



## A MODERN SURVEY OF *Tetralunata* (RHOPALODIALES, BACILLARIOPHYTA) SPECIES AND THEIR DISTRIBUTION IN LAKE TOBA, INDONESIA

### SURVEI TERKINI DAN DISTRIBUSI SPESIES *Tetralunata* (RHOPALODIALES, BACILLARIOPHYTA) DI DANAU TOBA, INDONESIA

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Submitted: 23 September 2025; Revised: 27 September 2025; Accepted: 4 February 2026

#### Abstract

*Tetralunata* is a genus of canal-bearing raphid diatoms (ordo *Rhopalodiales*) with the unique feature of cyanobacterial symbionts. A species assigned to the genus was originally reported and described from Lake Toba by Brun in 1891, and several species described by Hustedt in 1935 and 1937 under the name *Denticula* Kützing (1844) have also been transferred to *Tetralunata*. According to Hustedt, 14 species now in *Tetralunata* were originally found alive or as fossil forms. Firstly, this study aimed to reconfirm the presence of *Tetralunata* in Lake Toba after its first description more than 130 years ago. The second purpose was to examine *Tetralunata* habitats in Lake Toba, which were not described by Hustedt in his 1935 and 1937 reports. Samples were collected from various sites around the lake and observed using light microscopy after cleaning with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). As a result, 9 species of *Tetralunata* were found: *T. amphicephala*, *T. angusta*, *T. costata*, *T. elongata*, *T. lanceolata*, *T. obtusa*, *T. pelagica*, *T. cf. robusta*, and *T. vanheurckii*, indicating *Tetralunata* biodiversity in Lake Toba. Six species previously reported by Hustedt were not found in this study, while four species that had been reported as fossil forms were found alive in this study.

**Keywords:** Biodiversity; Diatoms; Lake Toba; *Rhopalodiales*; *Tetralunata*

#### Abstrak

*Tetralunata* merupakan genus diatom raphid (ordo *Rhopalodiales*) yang memiliki simbiosis cyanobacteria di dalam selnya. Genus asal Danau Toba ini pertama kali dilaporkan oleh Brun pada tahun 1891. Pada tahun 1935 dan 1937, Hustedt mendeskripsikan 14 spesies *Denticula* Kützing (1844) asal Danau Toba yang kemudian direvisi menjadi *Tetralunata* oleh Hamsher et al. pada tahun 2014. Penelitian ini bertujuan untuk mengecek kembali keberadaan 14 spesies *Tetralunata* yang dilaporkan oleh Hustedt lebih dari 130 tahun lalu. Tujuan lain adalah mendeskripsikan habitat *Tetralunata* di Danau Toba yang belum dilaporkan Hustedt pada tahun 1935 dan 1937. Sampel diatom dikumpulkan dari 10 lokasi yang mencakup bagian selatan dan utara danau. Sampel dibersihkan dengan hidrogen peroksida (H<sub>2</sub>O<sub>2</sub>) dan kalium dikromat (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>), lalu diamati menggunakan mikroskop cahaya. Hasilnya, ditemukan 9 spesies *Tetralunata*, yaitu *T. amphicephala*, *T. angusta*, *T. costata*, *T. elongata*, *T. lanceolata*, *T. obtusa*, *T. pelagica*, *T. cf. robusta*, dan *T. vanheurckii*, menunjukkan keanekaragaman hayati *Tetralunata* di Danau Toba. Enam spesies yang dilaporkan oleh Hustedt tidak ditemukan dalam penelitian ini, sementara empat spesies yang dilaporkan sebagai fosil ditemukan hidup di Danau Toba.

**Kata Kunci:** Danau Toba; Diatom; Keanekaragaman hayati; *Rhopalodiales*; *Tetralunata*

Permalink/DOI: <http://dx.doi.org/10.15408/kauniyah.v19i2.46515>

## INTRODUCTION

The genus *Denticula* was first identified by Kützing in 1844 and was subsequently classified within the order *Bacillariales*. Species within this group typically possess features such as valves that are generally linear to lanceolate in shape, ending in blunt or slightly rostrate ends. A defining trait of this genus is the presence of transapical ribs (costae) structures that span the valve surface and can be observed using light microscopy as they represent extensions of the fibulae (Round et al., 1990). *Denticula vanheurckii* (Brun) Hamsher et al. (2014) was classified in the order *Rhopalodiales*, along with the genera *Rhopalodia* and *Epithemia*, due to shared characteristics such as the canal raphe and in possessing blue-green algal (cyanobacterial) symbionts. Early researchers such as Hustedt (1930), Patrick and Reimer (1975) supported this classification and suggested that some species of *Denticula* were more closely related to *Epithemia* than to *Rhopalodia*.

