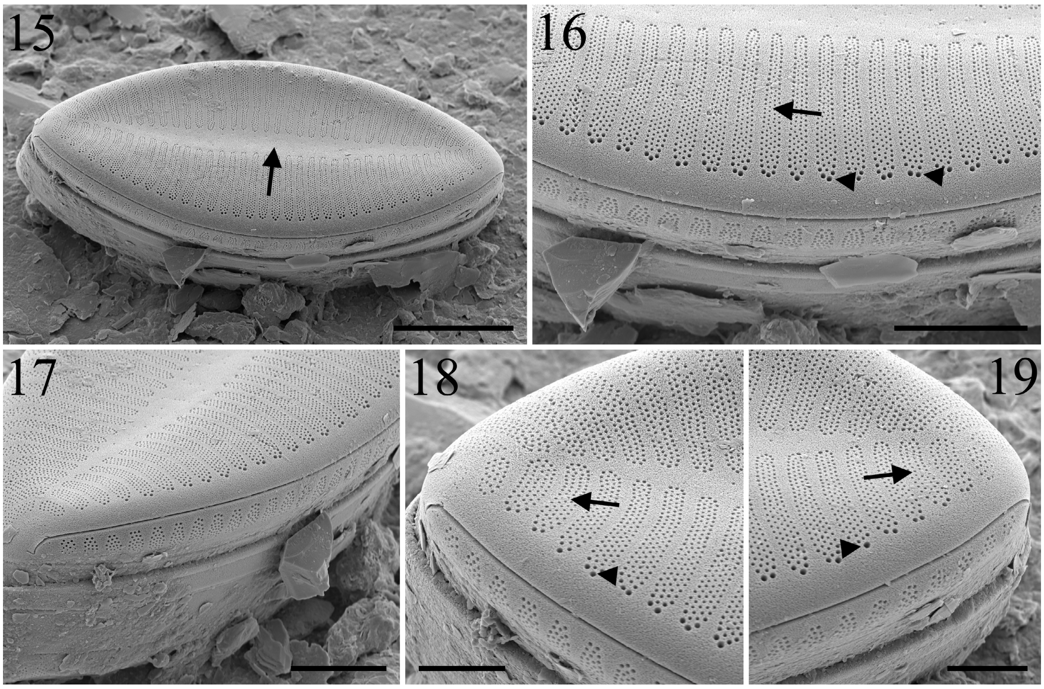
Type:—REPUBLIC OF KOREA, Suncheon-si: Collected at a depth of 2.5 m in the tidal flat of Suncheonman Bay wetland (34°52'10.1266" N, 127°29'27.5667" E) on June 11, 2018. Slide NNIBRDI24163 (holotype); Slides

NNIBRDI24164, NNIBRDI24165, NNIBRDI24166 (isotypes). The holotype and isotypes have been deposited in the NNIBR (Nakdonggang National Institute of Biological Resources) in the Republic of Korea (Figures 3–8).

