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# A new freshwater diatom, *Terpsinoe muninensis* sp. nov., from the Ogasawara Islands, Japan

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**Abstract.** A new freshwater diatom, *Terpsinoe muninensis*, is described as a new species. This taxon is close to *T. musica*, but is distinguished by its weak transapical bars at the apices and the ratio of each inflation. Since this taxon is the first reported freshwater *Terpsinoe* species in Japan, it may be related to taxa reported in other oceanic islands in the North Pacific.

**Key words:** endemism, *Terpsinoe muninensis*, *Terpsinoe musica*, Oceanic islands, Ogasawara Islands

## Introduction

The Ogasawara Islands are the only one oceanic islands in Japan, and many endemic organisms from these islands have been described. However, freshwater diatoms from these islands have never been investigated. The diatom flora from other oceanic islands in the North Pacific have been studied (Guam: Navarro and Lobban (2009), Hawaii: Massey (1978), Mcmillan and Rushforth (1985), Kubota (1999), Lowe *et al.* (2009) and Sherwood *et al.* (2014)), and we can evaluate the relationship between the Ogasawara Islands and other oceanic islands in the North Pacific. To elucidate the process by which freshwater diatoms spread, it is essential to investigate the flora in oceanic islands, with North Pacific oceanic islands including the Ogasawara Islands being an especially important place to investigate.

In this paper, I described a new *Terpsinoe* species from the Ogasawara Islands which seems to be related with species endemic to other North Pacific oceanic islands.

## Material and methods

1. Sampling

Fifty-four algal samples were collected from Chichi-jima Island and Haha-jima Island using small spoons, toothbrushes and a plankton net. A *Terpsinoe* species was found at one locality on Haha-jima Island and one locality on Chichi-jima Island.

a. Samples were collected from Funakiyama Waterfall on Haha-jima Island (latitude: 26°38.976′N, longitude: 142°10.193′E) on 12 and 14 September 2017. Samples are also collected from the outflow of Funakiyama Waterfall numbered TNS-AL-62917-62919. The chemical measurement of electrical conductivity was 460 $\mu$S/cm, the pH was 7.33, and the concentration of calcium ions was 86 ppm.

b: A samples was collected from Tokoyo Waterfall on Chichi-jima Island (latitude: 27°3.450′N, longitude: 142°12.358′E) on 15 September 2017 and numbered TNS-AL-62942. The chemical measurement of electrical conductivity was 440 $\mu$S/cm, the pH was 7.84, and the concentration of calcium ions was 44 ppm.

Some of the samples were added to culture medium (d medium: modified WC medium described in Tuji (2000)) and kept at room temperature before observation (living samples). All other collected samples were fixed with 5% formalin (fixed samples).

2. Microscopic observations

Living samples were observed and photographs were taken using light microscopy (LM; BX41, Olympus, Japan) equipped with water immersion lenses and a camera (EOS kiss X7i, Canon, Japan). Fixed samples were cleaned using concentrated nitric acid as described previously (Tuji and Tanimura, 2012). Cleaned samples were embedded using Zrax (Tuji and Tanimura, 2012). LM observation and imaging were done using a microscope (Axiophoto, Zeiss, Germany) equipped with a Charge-Coupled Device (CCD) camera (Infinity 4: Lumenera, Canada). A small part of the cleaned sample was filtered using a membrane filter (RTTP02500, Merck), placed on a scanning electron microscope (SEM) stub and dried at room temperature overnight. The stub was sputter-coated with platinum and examined with a SEM (JEOL-6390LV, JEOL, Japan) equipped with a lanthanum hexaboride cathode. All materials were deposited to TNS (Department of Botany, National Museum of Nature and Science).

3. Molecular analysis

DNA was extracted from a colony taken from a living sample using the Chelex method (Walsh *et al.*, 1991). Polymerase chain reaction and sequencing of *rbc*L and the 18S rRNA gene were then performed (Tuji *et al.*, 2014). Phylogenetic and molecular evolutionary analyses of the obtained sequences were conducted using the MEGA 7 computer program (Kumar *et al.*, 2016). Alignments were checked manually. Neighbor joining and maximum likelihood trees were calculated using MEGA software with the best fits model (T92 + G + I model) using Bayesian information criterion scores, and the substitution nucleotide matrix parameters were calculated by the software. A tree using 1000 bootstrap replicates was generated. All positions containing gaps and missing data were eliminated.

4. Examination of type materials for *Terpsinoe musica*

Original illustrations from Ehrenberg's collection in BHUPM (Museum für Naturkunde in Berlin (see Lazarus and Jahn (1998); Tuji and Tanimura (2006)) were examined for comparison with Japanese individuals.

