

# ***Entomoneis triundulata* sp. nov. (Bacillariophyta), a new freshwater diatom species from Dongting Lake, China**

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**Abstract** – The diatom genus *Entomoneis* includes species with a unique morphology, having panduriform frustules in girdle view, often twisted about its apical axis, with some unique characters such as a sigmoid raphe on elevated bilobate keel, differently shaped junction lines, numerous girdle bands and variously perforated striae. Most of the species belonging to this genus are found to be brackish or marine, with some occurrences in freshwater habitats, but not one species has been described as exclusively freshwater. In this study, the new freshwater diatom species *Entomoneis triundulata* sp. nov. is described based on a detailed morphological observation using light and scanning electron microscopy. The specific features of *E. triundulata* are its triundulate valve margin; the shape of the transition between keel and valve body having a proximal U-shaped end, a slightly curved middle segment and a distal V-shaped end; and five sub-compartments to the frustule cavity. Besides morphological features unique to *E. triundulata*, similarities with other *Entomoneis* species are reported and discussed.

**Keel / new species / reduced striae / reverse S-shape / Surirellales**

## **INTRODUCTION**

The name *Entomoneis* was first used by Ehrenberg as a subgenus (Ehrenberg, 1845a: 71), but was subsequently elevated to genus level (Ehrenberg, 1845b: 154) using *Navicula alata* Ehrenberg (1840: 212) as its type species, renamed as *Entomoneis alata* Ehrenberg (1845b: 154). Previously, Ehrenberg had established the genus *Amphiprora* (1843: 401) with *Amphiprora constricta* (1843: 25) as its type species, containing various species with panduriform shaped valves/cells. For some time, species of *Entomoneis* were nomenclaturally confused with those of *Amphiprora* until Reimer clarified the use of *Entomoneis*, established the Entomoneidaceae

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family and transferred some key species to *Entomoneis*, such as *E. robusta* (McCall) Reimer, *E. pulchra* (Bailey) Reimer, *E. ornata* (Bailey) Reimer and *E. paludosa* (W. Smith) Reimer (Reimer in Patrick & Reimer, 1975). Today, the family Entomoneidaceae comprises two diatom genera – *Entomoneis* and *Platichthys* (Lange-Bertalot, Kulikovskiy, Witkowski, Seddon & Kociolek in Lange-Bertalot *et al.* (2015: 137)), differing in few morphological features: species in *Platichthys* do not have twisted panduriform frustule, but possess only uniseriate striae, and they do not have a sigmoid keel (Lange-Bertalot *et al.*, 2015).

Species in *Entomoneis* share the following combination of features: i) the frustule appears panduriform and has numerous, open girdle bands; ii) the valves are often strongly compressed laterally and bear a high, narrow winged keel; iii) the areolae are occluded by hymens outside; iv) the raphe is located along the apex of the keel as a simple slit; the central raphe endings and external polar endings are all straight and not (or only slightly) expanded; and v) in the light microscope (LM) contoured junction lines are visible.

There are many misidentifications and taxonomic disagreements in *Entomoneis*, which can be appreciated from the data available in different databases: The WoRMS diatom database (*Diatombase*) (Kociolek *et al.*, 2017) lists 29 names in *Entomoneis*, the *Catalogue of Diatom Names* lists 39 names in *Entomoneis* (once duplicate synonyms are removed) (Fourtanier & Kociolek, 2011) and Algaebase lists 21 names in *Entomoneis* with 19 (including both species and intraspecific taxa) viewed as currently accepted (Guiry & Guiry, 2017), especially those described species by Osada & Kobayasi (*E. centrospinosa*, *E. decussata*, *E. pseudoduplex*) (Osada & Kobayasi, 1990a, b, c).

The two most recent descriptions of new species in *Entomoneis*, one of which is fossil, made morphological comparisons with a number of *Entomoneis* taxa and utilized molecular data to determine relationships within this genus, but there are still certain features for which no data was provided (Paillès *et al.*, 2014, Mejdandžić *et al.*, 2017). As publications that include scanning electron microscope (SEM) micrographs of species of *Entomoneis* often only provide images of low magnification, there are a number of ultrastructural details that require further studies (e.g. Osada & Kobayasi, 1990c; Clavero *et al.*, 1999; Reinke & Wujek, 2013). *Entomoneis* is clearly under-described and taxonomic studies describing new as well as existing species is much needed.

Round *et al.* (1990) noted that species in *Entomoneis* are mostly epipelagic and commonly found in brackish to marine sediments, only occasionally being found in freshwater. Since 1990, no exclusively freshwater species of *Entomoneis* have been described. In this study, numerous specimens of a freshwater *Entomoneis* species were found in Dongting Lake (China), which allowed detailed morphological and ultrastructural observations to be made. The unique triundulate valve margin and partitioned frustule cavity, along with other morphological features detailed below, supports the recognition of these specimens as a new species of *Entomoneis* that we have named *Entomoneis triundulata* sp. nov.

