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Taxonomy and distribution of two small negligible diatoms of *Plagiogrammopsis minima* and *Diploneis aestuarii* from Northeast Asian tidal flats

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Tidal flats are well developed in and around the Yellow Sea of Northeast Asia, and benthic diatoms are the most important primary producers in corresponding habitats. In the present study, the taxonomy and distribution of small negligible diatom species from Northeast Asian tidal flats are investigated for better understanding of the diversity of Korean marine benthic diatoms. The presence of *Plagiogrammopsis minima* and *Diploneis aestuarii*, which may have been ignored and/or misidentified due to their small size, were identified by both means of light microscopy (LM) and scanning electron microscopy (SEM). *Plagiogrammopsis minima* has never been reported from Korea before the present study, while *Diploneis aestuarii* was only once mentioned without any photographic information. Accordingly, we provide the morphological characteristics of the two species in detail with LM and SEM observation. Information on the regional distribution of the two species is also provided. Results of the present study contribute to the better understanding of the biodiversity of the Korean marine benthic diatoms.

Keywords: diatom, *Diploneis aestuarii*, distribution, *Plagiogrammopsis minima*, taxonomy

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Introduction

Diatoms are one of the most dominant groups of eukaryotic algae, thriving in a variety of aquatic habitats on Earth (Round, 1971). They play a crucial role in the global ecosystem, e.g. fixing CO_2 (Leblanc *et al*., 2018), stabilizing sediment (Vos *et al*., 1988), providing primary energy sources in the trophic level (Anu *et al*., 2018), and contributing to biogeochemical cycling (Benoiston *et al*., 2017). More than 200 genera of living diatoms and perhaps as many as 100,000 extant species have been identified and reported (Mann and Vanormelingen, 2013), and most recent estimations of the number of diatom species range from 12,000 to 30,000 (Malviya *et al*., 2016).

In terms of the history of taxonomic studies on marine benthic diatom in Korea, Skvortzow (1931) first reported 67 marine diatoms, of which only a few were benthic species. Skvortzow (1932) reported 83 diatom taxa from sediments of the Yellow and East Seas. Shim and Cho (1984) were the first Korean scientists to report on the marine benthic diatom communities in Korean tidal flats, followed by the work of Choi and Noh (1987) who made further taxonomic progress with the application of scanning electron microscopy (SEM). More recently, taxonomic studies of marine benthic diatoms have dramatically increased with reports of new taxa and new records in Korea (Park *et al*., 2012a; 2013). Through these efforts, total of 2332 diatom species, including about 520 marine species, have been compiled from Korean waters based on Lee and Joh (2015), yet the diversity of the Korean marine benthic diatoms seems to be still underestimated (Park *et al*., 2014).

Proper appreciation for the diversity of Korean marine benthic diatom has been limited because of the short history and lack of literature. Furthermore, small-sized diatoms are easily overlooked and/or misidentified particularly under light microscopy (LM). Thereafter, the correct identification of diatoms based on thorough morphological investigation is essential for better understanding of biodiversity of the Korean marine benthic diatoms. In the present study, we report the detailed information of the morphology and biogeography of two small-sized diatoms

