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Taxonomy and distribution of two small *Tryblionella* (Bacillariophyceae) species from the Northeast Asian tidal flats

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Species belonging to the genus *Tryblionella* are important members of the diatom assemblages of benthic environments such as tidal flats. Their proper identification is thus of great importance in terms of taxonomy, ecology, as well as environmental sciences. However, many species of the genus have been neglected and/or misidentified during diatom studies of Korean tidal flats possibly due to the small size of the species. Lack of proper references for the identification of the species is also noted. To better understand the diversity of *Tryblionella* species, sediment samples were taken from various tidal flats of the Yellow Sea. Light microscope (LM) and scanning electron microscope (SEM) observations have shown the presence of *Tryblionella adducta* and *T. hyalina*, which have not been previously reported from Korean tidal flats. The former was found mostly from sand flats and the latter from mudflats. It is expected that the present study would contribute to a better understanding of the diversity and ecology of benthic diatoms of the Korean tidal flats.

Keywords: diatom, distribution, Northeast Asia, taxonomy, tidal flat, *Tryblionella*

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Introduction

The primary production of benthic diatoms is ecologically important as major food sources for the benthic animals of tidal flats. Their ecological characteristics e.g. short life-cycles and quick responses to the changing environments allowed them to be excellent environmental indicators. They are also useful tools for palaeoecological research (Denys and De Wolf, 1999). While more than 200 genera of living diatoms have been classified with ca. 100,000 extant species worldwide (Round *et al*., 1990), 2000 diatom species including about 500 marine members have been reported from Korean waters (NIBR, 2015). In the meantime, Korean tidal flats are expected to have higher biodiversity than the number of reported species, considering the development of vast intertidal areas extending up to 10 km in width with a total area of about 2500 km^2 (Kellerman and Koh, 1999). It should be also noted that the history of Korean diatom studies is quite short compared to that of Europe and America. The diatom assemblages of Korean tidal flats were first reported from Incheon by Shim and Cho (1984). There have been many research papers mainly focused on assemblage and/or ecology of benthic diatoms since the 1980s (Park *et al*., 2014). In recent years, the number of taxonomic studies is increasing with reports of newly recorded diatom species (Chung *et al*., 2010; Yun and Lee, 2010; Lee *et al*., 2012; Joh, 2013; Lee and Park, 2015; Joh, 2017; Park *et al*., 2017; Kim *et al*., 2017; Joh and Lee, 2018; Lee *et al*., 2019) as well as new taxa (Park *et al*., 2012b; Park *et al*., 2013; Park and Lee, 2015).

The genus *Tryblionella* was first established by W. Smith (1853), however, it has long been considered to be congeneric with the genus *Nitzschia*. Later Round *et al*. (1990) resurrected the genus with the designation of a lectotype species (viz. *T. acuminata*) as well as the detailed light microscope (LM) and scanning electron microscope (SEM) observations of the genus. Valves of *Tryblionella* are broad and robust with the valve face often being undulated with external ridges. Raphe system is strongly eccentric with the development of keel and fibulae as in the genus *Nitzschia*. Striae are uniseriate to multiseriate, usually with interruption by one or more sterna. *Tryblionella* is a fairly large and widespread epipelic genus inhabiting