## MATERIAL AND METHODS

Dongting Lake is the second largest freshwater lake in China and is located between 28°30'–30°20' N/111°40'–113°40' E in the northeast part of Hunan Province (Fig. 1). It has four tributaries (Xiang, Zi, Yuan and Li Rivers) and the Yangtze

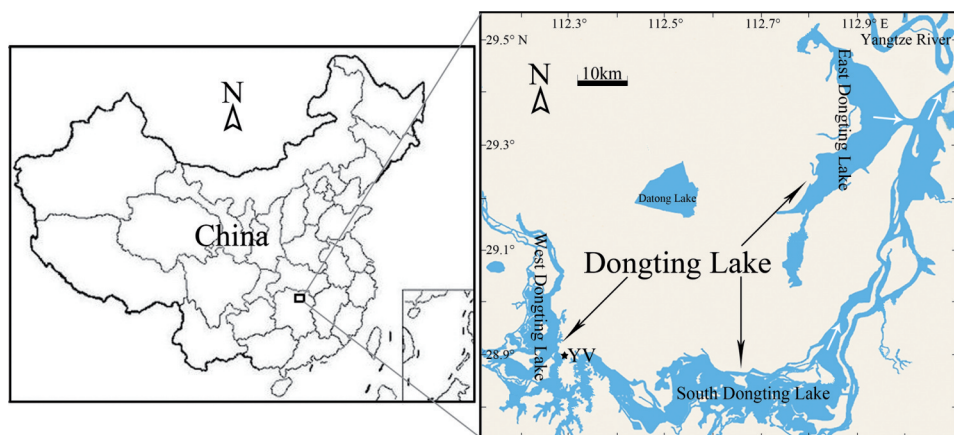


Fig. 1. Map of sampling site, Yang's Village (YV), West Dongting Lake, China.

River's three outlets (Songzi, Hudu, and Ouchi). Its outflow returns into the Yangtze River at Chenglingji, the sole outlet of Dongting Lake. Dongting Lake consists of three sub-lakes, i.e., East, South, and West Dongting Lake (Fig. 1). All those sub-lakes include permanent water catchment areas with large periodically inundated areas. Dongting Lake is located in the subtropical monsoon climate zone. The wet season is from May to October and the dry season is from November to April. The annual mean temperature of the lake water is ca. 16.4–17.0°C, and the annual total precipitation is ca. 1200–1400 mm (Cui *et al.*, 2012). To date, the diatoms of Dongting Lake have been neglected.

In April 2017, some benthic diatom samples were collected from one sampling site near Yang's Village (28°52'29.5" N/112°16'52" E, Fig. 1, YV). Epipellic diatoms were separated from the mud using lens tissue, which was removed from the mud surface after one day. The collected lens tissue was then processed with concentrated nitric and sulphuric acids (Mann *et al.*, 2004).

Permanent slides were prepared using Naphrax® mountant and examined using a Leica DM3000 (Germany) light microscope (LM) equipped with Leica DFC425C camera. The holotype slide is deposited in should be the Natural History Museum, London, United Kingdom while an isotype slide is kept in the Herbarium of Jishou University, Hunan, People's Republic of China.

For scanning electron microscopy, several drops of the cleaned diatom material were air-dried onto glass coverslips. The selected coverslips were attached to an aluminum stub using double-sided adhesive conductive carbon tape and sputter-coated with platinum (Cressington Sputter Coater 108auto, Ted Pella, Inc.). Samples were examined and imaged using a field emission scanning electron microscope (FE-SEM) Sigma HD (Carl Zeiss Microscopy, Germany) available at Huaihua University, China.

Diatom valve terminology largely follows Ross *et al.* (1979), Paddock & Sims (1981) and specifically for *Entomoneis* terminology, we follow Osada & Kobayasi (1985).

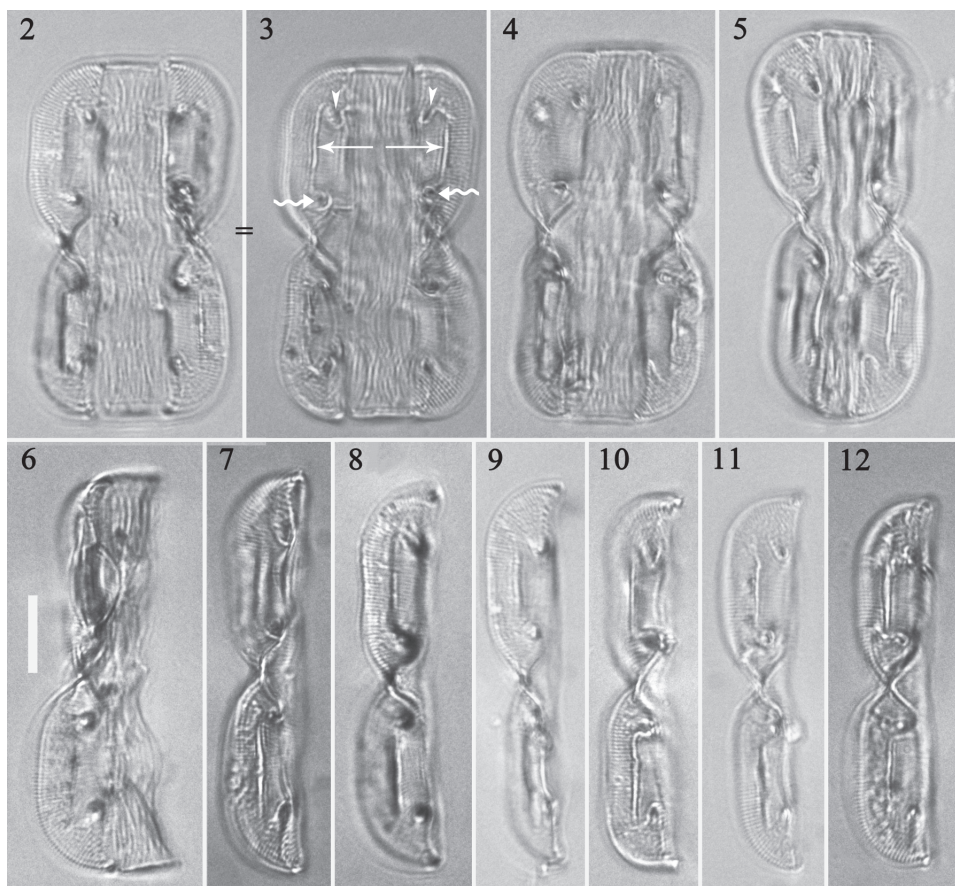
## RESULTS

Division Bacillariophyta  
 Class Bacillariophyceae  
 Subclass Bacillariophycidae  
 Order Surirellales  
 Family Entomoneidaceae  
 Genus *Entomoneis*

