



## ***Tabularia kobayasii*: a new araphid diatom (Bacillariophyta, Fragilariaceae) from Japan**

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### **Abstract**

A new species of *Tabularia*, *T. kobayasii*, was found on seaweeds collected from several estuaries in the Central Pacific coast of Japan. Its morphology was examined by light and scanning electron microscopy; details are described herein. This araphid diatom is characterized by: 1) tufted or band-like colony; 2) valves lanceolate, flat with steep mantle, striae consisting of cribrate areolae, a wide lanceolate sternum, single rimoportula, and an ocellulimbus at both poles; and 3) a cingulum consisting of a few non-fimbriated girdle bands including the valvocopula.

**Key Words:** araphid diatom, Bacillariophyta, estuary, Japan, new species, *Tabularia kobayasii*

### **Introduction**

*Tabularia* Williams & Round (1986: 320) (Bacillariophyta, Fragilariaceae) is one of the commonest marine ‘*Synedra*’ *sensu lato* forms. Its species are usually epiphytic or epilithic and attached to substrata by mucilage pads, usually in tufts. Fourteen species are listed in Algaebase (Guriry & Guiry 2015). *Tabularia* differs from the other similar genera, such as *Hyalosynedra* Williams & Round (1986: 318) and *Neosynedra* Williams & Round (1986: 332), by virtue of the structure of the vela and presence of an ocellulimbus. In addition, because of the fine structure of the striae, Williams & Round (1986) informally subdivided the genus into the following three groups: i) those with valves having biseriate striae with simple closing plates; ii) those with valves having cribra with heavily silicified cross-members; and iii) those with valves having a complex cribrate closing plate.

In this study, an unrecognized species of *Tabularia*, belonging to the second group, was found growing on seaweeds (e.g. *Caloglossa ogasawaraensis* Okamura (1897: 13)) from several estuaries in the Central Pacific coast of Japan. It is described using light (LM) and scanning electron microscopy (SEM) and comparisons are made to similar taxa. It has been named *Tabularia kobayasii* Hidek. Suzuki & Mitsuishi *sp. nov.*

### **Material and methods**

Samples were obtained from seaweeds collected from several estuaries in the Central Pacific coast of Japan as listed in Table 1. Samples and type slides were deposited at the Natural History Museum (BM 101792), the Museum of Fishery Sciences, Tokyo University of Marine Science and Technology, Japan (MTUF-AL 43058–43077), and the Micropaleontology Collection, National Museum of Nature and Science, Tokyo (MPC-RM-HK 4711 and 4718).

Samples were fixed with 2.5% glutaraldehyde or 10% formalin solution. The material was treated using the bleaching method as described by Nagumo & Kobayasi (1990), Nagumo (1995) and Osada & Nagumo (2001). Light and scanning electron microscopy techniques are described in Kuriyama *et al.* (2010). Prepared specimens were examined using HITACHI S-5000 SEM (The Nippon Dental University School of Life Dentistry at Tokyo).

**TABLE 1.** List of samples used in this study.

| No.  | Substrate                        | Estuary          | Site   | Latitude  | Longitude  | Date         | Collector    | Material and slide Number   |
|------|----------------------------------|------------------|--|-----------|------------|--------------|--------------|-----------------------------|
| I    | <i>Caloglossa ogasawaraensis</i> | Naka River       | Isohamacho, Oarai-machi, Higashiibaraki-gun, Ibaraki Pref. | 36° 20' N | 140° 35' E | 31 Jul. 2008 | K. Mitsuishi | MTUF-AL 43058               |
|      |                                  |                  |  |           |            | 30 Oct. 2008 | K. Mitsuishi | MTUF-AL 43059               |
| II   | n.d.                             | Arakawa River    | Kasai bridge, Nishikasai, Edogawa-ku, Tokyo                | 35° 39' N | 139° 50' E | Aug. 1994    | H. Kobayasi  | MPC-RM-HK 4711              |
| III  | n.d.                             | Sumida River     | Ryogoku, Sumida-ku, Tokyo                                  | 35° 41' N | 139° 47' E | undated 1994 | H. Kobayasi  | MPC-RM-HK 4718              |
| IV   | <i>Caloglossa ogasawaraensis</i> | Meguro River     | Keihin Canal, Konan, Minato-ku, Tokyo                      | 35° 37' N | 139° 45' E | 18 Jun. 2008 | K. Mitsuishi | MTUF-AL 43060               |
|      |                                  |                  |  |           |            | 16 Jul. 2008 | K. Mitsuishi | MTUF-AL 43061               |
|      |                                  |                  |  |           |            | 3 Aug. 2008  | K. Mitsuishi | BM 101792 and MTUF-AL 43062 |
|      |                                  |                  |  |           |            | 3 Sep. 2008  | K. Mitsuishi | MTUF-AL 43063               |
|      |                                  |                  |  |           |            | 13 Oct. 2008 | K. Mitsuishi | MTUF-AL 43064               |
|      |                                  |                  |  |           |            | 12 Nov. 2008 | K. Mitsuishi | MTUF-AL 43065               |
|      |                                  |                  |  |           |            | 13 Dec. 2008 | K. Mitsuishi | MTUF-AL 43066               |
|      |                                  |                  |  |           |            | 28 Mar. 2009 | K. Mitsuishi | MTUF-AL 43067               |
|      |                                  |                  |  |           |            | 24 Apr. 2009 | K. Mitsuishi | MTUF-AL 43068               |
|      |                                  |                  |  |           |            | 24 May 2009  | K. Mitsuishi | MTUF-AL 43069               |
| V    | <i>Caloglossa ogasawaraensis</i> | Tsurumi River    | Namamugi, Tsurumi-ku, Yokohama-shi, Kanagawa Pref.         | 35° 29' N | 139° 40' E | 4 Aug. 2008  | K. Mitsuishi | MTUF-AL 43070               |
|      |                                  |                  |  |           |            | 27 Oct. 2008 | K. Mitsuishi | MTUF-AL 43071               |
|      |                                  |                  |  |           |            | 10 Feb. 2009 | K. Mitsuishi | MTUF-AL 43072               |
|      |                                  |                  |  |           |            | 25 May 2009  | K. Mitsuishi | MTUF-AL 43073               |
| VI   | <i>Caloglossa ogasawaraensis</i> | Matsukoshi River | Nagasaka, Yokosuka-shi, Kanagawa Pref.                     | 35° 13' N | 139° 37' E | 2 Sep. 2008  | K. Mitsuishi | MTUF-AL 43074               |
| VII  | <i>Cladophora</i> sp.            | Ota River        | Kumomi, Matsuzaki-machi, Kamo-gun, Shizuoka Pref.          | 34° 43' N | 138° 44' E | 13 May 2001  | H. Suzuki    | MTUF-AL 43075               |
|      |                                  |                  |  |           |            | 23 Nov. 2008 | H. Suzuki    | MTUF-AL 43076               |
| VIII | <i>Caloglossa ogasawaraensis</i> | Kuroishi River   | Kogawa, Yaizu-shi, Shizuoka Pref.                          | 34° 50' N | 138° 19' E | 16 Jun. 2009 | H. Suzuki    | MTUF-AL 43077               |

