Two New Species of the Diatom Genus Praestephanos from the Pliocene Ueno Formation, Kobiwako Group, Mie Prefecture, Japan

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Two new species of the diatom genus *Praestephanos* from the Pliocene Ueno Formation, Kobiwako Group, Mie Prefecture, Japan

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Abstract. **Two new diatom species, *Praestephanos tokaiensis* sp. nov. and *P. miensis* sp. nov. are described from lacustrine deposits in the Pliocene Ueno Formation of the Kobiwako Group in Iga City, Mie Prefecture, Japan. The stratigraphic ranges of *P. tokaiensis* and *P. miensis* are approximately 3.9–3.8 Ma and 3.8–3.7 Ma, respectively. The morphology of these species is mainly characterized by a slightly doubly undulating central area; this feature is not found in previously described *Praestephanos* species or similar *Stephanodiscus* species. The stratigraphic distribution and morphology of the two new species suggest that *P. tokaiensis* and *P. miensis* have an ancestor–descendant relationship.**

Keywords: **Diatoms, Kobiwako Group, Paleo-lake Biwa, Pliocene, *Praestephanos*, Ueno Formation**

Introduction

The diatom genus *Praestephanos* Tuji and M. Julius (Tuji *et al*., 2014) is a freshwater planktonic taxon. Thus far, two extant and three fossil species belonging to the genus have been described (Tuji *et al*., 2014; Tuji and Ohtsuka, 2020). These species were originally described as members of the genus *Stephanodiscus* Ehrenberg (Grunow, 1878; Genkal and Kuzmin, 1978; Tuji and Kociolek, 2000; Saito-Kato *et al*., 2015), but were later assigned to *Praestephanos* because they share a unique valve morphology; in addition, molecular phylogenetic analysis revealed that the two extant species are monophyletic and clearly separate from *Stephanodiscus* (Tuji *et al*., 2014; Tuji and Ohtsuka, 2020).

Of the five *Praestephanos* species, *Praestephanos suzukii* (Tuji and Kociolek) Tuji, the type species of the genus, is extant and endemic to Lake Biwa, Japan (e.g. Tuji and Kociolek, 2000; Ohtsuka and Tuji, 2020). Fossils of *P. suzukii* and the ancestral species, *Praestephanos praesuzukii* (Saito-Kato) Tuji and Ohtsuka, *Praestephanos umbilicatus* (Saito-Kato) Tuji and Ohtsuka, and *Praestephanos* cf. *triporus* (originally described as *Stephanodiscus praesuzukii*, *S. umbilicatus*, and *S.* cf. *vestibulis*, respectively), have been found in a borehole core collected from Lake Biwa, and morphological evolution of these species over the past 240,000 years has been reconstructed from the fossil record (Saito-Kato *et al*., 2015). Diatoms similar to *P. suzukii* have also been found in the Pliocene–Pleistocene Kobiwako Group (Negoro, 1981; Tanaka and Nagumo, 2014; Ohtsuka *et al*., 2018; Tuji and Ohtsuka, 2020), which consists of lacustrine and fluvial deposits and is stratigraphically succeeded to the sediments accumulated beneath Lake Biwa (e.g. Satoguchi, 2020). In addition, Hattori *et al*. (2017) reported diatoms similar to *P. suzukii* from the Pliocene Kameyama Formation of the Tokai Group in the west coast area of Ise Bay, which once belonged to the same water system as the area of the Kobiwako Group (Yokoyama, 1995; Satoguchi, 2017). These diatoms have been also considered to be ancestral to *P. suzukii*, and their evolutionary history is of great interest (Tuji and Ohtsuka, 2018, 2020).

Lake Biwa is the largest lake in Japan and one of the ancient lakes in the world (e.g. Nakajima, 1994; Mackay *et al*., 2010). Its origin dates to about 4 million years ago or earlier (Satoguchi, 2015), and the location and scale of the lake basin have changed over time (e.g. Kawabe, 1994; Yoshikawa and Yamasaki, 1998; Satoguchi, 2017).

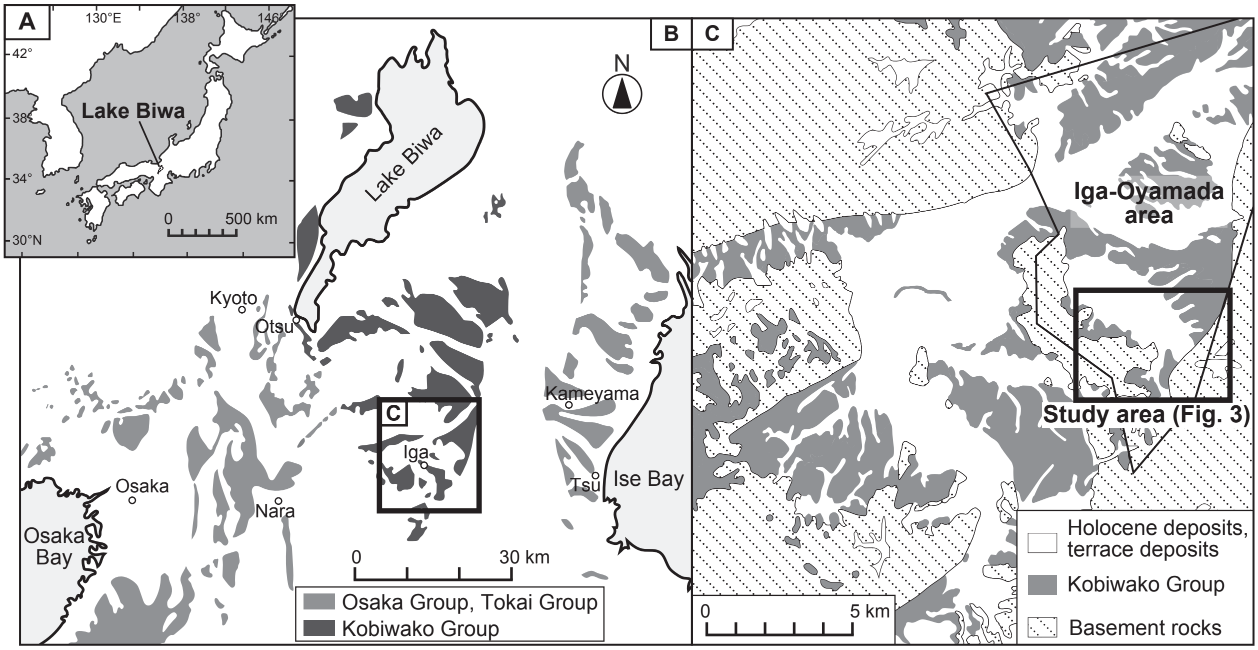

Figure 1. **A,** maps showing the location of Lake Biwa in Japan; **B,** the distribution of Pliocene–Pleistocene deposits around Lake Biwa, modified from Ichihara (1984); **C,** a geologic map of the Kobiwako Group around Iga City, Mie Prefecture, based on Hashimoto *et al*. (1998). The location of the Iga-Oyamada area is indicated by thin line enclosure (based on Kawabe, 1990), and that of the study area shown by the thick line.

The Pliocene–Pleistocene Kobiwako Group, which was formed in Paleo-lake Biwa and its surroundings, is distributed in the area around Lake Biwa (e.g. Kawabe, 1994; Satoguchi, 2009). The earliest Paleo-lake Biwa, also called "Paleo-lake Oyamada" (Kawabe, 1994; Satoguchi, 2020), existed in the Iga-Oyamada area (Figure 1; Kawabe, 1989) of Iga City, Mie Prefecture at 4.0–3.5 Ma (Satoguchi, 2017, 2020). The Ueno Formation of the Kobiwako Group, which was formed in Paleo-lake Oyamada, is distributed in the area (Kawabe, 1989; Kawabe *et al*., 1996), and diatoms similar to *P. suzukii* have been also found in the formation (Tanaka and Nagumo, 2014; Ohtsuka *et al*., 2018).

We investigated *Praestephanos* from the Ueno Formation in the Iga-Oyamada area. In this paper, we describe two new species of the genus *Praestephanos*, and discuss the phylogenetic relationship between these species based on their stratigraphic distribution and changes in valve morphology. These results provide valuable information to clarify the evolutionary history of *Praestephanos* in Paleo- and present Lake Biwa.