*Entomoneis triundulata* Bing Liu & D.M. Williams, **sp. nov.**

**Figs 2-50**

**Type:** Yang's Village, West Dongting Lake, China, lens tissue sample, leg. Bing Liu, coll. date April 23<sup>rd</sup>, 2017 (BM 101914, holotype, Natural History Museum, London, United Kingdom, here illustrated as Figs 2-3; JIU G201702, isotype, Department of



Figs 2-12. *Entomoneis triundulata* sp. nov., LM, girdle view. **2-3.** Illustrations of holotype specimen at different foci; note that the junction line contains a U-shaped proximal end (two curved arrows), a middle slightly curved segment (two arrows), and a V-shaped distal end (two arrowheads). **4-5.** Two additional frustules. **6-12.** Seven valves showing stria and junction line, Figure 6 with girdle bands. Scale bar = 10  $\mu$ m (in Fig. 6) for all figures.



Biology of Jishou University, Hunan, People's Republic of China, here illustrated as Fig. 16).

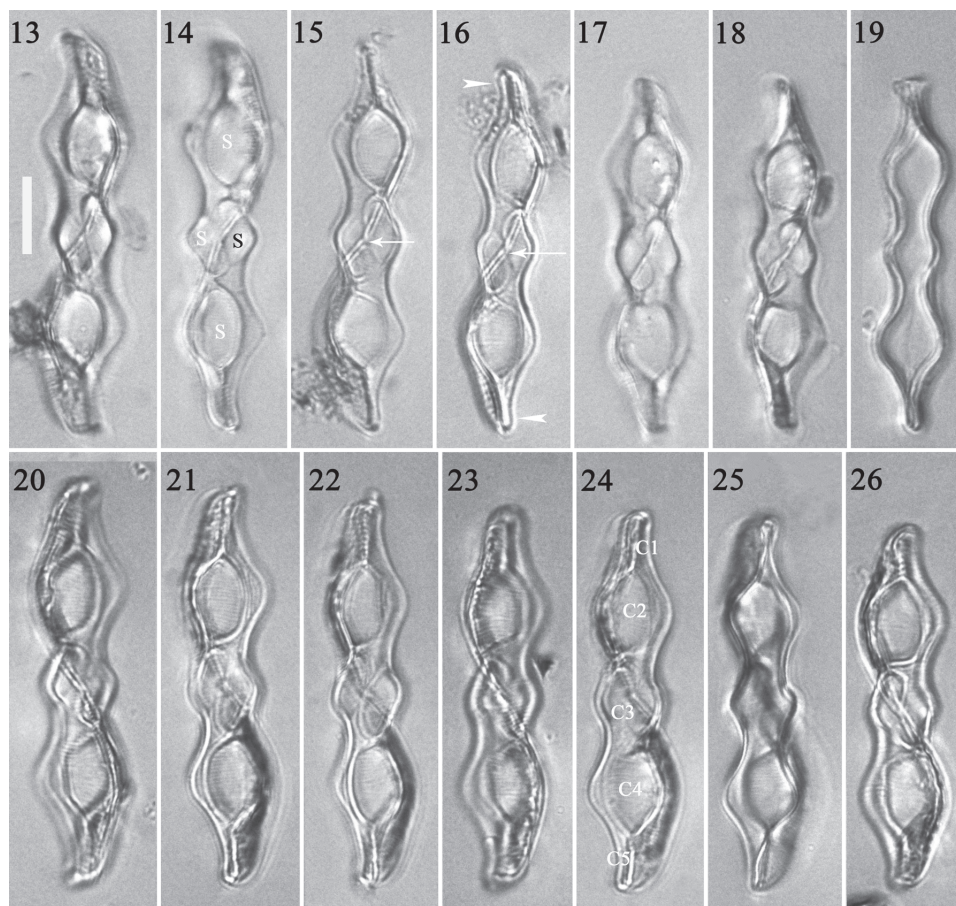
**Etymology:** From the Latin adjective *triundulata* referring to the triundulate valve and cingulum margin.

**Diagnosis:**

Valves triundulate, junction line having a slightly curved middle segment with a proximal U-shaped and distal V-shaped end, five sub-compartments to frustule cavity.