The valves' size and striae density were determined from measurements of 30 valves. For morphometric analysis, all scanning electron micrographs were taken under identical conditions to exclude potential mechanical errors being involved in SEM (with an accelerating voltage = 2 kV, magnification = 15,000–20,000). Measurements were made on 20 valves that were randomly selected from the type material under SEM. The measured variables were a) length of the minor axis of areola (= length of vimines) of the middle of each valve, which are cross connections of virgae forming areolae; and b) length of the major axis of ocellulimbus.

The temperature and salinity from the sampling sites were measured with a bar thermometer and a hand-held refractometer (IS/Mill-E, ATAGO Co., Ltd, Tokyo, Japan), respectively.

Terminology for the parts of the valve and girdle follow Anonymous (1975), Ross *et al.* (1979) and Cox & Ross (1981), with additional terms from Kobayasi *et al.* (2006).

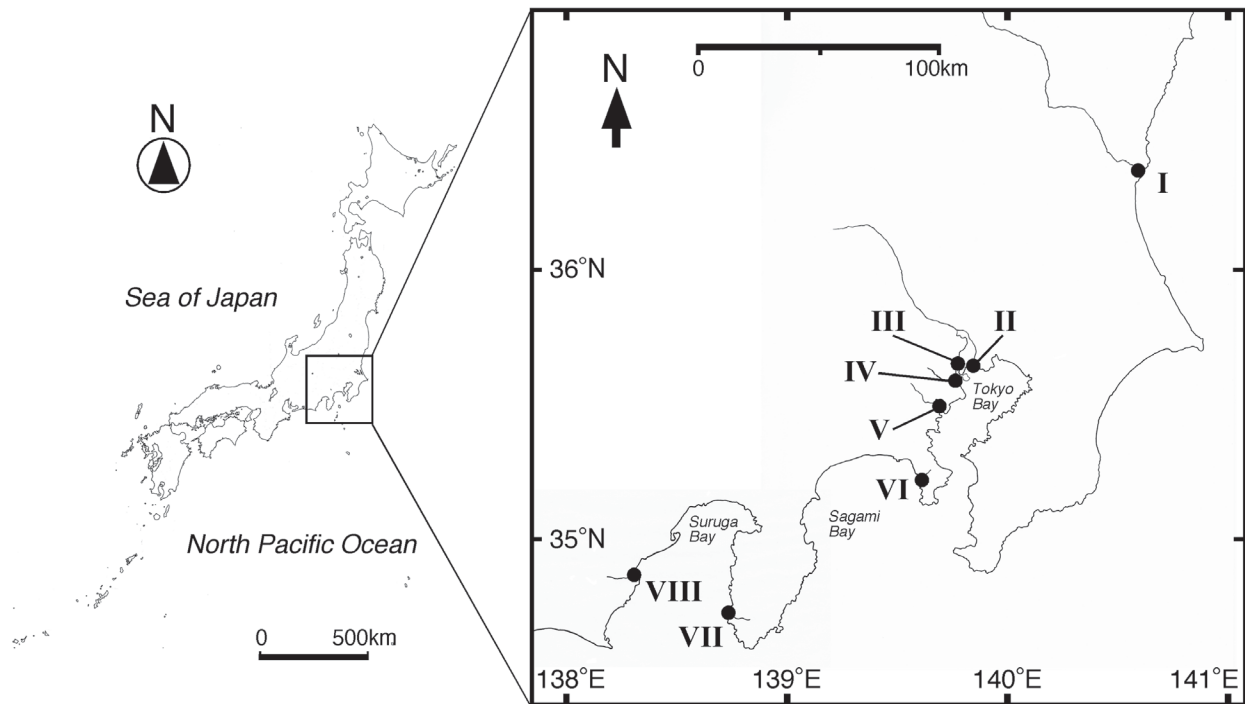


FIGURE 1. Map of the 8 sampling estuaries in the Central Pacific coast of Japan. Numbers (I–VIII) correspond to those in Table 1.