## Results

In Japan, *Terpsinoe americana* (Bailey) Grunow has been recorded (Schmidt (1874): No. 200) and used to determine the upper limit of marine facies in plaeo-environments (Maeda *et al.*, 1992). Since *T. americana* does not have rimoportula, the taxon found in Ogasawara is not *T. americana*. *T. japonica* Ehrenb. was also described from 'pflanzen cultur erden', Nagasaki, Japan (Ehrenberg, 1854). Both marine ('*Coccinodiscus radiolatus*' and '*Campylodiscus Echeneis*') and freshwater ('*Eunotia gibba*' and '*Gallionella granulata*') diatoms were reported from the same material (310. XIV. Japan). This material may have originated from a marine coast habitat. The published illustration (Ehrenberg, 1854) and Ehrenberg's original illustration (No. 1197) only show girdle views. The small frustule size in these illustrations and the marine habitat are consistent with *T. americana*, and *T. japonica* might be a synonym for *T. americana*. *T. japonica* is clearly distinguished from *T. musica* by its shallow frustule depth.

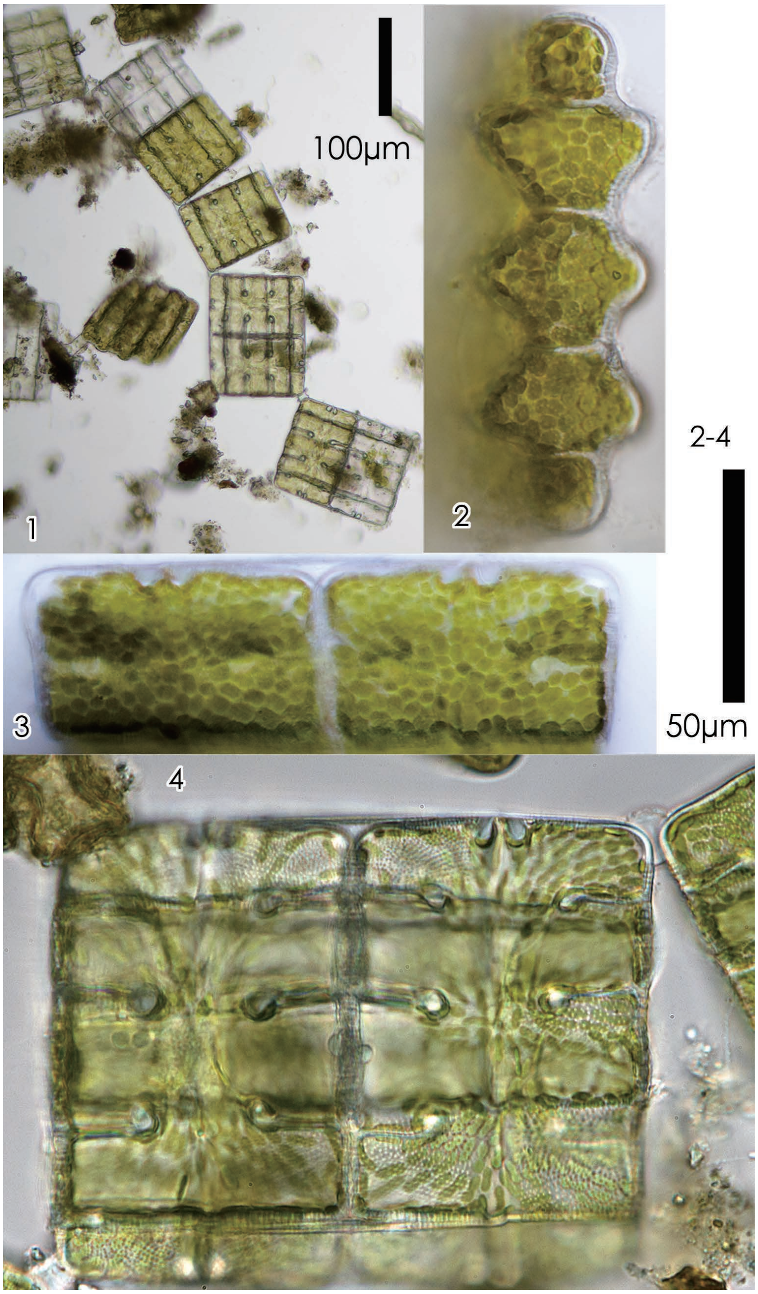
The *Terpsinoe* species found in the Ogasawara Islands is very close to *T. musica* Ehrenb. However, there are several morphological differences between this taxon and *T. musica*, and we described it as a new taxon.

***Terpsinoe muninensis*** Tuji sp. nov.

(Figs. 1–29)

Holotype: Slide numbered TNS-AL-62917d in TNS (Department of Botany, National Museum of Nature and Science).