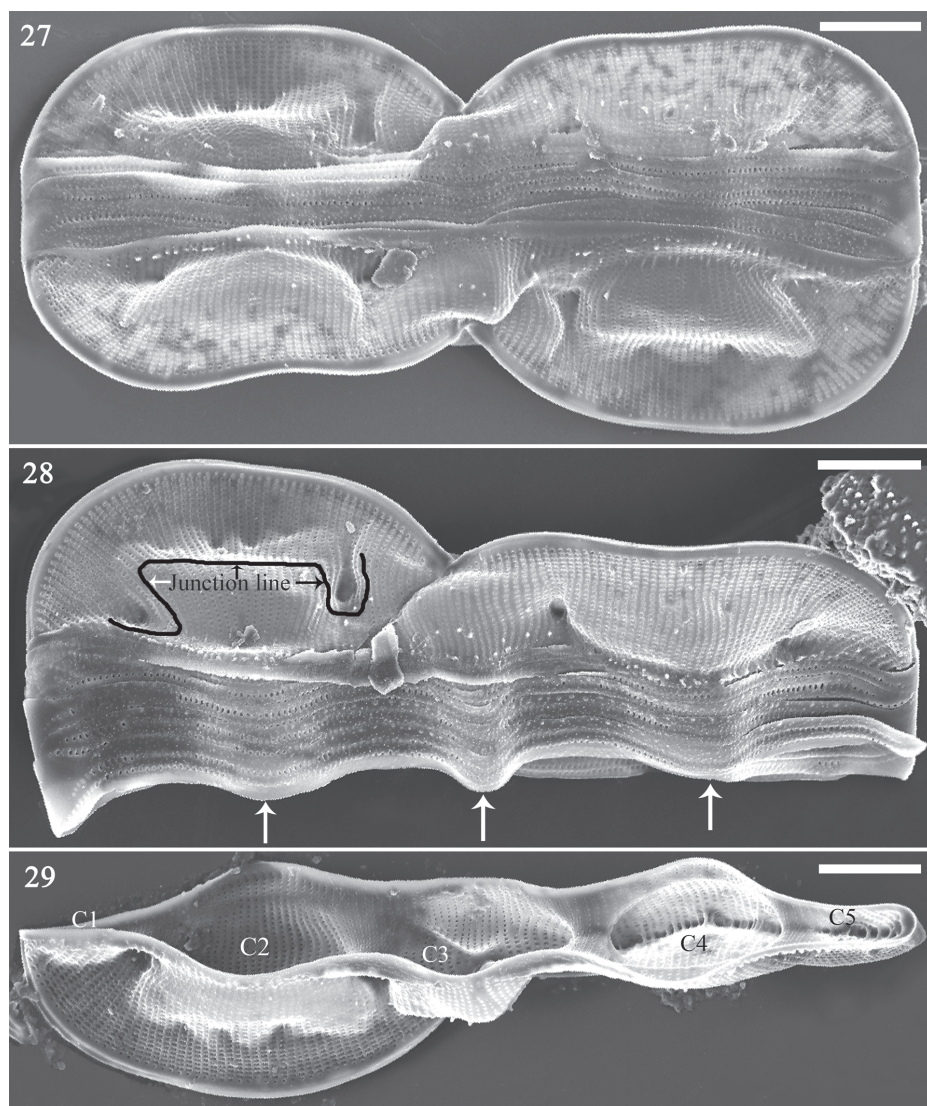
**Description:**

**LM: Girdle view:** frustule panduriform in girdle view, composed of epivalve, numerous girdle bands and hypovalve (Figs 2-5), length 45-52  $\mu\text{m}$ , width at constricted part 17-19  $\mu\text{m}$ , at widest part 23-26  $\mu\text{m}$ . Each valve separated by a



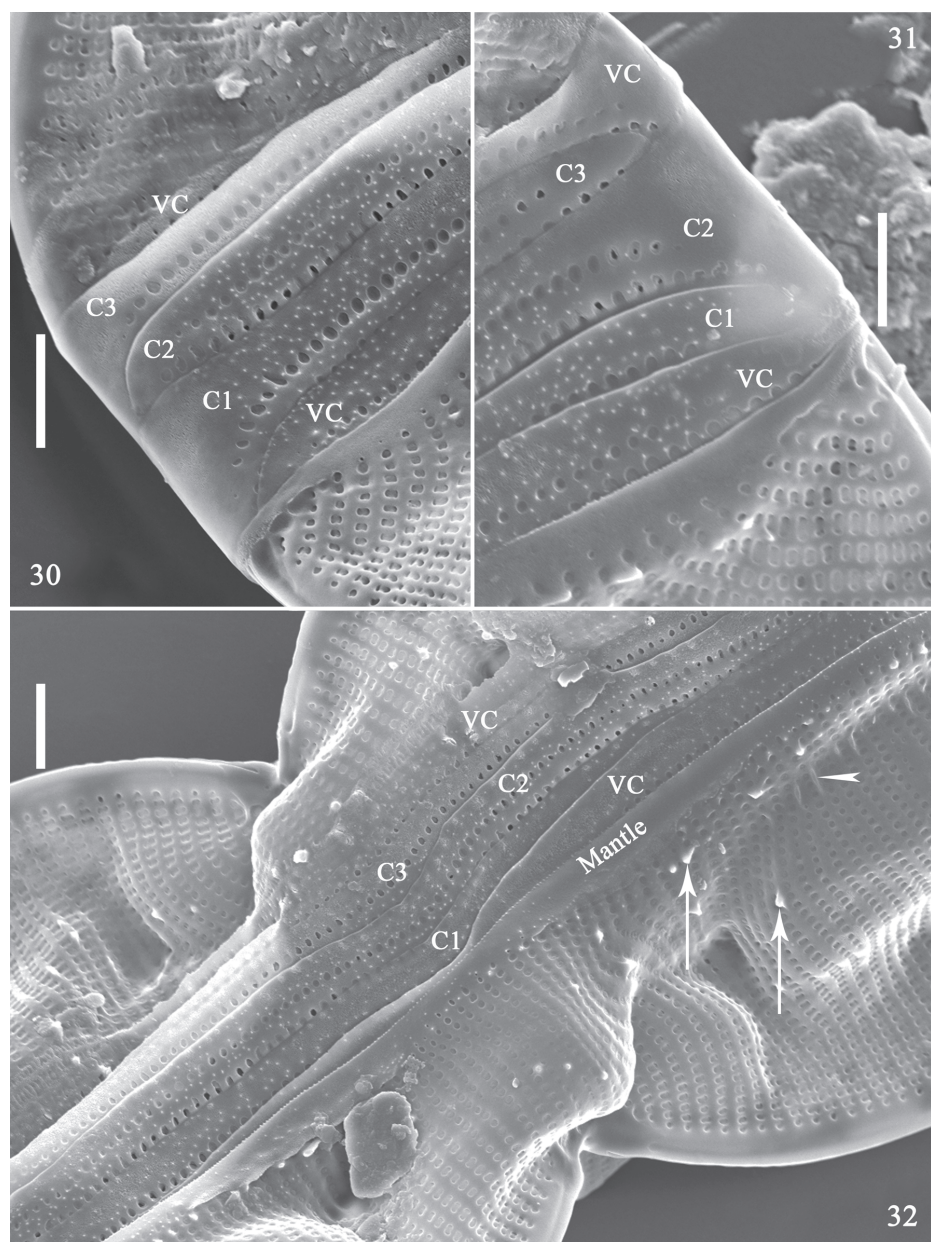
Figs 13-26. *Entomoneis triundulata* sp. nov., LM, valve view. **13-18.** Six valves in external view showing size diminution; note triundulate valve margin and 2-shapedly twisted keel. **14.** Valve showing its swollen portions (S). **15-16.** Two valves showing central nodule (arrows), proximal raphe endings and rostrate poles (arrowheads) (Figure 16 = isotype specimen). **19.** Cingulum with triundulate outline. **20-26.** Seven valves in internal view showing size diminution; note distinct five sub-compartments (labelled C1 to C5 in Figure 24). Scale bar = 10  $\mu\text{m}$  (in Fig. 13) for all figures.