## New species description

### Division Bacillariophyta

### Class Bacillariophyceae

### Order Fragilariales

### Family Fragilariaceae

### Genus *Tabularia*

#### *Tabularia kobayasii* Hidek. Suzuki & Mitsuishi *sp. nov.* (Figs 2–32)

Cells needle-like, grow either solitary or in tufted or band-like colonies attached to substrate by mucilage pads (Figs 2, 4, 5); valves only rarely joined face-to-face (Fig. 3). Each cell with several plate-like chloroplasts along valve inner wall (Fig. 3, arrowheads). Valves lanceolate with parallel to sub-parallel margins, tapering towards rounded poles (Figs 6–14, 17, 18); length 11.5–87.5  $\mu\text{m}$ , width 5.0–7.5  $\mu\text{m}$ . Cells elongated, rectangular in girdle view (Figs 15, 16), larger cells gently arcuate, possibly initial cells (Figs 6, 7). Valve face flat (Fig. 19), with transition to valve mantle gently steeped (Figs 19, 22). Transapical plane of valve rectangular to trapezoidal. Striae density at valve center 10–12 in 10  $\mu\text{m}$ . Striae parallel on valve face, becoming slightly radiate at pole (Figs 7–14, 17). Each stria (width: 0.2  $\mu\text{m}$ , ‘a’ in Fig. 33) consisting of a cribrate areola with several vimines (Figs 21, arrowheads, 22), continuing up to edge of valve (Figs 19, 22). Its outside edge bends through a right angle at junction between valve face and mantle (Figs 19, 22). Viewed internally, most of areolae sunk deeply between short ribs (virgae) (Figs 20, 21). Sternum wide lanceolate and occupying at least half of valve surface (Figs 7–14, 17). Single rimoportula at one end of sternum (Figs 17, 18, arrows), off-center and aligned with stria, opening to exterior as small rectangular slit pore (Fig. 23, arrow), internally as a shallow process (Fig. 24, arrow). Distinct ocellulimbus at both poles (0.47–0.80  $\mu\text{m}$ , asterisks in Figs 23–26 and ‘b’ in Fig. 36), swelling at center (Fig. 25, double arrowhead). Ocellulimbus composed of 6–8 rows of 8–9 poroids, enclosed by a small rim. Rim on its advalvar side is plain, without spines or flaps. Externally, areolae do not reach poles but terminate in a single row of unoccluded small pores (Figs 23, 25, arrowheads). Mature cingulum consisting of 4–6 girdle bands (Figs 27–29), open at one pole, divided into two types: Valvocopula (VC) has a crenulated edge (pars interior) fitting on top of valve ribs (Figs 29, 30, arrowheads), without areolae (Fig. 30); copulae (3) narrower and thinner than valvocopula, with uniseriate row of areolae (Fig. 29), without fimbriae.

**Type:**—JAPAN. Keihin Canal (35° 63' N, 138° 75' E), the estuary of Meguro River, Konan, Minato-ku, Tokyo, collected from surface of *Caloglossa ogasawaraensis*, K. Mitsuishi, 3 August 2008 (holotype BM! 101792, illustrated in Fig 6; isotype MTUF! AL 43062).

**Etymology:**—The specific epithet “kobayasii” is dedicated to the late Dr. Hiromu Kobayasi in recognition of his significant contribution to taxonomic research in diatoms.

**Ecology:**—With the sampling program undertaken, salinity ranges of 10.7–15.4, 8.1–25.6, 7.6–19.9 and 4.7–13.7, and temperature ranges of 8.3–27.2 °C, 15.0–32.3 °C, 11.5–28.0 °C and 18.8–27.5 °C were recorded in estuary of Naka River, Meguro River, Tsurumi River and Matsukoshi River respectively (Table 2), where *Tabularia kobayasii* was found growing abundantly (46.7–96.6% relative abundance) as an epiphyte on *Caloglossa ogasawaraensis* for a full year (predominantly summer and autumn months). *Tabularia kobayasii* can be described as a brackish-water, epiphytic diatom with high rate of productivity during summer months.

**TABLE 2.** Temperature and salinity in the four sampling estuaries in this study. Numbers (I, IV, V and VI) correspond to those in Table 1.