Material and methods

The Pliocene Ueno Formation of the Kobiwako Group is distributed around Iga City, Mie Prefecture (Kawabe, 1989). In the Iga-Oyamada area of Iga City (Figure 1C), the Ueno Formation is divided into the Hojiro Member, which consists of alternating sand and mud layers, and the Nakamura Member, which mainly consists of thick massive mud layers (Figure 2; Kawabe, 1989; Kawabe *et al.*, 1996). Volcanic ash layers are intercalated within both members (Figure 2; Yokoyama *et al*., 1980; Kawabe, 1989; Yoshikawa and Danhara, 1996; Satoguchi, 2015). The chronology of this formation has been established on the basis of magnetostratigraphy (Kawabe, 1989; Uchiyama, 1996) and correlation with widespread tephras (Yoshikawa and Danhara, 1996; Satoguchi and Nagahashi, 2012; Satoguchi, 2015). The Gilbert–Gauss geomagnetic reversal boundary has been identified in the upper part of this formation (Kawabe, 1989; Uchiyama, 1996; Satoguchi, 2015). In addition, the ages of the Hojiro II and the Hasuike (Ichibe) volcanic ash layers intercalated in this formation, which are widespread tephras, were estimated to be 3.9 Ma and 4.1 Ma (Satoguchi *et al*., 2005; Satoguchi, 2015), respectively. The age model in this formation was established by Satoguchi (2015), and we used it for this study (Figure 2).

Diatoms from the Ueno Formation in this area have been studied by Tomoda and Negoro (1979), Negoro (1981), Tanaka *et al*. (1984), Tanaka and Matsuoka (1985), Tanaka (1994, 1998), Mori and Usami (1996),

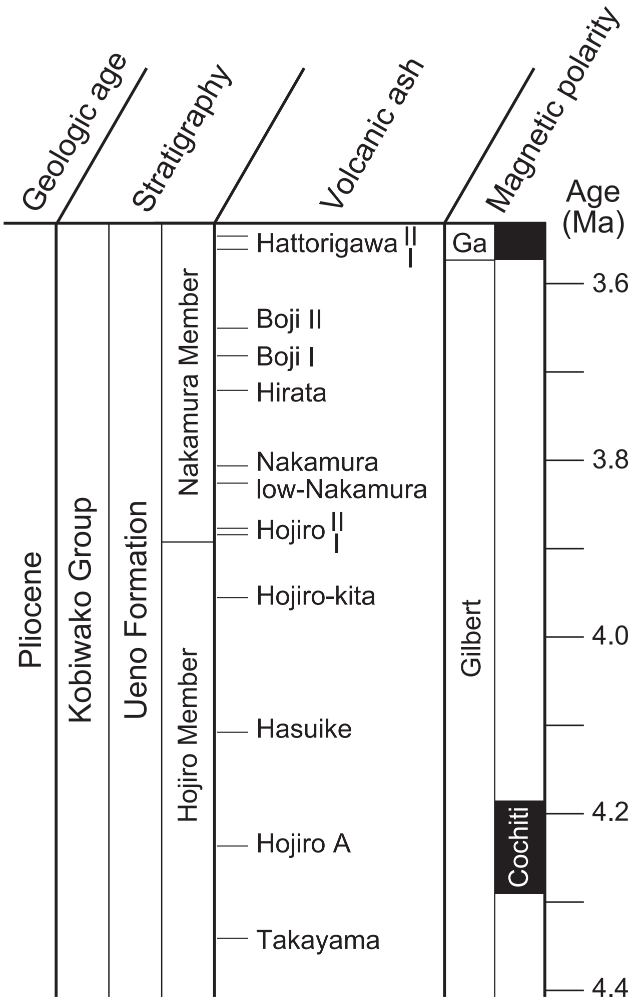

Figure 2. Stratigraphy and age model of the Ueno Formation of the Kobiwako Group in the Iga-Oyamada area, based on Satoguchi (2015). Ga, Gauss.

Tanaka and Nagumo (2014), Tanaka (2015), and Ohtsuka *et al*. (2018). Most of these studies reported dominance of freshwater planktonic diatoms such as *Aulacoseira* (*Melosira*) *praeislandica* (Jousé) Simonsen and *Stephanodiscus carconensis* Grunow, with small numbers of marine diatoms that were regarded as reworked fossils from the Miocene marine deposits (Tanaka and Matsuoka, 1983, 1985; Tanaka, 2015). In addition, Tanaka and Nagumo (2014) and Ohtsuka *et al*. (2018) reported diatoms similar to *Praestephanos suzukii*, but taxonomy and the stratigraphic distribution of these fossils are not clear in detail.

We surveyed outcrops along a stratigraphic section from near the Hojiro-kita volcanic ash layer to near the Hattorigawa II volcanic ash layer, distributed in the hills and along the Hattori River in the Iga-Oyamada area (Figure 3), and collected samples from 203 horizons (Figure 4). The stratigraphic position of each outcrop was determined according to the tephrostratigraphy (Kawabe, 1989; Satoguchi, 2015), columnar sections shown in Kawabe (1989), and geological mapping methods. Identification of volcanic ash layers was based on Kawabe (1989), Yoshikawa and Danhara (1996), and Satoguchi (2015). The volcanic ash layer described by Yoshikawa and Danhara (1996) as "volcanic ash layer about 10 m below Nakamura volcanic ash" is referred to herein as "low-Nakamura" for convenience; this abbreviation was also used by Uchiyama (1996). In addition, the ages of samples were estimated by interpolation on the age model (Figure 2; Satoguchi, 2015) based on their stratigraphic relationships with the volcanic ash layers.

For each sediment sample, a smear slide was made by putting a small amount of sediment suspended in water on a slide glass and covering with a cover slip. The smear slides were observed under a light microscope (LM), BX-51 (OLYMPUS, Tokyo) at 400× magnification to check for the presence of diatom fossils. Diatom fossils were obtained from 123 of the 203 samples (Figure 4). The genus *Aulacoseira* Thwaites was found in all of these samples, and diatoms considered to be of the genus *Praestephanos* were detected in 84 samples from the horizon 5.5 m above the Hojiro-kita volcanic ash layer to the horizon approximately 20 m above the Boji I volcanic ash layer (Figure 4). The sediment samples containing diatom fossils were cleaned with 3%–6% hydrogen peroxide and washed four times with water to obtain cleaned materials. Diatom fossils in the cleaned materials that were obtained from the horizons indicated by arrows beside columns in Figure 4 were collected on Millipore 0.45 µm mesh filters (HAWP04700, Merck KGaA, Darmstadt, Germany), and coated with gold for scanning electron microscopy (SEM). More than 20 individuals of *Praestephanos* taxon per sample were observed by using SEM with a JSM-6510 (JEOL, Tokyo, Japan). The cleaned type materials of new species were dropped on cover slips, dried, mounted on glass slides with Styrax, and observed using an LM at 1000× magnification.

Systematic description

Diatoms exhibiting the taxonomic characters of *Praestephanos* were found in all 22 samples that were examined under the SEM, along with *Aulacoseira praeislandica*. We separated the *Praestephanos* into two species based on the combination of their morphological characters, and here we describe these as new species. The terminology used herein follows Ross *et al*. (1979), Håkansson (2002) and Houk *et al*. (2014).