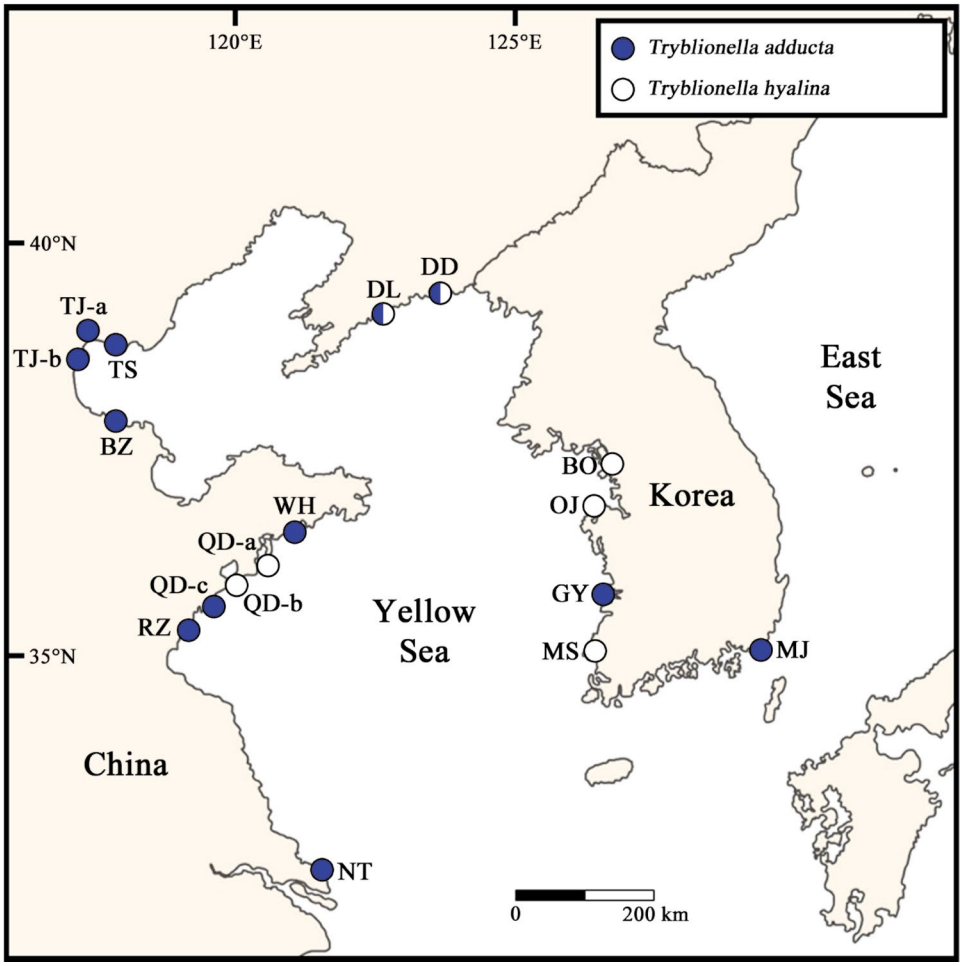

Fig. 1. Map showing the sampling locations along the coasts of the Yellow Sea where *Tryblionella adducta* and *T. hyalina* were observed.

Table 1. List of samples which have been analyzed in the present study.

Sampling date	Sites	Location	Latitude (N)	Longitude (E)
Oct. 27, 2006	BO	Buno-ri, Incheon, Korea	37°36′	126°31′
Jul. 03, 2007	OJ	Oji-ri, Chungcheongnam-do, Korea	36°57′	126°22′
Aug. 06, 2003	GY	Gyehwa-ri, Jeollabuk-do, Korea	35°46′	126°37′
May 24, 2005	MS	Manpung-ri, Jeollanam-do, Korea	35°07′	126°19′
Aug. 30, 2006	MJ	Myeongji-dong , Busan, Korea	35°04′	128°55'
Jul. 02, 2018	DD	Dandong, Liaoning, China	39°50′	123°39′
Jul. 02, 2018	DL	Dalian, Liaoning, China	39°39′	122°59′
Jul. 10, 2018	TS	Tangshan, Hebei, China	39°02′	118°21′
Jul. 10, 2018	TJ-a	Tianjin, China	39°01′	117°27′
Jul. 09, 2018	TJ-b	Tianjin, China	39°05′	117°43′
Jul. 08, 2018	BZ	Binzhou, Shandong, China	38°12′	118°00′
Jul. 01, 2018	WH	Weihai, Shandong, China	36°49′	121°27′
Jun. 30, 2018	QD-a	Qingdao, Shandong, China	36°15′	120°19′
Jun. 28, 2018	QD-b	Qingdao, Shandong, China	35°46′	119°55′
Jun. 28, 2018	QD-c	Qingdao, Shandong, China	35°44′	119°54′
Jun. 27, 2018	RZ	Rizhao, Shandong, China	35°17′	119°26′
Jul. 06, 2018	NT	Nantong, Jiangsu, China	31°56′	121°49′

brackish and marine sediments as well as high-conductivity freshwater habitats. While the species of the genus *Tryblionella* are common members of tidal flat diatom assemblages (Ohtsuka, 2005), they are often misidentified or only identified to the genus level due to taxonomic difficulties in proper identification. In the present study, two *Tryblionella* species have been reported from the Korean waters for the first time. Samples were collected from various tidal flats of the Korean and Chinese coast of the Yellow Sea. Distribution of the two *Tryblionella* species across the coast of the Yellow Sea was also investigated.