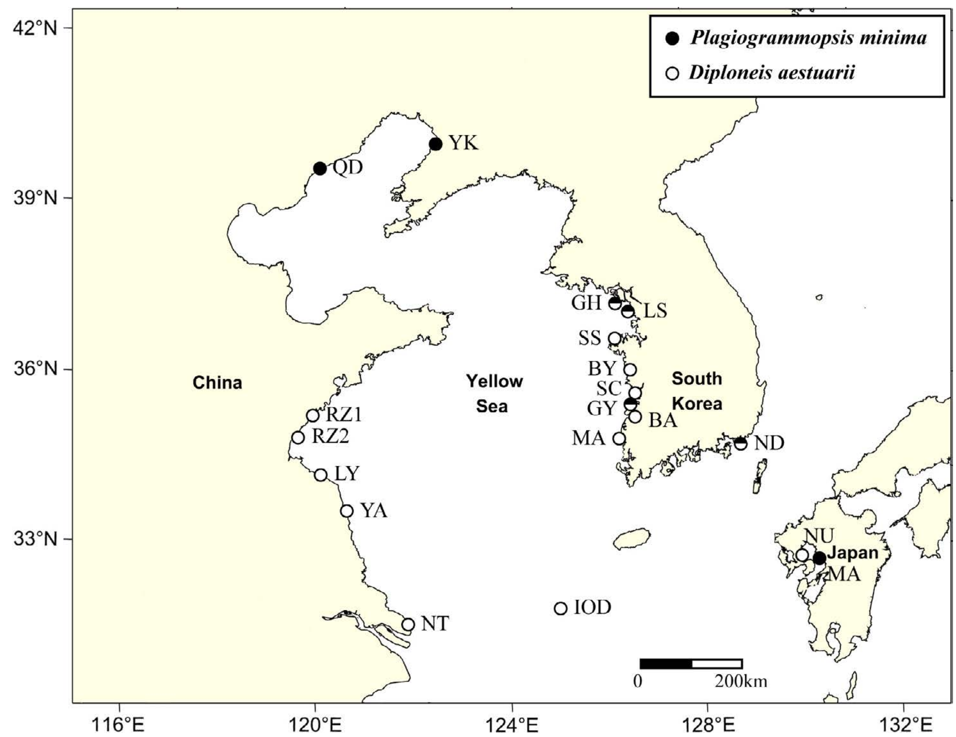

Fig. 1. The distribution of *Plagiogrammopsis minima* and *Diploneis aestuarii* in the Northeast Asia.

viz. *Plagiogrammopsis minima* and *Diploneis aestuarii*.

Materials and Methods

The upper layer of sediments (ca. 0–0.5 cm) were scarped with a stainless-steel spatula and collected into the 50 mL conical tube from ten stations in Korea (from 2006–2020), seven stations in China (from 2007–2018), and two stations in Japan (from 2002–2007) along the Northeast Asian coast (Fig. 1, Table 1). The sediments were fixed with 4% formaldehyde in the field then transferred to the laboratory for further treatment. The sediment samples were cleaned following the methods by Park (2011) and Kurzydłowski and Zgłobicka (2013). Sediment samples were sonicated with the addition of filtered seawater and decanted five times, consecutively for 10, 15, 30, 45, and 60 seconds in an ultrasonic water bath for the separation of diatom cells from sediment particles. The separated cells were then cleaned with HCl and H_2O_2 to remove calcium carbonate particles and organic substances, respectively. The cleaned materials were then mounted and dried on coverslips. Permanent slides were prepared using Naphrax resin (Brunel Microscopes Ltd., Chippenham, United Kingdom). Diatom frustules were observed and photographed under the Olympus BX51 and BX53 light microscope (Olympus, Tokyo, Japan) equipped with an Olympus Tech X Cam III (Olympus, Tokyo, Japan) and ZEISS Axiocam 305 color (Carl Zeiss, Oberkochen, Germany), respectively. A few drops of cleaned material were also dried onto aluminum stubs with carbon tape and coated with platinum to examine the fine structures of the diatom frustules using a field emission SEM (MIRA-3 and MIRA-4, Tescan, Brno, Czech).

Results

This is the first observation of *Plagiogrammopsis minima* from Korea, and the second report of *Diploneis aestuarii* with the first provision of photographic information and morphological description. Taxonomic characteristics of interest include the valve outline, microstructures (e.g. central area and raphe system), and the density of striae and areolae. Particularly SEM observations were made to clarify the ultrafine structure of the frustules. Finally, the distributions of two target species is also addressed in Northeast Asia.

***Plagiogrammopsis minima* (Salah) Sabbe & Witkowski, 2010 (Figs. 2, 3)**

Reference. Salah, 1955, Hydrobiologia. 7, p. 91, pl. I, fig. 15; Sabbe *et al*., 2010, Vie et Milieu, 60(3), 243–256.
Taxonomic remarks. The generic features of *Plagiogrammopsis* comprise a pseudoseptum, laterally opening

Table 1. List of sampling stations from the Northeast Asian tidal flats.