Brun (1891) and Hustedt (1935, 1937) described many *Denticula* species from Lake Toba, Sumatra, Indonesia. Later, Sims (1983) suggested that these species showed morphological similarities to *Epithemia*, although no supporting evidence was provided. Geitler (1977) found that one Indonesian species, *Denticula vanheurckii* Brun (1891), hosted blue-green algal symbionts, further reinforcing the idea that *Denticula* species in Indonesia may be more closely related to the genera in the order *Rhopalodiales* than to the *Bacillariales*.

While *Denticula* and *Tetralunata* are both canal raphe-bearing genera, the two are differentiated by *Denticula* species bearing a keel positioned offset from the valve center (Round et al., 1990), or for those species without a keel, the raphe is positioned on the valve mantle (Johansen et al., 1994; Van de Vijver et al., 2015). Areolae are occluded by internal hymenes. In *Tetralunata*, the raphe is placed medially on the valve face, is not elevated into a keel and the areolae are occluded externally by volae (Hamsher et al., 2014). Recent work using molecular methods has shown *Denticula* to be in the order *Bacillariales* (Mann et al., 2021; Kociolek et al., 2025a), far removed phylogenetically from the *Rhopalodiales* where *Tetralunata* is placed (Hamsher et al., 2014).

Taxonomic studies on the diatoms in Lake Toba are very limited. On the contrary, ecological research in Lake Toba has been very intensive, and most of the reports showed diatoms to represent a high proportion of the microalgal community structure (Purba & Nurhayati, 2022). Members of the orders *Surirellales*, *Bacillariales*, and *Fragilariales* were commonly found in lake water (Barus et al., 2008; Sulawesty, 2011), while only three members of *Rhopalodiales* had been reported, i.e., *Denticula vanheurckii*, *Epithemia turgida* (Ehrenberg) Kützing 1844, and *Rhopalodia gibba* Ehrenberg (Kützing) 1844 (Soeprbowati & Suedy, 2016). The study sites were scattered on the west (Sipisupisu, Haranggoal), east (Balige, Muara), north (Parapat and Ambarita), and south sides (Panguruan) of Lake Toba (Rahman et al., 2016; Soeprbowati & Suedy, 2016).

Many species of diatoms and other microorganisms are experiencing population declines or local extinctions due to their inability to adapt to the rapid environmental changes. These changes include rising temperatures, altered nutrient levels, pollution, and habitat modification, which can disrupt the delicate balance these organisms require to thrive. For instance, the freshwater diatom *Aulacoseira islandica* (O.Müll.) Simonsen 1979, once abundant in cold, stratified lakes, has declined in several regions due to warming water temperatures that affect its buoyancy and growth cycles (Rühland et al., 2015). Similarly, *Tabellaria fenestrata* (Lyngbye) Kützing 1844, a diatom sensitive to nutrient enrichment, has decreased in many freshwater ecosystems impacted by eutrophication.

Lake Toba has undergone significant changes from its ancient conditions, and these changes are particularly evident in its diatom communities (Rahman et al., 2016). In its ancient form, Lake Toba was an oligotrophic ecosystem, with clear, nutrient-poor waters that supported a rich diversity of diatom species, including those in the *Rhopalodiales* order, which are typically found in unpolluted environments (Ruck et al., 2016). However, over time, human activity, such as agricultural runoff, urbanization, and industrialization, has led to increased nutrient levels and eutrophication in the lake. Hastuti et al. (2024) survey reported total nitrogen >12,5 mg/L and total phosphorus >0,1 mg/L across multiple stations, classifying the lake as mesotrophic to eutrophic. These changes have altered the lake's water chemistry, favouring diatom species that thrive in nutrient-rich conditions. Hence, the investigation and intensive research on members of the order *Rhopalodiales* in Lake Toba has become

important to update our understanding of the lake's biodiversity, and from a conservation point of view.