Order Thalassiosirales Glezer and Makarova, 1986

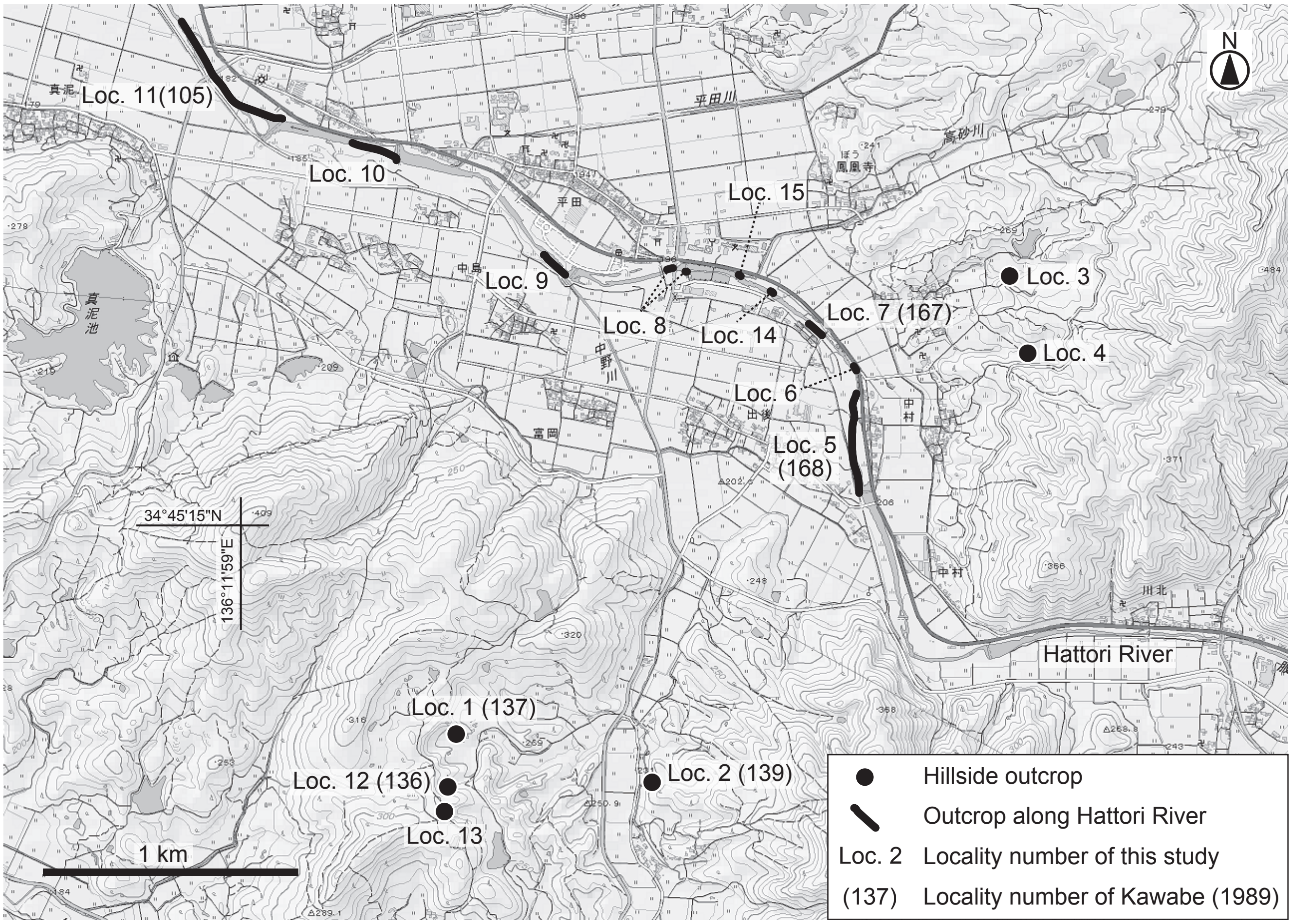

Figure 3. Map showing the locations of columnar sections and sampling points. This map is based on the GSI map published by the Geospatial Information Authority of Japan.

Genus ***Praestephanos*** Tuji and M. Julius in Tuji *et al.*, 2014

Type species.—*Praestephanos suzukii* (Tuji and Kociolek) Tuji in Tuji *et al.*, 2014.

Remarks.—Tuji *et al*. (2014) described the pattern of areolae and the location of marginal fultoportulae as unique characteristics of *Praestephanos*. That is, the former is that the pattern of the areolae changes at the level of the marginal fultoportulae but not at the level of the rimoportulae or spines, and the latter is that the marginal fultoportulae are often not on the extension of costae. These characters differ from the closely related genera *Stephanodiscus* Ehrenberg and *Cyclostephanos* Round. In these genera, the pattern of the areolae changes at the level of the rimoportulae or spines, and the marginal fultoportulae are on the extension of costae.

Praestephanos tokaiensis Kojima, K. Hattori, Ohtsuka and Saito-Kato sp. nov.

Figures 5A–E, 6, 7

Description.—Valve is circular (Figure 5A–E), 10–48 μm in diameter, with a single (Figures 6G, 7A, C, G) or slightly double (Figures 6A, B, D, E, J, 7E, M) concentric undulation of the valve face and a very shallow mantle (Figures 6C, E, H, 7B, D, J). Fascicles are composed of 3–9 rows of areolae at the valve face/mantle junction, 2–6 in 10 μm (Figure 7B, D, J). Areolae in a row on the valve face are 13–18 in 10 μm. The areolae become finer near the level of the marginal fultoportulae (Figures 6C, H, 7B, D, J), 4–5 in 1 μm. Externally, the fascicles of the concave valve usually bear grooves in the marginal area (Figure 6G–K). The costae are often strongly raised externally (Figure 6A, B, E, J). Sometimes a spine or its remnant is present at the slope of the valve face/mantle junction on the costa (Figure 6A, C, H, K). One to several

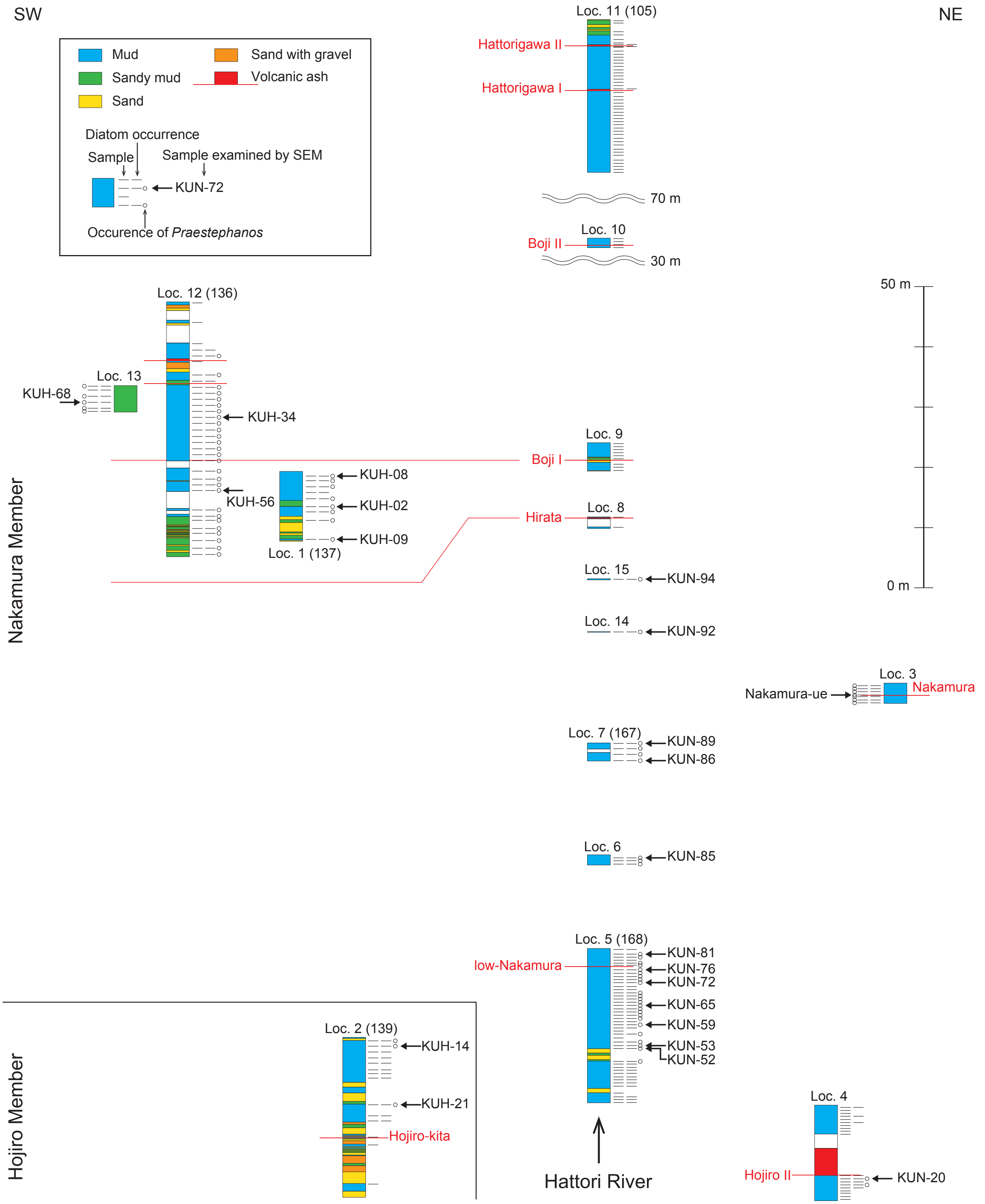

Figure 4. Columnar sections of the Ueno Formation in the study area, showing sampling horizons and the presence of diatom fossils in smear slides. The location of each column is shown in Figure 3.