(Figs. 6, 7, 11, 13, 15, 16)



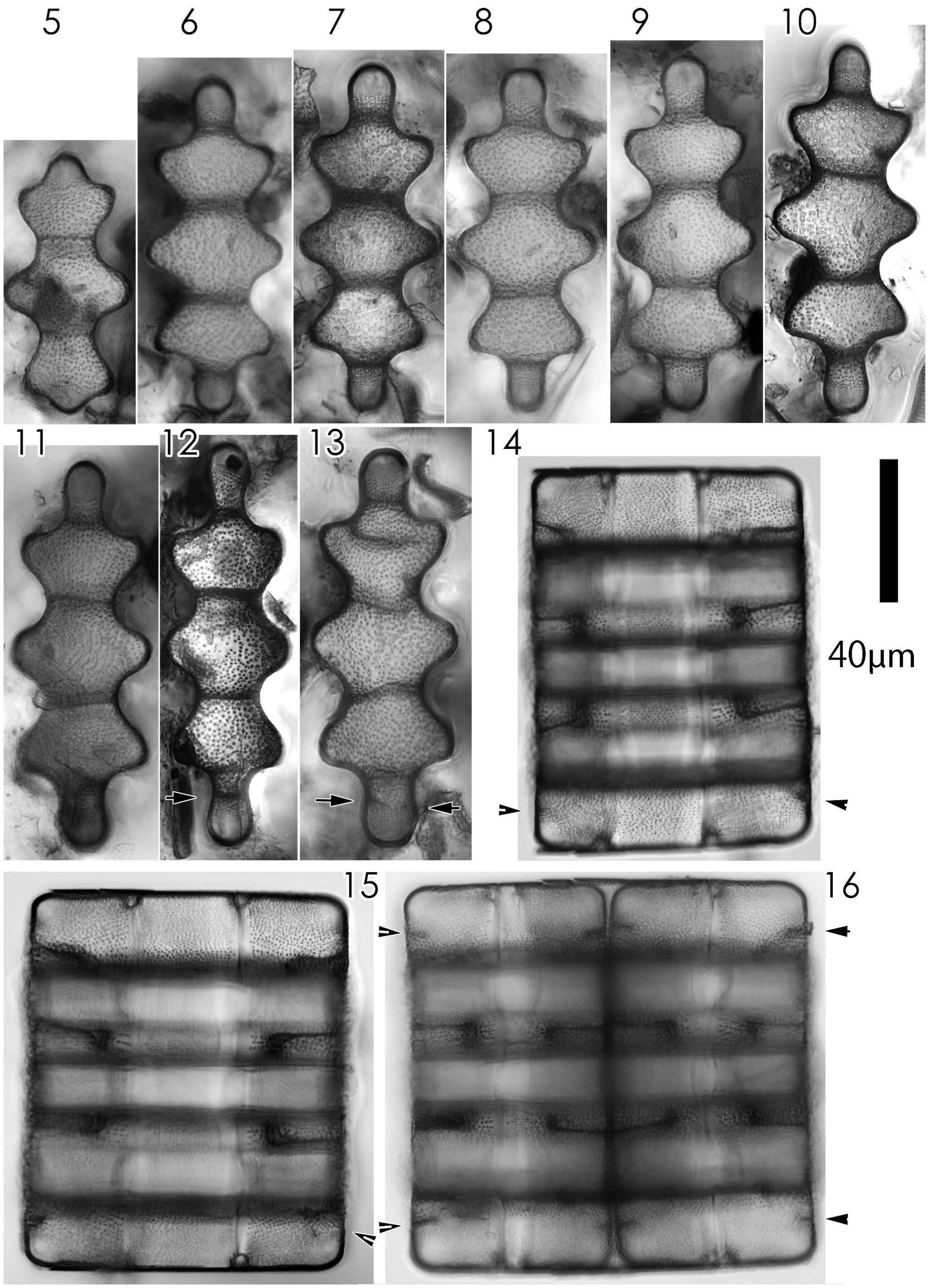

Figs. 1–4. *Terpsinoe muninensis* sp. nov. TNS-AL-62917. Funakiyama Waterfall, Haha-jima Island. LM. Living material.

Isotype: TNS-AL-62917m (raw material), 62917c (cleaned material) and 62917a–c, e–f (permanent slides) in TNS. Funakiyama Waterfall, Haha-jima Island, Ogasawara, Tokyo, Japan.