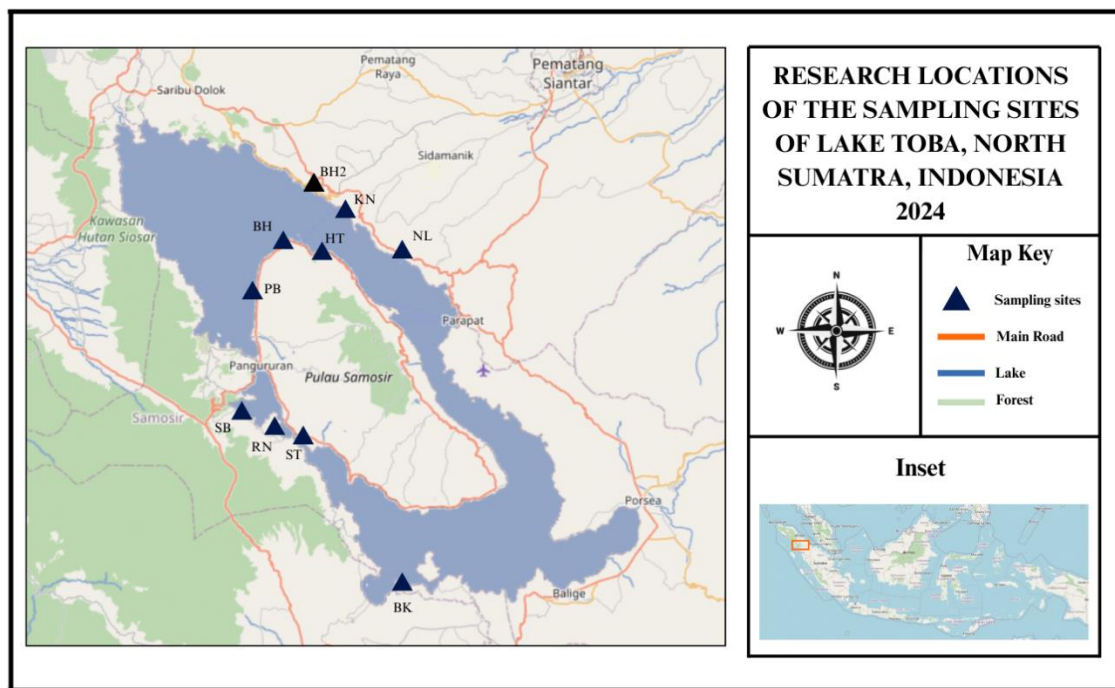
Except for the illustrations of *Tetralunata* species, Hustedt did not report in detail the sampling location and substrate type. Furthermore, there was no documentation of cells in a living state that indicated the presence of cyanobacterial endosymbionts (called spheroid bodies/SB). This study aims to provide a 2024 inventory regarding the number of *Tetralunata* species currently present in Lake Toba, and compare the present species richness of the genus with the inventory provided by Hustedt. The existence and documentation of *Tetralunata* (previously *Denticula*) in Lake Toba in 2024 is indispensable as a complement to Hustedt's reports of 1935 and 1937. A list of *Tetralunata* species in Lake Toba will provide the latest information and help diatomist exploration for *Tetralunata* genus in the future.

## MATERIALS AND METHODS

This research is a qualitative, descriptive account of the *Tetralunata* species in Lake Toba and was conducted from February 2024 to June 2025. The method used was the exploration method (Etikan et al., 2016). Species identification is carried out using a light microscope to observe morphological characteristics.

### Study Area

Sampling was carried out at 10 sampling sites (Figure 1). Locations were determined using the purposive sampling method (selected), based on the reference of Hustedt (1937), who received Lake Toba water samples around the Pangururan District in 1937. Various habitats were selected for site samplings to maximize species richness results (Table 1).



**Figure 1.** Research locations of the sampling sites of Lake Toba, North Sumatra, Indonesia

**Table 1.** Description of research sampling sites with geographic coordinates

Sampling sites	Sampling sites	Longitude	Latitude
BK	Bakkara area, Humbang Hasundutan Regency. This sampling site is located behind residential housing and close to shrimp and fish farming.	98° 85'57.10"E	2°33'8.216"S
ST	Sampurtoba, Pangururan District. This sampling site is located on a rocky beach.	98.726.008"E	2.535.945"S
RN	Aek Rangat Rianiate hot springs, Pangururan District. This sampling site is located near a small pier and filled with water hyacinth.	98.723.257"E	2.542.996"S

Sampling sites	Sampling sites	Longitude	Latitude
SB	Sibeabea (prominent hill), Samosir Regency. This sampling site is located on a cliff, near the pier where small boats are anchored.	98.677.305"E	2.552.969"S
PB	Parbaba in Huta Bolon, Pangururan, Samosir Regency. This sampling site is located on white sand beach surrounded by grass.	98.689.057"E	2.682.102"S
BH	Batuhoda Beach (west), Simanindo Regency. This sampling site is located on the west side of the island which is a sandy beach surrounded with large trees.	98.726.408"E	2.760.098"S
HT	Tigaras, Simalungun Regency. One of the main ports connecting the mainland of Sumatra to Samosir Island.	98.743.568"E	2.752.536"S
BH2	Batuhoda (north), Simanindo Regency. This sampling site is located on the north sides of the island which is surrounded by a sandy beach with clean water.	98.798.267"E	2.787.455"S
KN	Kenangan, Simalungun Regency. This sampling site is located in a tourist area, near several large rocks.	98.803.635"E	2.783.589"S
NL	Nauli, Simalungun Regency. This sampling site is located on the north sides of the island near a beach without much vegetation or substrate around it.	98.827.355"E	2.764.417"S

### Sampling

Attached diatom samples were obtained from rocks, plants, and other submerged substrates, such as trunks and plastics in shallow water (Taylor et al., 2007). Samples were taken from the surface of the substrates (rock or plastic) by scraping using a field knife. Sediment samples were taken using a turkey baster. Epiphytic samples from plants were taken by cutting the plant using a field knife, then putting it into a Whirl-Pak. To keep it from drying, lake water was added to the bag. All samples were labelled with information such as collection date and location code. After collections were made, the samples were kept cold by placing them in a cooler filled with ice cubes.