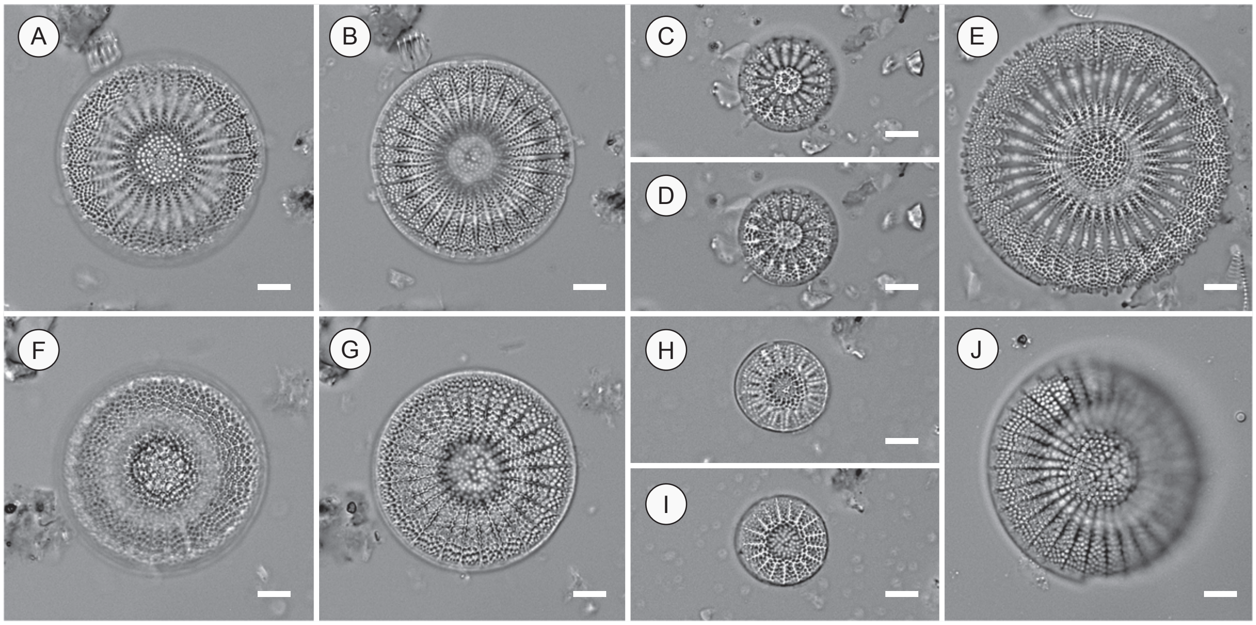

Figure 5. Light microscope images of *Praestephanos* species from the Ueno Formation. **A–E,** *Praestephanos tokaiensis* sp. nov. (A, B, Holotype, MPC-44433); **F–J,** *Praestephanos miensis* sp. nov. (F, G, Holotype, MPC-44434). Scale bars = 5 μm.

valve face fultoportulae are present in the central area, internally with three or rarely two satellite pores (Figure 7D, F, I, L). Three to five marginal fultoportulae per 10 μm are arranged near the valve rim. The marginal fultoportulae are located on both the extension of the fascicles and the extension of the costae (Figures 6C, H, 7B, D, J, M). Each marginal fultoportula internally has three satellite pores (Figure 7B, D, J), and externally appears as a simple pore in smaller valves (Figure 6H) and as a short tube in larger ones (Figure 6C, E). One rimoportula with an external tubular-like extension is present at the valve face/mantle junction (Figure 6F, K), which is also at the level of the spines (Figure 6K). Internally, the slit of the rimoportula is parallel or slightly oblique to the costa (Figure 7B, C, G). The costa bearing the rimoportula is often thicker than the other costae (Figure 7A, H).

Holotype.—MPC-44433 of the National Museum of Nature and Science (hereafter NMNS), Tsukuba, Japan (Figure 5A, B). England Finder locality, G48.

Type material.—RM-60211, NMNS. The material (sample no. KUH-14) was collected from the horizon 15.4 m above the Hojiro-kita volcanic ash layer at Loc. 2 (34° 44.691′ N, 136° 13.055′ E) on the hill in Hojiro of Iga City, Mie Prefecture (Figures 3, 4).

Etymology.—The species name refers to the Tokai Region (including Mie Prefecture), which is a region in central Honshu, Japan, facing the Pacific Ocean.

Stratigraphic occurrences.—This species occurs in the interval from the horizon (*ca.* 3.9 Ma) 5.5 m above the Hojiro-kita volcanic ash layer to the horizon (*ca.* 3.8 Ma) 2.1 m above low-Nakamura volcanic ash layers.

Remarks.—*Praestephanos tokaiensis* occurs near the bottom of the succession deposited in Paleo-lake Oyamada, the earliest Paleo-lake Biwa, and is also the oldest fossil *Praestephanos* species so far reported from Japan. Therefore, this species may be key to elucidating the evolutionary history of *Praestephanos* in the region around Lake Biwa and in Japan as a whole.

The *Stephanodiscus* species from the Iga Formation (which should correctly be called the Ueno Formation according to Tuji and Ohtsuka, 2018, 2020) of the Kobiwako Group reported by Tanaka and Nagumo (2014) is regarded as *P. tokaiensis* because the morphological features of the *Stephanodiscus* species correspond to those of the new species.

In addition, the morphological characteristics of *P. tokaiensis* correspond well with those of the *Praestephanos* taxon reported by Hattori *et al*. (2017) from the Pliocene Kameyama Formation of the Tokai Group that crops out in Tsu City of Mie Prefecture, on the west coast of Ise Bay (Figure 1B); thus, we regard them as the same taxon. Furthermore, the occurrence age of this taxon in the Kameyama Formation is *ca.* 3.8 Ma (Tuji and Ohtsuka, 2018, 2020), consistent with that in the Ueno Formation. A water system connection between the area of the Kobiwako Group and the west coast of Ise Bay is thought to have existed during deposition of the Ueno Formation (Yokoyama, 1995; Satoguchi, 2017); this paleo-water

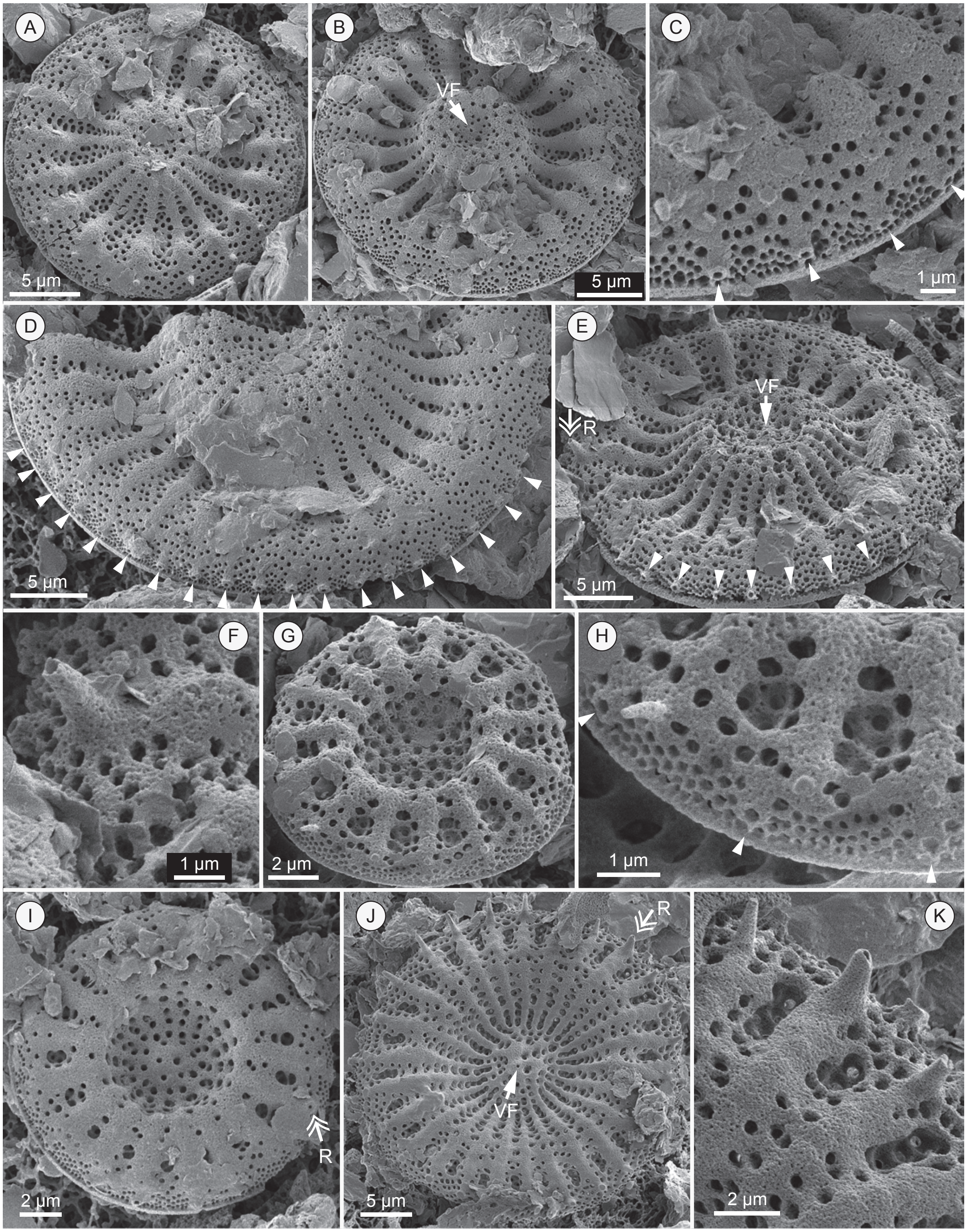

Figure 6. SEM images of external views of *Praestephanos tokaiensis* sp. nov. **A–F,** convex valves from KUN-52 (A) and KUH-14 (B–F); C, enlarged view of B, showing valve margin with marginal fultoportulae; F, enlarged view of E, showing rimoportula; **G–K,** concave valves from KUH-14 (G, H), KUN-72 (I) and KUN-76 (J, K); H, enlarged view of G, showing valve margin with marginal fultoportulae; K, enlarged view of J, showing rimoportula and spines. Arrows marked "VF" and "R" indicate the locations of the valve face fultoportula and the rimoportula, respectively. Triangles mark the locations of marginal fultoportulae.