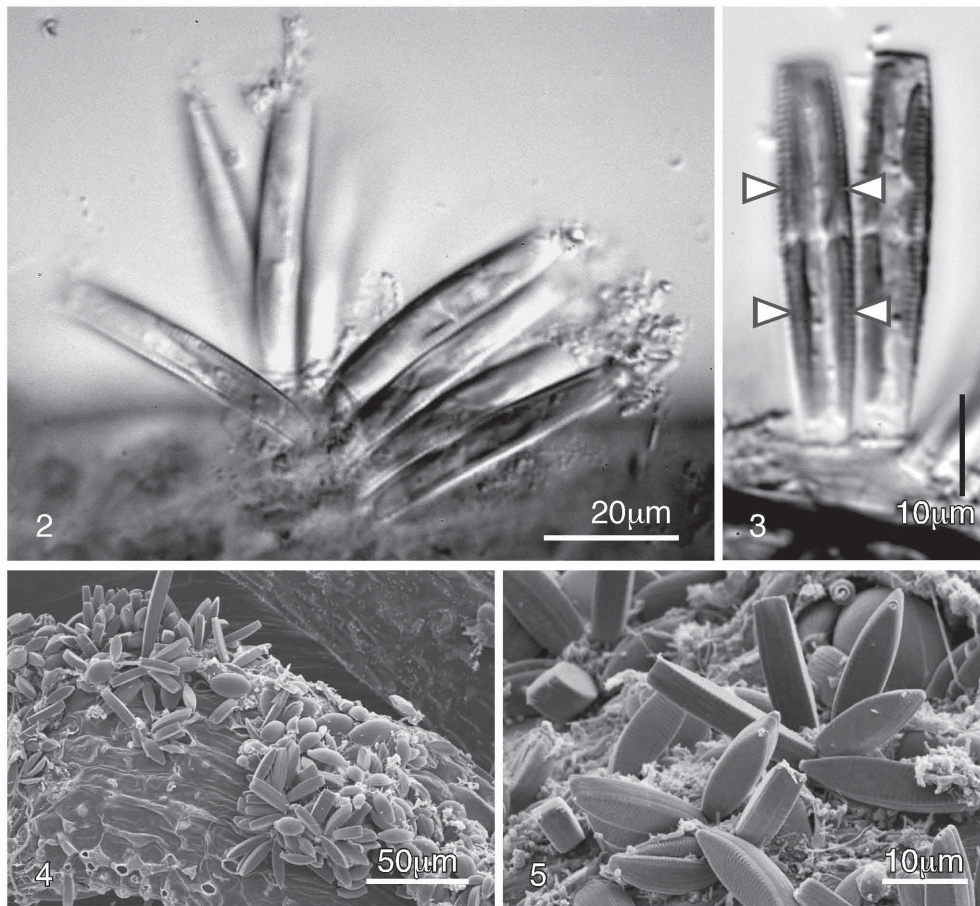
| No. | Estuary          |                  | Summer       | Autumn       | Winter       | Spring      |
|-----|------------------|------------------|--------------|--------------|--------------|-------------|
| I   | Naka River       | Date             | 31 Jul. 2008 | 30 Oct. 2008 | 11 Feb. 2009 | 10 May 2009 |
|     |                  | Temperature (°C) | 27.2         | 15.5         | 8.3          | 18.2        |
|     |                  | Salinity         | 10.7         | 15.4         | 12.8         | 10.7        |
| IV  | Meguro River     | Date             | 3 Aug. 2008  | 13 Oct. 2008 | 12 Feb. 2009 | 24 May 2009 |
|     |                  | Temperature (°C) | 32.3         | 25.0         | 15.0         | 19.5        |
|     |                  | Salinity         | 8.1          | 15.5         | 25.6         | 15.5        |
| V   | Tsurumi River    | Date             | 4 Aug. 2008  | 27 Oct. 2008 | 10 Feb. 2009 | 25 May 2009 |
|     |                  | Temperature (°C) | 28.0         | 20.3         | 11.5         | 20.2        |
|     |                  | Salinity         | 8.5          | 10.2         | 19.9         | 7.6         |
| VI  | Matsukoshi River | Date             | 2 Sep. 2008  | 28 Oct. 2008 | n.d.         | 9 May 2009  |
|     |                  | Temperature (°C) | 27.5         | 18.8         | n.d.         | 20.5        |
|     |                  | Salinity         | 9.6          | 13.7         | n.d.         | 4.7         |

n.d.: no data.

**Observations:**—*Tabularia kobayasii* can be compared to: *Tabularia barbatula* (Kützing) D. M. Williams & Round (1986: 322) (basionym: *Synedra barbatula* Kützing 1844: 68), *T. fasciculata* (C. Agardh) D. M. Williams & Round (1986: 326) (basionym: *Diatoma fasciculata* C. Agardh 1812: 35), *T. investiens* (W. Smith) D. M. Williams & Round (1986: 324) (basionym: *Synedra investiens* W. Smith 1856: 98), *T. parva* (Kützing) D. M. Williams & Round (1986: 324) (basionym: *Synedra parva* Kützing 1849: 46), *T. variostrata* M. A. Harper in Harper *et al.* (2009: 293) and *T. waernii* Snoeijs in Snoeijs & Kuylenstierna (1991: 352). The fine structures of the valves of *Tabularia*, such as areolae, ocellulimbus and cingulum, have been described in detail for these species (*Tabularia barbatula* in Williams & Round 1986; *T. fasciculata* in Williams & Round 1986, Snoeijs 1992 and Kuriyama *et al.* 2008, *T. investiens* in Williams & Round 1986 and Kuriyama *et al.* 2010, *T. variostrata* in Harper *et al.* 2009 and *T. waernii* in Snoeijs & Kuylenstierna 1991). Their characteristics can be summarized as following: they have araphid valves; the valves are elongate, linear or lanceolate; the striae are transverse, separated internally by distinct ribs; the areolae are occluded externally by cribra with distinct cross bars; they have an ocellulimbus, which is small, with few poroids per plate; there is one rimoportula situated close to ocellulimbus; and the cingulum consists of a few open, ligulate bands.