Materials and Methods

Surface sediments (0–0.5 cm depth) were sampled from five locations in Korea (from 2003–2007) and 12 locations in China (from 2018) along the coast of the Yellow Sea (Fig. 1, Table 1). Samples were transferred to the laboratory then treated with concentrated HCl and H_2O_2 for the removal of calcium carbonate particles and organic substances, respectively. The cleaned diatom frustules were dried on microscope cover glass then mounted on slide glass using Naphrax resin to prepare permanent slides. Diatoms were observed and photos taken using a BX53 light microscope (Olympus, Tokyo, Japan) equipped with an Axiocam 305 color camera (Carl Zeiss, Oberkochen, Germany). A few drops of cleaned material were also dried onto aluminum stubs and coated with platinum, then examined at 5 kV in a MIRA-3 (Tescan, Brno, Czech) for SEM.

Results

Two diatom taxa viz. *Tryblionella adducta* and *T. hyalina* were newly observed from Korean tidal flats. Their morphological characteristics, taxonomic remarks and

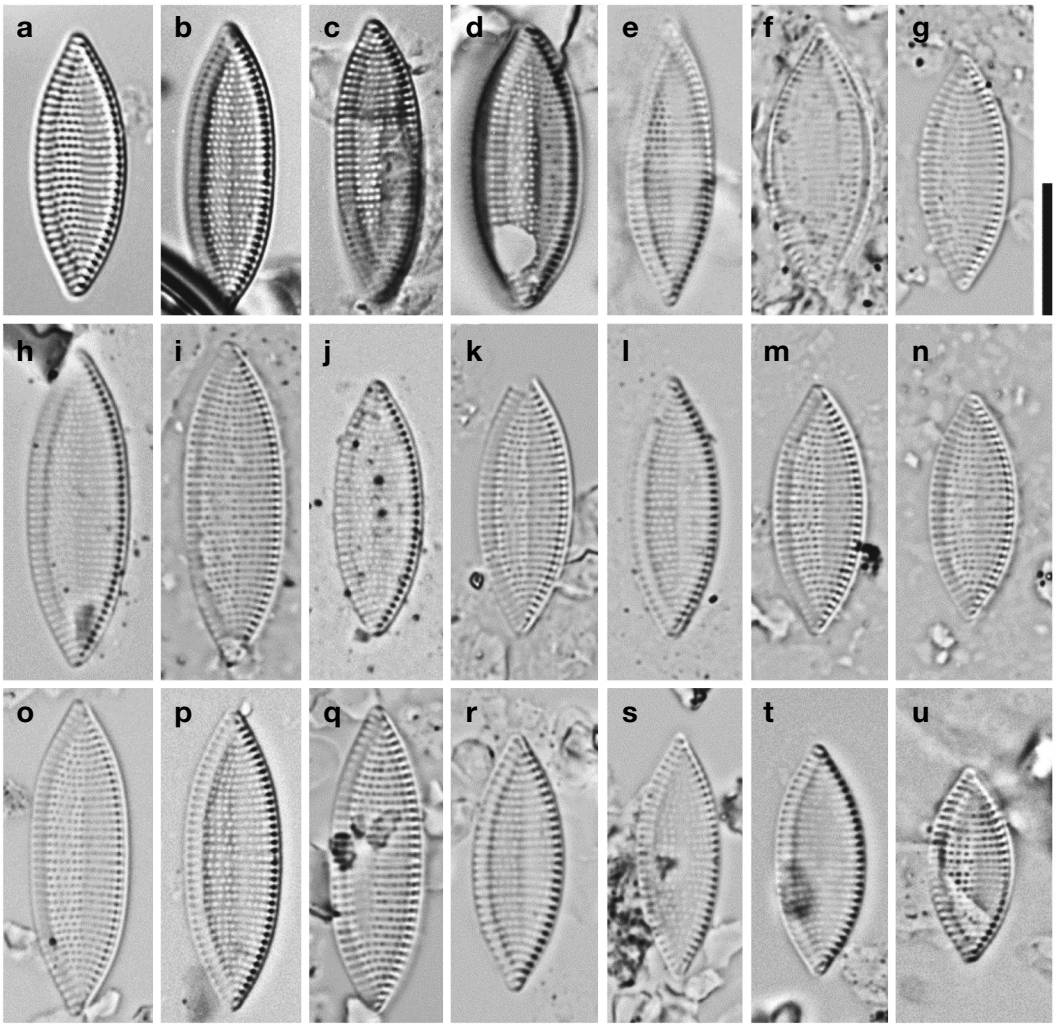

Fig. 2. Light microscope photographs of *Tryblionella adducta* from Korea (a–d) and China (e–u). Scale bar = 10 μm.

distributions in Northeast Asia have been addressed in the present study.

Tryblionella adducta
(Hustedt) D.G. Mann 1990 (Figs. 2, 4)

Reference. Hustedt 1955, p. 43, pl. 15, figs. 21–22.
Description. Valves are elliptic-lanceolate with sharply rounded apices with the length of 16.6–24 μm and width of 6–7.6 μm. The valve face is transapically undulated. Striae are parallel in the middle and slightly radiate towards the ends, 15–21 in 10 μm. Fibulae are eccentric, developed along the edge of the valve, 15–21 in 10 μm. Areolae are punctuated and resolvable under light microscopy, 15–18 in 10 μm.