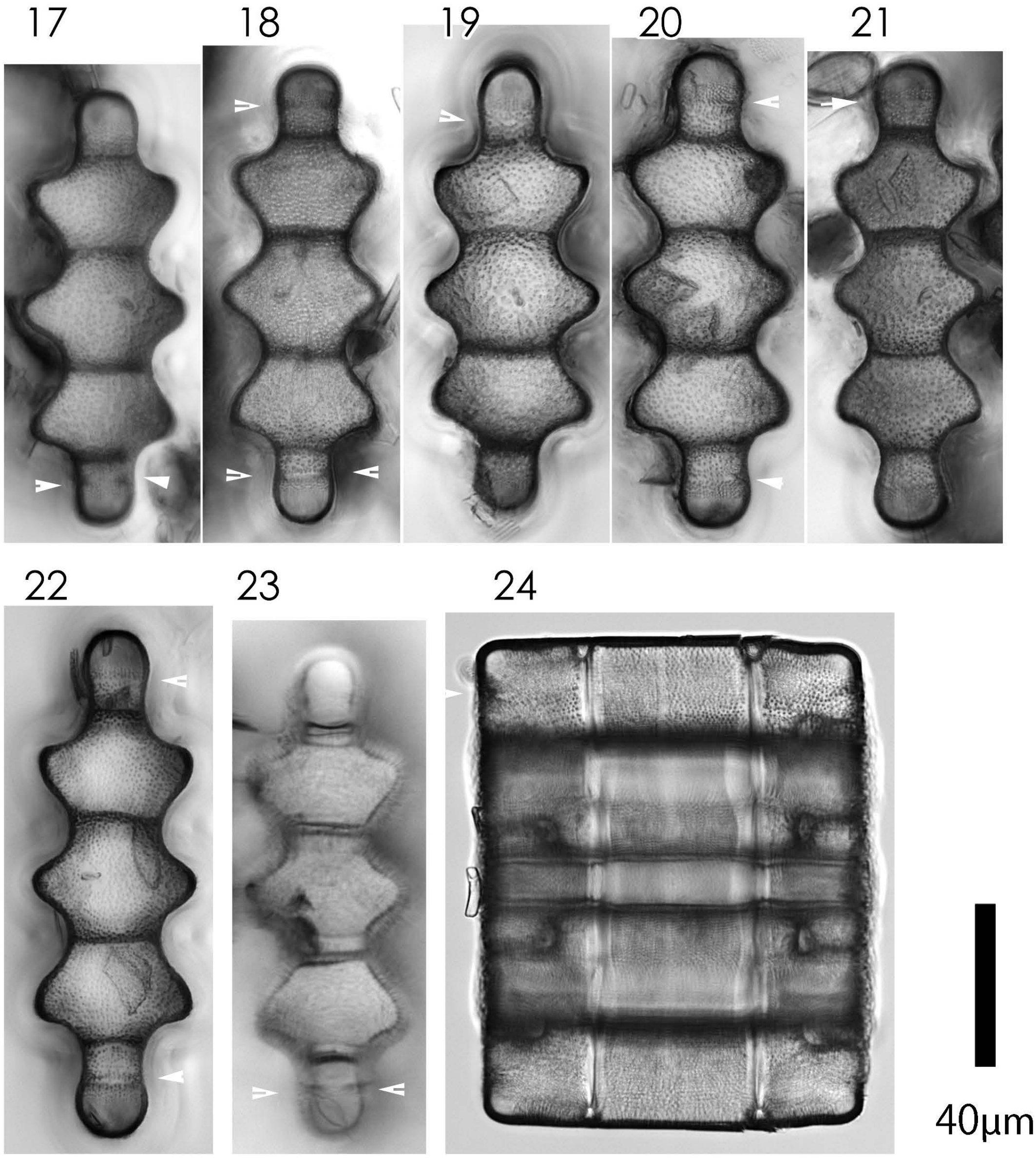
Another locality: Tokoyo Waterfall, Chichi-jima Island, Ogasawara, Tokyo, Japan.

Etymology: 'Munin' is an old name for the Ogasawara Islands.

Description;