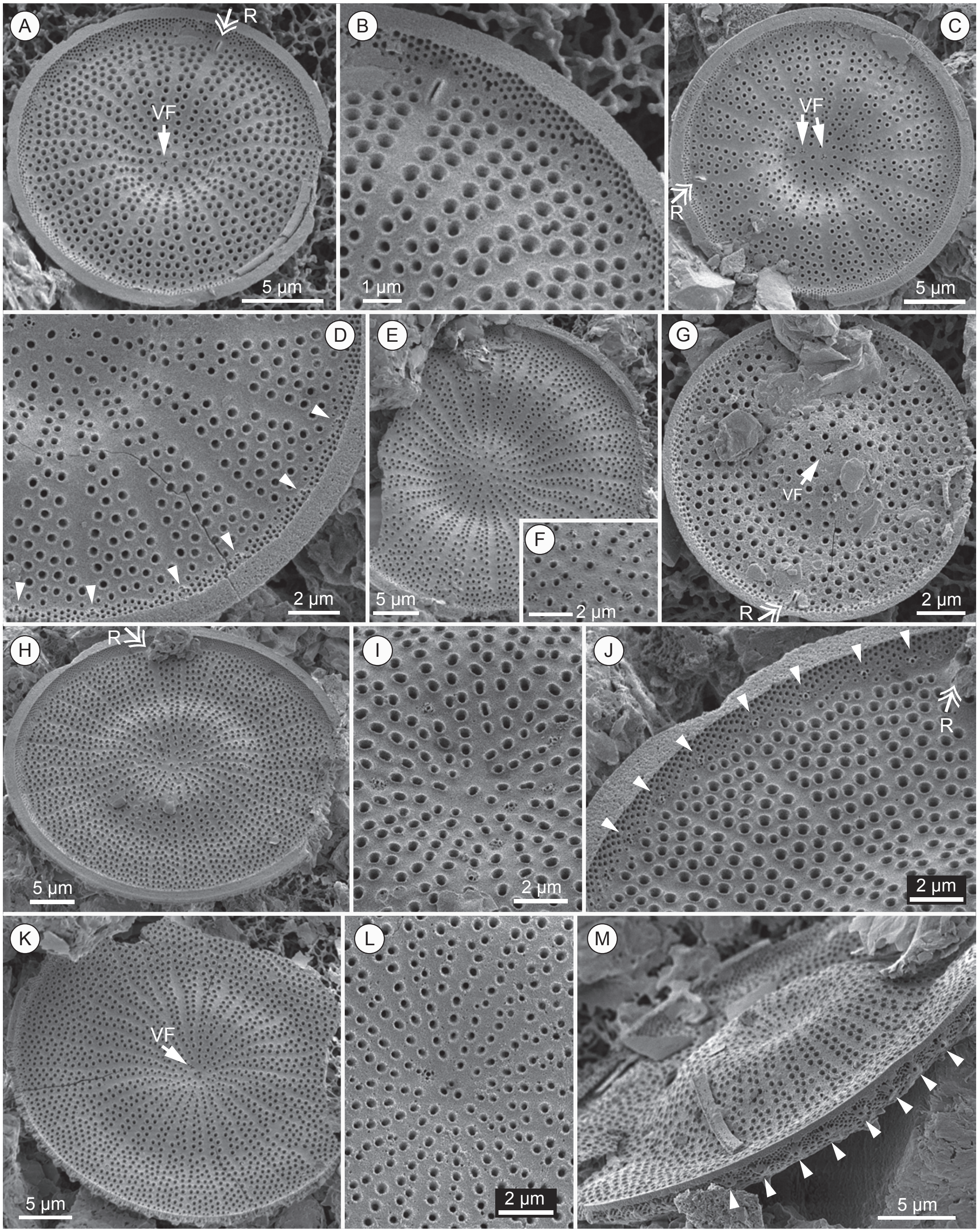

Figure 7. SEM images of internal views of *Praestephanos tokaiensis* sp. nov. **A–F,** convex valves from KUN-52 (A, B), KUN-72 (C, D) and KUN-76 (E, F); B, enlarged view of A, showing valve margin with marginal fultoportulae and rimoportula; F, enlarged view of E, showing valve face fultoportulae; **G–M,** concave valves from KUN-81 (H–J), KUN-53 (K, L) and KUH-14 (G, M); I, J, enlarged views of H, showing central area with valve face fultoportulae and valve margin with marginal fultoportulae and rimoportula, respectively; L, enlarged view of K, showing central area with valve face fultoportula. Arrows marked "VF" and "R" indicate the locations of the valve face fultoportula and the rimoportula, respectively. Triangles mark the locations of marginal fultoportulae.

system would have allowed *P. tokaiensis* to inhabit both areas.

Mori and Usami (1996) reported that *Stephanodiscus carconensis* and *S. carconensis* var. *pusilla* occur in the Ueno Formation at site 1 and site 2 of their study in Hojiro of Ueno City and in Nakamura of Oyamada village, respectively (both currently Iga City). These diatoms are also likely to be *P. tokaiensis* on the basis of their locality, horizon, and morphological description, although no photographs were provided.

Praestephanos miensis Kojima and Saito-Kato sp. nov.

Figures 5F–J, 8, 9

Description.—Valve is circular (Figure 5F–J), 11–62.5 μm in diameter, with a single (Figures 8G, J, 9A, H) or slightly double (Figures 8E, H, K, 9B) concentric undulation of the valve face and a very shallow mantle (Figures 8B, F, I, L, 9D, G, J, M). Fascicles are composed of 3–8 rows of areolae at the valve face/mantle junction, 2–6 in 10 μm (Figure 9D, G, J, M). Areolae in a row on the valve face are 12–18 in 10 μm. The areolae become finer near the level of the marginal fultoportulae (Figures 8F, I, L, 9D, G, J, M), 4 in 10 μm. Externally, the fascicles of the concave valve usually bear grooves in the marginal area (Figure 8H–L). The costae are often strongly raised externally (Figure 8A, E, K). Sometimes a spine or its remnant is present at the slope of the valve face/mantle junction on the costa (Figure 8F, I, L). One to several valve face fultoportulae are present in the central area, internally with three or very rarely two satellite pores (Figure 9C, F, I, L). Greater numbers of valve face fultoportulae are observed on valves with larger diameters (Figure 10). Two to four marginal fultoportulae per 10 μm are arranged near the valve rim. The marginal fultoportulae are usually located on the extension of the costae (Figures 8C, E, 9B, J), but often on the extension of fascicles only near the rimoportula (Figures 8B, D, F, I, L, 9D, M). They are rarely located on the extension of fascicles except near the rimoportula (Figure 9G). Each marginal fultoportula internally has three satellite pores (Figure 9D, G, J, M), and externally appears as a simple pore in smaller valves (Figure 8B) and a short tube in larger ones (Figure 8F, I, L). One rimoportula with an external tubular-like extension is present at the valve face/mantle junction (Figure 8D), which is also at the level of the spines (Figure 8F, L). Internally, the slit of the rimoportula is parallel or slightly oblique to the costa (Figure 9A, J, M). The costa bearing the rimoportula is often thicker than the other costae (Figure 9H, K).

Holotype.—MPC-44434 of NMNS, Tsukuba, Japan (Figure 5F, G). England Finder locality, P45/1.

Type material.—RM-60212, NMNS. The material (sample: Nakamura-ue) was collected from the horizon immediately above the Nakamura volcanic ash layer from Loc. 3 (34° 45.780′ N, 136° 13.971′ E) on the hill in Nakamura of Iga City, Mie Prefecture (Figures 3, 4).