Taxonomic remarks. *Tryblionella adducta* is superficially similar to *Nitzschia punctata* particularly in valve outline and punctate areolae, however the latter has a much larger size and more sparse striae. In the original description of *N. punctata* by W. Smith (1853), the length of the frustule was ca. 31–53 μm (0.012–0.021 inch) with striae of 12 in 10 μm (31 in 0.001 inch). Later Cleve and Grunow (1880) have also provided the striae density of the same species as of 7–9.5 in 10 μm. In fact, there was a record of *T. adducta* from the Nakdong Estuary, yet with misidentification of the species as *N. punctata* var. *minor* Tempère & Peragallo (Cho, 1989).
Distribution. In Korea, *T. adducta* was observed from Gyehwa sandflat of Mangyeong-Dongjin River Estuary and Myoengji sandflat of Nakdong River Estuary. In the present study *T. adducta* was observed from 10 locations along the Chinese coast of the Yellow Sea (Fig. 1). Considering the fact that *T. adducta* was also reported from mudflats in Ariake Sea, Japan (Ohstuka, 2005; Park *et al.*, 2012a), it is likely that the species is widespread along the coasts of Northeast Asia.

Tryblionella hyalina
(Amossé) Ohtsuka 2005 (Figs. 3, 4)

Reference. Amossé 1924, p. 165, fig. 1.
Description. Valves are broadly lanceolate with acute apices, length 13.3–38.6 μm, width 7.3–13.3 μm. Fibula is extremely eccentric to the valve margin, indistinct. Valve face has a broad hyaline area along the apical axis. Striae are composed of distinct coarse puncta, irregularly scattered at the margin, 7–9 in 10 μm.
Taxonomic remarks. *Tryblionella hyalina* is similar to *Nitzschia navicularis* (Brébisson) Grunow, which also exhibits punctate areolae as well as transapical hyaline area. However the former has monoseriate striae yet the striae of *N. navicularis* are biseriate. In addition, *T. hyalina* is a marine species while *N. navicularis* is a freshwater species. For example, Choi (1988) has reported *N. navicu-*