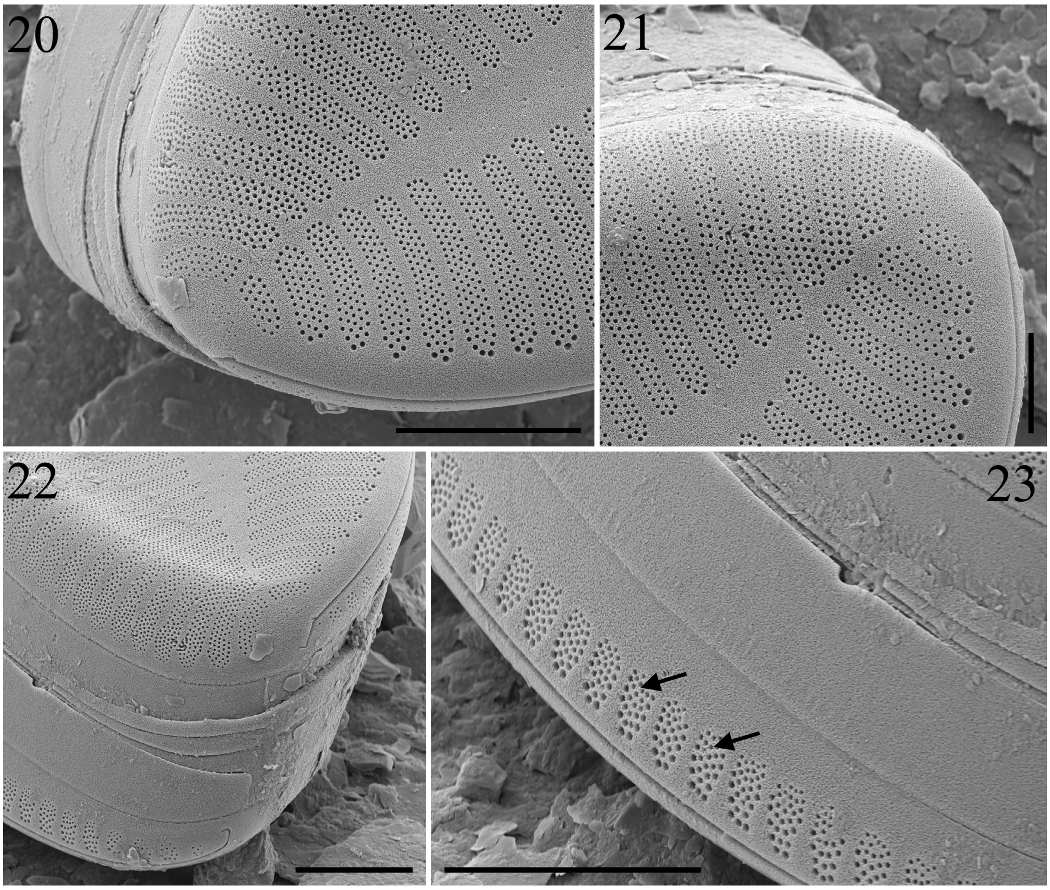


FIGURES 9–14. SEM images of *Giffenia koreana sp. nov.* featuring type materials. Fig. 9: A whole cell displaying the thin lip-shaped sternum (arrow), accompanied by numerous alveoli consisting of rows of pores. Fig. 10: The valve face with disrupted rows of pores and the thin lip-shaped sternum (arrow). Fig. 11: Side of the valve showing the continuous, whole raphe slit. Fig. 12: Internal valve face featuring the internal openings of the alveoli. Fig. 13: The valve face with a central thin lip-shaped sternum. Fig. 14: The alveoli with multiseriate pores on the external valve face. Scale bars: 10 µm for Figs 10–12, 14; 20 µm for Figs 9, 13.

Ecology and Distribution:—*Giffenia koreana sp. nov.* is a benthic and epipelic diatom, as the taxon is distributed from the surface to a sedimentary layer at a depth of 6.0 m in the tidal flat of Suncheonman Bay, Republic of Korea, according to this study. The sampling site, Suncheonman Bay, has brackish water conditions with a salinity of 25.87 PSU, a temperature of 20.3 ºC, slightly neutral pH (7.63), high conductivity (40,395 µS/cm), and dissolved oxygen (DO) levels of 5.06 mg/L. The previously known *G. cocconeiformis* is distributed in Mexico (Torres-Ariño *et al*. 2019), Iraq (Al-Saedy *et al*. 2020), Kuwait (Al-Kandari & Suburova 2019), India (Gupta & Das 2020), the Philippines (Ohtsuka *et al*. 2009, Martinez-Goss 2021), the Republic of Korea (Lee *et al*. 2012), and Chuuk Lagoon (Park *et al*. 2022). *Nitzschia cocconeiformis*, a synonym of *G. cocconeiformis*, is found in South Africa (Giffen 1967), Iraq (Maulood *et al*. 2013, Al-Saboonchi & Al-Shawi 2016), the Philippines (Martinez-Goss 2021), China (Liu 2008), and Taiwan (Shao 2003–2014). *Tryblionella cocconeiformis*, another synonym, has been reported in Turkey (Taskin *et al*. 2019), India (Gupta & Das 2020), Singapore (Pham *et al*. 2011), Taiwan (Shao 2003–2014), Australia (McCarthy 2013), and Kuwait (Al-Yamani & Saburova 2011) (Table 2).



FIGURES 15–19. SEM images of *Giffenia koreana sp. nov.* on the type materials. Fig. 15: A whole cell displaying the central thin lip-shaped sternum (arrow), numerous alveoli, and raphe slit. Figs 16–19: A hyaline area between the raphe slit and the alveoli (arrow). Figs 17–19: Valve apex featuring a downturned raphe apex, and with curved alveoli (arrow). Scale bars: 5 µm for Figs 18, 19; 10 µm for Figs 15–17.