Sampling date	Country	Station	Location	Latitude/Longitude	Sediment type
Oct. 24, 2006	Korea	GH	Yeocha-ri, Hwado-myeon, Ganghwa-gun, Incheon	37°35′N 126°22′E	Muddy sand
Jul. 16, 2018	Korea	LS	Lake Sihwa, Siheung-si, Gyeonggi-do	37°20′N 126°41′E	Mud
Jul. 03, 2007	Korea	SS	Oji-ri, Daesan-eup, Seosan-si, Chungcheongnam-do	36°57′N 126°22′E	Mud
Feb. 28. 2016	Korea	BY	Soseong-ri, Ocheon-myeon, Boryeong-si, Chungcheongnam-do	36°26′N 126°31′E	Mud
Jul. 14, 2018	Korea	SC	Dosam-ri, Maseo-myeon, Seocheon-gun, Chungcheongnam-do	36°01′N 126°44′E	Mud
Aug. 06, 2003	Korea	GY	Gyehwa-ri, Gyehwa-myeon, Buan-gun, Jeollabuk-do	35°46′N 126°34′E	Muddy sand
Jul. 14, 2018	Korea	BA	Upo-ri, Julpo-myeon, Buan-gun, Jeollabuk-do	35°34′N 126°39′E	Mud
May. 24, 2005	Korea	MA	Yongjeong-ri, Hyeongyeong-myeon, Muan-gun, Jeollanam-do	35°07′N 126°19′E	Mud
Apr. 14, 2020	Korea	ND	Nakdong river, Saha-gu, Busan	35°02′N 128°58′E	Muddy sand
Jun. 28, 2019	Korea	IOD	Ieodo Island, Seogwipo-si Jeju Province	32°08′N 125°11′E	Mud
Jul. 04, 2018	China	YK	Limoluocun, Yingkou prefecture, Liaoning province	40°25′N 122°17′E	Mud
Jun. 07, 2007	China	QD	Chengyang, Qingdao prefecture, Shandong province	36°15′N 120°19′E	Mud
Jun. 27, 2018	China	RZ1	Donggang, Rizhao prefecture, Shandong province	35°17′N 119°26′E	Muddy sand
Jun. 27, 2018	China	RZ2	Ganyu, Lianyungang prefecture, Jiangsu province	35°04′N 119°18′E	Muddy sand
Jul. 01, 2018	China	LY	Qiweigang, Lianyungang prefecture, Jiangsu province	34°30′N 119°46′E	Mud
Jul. 02, 2018	China	YA	Shawan harbor, Yancheng Prefecture, Jiangsu Province	33°48′N 120°28′E	Mud
Jul. 07, 2018	China	NT	Tongqi canal, Haimen, Nantong prefecture, Jiangsu province	31°56′N 121°49′E	Mud
Oct. 17, 2002	Japan	NU	Nanaura, Kashima, Saga Prefecture, Kyushu	33°00′N 130°10′E	Mud
May. 17, 2007	Japan	MA	Minami-arao, Kumamoto Prefecture, Kyushu	32°58′N 130°26′E	Sand

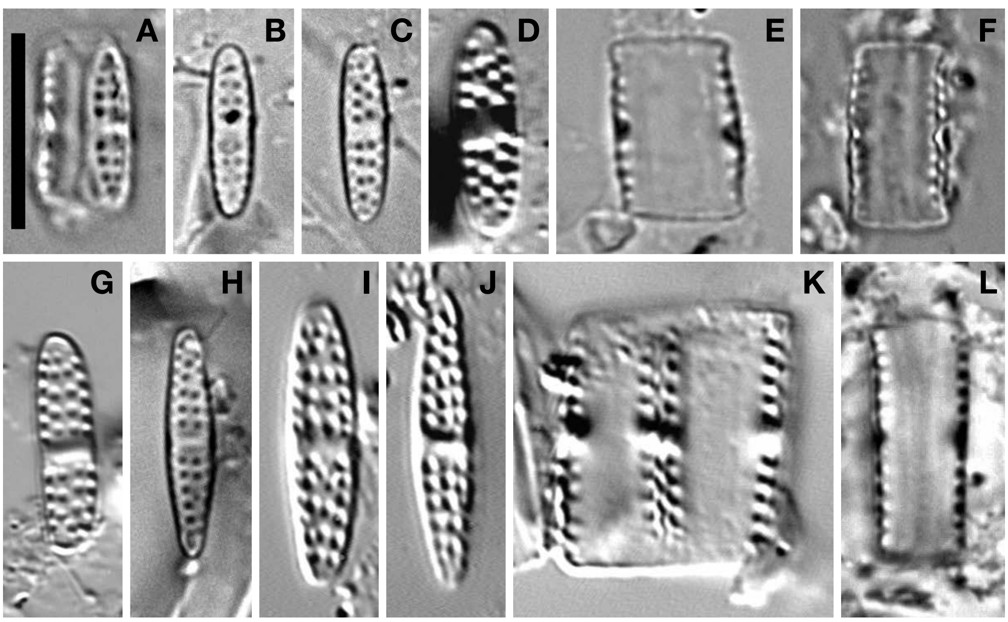

Fig. 2. Light microscope (LM) photographs of *Plagiogrammopsis minima*: A–D and G–J. Size range in valve view; E, F and K, L. Size range in girdle view. Scale bar = 10 μm.