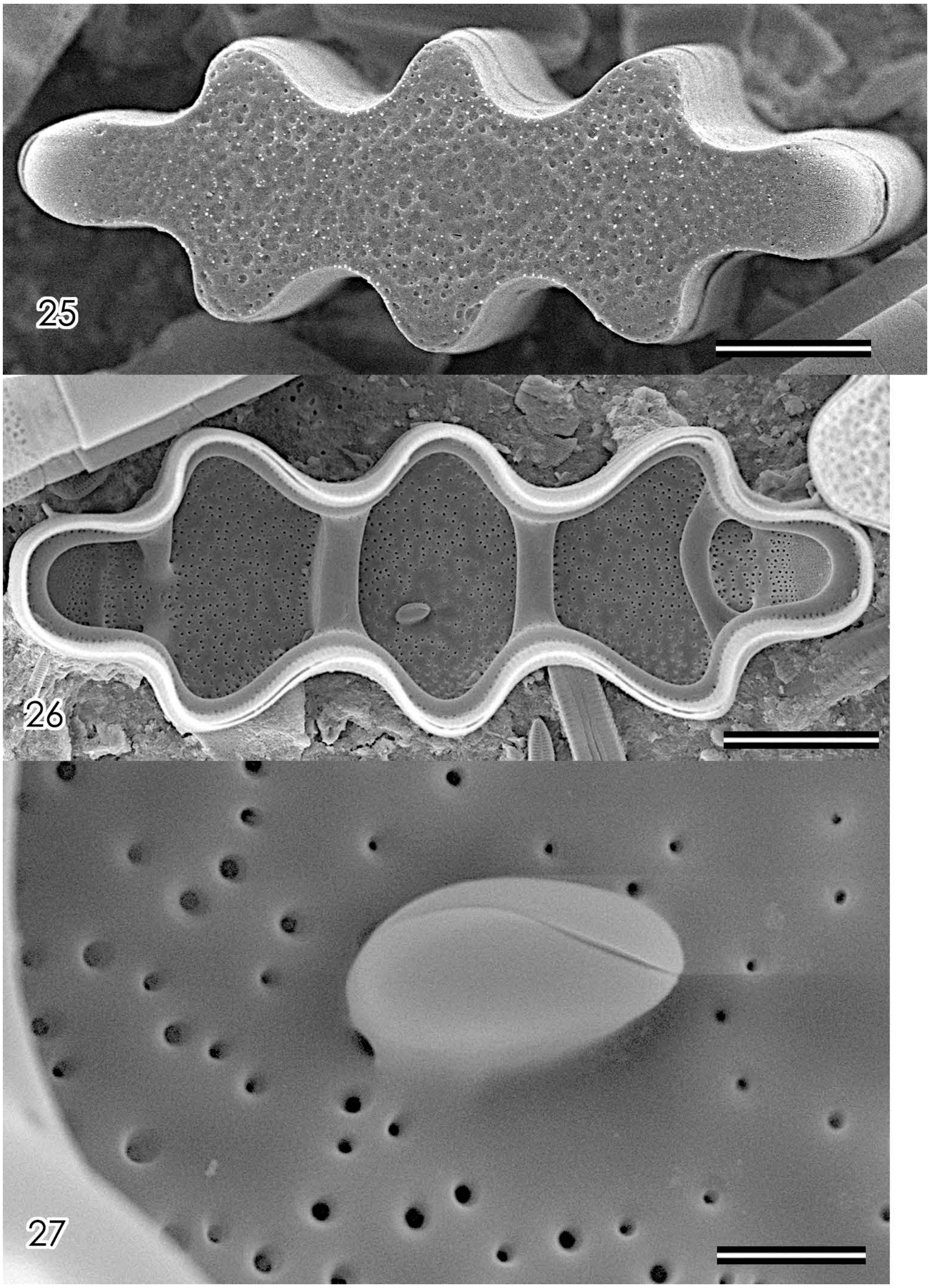



Figs. 5–16. *Terpsinoe muninensis* sp. nov. TNS-AL-62917. LM. 5. slide 62917b. 6, 7, 11, 13, 15, 16. slide 62917d. 9, 10, 12, 14. slide 62917e. Arrows. Two weak transapical bars on each valve apex.




Figs. 17–24. *Terpsinoe muninensis* sp. nov. TNS-AL-62942. Tokoyo Waterfall, Chichi-jima Island. LM. Arrows. Two weak transapical bars on each valve apex.