FIGURES 20–23. SEM images of *Giffenia koreana sp. nov.* using type materials. Figs 20, 21: Valve face displaying the terminal valve apex with a hyaline area. Fig. 22: The apex of the epi/hypo-valve featuring a terminal hooked raphe and numerous alveoli. Fig. 23: The mantle showing numerous nail-shaped pores clusters positioned below the raphe slit (arrow). Scale bars: 5 µm for Fig. 21; 10 µm for Figs 20, 22, 23.

TABLE 2. Distribution of *Giffenia* taxa.

Species	Distribution	Habitat	Reference
Giffenia cocconeiformis	Mexico	Marine	Torres-Ariño *et al*. 2019
	Iraq	Marine to brackish water	Al-Saedy *et al*. 2020
	Kuwait	Marine	Al-Yamani & Saburova 2019
	India	Salt lake	Gupta & Das 2020
	Philippines	Marine	Ohtsuka *et al*. 2009, Martinez-Goss 2021
	Korea	Brackish and coastal water	Lee *et al*. 2012
	New Zealand	-	Harper *et al*. 2012
	Australia	-	John 2016
	Chuuk Lagoon	Marine	Park *et al*. 2022
Nitzschia cocconeiformis (as syn. of *G. cocconeiformis*)	South Africa	Brackish, Marine	Giffen 1967
	Iraq	Marine	Maulood *et al*. 2013, Al-Saboonchi & Al-Shawi 2016
	Philippines	Marine	Martinez-Goss 2021
	China	Marine	Liu 2008
	Taiwan	Marine	Shao 2003–2014
Tryblionella cocconeiformis (as syn. of *G. cocconeiformis*)	Turkey	-	Taskin *et al*. 2019
	India	Salt lake	Gupta & Das 2020
	Singapore	Marine	Pham *et al*. 2011
	Taiwan	Marine	Shao 2003–2014
	Australia	Marine	McCarthy 2013
	Kuwait	Marine	Al-Yamani & Saburova 2011
G. koreana sp. nov.	Republic of Korea	Brackish	This study

Etymology:—The specific epithet *koreana* refers to the type locality in the Republic of Korea.

Discussion

The new diatom species, *Giffenia koreana sp. nov.*, was initially reported as an unidentified species (*Giffenia* sp.) in a previous study (Park *et al*. 2021). Subsequently, the species was confirmed to be novel through extensive electron microscopic analyses and a review of references.

G. cocconeiformis and *G. koreana sp. nov.* exhibit different morphological characteristics. *G. koreana sp. nov.* (Length: 47.7–80.6 µm, Width: 37.5–40.6 µm) is approximately 1.5 times larger than *G. cocconeiformis* (Length: 39–65 µm, Width: 22–29 µm). The number of striae for *G. cocconeiformis* is 6.5–10 per 10 µm, compared to 11 per 10 µm for *G. koreana sp. nov.* Additionally, *G. koreana sp. nov.* (rows of pores in an alveolus: 4–5; copulae: 3) has two more rows of pores in an alveolus and one fewer copula than *G. cocconeiformis* (rows of pores in an alveolus: 3; copulae: 4). *G. cocconeiformis* has an indented sternum, while *G. koreana sp. nov.* has a thin, lip-shaped sternum. Both species possess a lateral raphe, but in *G. cocconeiformis*, it forms an upturned apex, whereas in *G. koreana sp. nov.*, it assumes a J or square root symbol (√) shape. However, both species share the same valve face, which is transapically undulated, and the same number of alveoli: 7 per 10 µm and bisymmetrical lanceolate overall shape (Table 3).

Round & Basson (1997) proposed the genus *Giffenia* as a new genus, observed the indented sternum in *G. cocconeiformis*, and described this feature as one of the most important morphological characteristics of the genus *Giffenia*. In this study, *G. koreana* displayed a thin, lip-shaped sternum that was not indented. Therefore, we propose that the indented sternum is a morphological feature specific to *Giffenia cocconeiformis* rather than a general characteristic of the genus *Giffenia*.