ocelluli, long spines, and an external cribrum with spinules (Sabbe *et al*., 2010). *Plagiogrammopsis minima* has a somewhat complex taxonomic history. The species was first described as new species to science by Salah (1955) under the name of *Plagiogramma minimum*. In the meantime, certain specimens of the very species were also newly described and reported as *Plagiogrammopsis crawfordii* by Witkowski *et al*. (2000), yet the taxonomic characteristics of *P. crawfordii* were not properly defined. The description of the species did not match with photo-

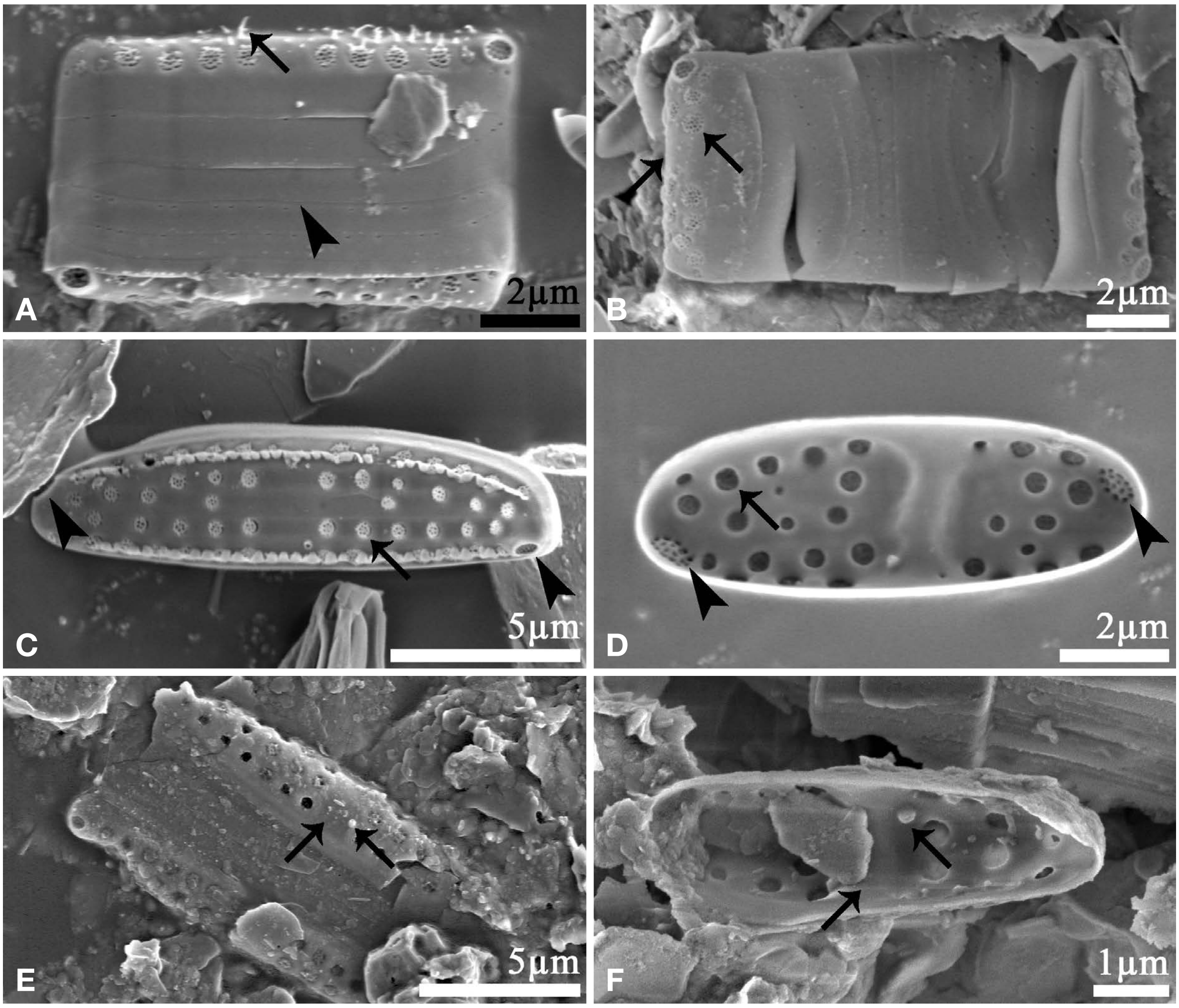

Fig. 3. Scanning electron microscope (SEM) photographs of *Plagiogrammopsis minima* from A−D (Minami-arao, Japan), E, F (Nakdong, Busan, Korea): A. Entire view of the girdle. Arrow indicates row of the short spines on the valve face. Large arrowhead indicates girdle bands ornamented by a row of poroids; B. External view of the valve face with girdle bands. Right arrow indicates pseudoseptum extends from margin to margin in the central fascia. Left arrow indicates the cribrum occlusion; C. External view of the whole valve. Large arrowheads indicate the two ocelluli which are twisted to opposite directions along the valve axis. Arrow indicates the cribrum occlusion; D. Internal view of the whole valve. Large arrowheads indicate the two ocelluli which are twisted to opposite directions at valve apex. Arrow indicates internal view of the cribrum occlusion located outside of the areolae; E. Right arrow indicates the pseudosptum while left arrow indicates external aperture of the rimoportula; F. Right arrow indicates the pseudosptum while left arrow indicates a rimoportula.

graphic information provided in the literature. Sabbe *et al.* (2010) found that part of the '*P. crawfordii*' specimens were conspecific with *P. minimum*; since *P. crawfordii* represents more than one species, it is taxonomically invalid. Sabbe *et al.* (2010) also found that *Plagiogramma minima* should be a member of the genus *Plagiogrammopsis* judging from its ultrastructure under SEM observation, thus the generic transfer of the species has been made under the nomenclature of '*Plagiogramopsis minima*'. In the meantime, the authors have also described a new species viz. *Cymatosira minutissima* based on certain other specimens of '*P. crawfordii*'.