Etymology.—The species name refers to Mie Prefecture, where the type locality is located.

Stratigraphic occurrences.—This species occurs from the horizon (*ca.* 3.8 Ma) approximately 18 m above the low-Nakamura volcanic ash layer to the horizon (*ca.* 3.7 Ma) approximately 10 m above the Boji I volcanic ash layer.

Stratigraphic changes in the valve morphology and ranges of *Praestephanos* species

The major morphological difference between *P. tokaiensis* and *P. miensis* is the arrangement pattern of marginal fultoportulae. The marginal fultoportulae of *P. tokaiensis* are often not located on the extension of costae, but those of *P. miensis* are commonly located on the extension of costae except for ones near to the rimoportula. For convenience, we refer to the former arrangement pattern of the marginal fultoportulae as "Unmatched Pattern" and the latter as "Matched Pattern". Additionally, there are also differences in the number of valve face fultoportulae (Figure 10). The maximum number of valve face fultoportulae of *P. miensis* is larger than that of *P. tokaiensis*. Furthermore, the number of valve face fultoportulae of *P. miensis* depends on valve size, i.e., greater numbers of valve face fultoportulae are observed on valves with larger diameters. In contrast, that of *P. tokaiensis* seems to be independent of size, and even large specimens (e.g. Figure 7K) often have a small number of valve face fultoportulae.

The number of valve face fultoportulae and the arrangement pattern of marginal fultoportulae in specimens from each horizon are shown in Figure 11B and C, respectively. The number of valve face fultoportulae is always one at *ca.* 3.9 Ma between the Hojiro-kita and the Hojiro I volcanic ash layers, one to four at *ca.* 3.9 Ma immediately beneath the Hojiro II volcanic ash layer, one (rarely two) at *ca.* 3.8 Ma between the Hojiro II and low-Nakamura volcanic ash layers (the horizons of KUN-52, 53 samples), and one to several from *ca.* 3.8 Ma near the low-Nakamura volcanic ash layer to *ca.* 3.7 Ma near the Boji I volcanic ash layer (Figure 11B). The arrangement pattern of marginal fultoportulae is "Unmatched Pattern" from *ca.* 3.9 Ma above the Hojiro-kita volcanic ash layer to *ca.* 3.8 Ma near the low-Nakamura volcanic ash layer, and "Matched Pattern" from *ca.* 3.8 Ma near the Nakamura volcanic ash layer to *ca.* 3.7 Ma near the Boji I volcanic ash layer (Figure 11C).

According to the classification criteria described above, the two *Praestephanos* species had been replaced at *ca.*

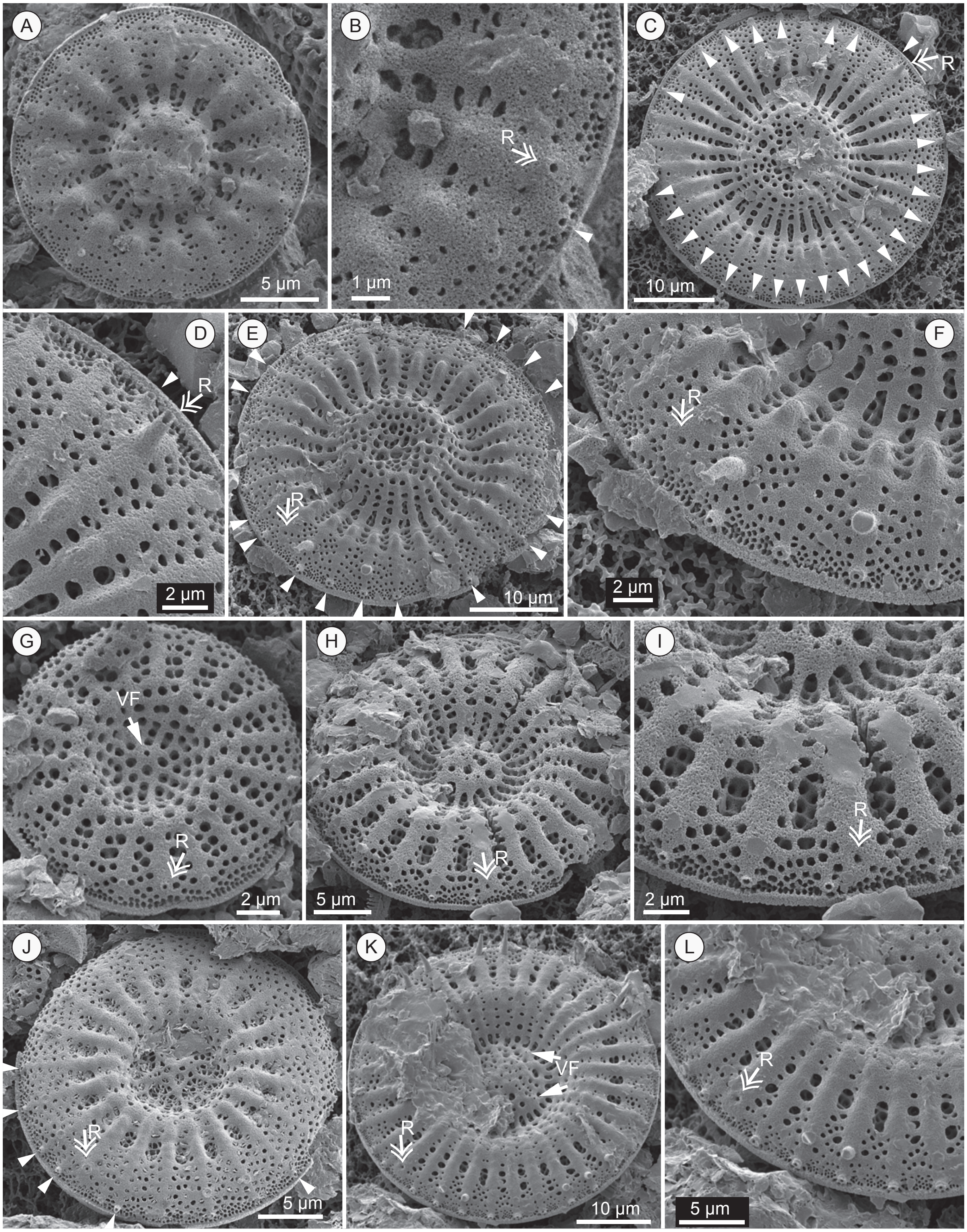

Figure 8. SEM images of external views of *Praestephanos miensis* sp. nov. **A–F,** convex valves from KUN-85 (A, B, E, F) and KUH-02 (C, D); **G–L,** concave valves from KUN-89 (G), KUN-85 (H, I, K, L) and Nakamura-ue (J); B, D, F, I, L, enlarged views of A, C, E, H, K, respectively, showing valve margin with marginal fultoportulae and rimoportula. Arrows marked "VF" and "R" indicate the locations of the valve face fultoportula and the rimoportula, respectively. Triangles mark the locations of marginal fultoportulae.