Our SEM observations on *Tabularia kobayasii* confirm it is a member of *Tabularia*. It is most similar to *T. investiens* and *T. variostrata* as these species have cribra with heavily silicified cross-members in structure of areolae. All three species, *Tabularia kobayasii*, *T. investiens* and *T. variostrata*, belong to “Group 2” of Williams & Round (1986). The cingulum of the three species is typical in having a non-areolate open valvocopula and several open copulae with a single row of poroids.

The following features distinguish *T. kobayasii* from *T. investiens* and *T. variostrata*, are thus diagnostic for *T. kobayasii* (listed in Table 3): a) lanceolate valves; b) narrow striae; c) small ocellulimbus; and d) denser vimines.



**FIGURES 2–5.** *Tabularia kobayasii*. Epiphytic on *Caloglossa ogasawaraensis* from Keihin Canal, Meguro River Estuary collected in 18 June 2008, LM (2, 3) and SEM (4, 5). 2. Living tufted and band-like colonies. 3. Living cells attached by small mucilage pads. Arrowheads indicate four plastids after cytokinesis. 4, 5. Tufted colonies.

**TABLE 3.** Comparison of *Tabularia kobayasii* with *T. investiens* and *T. variostrata* in morphological attributes.

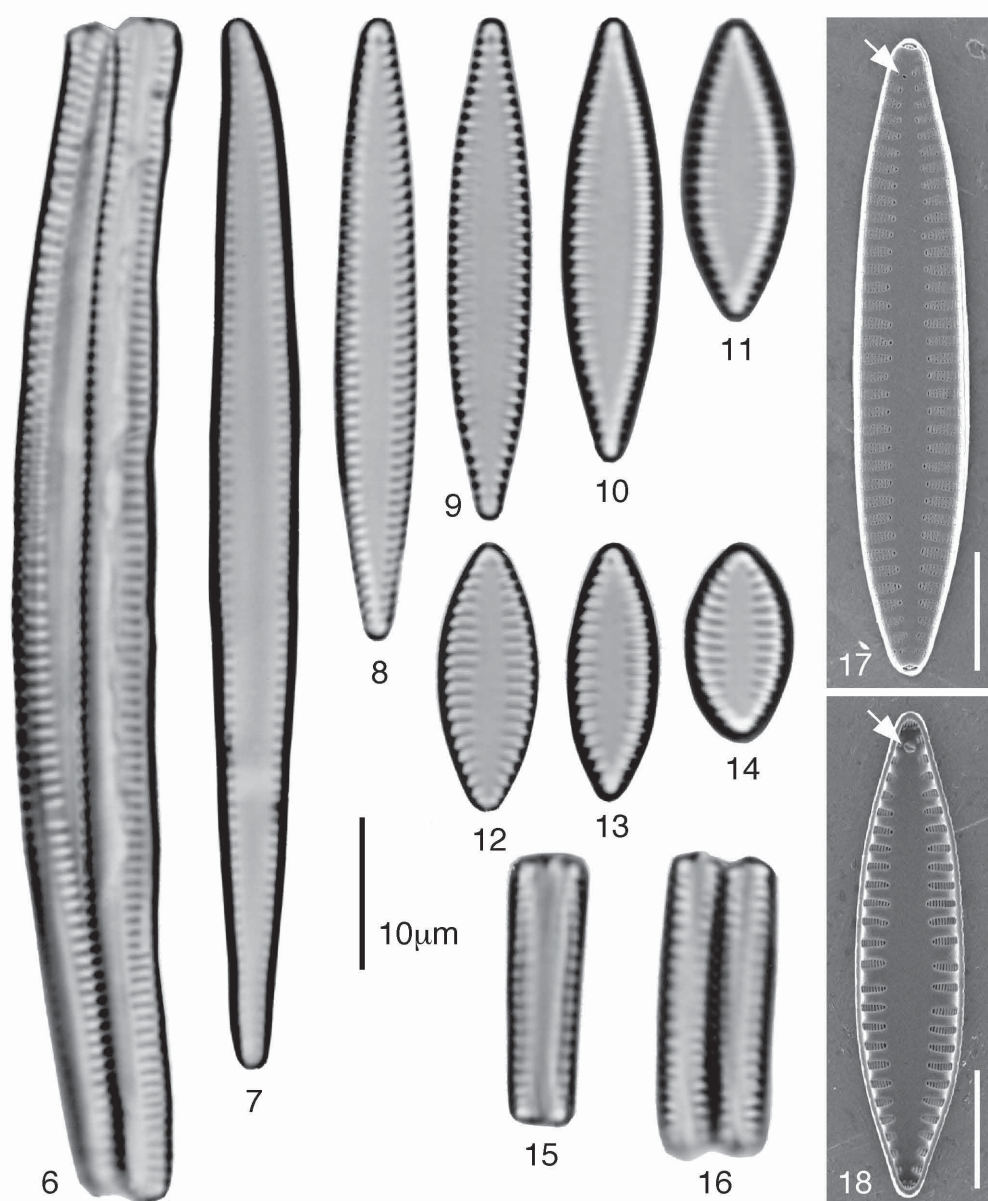
| Characteristics                    | <i>T. kobayasii</i> <sup>a</sup> | <i>T. investiens</i> <sup>a, b, c</sup>           | <i>T. variostrata</i> <sup>d</sup>                                     |
|------------------------------------|----------------------------------|---|--|
| Structure of colony                | tufted or band-like              | tufted or band-like                               | tufted   |
| Length of valve (µm)               | 11.5–87.5                        | <sup>b</sup> 25.0–35.0, <sup>c</sup> 15.0–97.0    | 60.0–200.0   |
| Width of valve (µm)                | 5.0–7.5                          | <sup>b</sup> 2.0–4.0, <sup>c</sup> 3.0–5.0        | 5.0–10.0   |
| Valve                              |                                  |   |  |
| Valve outline                      | lanceolate                       | linear–elliptical, tapering towards rounded poles | linear to linear–lanceolate, nearly isopolar to distinctly heteropolar |
| Girdle view                        | elongate and rectangle           | elongate and rectangle                            | elongate and rectangle   |
| Valve face                         | plane                            | convex  | plane  |
| Mantle                             | present                          | absent  | present  |
| Shape of sternum                   | wide lanceolate                  | narrow lanceolate                                 | wide lanceolate, with undulated edge                                   |
| Density of striae in 10µm          | 10–12                            | <sup>b</sup> n.d., <sup>c</sup> 7–13              | 11–16  |
| Structure of stria                 | a cribrate areola with vimines   | a cribrate areola with vimines                    | a cribrate areola with vimines   |
| Width of stria (a in Fig. 33) (µm) | 0.2                              | 0.6 <sup>a</sup>                                  | 0.4  |
| Density of vimines in 1µm          | 10                               | 5   | 3  |
| Number of rimopotula               | 1                                | 1 or 2  | 2  |