The size of *P. minima* specimens from the present study is slightly larger compared to the original description of the species in Salah (1955), where the frustules were 6−14 μm in length and 2.5−3 μm in width. *Plagiogrammopsis minima* is similar to *C. minutissima* in terms of length (3−9 μm) and width (1.5−3 μm) with striae of 12−15 in 10 μm (Garcia, 2016). The central area of *P. minima* is distinctly constricted to pseudoseptum in valve view, and two ocelluli are laterally twisted in opposite directions at valve apices. *Cymatosira minutissima* is easily differentiated from *P. minima* in terms of the circular central area and centrally located two ocelluli.

LM observation. Outline of the valves is narrowly lanceolate with rounded apices, 8.3–16.0 μm in length, 2.0–3.7 μm in width (Fig. 2A–D, G–J). The frustules are rectangular with slightly produced apices in girdle view (Fig. 2E, F, K, L). Central area of the valve is distinctly constricted in LM due to the presence of pseudoseptum. No areolae are present at the apices or in the center of the valve. The density of striae is 12–15 in 10 μm, and the striae are rather irregular (Fig. 2A–D, G–J).
SEM observation. External view of the valve face is slightly curved along the edge, merging almost indiscernible with a rather deep mantle ornamented by a row of poroids (Fig. 3A, B). Two ocelluli are laterally twisted in opposite directions at valve apices (Fig. 3C, D). The central area presents a hyaline area and areolae are occluded by an external cribrum (Fig. 3C). Internal and external view of the pseudoseptum extends from margin to margin in the central fascia and has one rimoportula on one side of the pseudoseptum (Fig. 3D–F). The striae are developed irregularly one on the valve face and one along the valve margin (Fig. 3C, D).
Distribution. *Plagiogrammopsis minima* was firstly reported from salt marshes at Blakeney Point, Norfolk, England by Salah (1955). Sabbe *et al*. (2010) reported the species from sandy sediments at Westerschelde Estuary, North Sea, and the Portugal Atlantic Coast. The species was also observed from Bombas, State of Santa Catarina, Brazil by Garcia (2016). The present study is the very first report of *P. minima* from Korean waters. In the present study, the species was observed from four locations from Korea, two locations from China, and one location from Japan (Fig. 1).