junction line into two parts: keel and valve body (Figs 6-12). Junction line composed of three parts: proximal U-shape end (Fig. 3, two curved arrows), middle slightly curved segment (Fig. 3, two arrows), distal V-shaped end (Fig. 3, two arrowheads), i.e., junction line at its middle a slightly curved segment plus a U-shaped end and a V-shaped end. Two ends not always distinct (e.g., Figs 6, 12). Striae parallel but becoming radiate towards poles, 22-26 in 10  $\mu\text{m}$ .



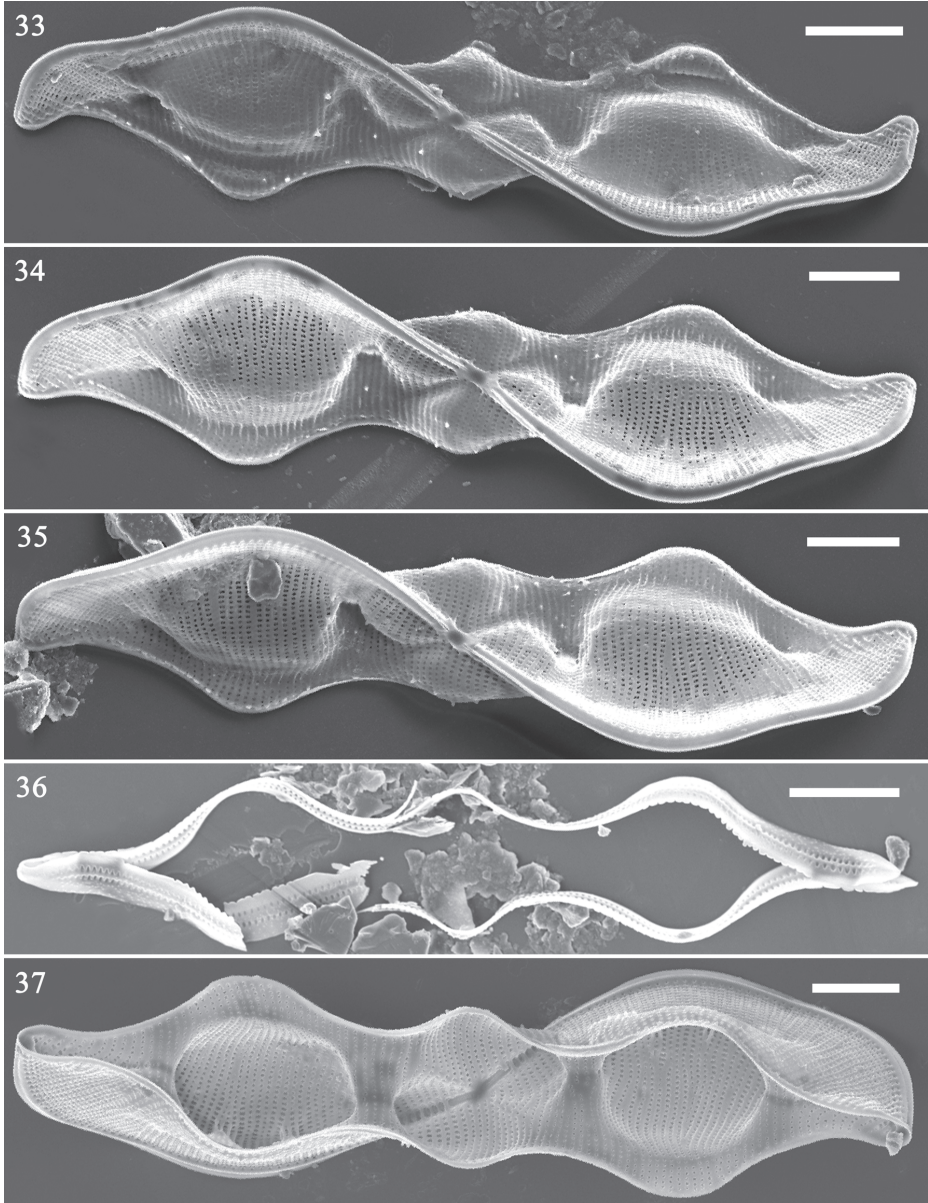
Figs 27-29. *Entomoneis triundulata* sp. nov., SEM. **27.** A whole frustule. **28.** Hypotheca showing junction line (indicated by black sketch line) and triundulate cingulum margin (three arrows). **29.** A valve in side view showing five sub-compartments of frustule cavity (labelled C1-C5). Scale bars = 5  $\mu\text{m}$ .





Figs 30-32. *Entomoneis triundulata* sp. nov., SEM, details of specimen in Figure 27. **30-31.** Two poles showing two valvocopulae (VC) and three copulae (C1-C3). **32.** The middle portion showing two valvocopulae (VC) and three copulae (C1-C3), warts (two arrows) and thickened ribs (arrowhead). Scale bars = 2  $\mu$ m.