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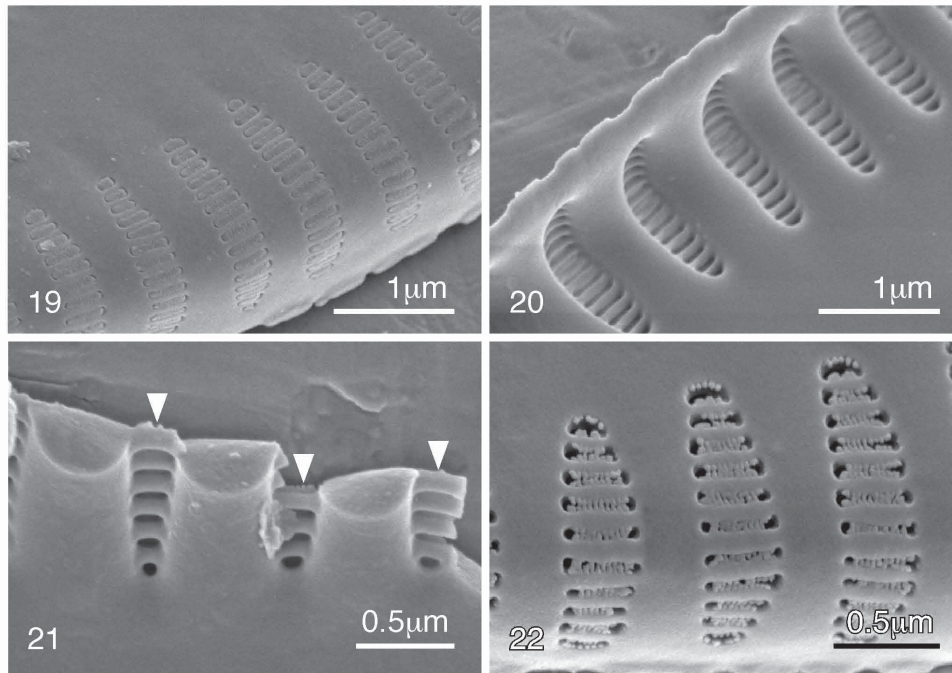
**TABLE 3.** (Continued)

| Characteristics                           | <i>T. kobayasi</i> <sup>a</sup>         | <i>T. investiens</i> <sup>a, b, c</sup> | <i>T. variostrata</i> <sup>d</sup>      |
|---|---|---|---|
| Width of ocellulimbus (b in Fig. 36) (µm) | 0.47–0.80                               | 1.00–1.87 <sup>a</sup>                  | n.d.                                    |
| Width of ocellulimbus / width of valve    | 0.10–0.24                               | 0.29–0.51 <sup>a</sup>                  | n.d.                                    |
| Spines overhang ocellulimbus              | absent                                  | absent                                  | present                                 |
| <b>Girdle bands</b>                       |   |   |   |
| Number of bands                           | 4–6                                     | 5–7                                     | 4                                       |
| Valvocopula                               | non-areolate open band                  | non-areolate open band                  | non-areolate open band                  |
| Shape of inner edge                       | slightly crenulate                      | slightly crenulate                      | slightly crenulate                      |
| Copulae                                   | open bands with a single row of poroids | open bands with a single row of poroids | open bands with a single row of poroids |
| Shape of inner edge                       | smooth                                  | smooth                                  | n.d.                                    |