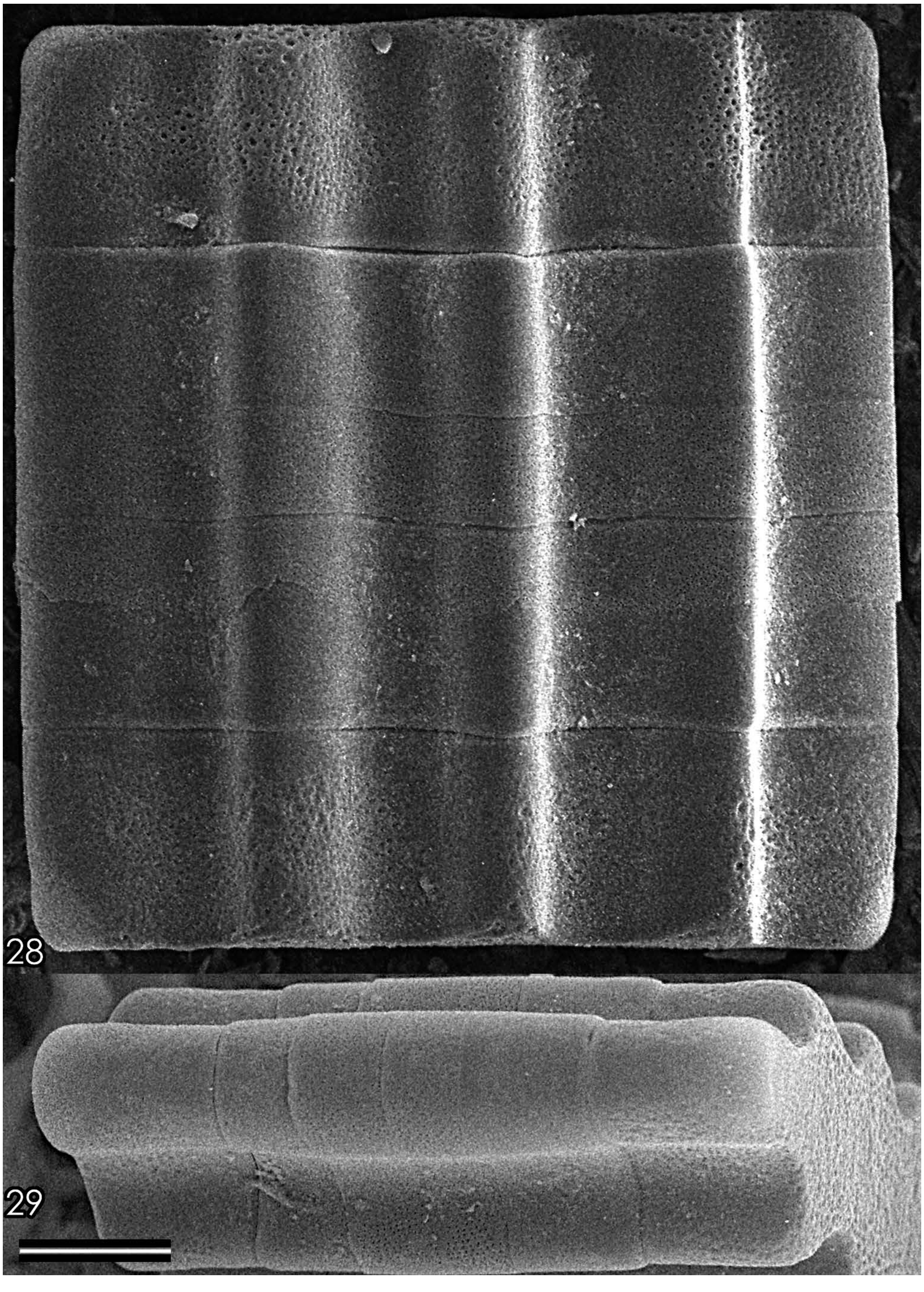
Living cells form zig-zag colonies (Fig. 1) with mucilage pads secreted from pseudocelli at valve apices (Figs. 4, 25, 26), and possess numerous discoid chloroplasts (Figs. 1–4). The valves are triundulate, with lengths of 70–115 $\mu$m, widths of 35–42 $\mu$m, and mantle heights of 25–30 $\mu$m. The lengths and widths of the inflations are almost the same. The middle inflation is symmetrical, but the other inflations are asymmetrical (Figs. 5–13, 17–23). The valve apices are rostrate to subcapitate with