***Diploneis aestuarii* Hustedt, 1939 (Figs. 4, 5)**

Reference. Hustedt, 1939, p. 612, figs. 41, 42; Simonsen, 1987, p. 254, figs. 376: 21–26.
Taxonomic remarks. The members of the genus *Diploneis* Ehrenberg ex Cleve are mostly marine or brackish species, with only a small number being freshwater species. Valve faces of the *Diploneis* species are to be divided into two parts, firstly longitudinal canal and also the marginal part (Jovanovska *et al*., 2015). The size of our specimens is slightly larger compared to the description of *Diploneis aestuarii* in Hustedt (1939) where the frustules were 15–20 μm in length and 6–7 μm in width. The density of striae, shape of frustules, square central nodule, and narrowly linear longitudinal canal are well matched with the original description of *D. aestuarii*. The majority of *Diploneis* species reported from Korea (Noh and Choi, 1992; Cho, 2015) are much larger in size compared to *D. aestuarii* and readily distinguishable. The size range of *Diploneis rimosa* Pennesi et Caputo sp. nov. (Figs. 98–107) (Pennesi *et al*., 2017) is similar to *D. aestuarii* both in terms of length (12–21 μm) and width (6.3–9.7 μm) with transapical striae of 16–20 in 10 μm. However, *D. aestuarii* has a much longer valve length compared to valve width and characteristic in having a central nodule with four areolae as C-shaped foramen.
LM observation. Outline of the valves is linear-elliptic, slightly constricted in the central shape with bluntly rounded apices, 10–21.3 μm in length, 5.3–7.3 μm in width. Raphe-sternum along with longitudinal canal is narrowly linear. The central nodule is rarely distinct but small and square. Transapical striae are almost parallel

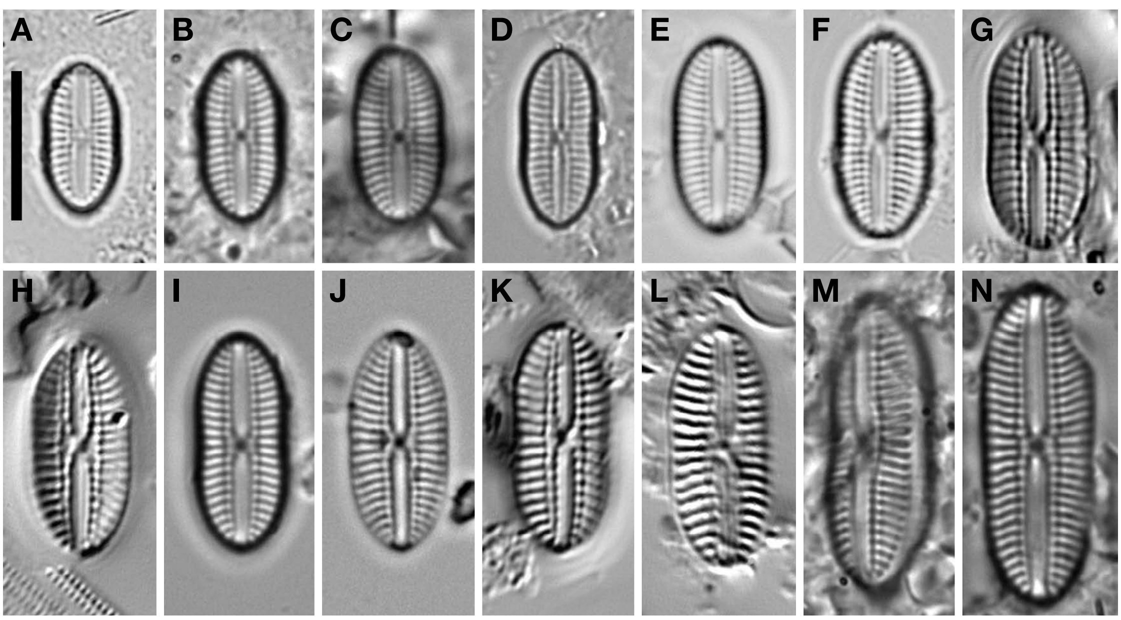

Fig. 4. Light microscope (LM) photographs of *Diploneis aestuarii*: A–N. Size range in valve view. Scale bar = 10 μm.