**Valve view:** valve margin triundulate, 42.2-52.0  $\mu\text{m}$  long ( $n = 54$ ), 6.8-8.7  $\mu\text{m}$  wide at centre ( $n = 24$ ), with elevated sigmoid bilobate keel about its apical axis (as a twisted Z-shape) (Figs 13-18, as a reverse S-shaped (Z), see below). Valve apices acuminate/rostrate (excluding keel) (Fig. 16, two arrowheads). Inner valve reveals

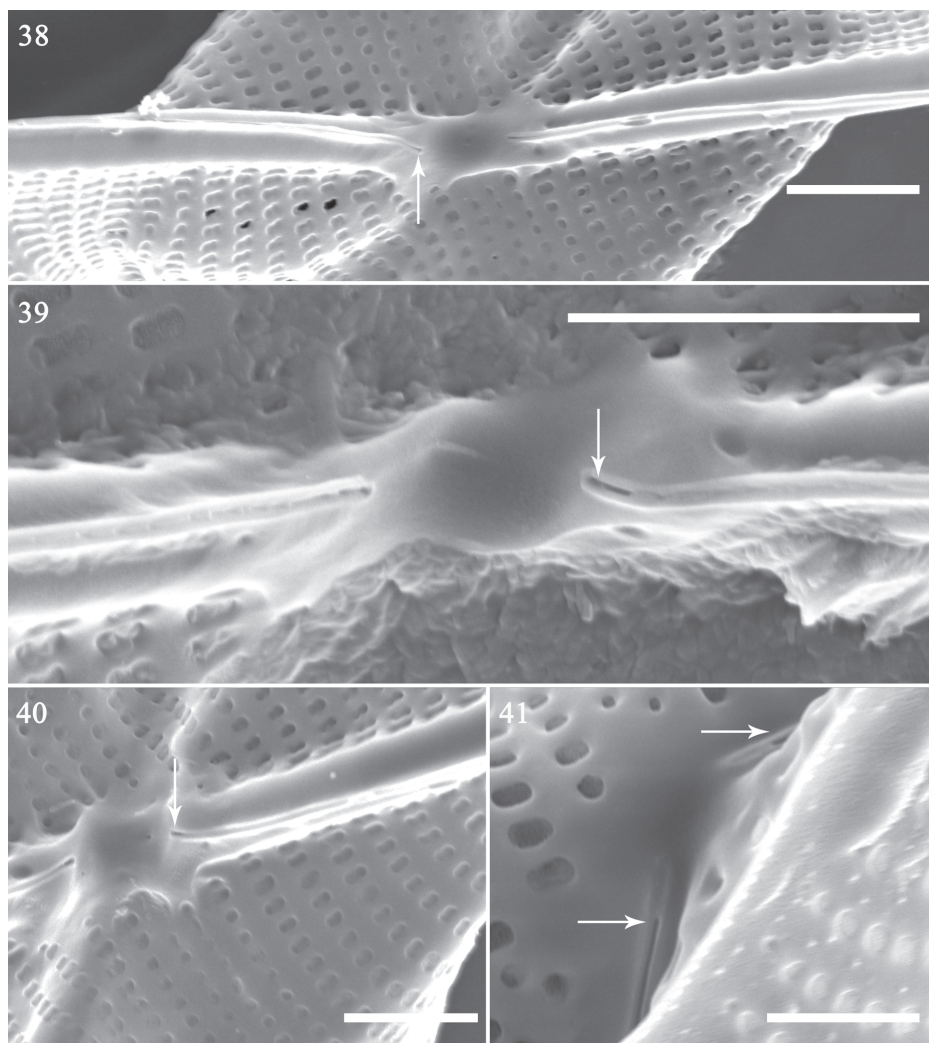


Figs 33-37. *Entomoneis triundulata* sp. nov., SEM, valve view. **33-35.** Three valves showing reverse S-shaped keel outline. **36.** One girdle band (copula) showing its triundulate outline and open nature. **37.** Valve in internal view showing triundulate valve margin and frustule cavity. Scale bars = 5  $\mu\text{m}$ .