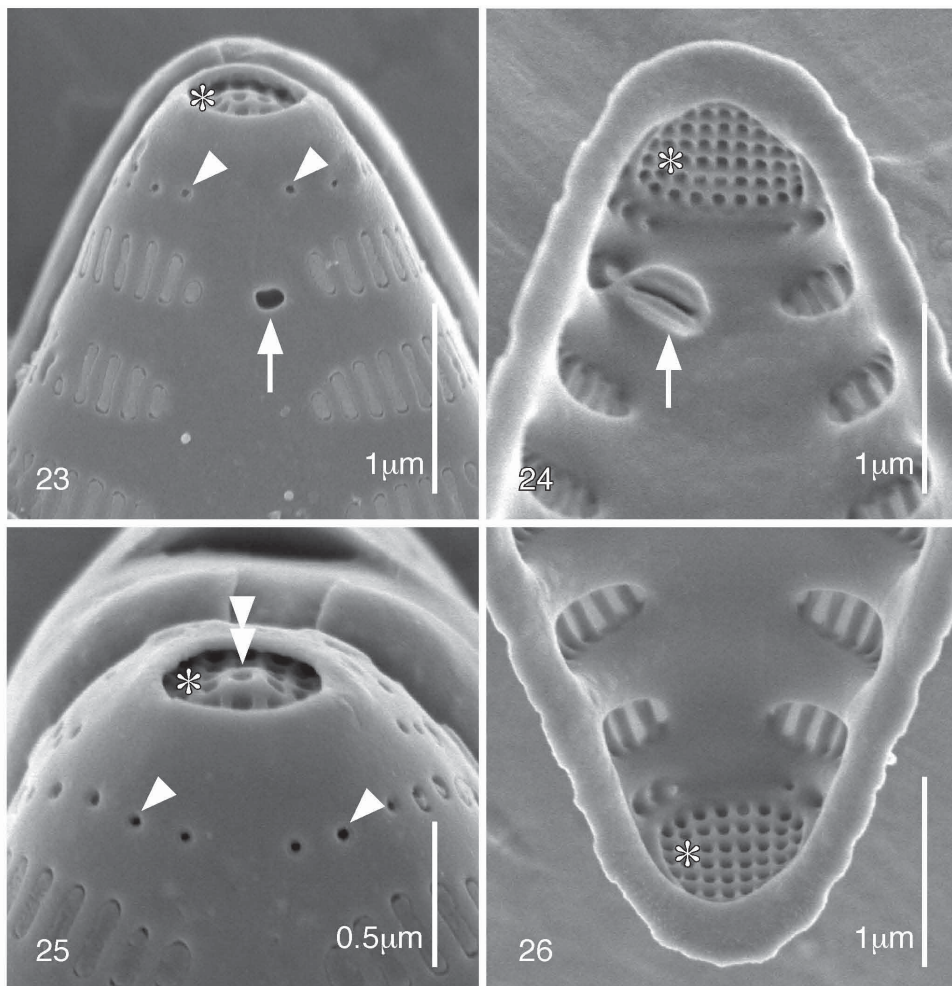
<sup>a</sup>This study, <sup>b</sup>Williams and Round (1986), <sup>c</sup>Kuriyama *et al.* (2010), <sup>d</sup>Harper *et al.* (2009), n.d.: no data.



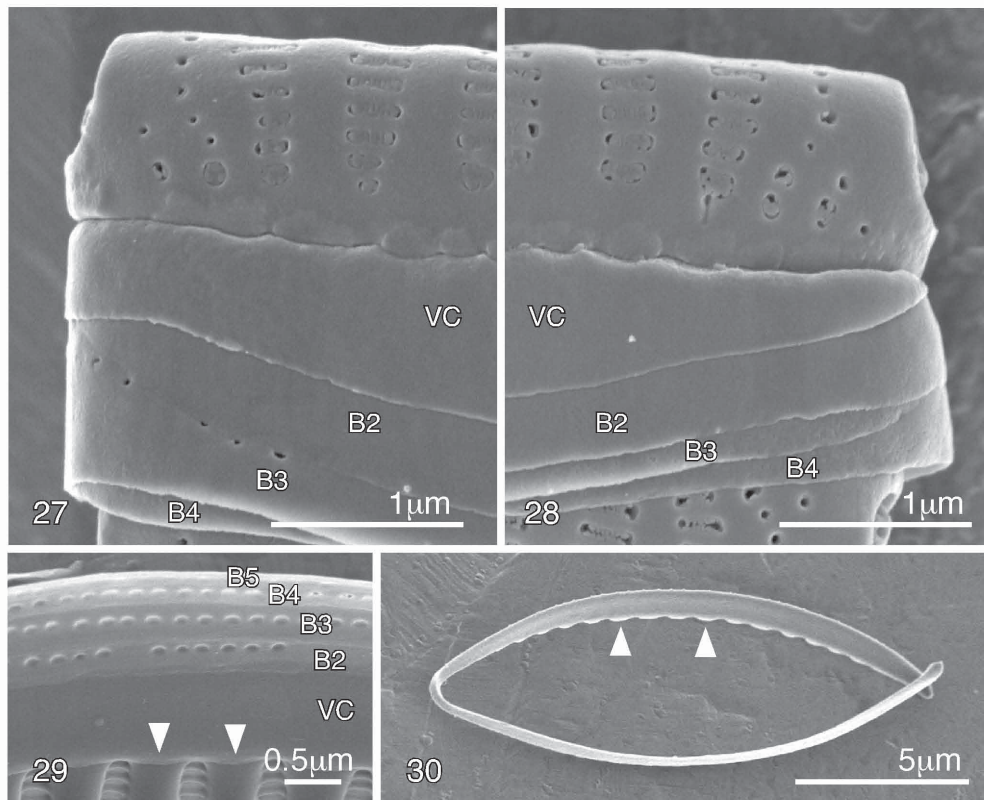
**FIGURES 6–18.** *Tabularia kobayasi*. LM (6–16) and SEM (17, 18), holotype and other specimens. 6, 16. Girdle views of two frustules. 7–14. Valve views. 6, 7. Initial valves. 8. Specimen from holotype slide. 15. Girdle view of a frustule. 17. External view of a whole valve. Arrow indicates the opening of rimoportula. 18. Internal view of a whole valve. Arrow indicates the rimoportula. Scale bars = 5 µm (17, 18).