The important morphological characteristics of *G. koreana* are a thin lip-shaped sternum, elongated alveoli, and an eccentric raphe slit. The sternum is a longitudinal silica element of the valve of pennate diatoms (Round *et al*. 1990). It is typically thickened and hyaline. In many genera, such as *Navicula*, the sternum is positioned along the apical axis, but in others, like *Eunotia*, it is positioned along the valve margin (Round *et al*. 1990). In many raphid diatoms, the sternum houses the raphe and is synonymous with the axial area (Round *et al*. 1990). The sternum serves as the ontogenic center of pennate valves; it is the first silica deposited in valve formation following cell division (Round *et al*. 1990). In this study, *G. koreana* features a thin lip-shaped sternum.

TABLE 3. Morphological Differences Between *G. cocconeiformis* and *G. koreana sp. nov.* (This Study).

Morphology	***G. cocconeiformis***	***G. koreana sp. nov.***
Long (μm)	39–65	67.7–80.6
Width (μm)	22–29	37.5–40.6
Valve face	Transapically undulate	Transapically undulate
Striae (per 10 μm)	6.5–10	11
Alveoli (per 10 μm)	7	7
Interstriae	Raised	Flat
Rows of pores in an alveolus	3	4–5
Copula	4	3
Sternum	Indented	Thin lip-shaped
Raphe	Lateral raphe, Upturned apex	Lateral raphe, Epivalve: J shape or √ shape

The alveolus is an elongated chamber that forms part or all of a stria (Round *et al*. 1990, Cox 2011). Genera such as *Pinnularia* and *Caloneis* possess alveoli. The external wall of an alveolus is covered by a plate of fine pores, while the internal wall has at least one elongated opening (Round *et al*. 1990, Cox 2011). Elongated alveoli were observed in *G. koreana sp. nov.* in this study.

The raphe is an opening, or slit, within the silica cell wall, consisting of the slit itself and the mucilaginous material secreted from the cell, which is used for cell motility. A raphe can contain one or two slits that penetrate the valve of some diatoms. If two slits are present, each is termed a branch of the raphe, and branches may be separated by a central nodule (Round *et al*. 1990, Cox 2012). The raphe of *Giffenia koreana sp. nov.* comprises a single slit without a central nodule. The position of the raphe can be (1) axial, along the apical axis; (2) eccentric, along one margin; or (3) circumferential, around the whole margin of the valve (Round *et al*. 1990, Cox 2012). An eccentric raphe was identified in *G. koreana sp. nov.* in this study.

Previously known *G. cocconeiformis* distributions range from Chuuk Lagoon (Park *et al*. 2022), near the equator, to South Korea (Lee *et al*. 2012), situated in temperate regions. Additionally, the species has been found in the southern hemisphere, specifically in New Zealand (Harper *et al*. 2012) and Australia (John 2016). *Nitzschia cocconeiformis* and *Tryblionella cocconeiformis*, synonyms of *G. cocconeiformis*, have also been found in both the northern and southern hemispheres, from tropical to temperate regions. If further studies are conducted, *Giffenia* taxa could serve as indicator species for global warming and become important for assessing rising seawater temperatures or estimating climate change through continuous monitoring.

Ohtsuka *et al*. (2009) reported *Giffenia cocconeiformis* based on the illustration of light microscopic photographs from Laguna de Bay, the largest lake in the Philippines. Upon comparing the illustrations of *Giffenia cocconeiformis* in that study with the microphotographs of *G. koreana* in this study, it was determined that the morphological characteristics were nearly identical. Therefore, it is highly likely that the species is *G. koreana*, not *G. cocconeiformis*. Additional LM and SEM analyses of samples from Laguna de Bay in the Philippines are needed for further study.

Giffenia koreana sp. nov. is found in all sections from 0.1 m to 6.0 m at 0.5 m intervals in core SCW03, both in this study and the previous report (as *Giffenia* sp.) (Park *et al*. 2021). Considering the AMS age-dating results of core SCW03 (Park *et al*. 2021), this species, identified as *G.* sp., appears to have continuously adapted to this area for at least 1,400 years, despite periods of global temperature anomalies such as the Medieval Warm Period (MWP) and the Little Ice Age (LIA) (Loehle 2007, Ma *et al*. 2011, Wassenburg *et al*. 2013). This suggests that the temperature range in which this species can survive is broad and indicates that this species may be found in regions or countries other than Suncheonman Bay in the Republic of Korea. Future studies should consider isolating and culturing living *G. koreana sp. nov.* to confirm the temperature, salinity, and pH ranges in which this species can thrive.

Acknowledgments

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