### Samples Observation

Fresh samples were immediately observed by placing one drop of the sample onto the slide using a glass pipette and covering with a cover glass. Observation was also carried out using treated or cleaned samples. Fresh samples were cleaned using hydrogen peroxide and potassium dichromate before being permanently mounted on microscope slides using Naphrax (Van Der Werff, 1955). Specifically, 5 to 10 mL of each sample was cleaned using 30% hydrogen peroxide ( $H_2O_2$ ) and potassium dichromate ( $K_2Cr_2O_7$ ), then rinsed and settled for 24 hours several times with distilled water. After this processing, the samples were air-dried onto coverglasses and mounted onto glass microscope slides using Naphrax.

Both fresh and cleaned sample types were made into slides. The slides were observed under a light microscope (LEICA DM 1,000) with 40× and 100× (oil immersion) objectives, yielding 400× and 1,000× magnification, respectively. The 100× objective is an immersion oil lens, which is applied between the front lens of the objective and the cover slip. Each sample was observed three times to confirm identification based on the shape of the valves and the visible raphe structure in the diatom cells. Digital images were captured in TIFF format using LEICA software. The measurement of cell size was carried out by GRYPHAX software. The images were taken with GRYPHAX software, which immediately measured the length and width, providing a scale to each image taken.

### Data Analysis

Species *Tetralunata* found in the samples were characterized based on the frustules, valve shape, raphe structure, and apices shape (Taylor et al., 2007). References for species identification used in this study were Brun (1891), Hustedt (1935, 1937, 1942), Simonsen (1987), and Hamsher et al. (2014). Comparison of morphological characters was carried out according to the characteristics of each species found and presented in the tables.

### RESULTS

A total of 49 samples were taken from ten (10) sampling sites. After screening with light microscopy, the 49 samples were grouped into low, medium, and high priority based on the

occurrence of members from *Rhopalodiales*. High priority samples were designated as those having all members of *Rhopalodiales* with more than five species of *Tetralunata*, medium priority with fewer than five species of *Tetralunata*, and low priority with no species of *Tetralunata*. As a result, there were 16 high-priority samples, 10 low-priority samples, 19 low-priority samples, and four (4) samples were discarded. Only samples with medium and high priority were further observed by light microscopic observation. The final 26 samples (medium and high priority) covered northern (20 samples) and southern (6 samples) areas of the lake. This suggests that geographic distribution and local environmental conditions may influence the observed species of *Tetralunata* in Lake Toba.

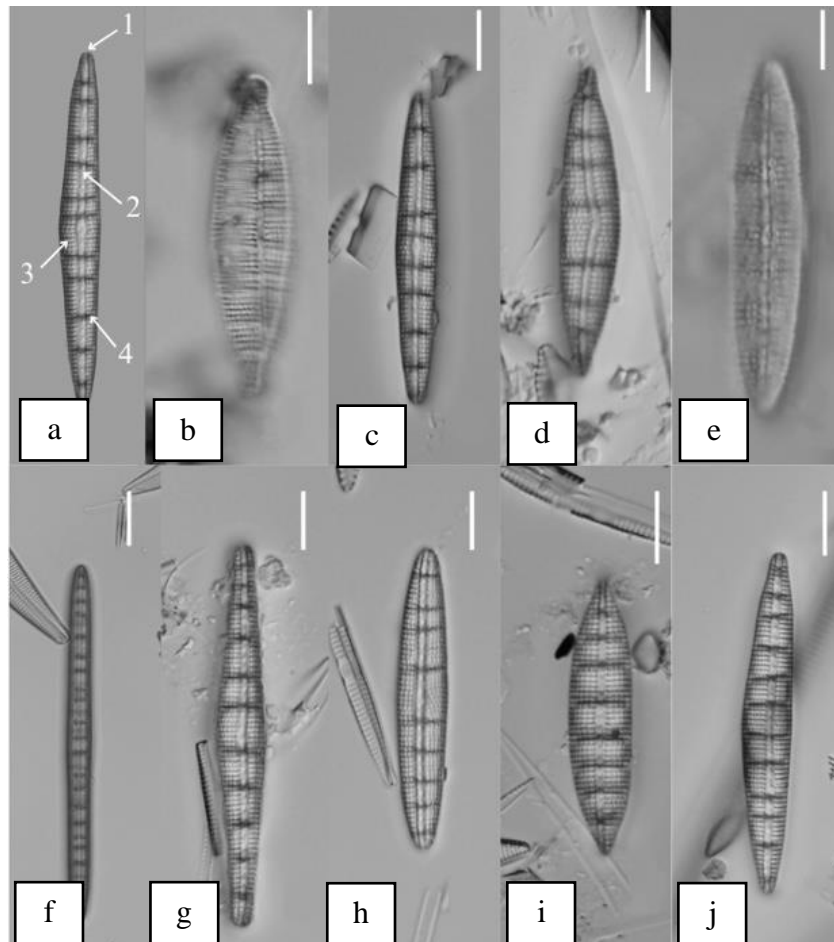
Nine species of *Tetralunata* were identified from collected samples (Table 2; Figure 2). The nine identified species of *Tetralunata* have varying lengths and widths of frustules. *Tetralunata elongata* features the largest frustule dimensions, ranging from 180 to 200  $\mu\text{m}$  in length and 10 to 11  $\mu\text{m}$  in width, while the smallest is *Tetralunata amphicephala*, measuring 25 to 30  $\mu\text{m}$  in length and 11 to 12  $\mu\text{m}$  in width. The valve shapes of the nine *Tetralunata* species show a transition from linear in *T. elongata* to elliptical (*T. pelagica* and lanceolate in *T. lanceolata*). The majority of *Tetralunata* species had either very rounded or slightly rounded apices of valves. The only exception is *T. amphicephala*, which had a bluntly rounded apex. Another distinguishing characteristic was the raphe, which is a slit or opening in the diatom frustule's cell wall. The ending of raphe was either curved in *T. cf. robusta* and *T. lanceolata* or straight in the other seven species. Morphological determination key for *Tetralunata* species is as follows.