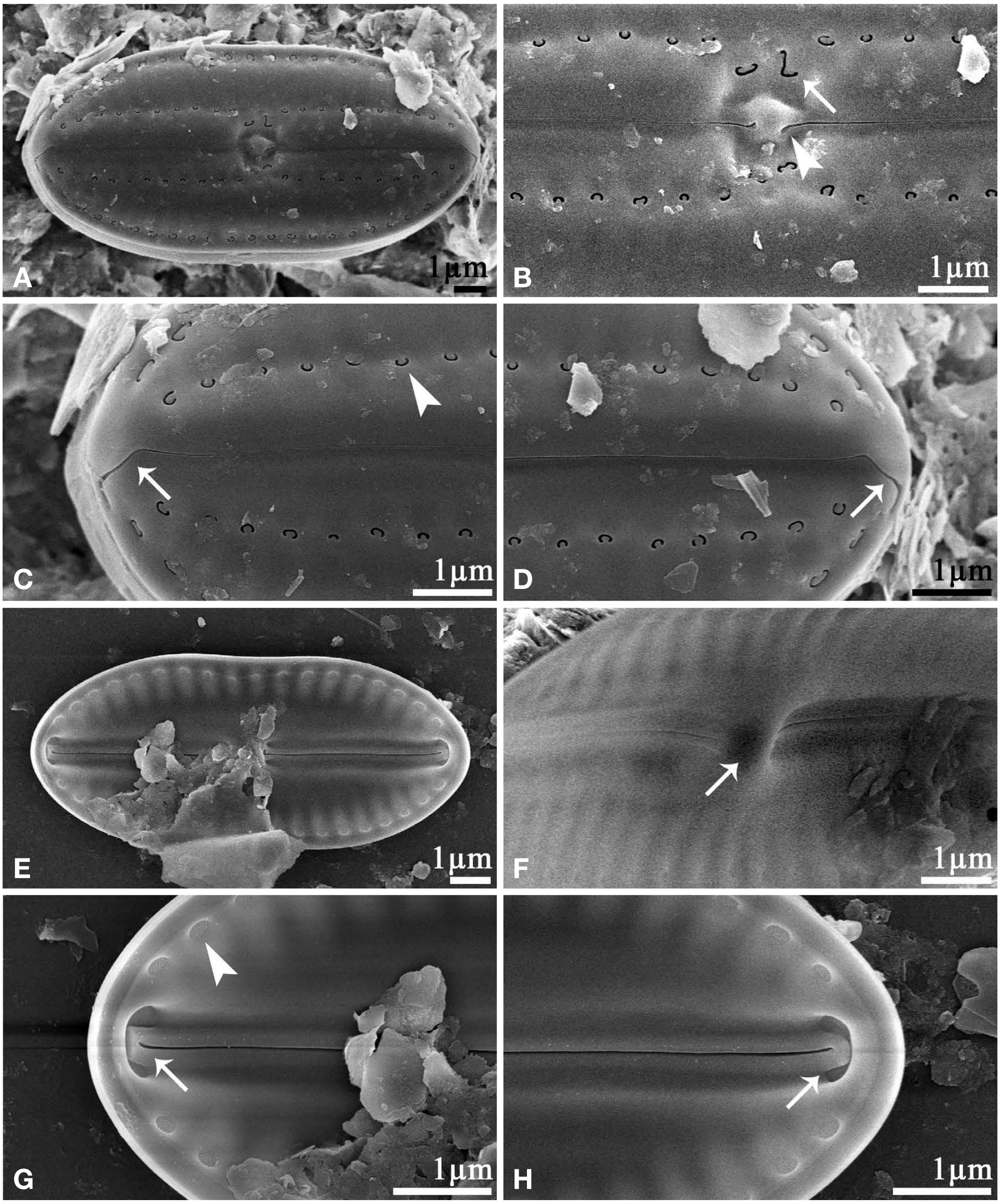

Fig. 5. Scanning electron microscope (SEM) photographs of *Diploneis aestuarii*: A. External view of the whole valve; B. Arrow indicates central nodule with four areolae. Large Arrowhead indicates central raphe endings which are bent to the opposite direction; C, D. Large arrowhead indicates C-shaped foramen areolae. Arrows indicate terminal raphe endings which are bent to the same direction; E. Internal view of the whole valve; F. Arrow notes central raphe endings; G, H. Large arrowhead indicates rounded perforations of cribrum. Arrows indicate internal terminal fissures.

in the central region and gradually radiate to both apices. The density of striae is 15–18 in 10 μm, and areolae are inconspicuous (Fig. 4A–N).