pseudocelli. Four straight transapical bars exist per frustule, two are between the inflations, and two are between the inflations and the apices (*in Figs. 30–33). Two more weak transapical bars are observed on each valve apex (arrows in Figs.




Figs. 25–27. *Terpsinoe muninensis* sp. nov. TNS-AL-62917. Bar = 20 $\mu$m. SEM.

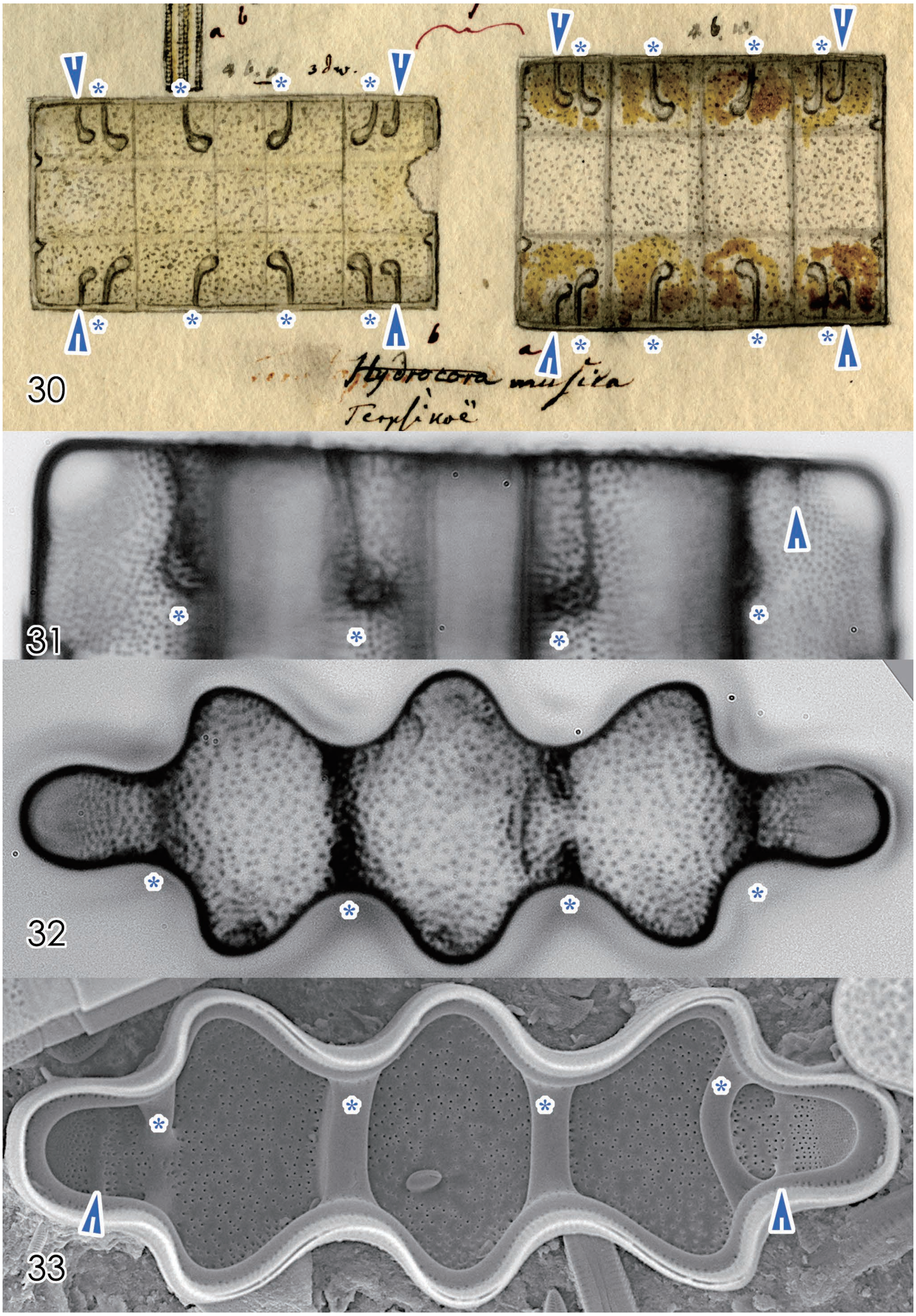
12–16, 18–24, 30–33). A rimoportula exists on the middle inflation, and it's visible in LM (Figs. 5–13, 17–23, 26–27). Three closed girdle bands exist per frustule (Figs. 28–29).