1a	Frustule shape planar; Valve shape linear or linear-lanceolate.....	2
1b	Frustule shape planar; Valve shape elliptical or linear-elliptical.....	6
2a	Length of valve 80–223 $\mu\text{m}$ , width 8–13 $\mu\text{m}$ .....	<i>T. elongata</i>
2b	Valve shape linear-lanceolate.....	3
3a	Length of valve <75 $\mu\text{m}$ .....	4
3b	Length of valve 45–86 $\mu\text{m}$ , width 7–10 $\mu\text{m}$ .....	<i>T. lanceolata</i>
4a	Length of valve 31–100 $\mu\text{m}$ , width 8–9 $\mu\text{m}$ .....	<i>T. vanheurckii</i>
4b	Length of valve smaller than 100 $\mu\text{m}$ .....	5
5a	Length of valve 40–99 $\mu\text{m}$ , width 7–12 $\mu\text{m}$ .....	<i>T. cf. robusta</i>
5b	Length of valve 40–71 $\mu\text{m}$ , width 7–9 $\mu\text{m}$ .....	<i>T. angusta</i>
6a	Valve shape elliptical, length <50 $\mu\text{m}$ .....	7
6b	Valve shape linear-elliptical, length >50 $\mu\text{m}$ .....	8
7a	Length 30–40, width 11–12 $\mu\text{m}$ .....	<i>T. amphicephala</i>
7b	Length 17–40, width 7–10 $\mu\text{m}$ .....	<i>T. pelagica</i>
8a	Length of valve 40–55 $\mu\text{m}$ , width 8–10 $\mu\text{m}$ .....	<i>T. costata</i>
8b	Length of valve 28–48 $\mu\text{m}$ , width 7–10 $\mu\text{m}$ .....	<i>T. obtusa</i>

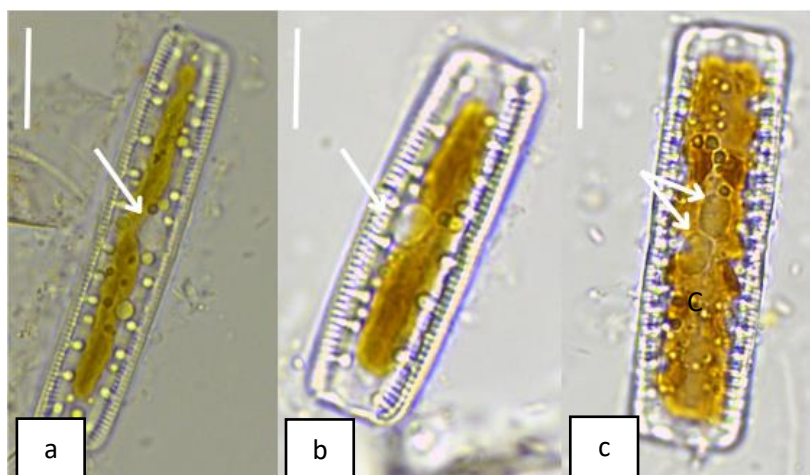
The presence and number of spheroid bodies (SB) in *Tetralunata* were clearly visible under light microscope observation (Figure 3). The SB was located mostly in the center of the cell. One to two SBs were found in individual specimens.