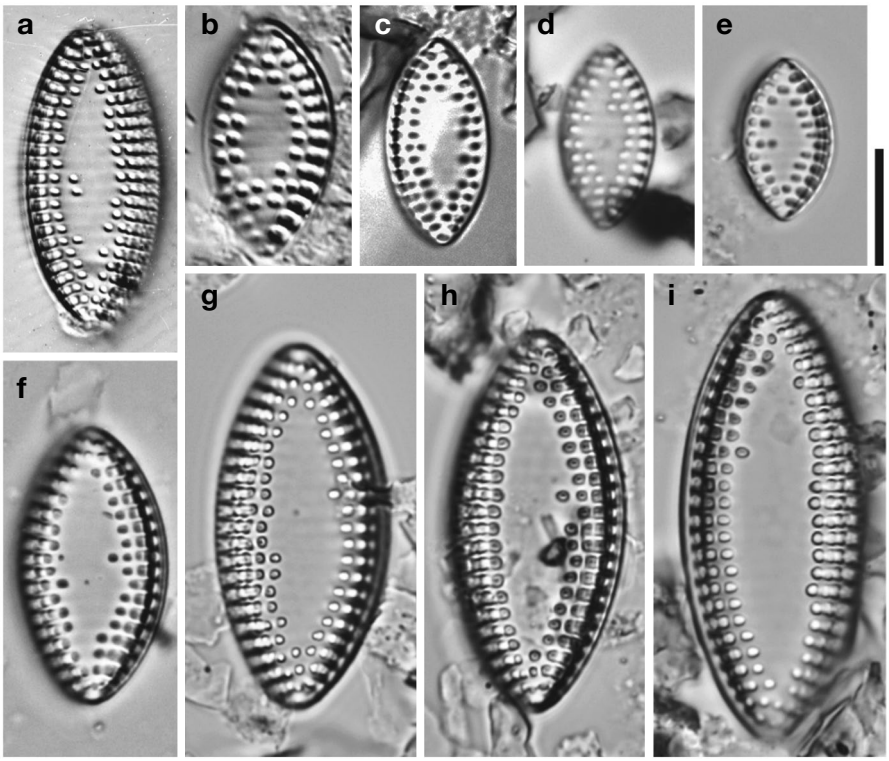

Fig. 3. Light microscope photographs of *Tryblionella hyalina* from Korea (a–c) and China (d–i). Scale bar = 10 μm.