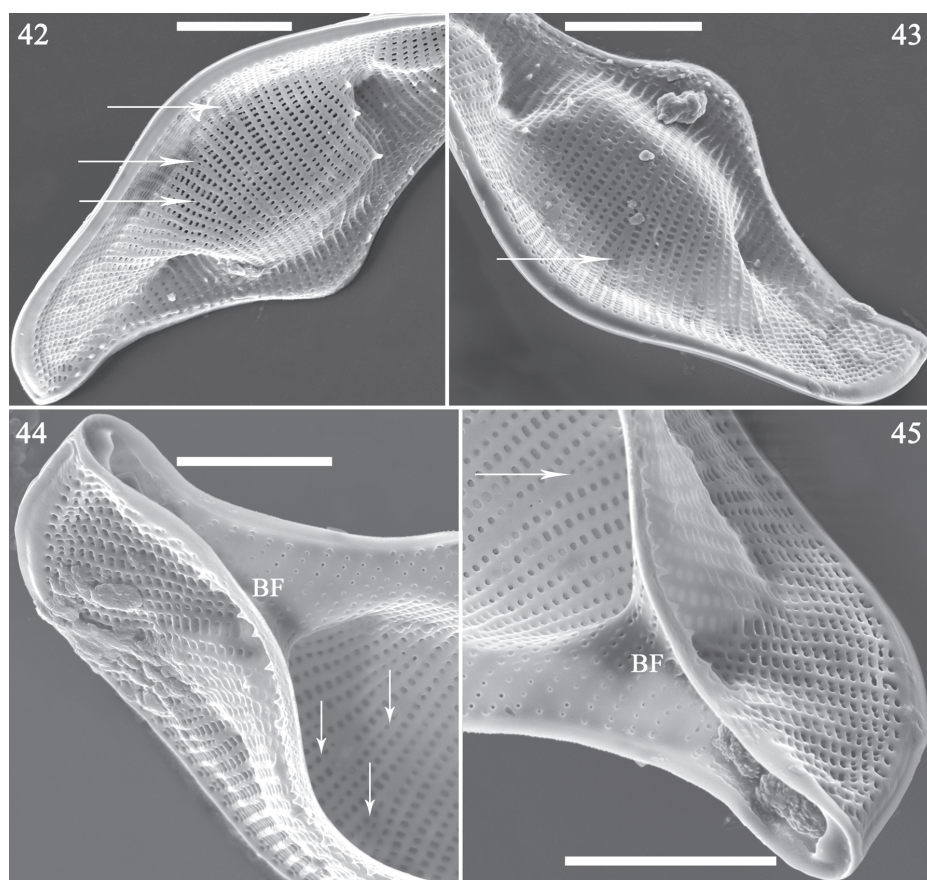
specific cavities visible at outer valve as swellings (Fig. 14). Raphe located at keel apex, proximal raphe endings terminate before central nodule (Figs 15-16, arrows). Cingulum, in valve view, triundulate (Fig. 19). Internally, valve margin triundulate (Figs 20-26). Valve cavity divided into five sub-compartments (Fig. 24, labelled C1 to C5).

**SEM observation:** frustule panduriform, consisting of epivalve and hypovalve; each frustule with two valvocopulae and three copulae (Figs 27-29, 30-32, labelled VC, C1 to C3). Valve triundulate with elevated winged sigmoid keel (Figs 33-35, 37). Each valve divided into five distinct parts observed as outer swellings, or



Figs 38-41. *Entomoneis triundulata* sp. nov., SEM. **38-40.** Three central parts of valve in external view, showing central nodule and one slightly bent proximal raphe ending (arrows). **41.** One central part in internal view; note internal central nodule and two straight raphe endings (two arrows). Scale bars = 2  $\mu$ m.

cavities from inner valve (Figs 27, 29). Swellings located between winged keel and valve body. Junction line composed of three portions: proximal U-shaped end, slightly curved middle segment, and distal V-shaped end (Fig. 28, indicated by black sketch line). Striae parallel, but radiate at approaching apices (Figs 27-29). Stria composed of apically elongated areolae occluded by hymens externally (Figs 30-32), areolae ca. 40 in 10  $\mu\text{m}$ . Mantle not perforated (Fig. 32). Scattered siliceous warts on valve face (Fig. 32, arrows), thickened costae between swollen portions and valve surface (Fig. 32, arrowhead). Raphe located along keel apex, proximal raphe endings terminated before central nodule (Figs 33-35). Two proximal raphe endings not expanded, one ending straight, the other slightly bent (Figs 38-40, arrows). Cingulum composed of two valvocopulae and three copulae, each possessing triundulate outline (Fig. 28), also triundulate in valve view (Fig. 36), each with two rows of areolae (Figs 30-32, 36). Number of areolae in each girdle band ca. 32-35 in 10  $\mu\text{m}$ . Some reduced striae exist (Figs 42-45). Internally, frustule cavity divided into five sub-compartments (Fig. 46, labelled C1 to C5) by four large plate-like basal