**Table 2.** Features of *Tetralunata* species from Lake Toba in 2024

Taxa	Features				
	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Raphe ending	Valve shape	Valve apices
<i>T. amphicephala</i>	25–30	11–12	Straight	Elliptical	Bluntly rounded
<i>T. angusta</i>	41–71	7–9	Straight	Linear-lanceolate	Broadly rounded
<i>T. cf. robusta</i>	50–60	8–11	Curved	Linear-lanceolate	Slightly rounded
<i>T. costata</i>	35–45	9–10	Straight	Linear-elliptical	Slightly rounded
<i>T. elongata</i>	180–200	10–11	Straight	Linear	Slightly rounded
<i>T. lanceolata</i>	60–75	7–8	Curved	Lanceolate	Rounded
<i>T. obtusa</i>	55–65	10–11	Straight	Linear-elliptical	Rounded
<i>T. pelagica</i>	20–45	13–17	Straight	Elliptical	Rounded
<i>T. vanheurckii</i>	90–110	9–10	Straight	Linear-lanceolate	Broadly rounded



**Figure 2.** Key morphological characteristics of *Tetralunata* (a), *Tetralunata amphicephala* (b), *Tetralunata angusta* (c), *Tetralunata* cf. *robusta* (d), *Tetralunata costata* (e), *Tetralunata elongata* (f), *Tetralunata lanceolata* (g), *Tetralunata obtusa* (h), *Tetralunata pelagica* (i), *Tetralunata vanheurckii* (j). Apices (1), raphe (2), striae (3), costae (4). Scale bars= 10  $\mu$ m (a-e, g-i), 20  $\mu$ m (f)



**Figure 3.** Spheroid bodies (arrow) found in species of *Tetralunata vanheurckii* (a), *Tetralunata obtusa* (b), *Tetralunata* cf. *robusta* (c) (girdle view). Scale bars= 10  $\mu$ m

The distribution of *Tetralunata* in ten sampling points is shown in Table 3. Of the 26 samples examined, 20 were collected from the northern part (across 6 sampling sites), while the remaining six (6) samples were obtained from the southern part (across 4 sampling sites). Six to 8 species of *Tetralunata* were found on the northern side of the lake, while 4 to 7 species were found on the southern side. Parbaba (PB) and Tigaras (HT) were the sampling sites with the highest species

occurrence (8 species), while the lowest (4 species) was observed at the sites Sampurtoba (ST) and Rianite (RN). *Tetralunata amphicephala* and *T. costata* were rarely found, and their presence was only in the Parbaba (PB) and Tigaras Harbour (HT) samples.

**Table 3.** Presence of *Tetralunata* species in sampling sites from Lake Toba

Species	South					North				
	BK	ST	RN	SB	PB	BH	HT	BH2	KN	NL
<i>T. amphicephala</i>	-	-	-	-	+	-	+	-	-	-
<i>T. angusta</i>	-	+	-	-	+	-	+	+	-	+
<i>T. cf.robusta</i>	+	-	+	-	-	+	-	+	+	+
<i>T. costata</i>	-	-	-	-	+	-	+	-	+	-
<i>T. elongata</i>	-	+	-	+	+	+	+	+	+	+
<i>T. lanceolata</i>	+	+	+	+	+	+	+	+	+	+
<i>T. obtusa</i>	+	-	-	+	+	+	+	+	+	+
<i>T. pelagica</i>	+	+	+	+	+	+	+	+	+	+
<i>T. vanheurckii</i>	+	-	+	+	+	+	+	+	+	+
Total species found	5	4	4	5	8	6	8	7	7	7
Total samples analysed	2	2	1	1	3	3	3	5	1	5

Note: Sibeabea (SB), Batuhoda (BH), Harbour Tigaras (HT), Batuhoda 2, (BH2), Nauli (NL), Kenangan (KN), Parbaba (PB), Rianite (RN), Sampurtoba (ST), Bakkara (BK). (+)= present, (-)= absent

The number of species identified at each sampling site appears to correlate to some extent with the number of samples analysed. For example, Parbaba and Tigaras (3 samples each) yielded 8 species, while sites with only 1–2 samples (e.g., Rianite, Sibeabea) showed slightly fewer species (4–5 species). However, Kenangan, despite only one (1) sample, yielded 7 species, suggesting that species richness may not be solely a function of sample size. Therefore, while differences in sampling effort could have influenced the observed species richness, the relatively high number of species found in some minimally sampled sites (e.g., Kenangan) indicates that real biological differences between sites may also contribute significantly to the observed variation.

Comparison of fossil or living *Tetralunata* reported by Hustedt (1935–1938) and the recent study is shown in Table 4. Of the 14 species reported by Hustedt, this study found eight species were alive. One species of *Tetralunata cf. robusta* was not discovered in Hustedt and is considered a putative new species.