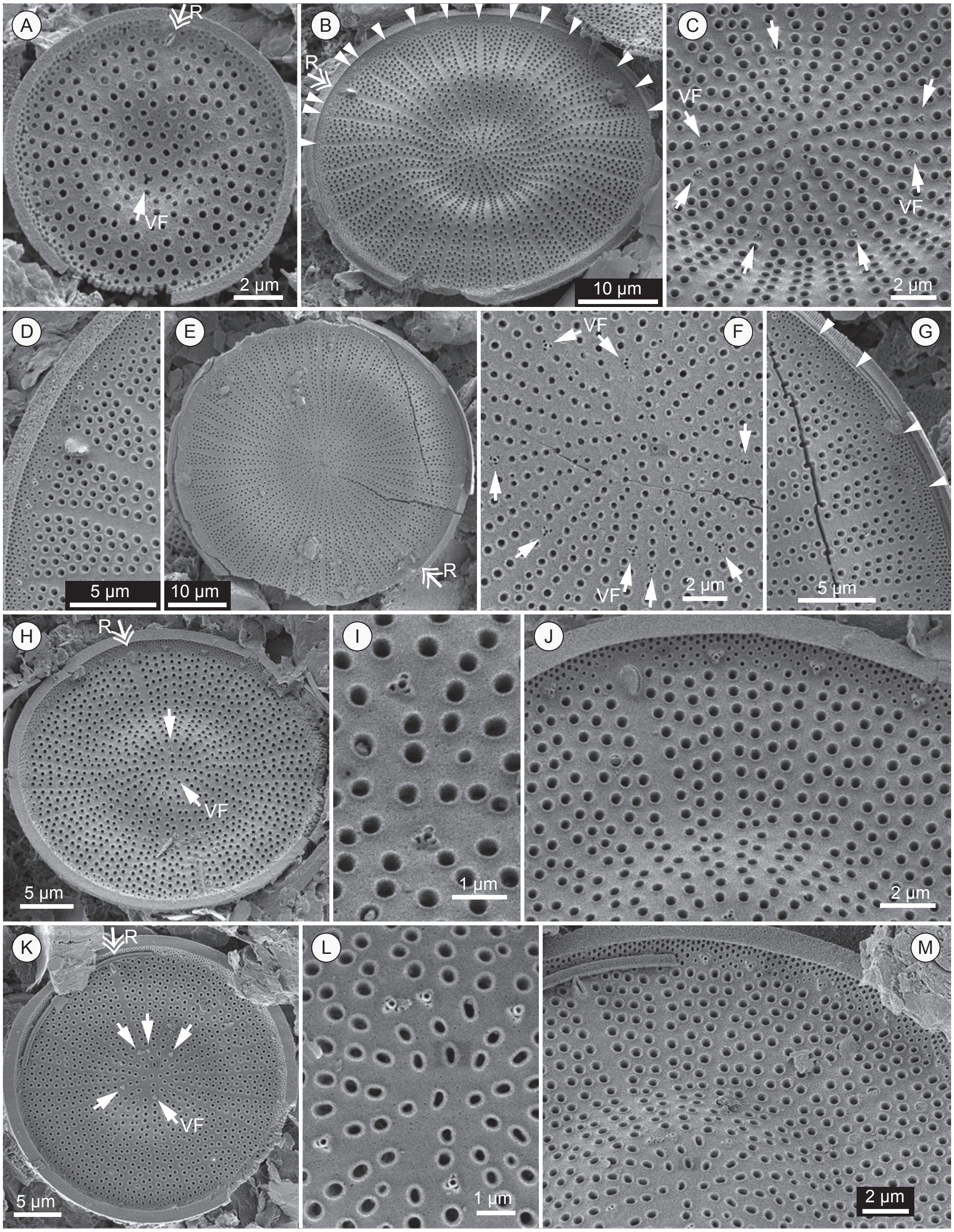

Figure 9. SEM images of internal views of *Praestephanos miensis* sp. nov. **A–G,** convex valves from KUN-85 (E–G), KUN-92 (A), and KUH-56 (B–D); C, D, enlarged views of B, showing central area with valve face fultoportulae and valve margin with marginal fultoportulae and rimoportula, respectively; F, G, enlarged views of E, showing valve face fultoportulae and valve margin with marginal fultoportulae, respectively; **H–M,** concave valves from KUH-56; I, J, enlarged views of H, showing valve face fultoportulae and valve margin with marginal fultoportulae and rimoportula, respectively; L, M, enlarged views of K, showing valve face fultoportulae and valve margin with marginal fultoportulae and rimoportula, respectively. Arrows marked "VF" and "R" indicate the locations of the valve face fultoportula and the rimoportula, respectively. Triangles mark the locations of marginal fultoportulae.

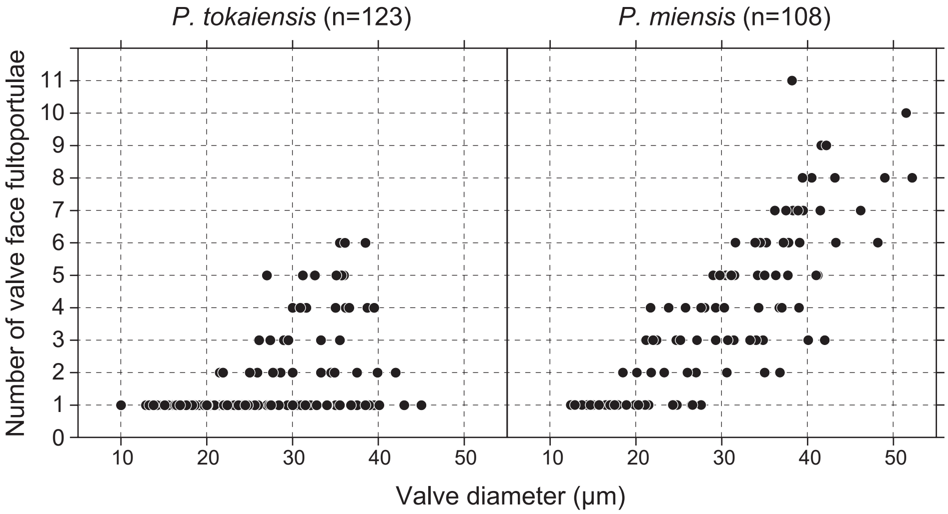

Figure 10. Relationship of the number of valve face fultoportulae to valve diameter in two new *Praestephanos* species from the Ueno Formation.

3.8 Ma, the horizon between KUN-81 and KUN-85 samples (Figure 11). The stratigraphic ranges of *P. tokaiensis* and *P. miensis* are *ca.* 3.9–3.8 Ma and *ca.* 3.8–3.7 Ma, respectively (Figure 11A).

Discussion

Comparison with allied species and similar *Stephanodiscus* species

Five *Praestephanos* species—*P. suzukii*, *P. praesuzukii*, *P. umbilicatus*, *Praestephanos carconensis* (Grunow) Tuji, and *Praestephanos triporus* (Genkal and G. V. Kuzmin) Tuji and J.-S. Ki—have been described so far (Tuji *et al.*, 2014; Tuji and Ohtsuka, 2020). In addition, *Stephanodiscus miyagiensis* H. Tanaka and Nagumo and *Stephanodiscus giganteus* Houk *et al.* (syn. *Stephanodiscus carconensis* f. *maxima* Fricke in Schmidt *et al.*) seem to have features that can be identified with *Praestephanos* (Tanaka and Nagumo, 2006; Houk *et al.*, 2014), namely “the marginal fultoportulae are often not on the extension of costae” and “the pattern of the areolae changes at the level of the marginal fultoportulae but not at the level of the rimoportulae or spines” (Tuji *et al.*, 2014). Here, two new *Praestephanos* species from the Ueno Formation are compared with these seven species, as shown in Table 1.

Praestephanos tokaiensis and *P. miensis* characteristically often exhibit a slight double undulation in the central area, whereas the central area of all similar species exhibits a single undulation or is flat (Tuji and Kociolek, 2000; Kato *et al.*, 2003; Tanaka and Nagumo, 2006; Kiss *et al.*, 2013; Houk *et al.*, 2014; Tuji *et al.*, 2014; Saito-Kato *et al.*, 2015). Furthermore, the valve face fultoportulae of *P. suzukii*, *P. praesuzukii*, and *P. umbilicatus* are located in deep depressions (Tuji and Kociolek, 2000; Kato *et al.*, 2003; Saito-Kato *et al.*, 2015), but no such depression is found in *P. tokaiensis* and *P. miensis*. *Praestephanos triporus* has a slit below the external opening of the marginal fultoportula (Kiss *et al.*, 2013), which is not found in *P. tokaiensis* and *P. miensis*. The fascicles of *P. triporus* and *P. umbilicatus* are composed of two areolae rows at the valve face/mantle junction and are thinner than those of *P. tokaiensis* and *P. miensis* (Kiss *et al.*, 2013; Saito-Kato *et al.*, 2015). The costae of *P. praesuzukii* have externally complex connections (Saito-Kato *et al.*, 2015), whereas those of *P. tokaiensis* and *P. miensis* do not. *Praestephanos carconensis*, *S. giganteus*, and *S. miyagiensis* can have more than one rimoportulae (Tanaka and Nagumo, 2006; Houk *et al.*, 2014; Tuji *et al.*, 2014), but *P. tokaiensis* and *P. miensis* always have one. The mantle of *S. miyagiensis* is high (Tanaka and Nagumo, 2006), but that of *P. tokaiensis* and *P. miensis* is very shallow.

As described above, no species was found that matched the combination of morphological characteristics of *P. tokaiensis* and *P. miensis*; therefore, we conclude that these are new species.