SEM observation. External view of the transapical striae is shown under LM but not observed on the valve face (Fig. 5A–D). Areolae, as C-shaped foramen, were aligned both along the valve margin and raphe sternum (Fig. 5A). Raphe system is very narrow (Fig. 5B), and terminal raphe endings were bent to the same direction (Fig. 5C, D). Central raphe endings were bent in the opposite direction, and the area around the central nodule was deeply depressed with four areolae (Fig. 5B). Internally areolae are rounded perforations of cribra (Fig. 5E, G, H). Internal terminal fissures were bent to the same side (Fig. 5G, H) as the central raphe endings (Fig. 5F).

Distribution. *Diploneis aestuarii* was first reported from the German coast by Hustedt (1939). Simonsen (1987) observed the species from Rheiderland Shore, Bingum in East Frisia Island, Germany. The species is also widespread along the European coasts of Atlantic Ocean and western Baltic Sea (Witkowski *et al*., 2000). *Diploneis aestuarii* was also reported from a saltern in Sinan, Korea (Bae *et al*., 2020), and Isahaya tidal flat in the Ariake Sea, Japan (Ohtsuka, 2005). The present study would be second report of the occurrence of the species from Korea, and the very first report of detailed morphology of the species. In the present study, *D. aestuarii* was observed from 10 locations in Korea, five locations in China, and one location in Japan (Fig. 1).

Discussion

Studies of systematics for the marine benthic diatoms have developed largely by the beginning of the late 19th century from the Sea of Marmara in Europe, and more recently Witkowski *et al*. (2000) published a most comprehensive reference on marine benthic diatoms based on light microscopy. Despite the efforts of many phycologists (Mann, 1999), diatom taxonomy and nomenclature from Europe and America have long been adopted for the study of Northeast Asian diatoms without verifying validity of the literature, which greatly hindered the proper appreciation of biodiversity of marine benthic diatoms of the region (Park *et al*., 2012b; 2014). The problem was further aggravated, due to a short history in diatom taxonomy, lack of experienced experts, and unavailability of relevant literature. For example, *Navicula spartinetensis* was misidentified in Northeast Asia as *Navicula flantica* (Kim *et al*., 2020). *Berkeleya scopulroum* has also been reported from Korea for a long time, however, it was found that reports of Korean '*Berekeleya scopulorum*' were misidentifications of a *Climaconeis* species which has later been described as a new species under the nomenclature of *Climaconeis mabikii* (Park *et al*., 2016).

Small-sized diatom species are easily overlooked during LM observation particularly when there are plenty of diatom frustules and sediment particles. Besides small members of the genus *Diploneis* and *Plagiogrammopsis* as shown in the present study, small monoraphid forms such as *Planothidium* and *Cocconeis* are also reported to be easily overlooked (Witkowski *et al*., 2016). Besides, many small and fragile diatom frustules, particularly of the genera *Navicula* and *Nitzschia*, are readily observed from tidal flat samples, yet it is very difficult to discern their fine structures under LM (personal observation). There have also been misidentifications of certain diatom species as smaller specimens of well-known diatom species. For example, Im *et al*. (2020) reported the small sized-diatom species of *Tryblionella adducta* along the coasts of Northeast Asia, while the species was commonly misidentified as *Nitzschia punctata*, which is larger in size.

In the present study, we have identified two small negligible diatom species of *Plagiogrammopsis minima* and *Diploneis aestuarii*. Detailed morphological descriptions as well as geographical distribution of the two species have also been addressed. The results of the present study suggest that there could be numerously misidentified and/or unidentified diatom species in Korean tidal flats, particularly for small-sized diatoms. Witkowski *et al*. (2016) clearly noted the possible underestimation of diatom biodiversity in Bohai and Yellow Sea coasts with the provision of both morphological and molecular data. They also mentioned the number of novel taxa would be ca. 20−30% out of the total diatom assemblages. To avoid unintentional negligence of small-sized diatoms, it is recommendable for diatomist to use a fine LM system with ×100 objective lens preferably with DIC optics for better discernment of small fragile frustules, and also SEM in high magnification for proper observation of very fine ultrastructures. In conclusion, the present study has widened our understudying of biodiversity of the benthic diatom from Korean tidal flats through critical taxonomic investigations based on careful LM and SEM observation.

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