**Table 4.** Comparison of fossil/living among *Tetralunata* species from Hustedt (1935–1938) and a recent study (2024)

Species	Fossil or living as reported by Hustedt	Presence (+) or absence (-) in the 2024 survey
<i>Tetralunata amphicephala</i>	Fossil	Living
<i>Tetralunata angusta</i>	Living	Living
<i>Tetralunata bicuneata</i>	Living	Not found
<i>Tetralunata cf. robusta</i>	Not found	Living
<i>Tetralunata costata</i>	Fossil	Living
<i>Tetralunata dubia</i>	Living	Not found
<i>Tetralunata elongata</i>	Living	Living
<i>Tetralunata lanceolata</i>	Fossil	Living
<i>Tetralunata lata</i>	Living	Not found
<i>Tetralunata obtusa</i>	Living	Living
<i>Tetralunata pelagica</i>	Fossil	Living
<i>Tetralunata rhynchocephala</i>	Living	Not found
<i>Tetralunata spathulifera</i>	Living	Not found
<i>Tetralunata subrobusta</i>	Living	Not found
<i>Tetralunata vanheurckii</i>	Living	Living

## DISCUSSION

The *Rhopodiales* have received increased attention over the last several years, as interest in the symbiotic relationship between the host diatom and symbiont cyanobacterium has increased

(Schvarcz et al., 2022, 2024; Moulin et al., 2024; Frail et al., 2025). A taxonomic revision of *Epithemia* (Kociolek et al., 2025b) and description of new taxa in the genus *Rhopalodia* (e.g. Moore et al., 2025) also contribute to our understanding of the diversity of the group. Within this context, more information about the genus *Tetralunata* has been a priority for understanding the diversity and interactions of the symbiosis.

Morphological characters for diatom identification have been demonstrated by many researchers. Nine species of *Tetralunata* found in this study showed morphological differences under microscopic observation. *Tetralunata elongata* is easily identified by its huge cell size (180–200 µm), and *T. amphicephala* is the smallest (25–30 µm). Among *Rhopalodiales*, *Rhopalodia gibba* and *E. turgida* are also known to have large frustule dimensions (205 µm and 166 µm, respectively). For small *Tetralunata*, a combination of valve apices and frustule shape would give more information than valve apices or valve shape alone. For example, *T. angusta*, *T. obtusa*, and *T. pelagica* are difficult to distinguish from their valve apices. However, by combining with the shape of valves, identification is more reliable. Microscopic observation showed that many *Tetralunata* species have a straight raphe ending. As a consequence, this character cannot be counted as a strong morphological feature of *Tetralunata*.

A total of 26 samples were analysed from 10 sampling sites around Lake Toba, with 20 samples collected from the northern region and 6 from the southern region. Species richness varied across sites, with some evidence of correlation between the number of samples collected and the number of species observed. For instance, sites with greater sampling effort, such as Parbaba and Tigaras (3 samples each) yielded a relatively high number of species (8 species each), whereas sites with only one or two samples, such as Rianiate and Sibeabea, recorded slightly fewer species (4–5 species). However, this relationship was not consistent across all sites. Notably, Kenangan, with only one sample collected, exhibited a high species richness (7 species), comparable to Nauli (5 samples, 7 species). This suggests that while sampling effort may influence observed species diversity, it is unlikely to be the sole determinant. Further research is needed to clarify the differences in species numbers between sites around Lake Toba.

The variation in species richness observed across sampling sites in Lake Toba may be influenced not only by sampling effort but also by several underlying ecological and environmental factors. One key factor is that **habitat heterogeneity** sites with more complex or diverse microhabitats, such as varying substrate types or aquatic vegetation, can support a wider range of diatom species by providing more ecological niches (Passy, 2007; Stevenson et al., 2010). **Water quality parameters**, including pH, temperature, dissolved oxygen, and nutrient concentrations (e.g., nitrate and phosphate), also play a critical role in shaping diatom communities (Smol & Stoermer, 2010). **Light penetration and water depth** further influence diatom distribution, with clearer, optimally shallow areas supporting greater photosynthetic activity and diversity (Round et al., 1990). These ecological differences of abiotic parameters likely contribute to the high species richness recorded in certain minimally sampled sites, such as Kenangan, and suggest that biological variation among sites is an important factor alongside sampling effort in determining diatom diversity.

Benthic diatoms are ubiquitously found on nearly all stable substrates, including rocks (epilithon), sand (epipsammon), woody debris (epidendron), sediment (epipelon), aquatic vegetation (epiphyton), and decomposed leaves (Lowe & Laliberte, 1996). The substrate is significant for diatoms as it furnishes a necessary surface for attachment and movement. Numerous benthic diatoms depend on stable surfaces such as rocks, sediments, or aquatic flora for colonization and growth (Townsend & Gell, 2005). The nature of the substrate also exerts an influence on the species composition of diatom communities, since certain species favor hard surfaces, whereas others flourish in soft or organic-rich sediments (Battarbee et al., 2001). Furthermore, substrates can impact the localized availability of nutrients and the intensity of light that diatoms are exposed to, both of which are vital for their photosynthetic processes and overall survival (Vadeboncoeur & Steinman, 2002).