Notes on identification of the genus *Praestephanos*

The marginal fultoportulae of *P. miensis* are mostly on the extensions of costae, unlike those of typical *Praestephanos* taxa such as *P. suzukii*. *Praestephanos miensis*, however, has the characters that “the pattern of the areolae changes at the level of the marginal fultoportulae but not at the level of the rimoportulae or spines” (Tuji *et al.*, 2014), and the marginal fultoportulae near the

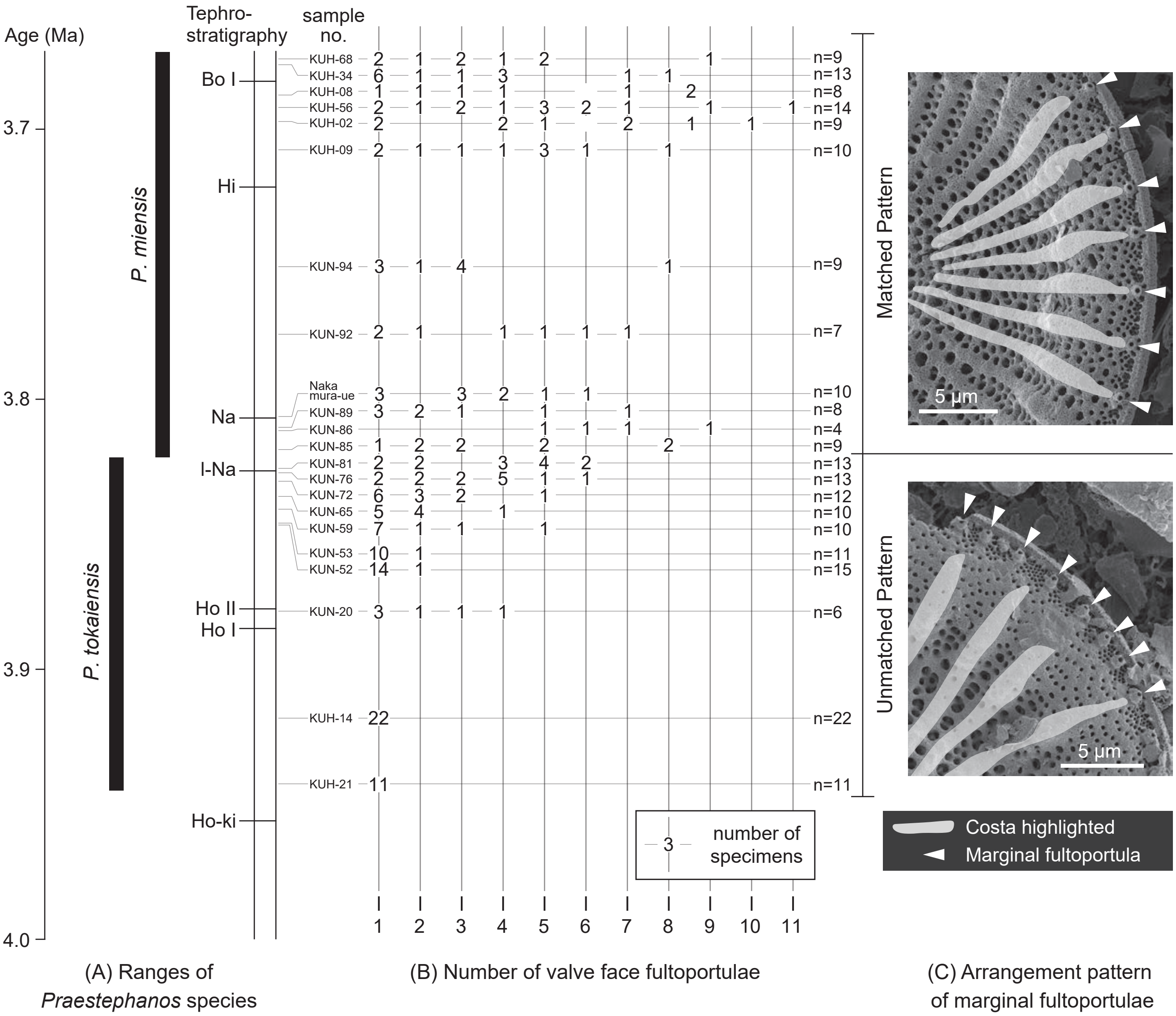

Figure 11. Stratigraphic ranges and changes in valve morphology of *Praestephanos* species from the Ueno Formation. Age and tephrostratigraphy are based on Satoguchi (2015). Bo I, Boji I; Hi, Hirata; Na, Nakamura; l-Na, low-Nakamura; Ho II, Hojiro II; Ho I, Hojiro I; Ho-ki, Hojiro-kita.

rimoportula are often not on the extension of the costa. Additionally, as mentioned below, *P. miensis* is clearly a descendant of *P. tokaiensis* which exhibits a combination of characters typical of *Praestephanos*. Therefore, *P. miensis* belongs without doubt, to the genus *Praestephanos*.

Tuji and Ohtsuka (2020) pointed out that the unique characteristic of the genus *Praestephanos*, which is that the marginal fultoportulae are often not on the extensions of costae, is easily observed using LM. Indeed, it seems that such fultoportulae can be recognized in *P. tokaiensis* by LM (Figure 5A–E); however, it is difficult to observe the marginal fultoportula that is not on the extension of the costa in *P. miensis* using only LM (Figure 5F–J). This finding suggests that careful observation using SEM may be necessary for assessing whether diatoms are the genus *Praestephanos* or other thalassiosiroid genera, even if the marginal fultoportulae of the diatoms can be seen to be located on the extensions of costae under LM.

Intraspecific variation of *Praestephanos tokaiensis*

Praestephanos tokaiensis has two patterns in the num-

Table 1. Descriptive and morphometric features of *Praestephanos tokaiensis* and *P. miensis* and comparison to the other *Praestephanos* and *Praestephanos*-like *Stephanodiscus* species.

Species	Diameter (μm)	Central area	Mantle	Number of areolae rows in fascicle at margin	Number of valve face fultoportulae [satellite pores]	Number of rimoportulae	Reference
P. tokaiensis	10–48	single or slightly double undulation	shallow	3–9	1–6 [3 (rarely 2)]	1	This study
P. miensis	11–62.5	single or slightly double undulation	shallow	3–8	1–11 [3 (rarely 2)]	1	This study
P. suzukii	5–50	single undulation	shallow	4–6	0–3 [3–4]	1	Kato *et al.* (2003), Tuji *et al.* (2014)
P. praesuzukii	4–24	single undulation	shallow	3–4	1?	1?	Saito-Kato *et al.* (2015)
P. umbilicatus	4–20	single undulation	shallow	2	1 [3]	1	Saito-Kato *et al.* (2015)
P. carconensis	12–100	single undulation	high?	4–6	1–several [2–3]	1–several	Tuji and Kociolek (2000), Tuji *et al.* (2014)
P. triporus	3.7–12.5	single undulation or flat	shallow	2–3 (rarely 1, 4)	1 [3 (rarely 2, 4)]	1	Kiss *et al.* (2013)
S. miyagiensis	20–68	single undulation	high	3–15	3–6 [1–4]	1 (rarely 2)	Tanaka and Nagumo (2006)
S. giganteus	25–180	flat	high?	–	many [2–3]	2	Houk *et al.* (2014)

ber of valve face fultoportulae depending on the stratigraphic occurrences. One of the patterns is almost always one fultoportula on a valve, and another one is one to several fultoportulae on a valve. The valves of the former pattern occur at the horizons (KUH-21, 14 samples) between the Hojiro-kita and the Hojiro I volcanic ash layers, and at the horizons (KUN-52, 53 samples) between the Hojiro II and the low-Nakamura volcanic ash layers (Figure 11B). The valves of the latter pattern occur at the horizon (KUN-20 sample) immediately below the Hojiro II volcanic ash layer and the horizons (KUN-59, 65, 72, 76, 81 samples) around the low-Nakamura volcanic ash layer (Figure 11B). Thus, the valves of the two patterns occur alternately during the interval of *ca.* 3.9–3.8 Ma (Figure 11B). In addition, there are no differences in the morphology other than the valve face fultoportulae between the valves of each pattern. Therefore, the difference can be interpreted as morphological variation over time.

Phylogenetic relationship between *Praestephanos tokaiensis* and *P. miensis*

On the basis of the morphology and their stratigraphic distribution, we suggest that *P. tokaiensis* and *P. miensis* are phylogenetically related. The combination of morphological characteristics of *P. tokaiensis* and *P. miensis* is consistent, except for the arrangement pattern of marginal fultoportulae and the number of valve face fultoportulae. Additionally, they occur successively in the strata deposited in a series of lake. Furthermore, around the horizon at which they are replaced, the maximum number of valve face fultoportulae, which is one of the differences between the two species, roughly increases gradually toward the upper horizons (Figure 11B), indicating that there seems to be transitional changes in the valve morphology from *P. tokaiensis* to *P. miensis*. These findings suggest that *P. tokaiensis* evolved into *P. miensis*, i.e., *P. tokaiensis* and *P. miensis* have an ancestor–descendant relationship.

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Author contributions

TK, MSK, and TO were responsible for the description and taxonomic aspects. TK and YS carried out the field work and collected the material. All authors contributed to the writing of the manuscript.