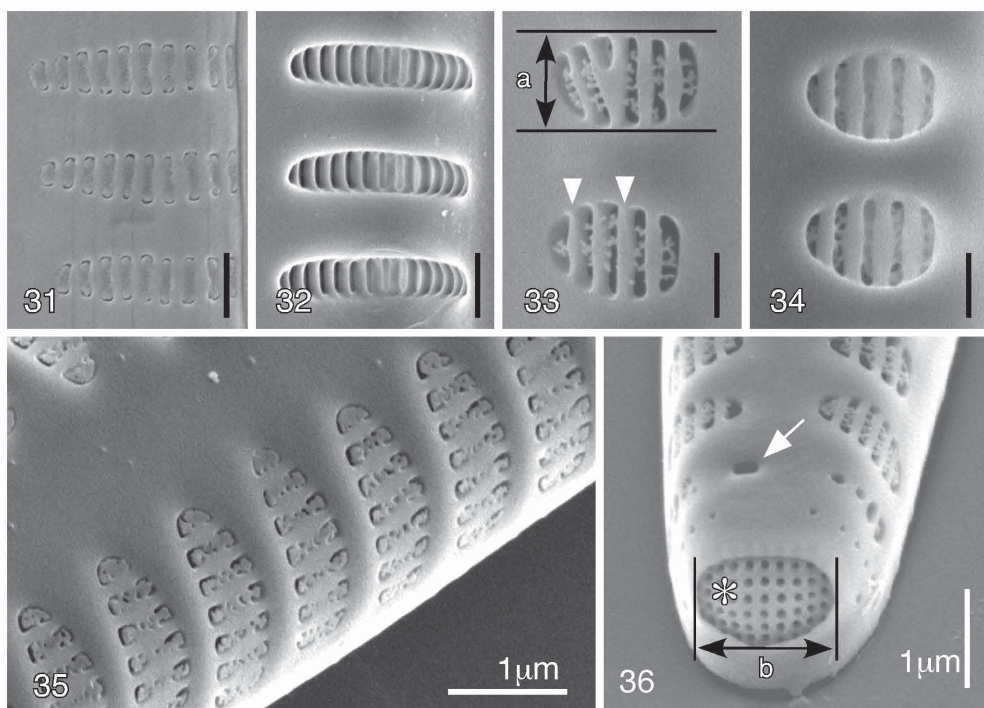
**FIGURES 19–22.** *Tabularia kobayasii*. SEM. 19. External view of valve mantle showing the areolae with cross-bars (= vimines). 20. Internal view of the areolae. 21. Detail of the cross section of areolae. Arrowheads indicate cross-bars (= vimines). 22. External view of immature areolae.



**FIGURES 23–26.** *Tabularia kobayasii*. SEM. 23, 25. External polar views showing the ocellulimbus (asterisks), the opening of rimoportula (arrow) and the tiny simple pores (arrowheads). 25. Double arrowhead indicates a small bulge of ocellulimbus. 24, 26. Internal polar views showing the ocellulimbus (asterisks) and a rimoportula (arrow).



**FIGURES 27–30.** *Tabularia kobayasii*. SEM. 27, 28. Terminal views of the same cingulum composed of a valvocopula (VC) and three bands (B2–B4). 29. Internal view of a valvocopula (VC) and four bands (B2–B5). Arrowheads indicate the crenulated parts interior of valvocopula. 30. Valvocopula. Arrowheads indicate the crenulated parts interior.



**FIGURES 31–36.** *Tabularia kobayasii* (31, 32) and *T. investiens* (33–36). SEM. 31, 33. Detail of external views of cribrate areolae. Arrowheads indicate the cross-bars (= vimines). 32, 34. Detail of internal views of areolae. 35. External view of valve face showing the areolae. 36. External polar view showing the ocellulimbus (asterisk) and the opening of rimoportula (arrow). Scale bars = 0.5  $\mu\text{m}$  (31–34). 'a': length of the minor axis of areola, 'b': length of the major axis of ocellulimbus. See Material and Methods for details.



*Tabularia kobayasii* can be readily distinguished from *T. investiens* as the latter has a linear-elliptical valve outline, a convex valve plane (Fig. 35), a narrow lanceolate sternum, wider striae (0.6 µm, Figs 33, 34), a larger ocellulimbus (1.00–1.87 µm, Fig. 36) and less dense vimines (5 in 1 µm, Fig. 33); it can be also differentiated from *T. variostrata* by the latter having a different valve outline and shape of its sternum, and the rim structure of the ocellulimbus: *T. variostrata* has nearly isopolar to distinctly heteropolar valves, striae of various lengths forming an undulated edge to wide lanceolate sternum, and two to four advalvar spines on the rim of the ocellulimbus-type pore-fields (Harper *et al.* 2009).

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