This taxon is close to *T. musica*. However, two transapical bars on the valve apices of *T. musica* (arrow in Fig. 30) are shaped like musical notes (*in Fig. 30) in the original illustrations. *T.*




Figs. 28–29. *Terpsinoe muninensis* sp. nov. TNS-AL-62917. Bar = 20 $\mu$m. SEM images showing band structures.

*musica* presented by Wu (2013) is not shaped like musical notes, but two transpical bars on the valve apices are always visible on the valve face views of LM images. The middle inflations of *T. musica* presented by Wu (2013) and Luttenton *et al.* (1986), are longer than other inflations, and differ from *T. muninensis*. The transapical bars which form the middle inflation presented in




Figs. 30–33. *Terpsinoe* taxa showing the positions of the bars. 20. *T. musica,* Ehrenberg's original illustration (No. 1197). 21–23. *T. muninensis* sp. nov. TNS-AL-62917.

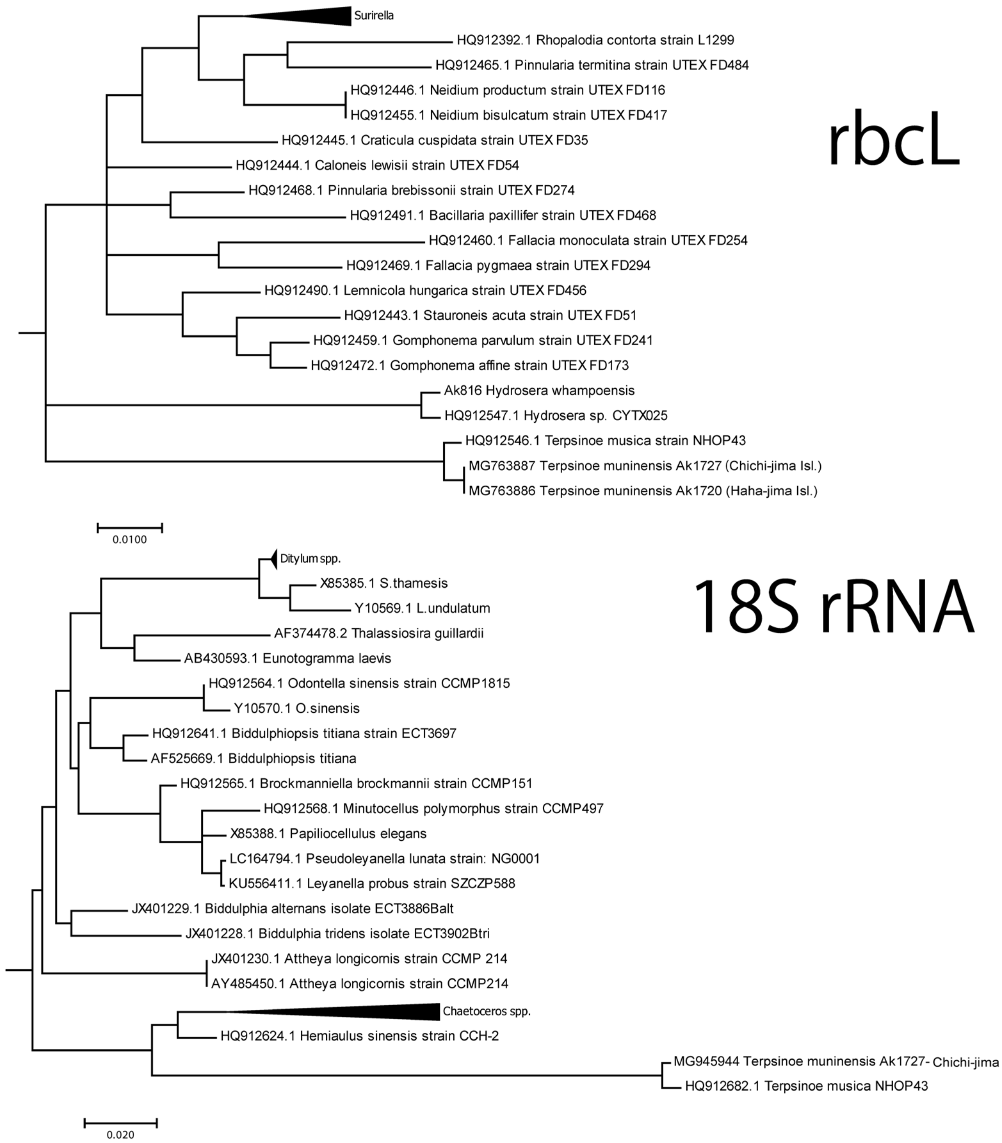



Fig. 34. Phylogenetic trees of *Terpsinoe muninensis* sp. nov. and related taxa using *rbc*L (a) and 18S rRNA (b).

these papers are swollen, and also differ from the straight bars of *T. musica*.

Phylogenetic trees using 18S rRNA and *rbc*L are shown in Figure 24. There is an 8-bp difference in the 18S rRNA and a 10-bp difference in *rbc*L between *T. muninensis* and *T. musica*. There are no differences in 18S rRNA or rbcL between a Haha-jima individual and Chichi-jima individual. However, only one sequence for 18S rRNA and *rbc*L was deposited using the strain NHOP43 which originated from Brackenridge Field Lab, Austin, Texas, U.S.A. (Theriot *et al.*, 2010). It is difficult to assess the phylogenetic variation of *T. musica*.

## Discussion

*T. muninensis* is the first recorded freshwater *Terpsinoe* species in Japan. I have also examined diatom flora from Okinawa Island, the Yaeyama

Islands, Yaku-shima Island and the Izu Islands, which are at almost the same latitude as the Ogasawara Islands in Japan, but I did not find any freshwater *Terpsinoe* species in these areas.

*T. musica* is most frequently found in hard waters with warm temperatures (Sterrenburg, 1994). It is widely distributed and can be found in freshwater to marine habitats (Wehr and Sheath, 2003). Navarro and Lobban (2009) also reported that this species is a common species in Guam. Kubota (1999) reported this species from Alewa Heights Spring in the southern part of Oahu Island, Hawaii.

Based on the difference between the original description of *T. musica* and *T. musica* sensu Wu (2013), *T. musica* shows great morphological variation and appears to be a species complex (flock). Thus, type locality is an important factor in the taxonomical treatment. *T. musica* was described from Atotonilco el Grande, Mexico (Ehrenberg, 1841). Since the sequences of strain NHOP43 originated from Texas, North America, which is geographically close to the type locality in Mexico, differences in the sequences of the two taxa also support the existence of a new taxon.

The Ogasawara Islands are located between the Micronesian biogeographic province, in the Oceanic realm, and the Japanese evergreen forest, in the Palearctic realm (Udvardy, 1975). Many higher plants located in the Ogasawara Islands are of Southeast Asian origin, and are associated with locations in Oceania (Micronesia, Polynesia, etc.) (Suzuki *et al.*, 2010). Since *T. muninensis* is the first recorded freshwater *Terpsinoe*, it did not originate in other areas in Japan. It should have its origin in Oceania. Hawaiian (*T. musica* sensu Kubota (1999)) and Guam individuals (*T. musica* sensu Navarro and Lobban (2009)) seem to be *T. muninensis*. Since these previous authors presented only limited photographs, I have not fully assessed the synonymy. The distribution of freshwater diatoms in oceanic islands is not well understood. Research on the morphological variation and molecular phylogeny of *T. muninensis* and *T. musica* in these oceanic islands is an important and interesting topic for further research.

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# 小笠原諸島から見いだされた淡水棲珪藻の一新種, *Terpsinoe muninensis* sp. nov.

辻　彰洋

（国立科学博物館・植物研究部）

淡水棲珪藻の一新種―*Terpsinoe muninensis* を記載した. 本分類群は *T. musica* に類似するが殻端の横走肋が弱いことや被殻の膨らみの比が異なることから区別できる. 本分類群は本邦の淡水域からの新産報告となる. また, 北太平洋の海洋島に分布する類似種との関連が考えられた.