The presence of infrequent species can be explained by data presented by Hustedt, which indicates that there are geographical restrictions for some of the species found. Hustedt (1937) reported several species of *Denticula* (now reclassified as *Tetralunata*) along with various notes on

their habitat preferences or locations where they were found. For example, *Tetralunata amphicephala* was found exclusively in the muddy bottom of the northern coastal area of Pangururan. Another infrequent species is *Tetralunata costata* is more commonly found on the eastern side of the lake, such as the Porsea and Balige areas (Hustedt, 1938). In this study, it is expected that *T. costata* was found in low numbers likely because sampling was conducted only in the northern and southern regions of Lake Toba.

Hustedt (1935) stated that four species, *T. amphicephala*, *T. costata*, *T. lanceolata*, and *T. pelagica*, were found in deeper waters in sediments in the form of fossils. **Fossil diatoms are the remnants or traces of frustules (silica cell walls) from diatoms that have been preserved in geological sediments**, ranging in age from hundreds to hundreds of thousands of years old (Siemińska, 2000). These frustules have unique shapes and structures that allow identification down to the genus or species level (Sims et al., 2006). In this study, the four species are found alive. This evidence shows that species of *Tetralunata* survived for more than 90 to 130 years after their first encounter in Lake Toba. This finding suggests that some *Tetralunata* species possess ecological resilience and adaptive capacity that allow survival amid the lake's environmental transitions from the more oligotrophic conditions of the early 20<sup>th</sup> century to the present, more eutrophic state. Their persistence may be facilitated by the ability to form resting stages or occupy microhabitats with stable physicochemical conditions, such as deeper sediments or sheltered littoral zones (Round et al., 1990).

In this study, *T. costata* and *T. amphicephala* are the species with the lowest presence. Both species were found in shallow waters at the edge of the harbor. The other two species, *T. pelagica* and *T. lanceolata*, were found with moderate presence in various habitats, such as shallow waters near the harbor, ponds near the west coast of Parbaba, and attached to rock substrates on Kenangan Beach. Divergences of sampling sites and equipment, again, could be a consideration to confirm the diversity of *Tetralunata* in Lake Toba, especially for the six species that have not been found in this study (Table 4). One of the six species aforementioned is *T. spathulifera*. *Tetralunata spathulifera* was found on the bottom of mud with a depth of 21 m in the Porsea area (east side of the lake) (Hustedt, 1937), which was not covered in recent study. Another interesting result obtained from the recent study is *T. rhynchocephala*. *Tetralunata rhynchocephala* was reported in 1940 in small number population and no longer existed in 1942 (Hustedt, 1942). This species may have narrow adaptation to environmental changes so that it cannot survive until now.

This research also provides new information regarding the existence of a possibly new *Tetralunata* species that have never been reported before, (listed herein as *T. cf. robusta*). This putative new species was quite common, being found at almost all sampling locations. The absence of prior records may be due to its strong morphological resemblance to other *Tetralunata* species. Morphologically, *T. cf. robusta* is similar to *T. robusta*, but differs in several key aspects: the proximal raphe endings in *T. cf. robusta* are distinctly curved, the valve outline is linear-lanceolate (rather than elliptical-lanceolate), and the frustule length is consistently smaller (50–60  $\mu\text{m}$  versus 60–100  $\mu\text{m}$  in *T. robusta*).

Although these differences may suggest taxonomic distinctiveness, variation in raphe structure and valve dimensions can sometimes occur within species as a result of environmental influences or intraspecific variability (Pouličková et al., 2009; Cantonati et al., 2020). Therefore, the observed differences while morphologically consistent require further validation. Additional analyses, particularly using scanning electron microscopy (SEM) to examine areolar ultrastructure and, if possible, molecular data, are necessary to confirm whether *T. cf. robusta* represents a distinct taxon or a morphological variant of *T. robusta*.

## CONCLUSION

Based on the research conducted, it was found that there are 9 species of *Tetralunata* found alive in Lake Toba, North Sumatra, namely *T. amphicephala*, *T. angusta*, *T. cf. robusta*, *T. costata*, *T. elongata*, *T. lanceolata*, *T. obtusa*, *T. pelagica*, and *T. vanheurckii*. The most frequently reported species are *T. lanceolata*, *T. pelagica*, and *T. vanheurckii*. These species can be found in almost all locations because they can easily attach to various substrates (hard and soft). The infrequent species

are *T. amphicephala*, *T. costata*, and *T. angusta* whose number of presences is very low. These species were found attached to vascular plant substrates. All species that were documented as fossils in 1935 were found living in 2024. For a better understanding of the interactions between *Tetralunata* and their habitats, future research needs more extensive sampling and measurement of environmental parameters that critically shape diatom communities.

## ACKNOWLEDGEMENTS

The study was supported by The Universitas Indonesia PUTI Q1 Grant (NKB-398/UN2.RST/HKP.05.00/2024) and grant 2222944 of the United States National Science Foundation.

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