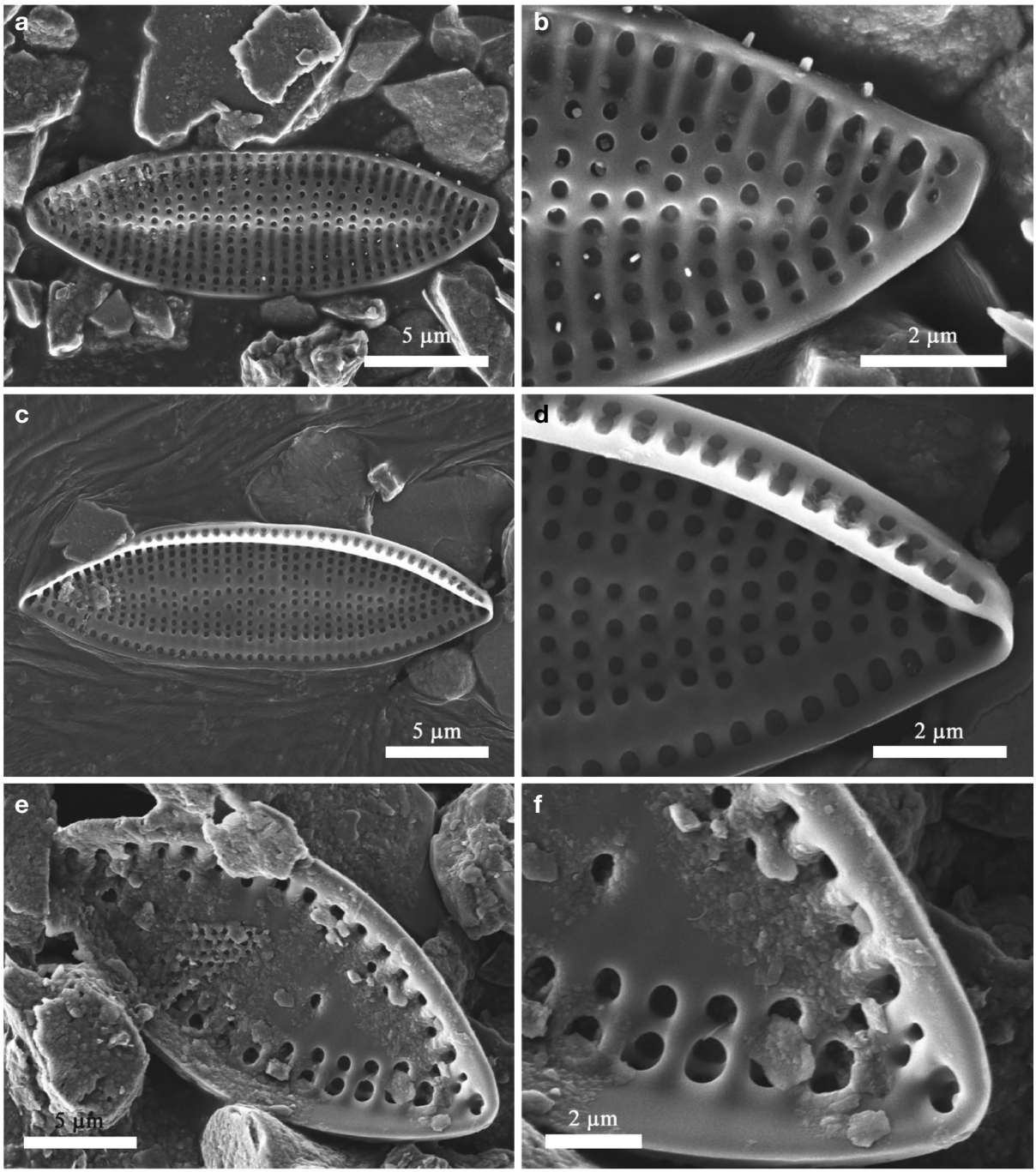

Fig. 4. Scanning electron microscope photographs of two newly recorded species. (a–d) *Tryblionella adducta* (a: external view of the whole valve, b: external view of the valve apex with slightly radiating striae, c: internal view of the whole valve, d: internal valve of valve apex with conspicuous fibulae); (e, f) *Tryblionella hyalina* (e: internal view of the whole valve, f: internal view of valve apex).

laris from the Songdo tidal flats in Korea, however the species should be identified as *N. granulata* var. *hyalina* considering its monoseriate striae.

Distribution. *T. hyalina* has been recorded from the mudflats of China (Jin *et al*., 1985; Jin *et al*., 1991) and Japan (Ohstuka, 2005; Park *et al*., 2012a). In the present study, this species was observed from the Buno tidal flat of Ganghwa Island (2006, October), Oji tidal flat of Garorim Bay (2007, June), and Manpung tidal flat of Hampyeong Bay (2005, May). In the present study *T. hyalina* was observed from four locations along the Chinese coast of the Yellow Sea (Fig. 1).

Discussion

Taxonomy as a fundamental science has long been based on the rich historical legacy of so-called Western countries, and the study of the diatom is not an exception. When scientists are to investigate the biodiversity of a certain area, they largely have relied on the literature from Europe and America. This also have resulted in force-fitting of Northeast Asian diatom taxa into European and/or American species (Park *et al*., 2012a), thus true appreciation of the diatom biodiversity of the area has been much hindered. In the meantime, recent studies have suggested that the diatom assemblages of the Yellow Sea are very unique with rich biodiversity as well as many prospective new taxa to the science (Park *et al*., 2014). The result of our study has also supported the above argument - a close look of diatom samples has revealed the presence of *Tryblionella adducta* and *T. hyalina*, which have not been previously reported from Korean waters. The present study has also shown that the two taxa are quite widespread even among the tidal flats of Northeast Asia. Particularly, *T. adducta* was common among Korean and Chinese sandflats, while *T. hyalina* was comparably rare from mudflats. These two species may have been neglected due to their small size, particularly as for *T. adducta* and/or misidentified as similar other species such as *Nitzschia punctata* and *N. navicularis*. It should be also noted that not only the overall feature of diatoms, such as valve outline and the detailed structures such as striae and areolae, should be well examined for diatom identification. Ecology should also to be considered because many freshwater species have been reported from Korean coastal areas, yet their true existence is of question often with a lack of critical examination of taxonomic information. In conclusion, our study has supported the high biodiversity of benthic diatoms from Korean tidal flats. While there has been force-fitting of European and American diatom taxa to the Northeast Asian taxa, future studies with the critical implementation of careful taxonomic studies would further contribute to the better appreciation of biodiversity of benthic diatoms not only from Korean coasts but also from the marine habitats of Northeast Asia.

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