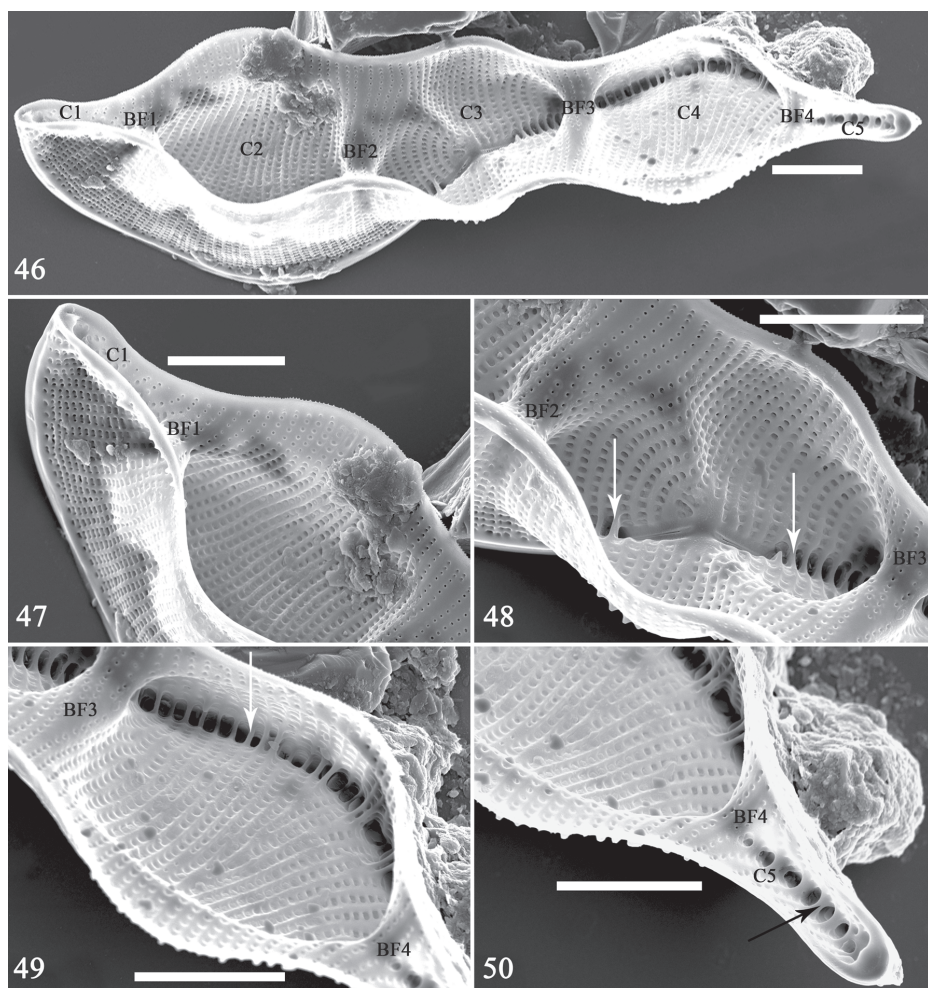


Figs 42-45. *Entomoneis triundulata* sp. nov., SEM. **42-43.** Both poles in external view, showing reduced striae (arrows). **44-45.** Both poles in side view, showing reduced striae (arrows) and two basal fibulae (BF). Scale bars = 5  $\mu\text{m}$ .

fibulae (Fig. 46, labelled BF1 to BF4). Fibulae at different levels (Figs 48-50, arrows). Central raphe endings straight, not expanded (Fig. 41).

**Distribution:** Known only from the type locality.

**Ecology:** *Entomoneis triundulata* was the dominant species in the surface sediment collected in West Dongting Lake. Associated species comprised *Nitzschia* spp., *Surirella* spp., *Navicula* spp., *Bacillaria paxillifera* (O.F. Müller) Hendey, *Sellaphora* spp., *Tryblionella* sp., *Cymatopleura solea* W. Smith, and others. The following environmental parameters were measured in the field. Conductivity was  $109.3 \pm 0.1 \mu\text{S/cm}$ , pH was  $8.3 \pm 0.2$  and water temperature was  $23.5 \pm 0.3^\circ\text{C}$ . Since the diatom sample was collected using lens tissue from the mud surface and the water conductivity is above  $100 \mu\text{S/cm}$ , *Entomoneis triundulata* can be considered an epipelagic alkaliphilous diatom characteristic of moderate electrolyte content in fresh waters.



Figs 46-50. *Entomoneis triundulata* sp. nov., SEM, internal view. **46.** One whole valve showing four basal fibulae (labelled BF1-BF4) dividing frustule cavity into five sub-compartments (labelled C1-C5). **47-50.** Details of Fig. 46; note fibulae at different levels (arrows). Scale bars = 5  $\mu\text{m}$ .



## DISCUSSION

*Entomoneis triundulata* possess the common should be features in the genus *Entomoneis*: elevated sigmoid bilobate keel about its apical axis; and its specific features: triundulate valve margin; the shape of the transition between keel and valve body having a proximal U-shaped end, a slightly curved middle segment and a distal V-shaped end; and five sub-compartments to the frustule cavity. Round *et al.* (1990) pointed out that valves of species in *Entomoneis* are either lanceolate or linear with acute poles. However, only a few taxa were provided with valve view SEM illustrations and most have been illustrated only with LM images (see Table 1). So far, the outline of the winged keel has been described sigmoid, but this is not correct as clear SEM images of valve view for most published *Entomoneis* are lacking (see Table 1). From our clear SEM images of valve view (both external and internal) for *E. triundulata* (Figs 33-35, 37), we find that there only one type valve exists, i.e. the valve with 2-shaped keel outline. Furthermore, we checked those species listed in Table 1 and found there are two types of valves existing in the genus *Entomoneis*: one type with the 2-shaped keel outline, the other with S-shaped keel outline. This feature of *Entomoneis* is completely different from other diatoms that possess a sigmoid valve outline, such as taxa in the genus *Gyrosigma* Hassall. Liu *et al.* (2015) provided a good example in their description of the new species *Gyrosigma xiamenense* Liu, Sterrenburg & Huang. *G. xiamenense* possesses two type valves, one type with 2-shaped outline (Liu *et al.*, 2015, p. 261, figs 2-4) and the other with S-shaped outline (Liu *et al.*, 2015, p. 261, figs 5-7). As for the frustule, the outlines of epivalve and hypovalve are completely different in external valve view (compare figs 2-4 with figs 5-7 in Liu *et al.*, 2015, p. 261). However, in *Entomoneis*, the keel outlines of epivalve and hypovalve from the same frustule are the same in valve view (e.g. Figs 13-18, 33-35).

*Entomoneis* taxa are mostly found in low abundance with very few records of their occurrence in large numbers in the benthos (Dalu *et al.*, 2015). Therefore, because of their rarity, details of the morphology of most species have never been studied. For example, knowledge of the valve elements is very limited (see the references listed in Table 1). Concerning the outline of the valve, when seen in valve view and the shape of winged keel, also when seen in valve view, no comparative data was available. Data from 12 taxa, which had clear illustrations, showed that 10 have lanceolate, linear-lanceolate or linear valve margins, when in valve view; only one species, *E. japonica* (Cleve) Osada & Kobayasi, has a panduriform valve margin and *E. triundulata*, has a triundulate valve margin (Table 1). The juncture of the keel with the valve body forms a variously contoured “line” called a “junction line” by Cleve (1894) and a “Trennungslinie” (separation line) by Hustedt (1930). The junction line is observed in LM but in SEM its shape can be determined. There are few junction lines documented with SEM images, so the images here provide the first clear example of its structure (Fig. 28). We carefully checked the data from the 12 taxa in Table 1, unfortunately however, we cannot find a figure showing the complete internal view of valve. Thus the whole internal view of the valve (Figs 29, 37, 46) once again provide the first clear example of its structure.

Most taxa described so far in the genus *Entomoneis* are found in brackish to marine sediments – some have been referred to as occasionally found in freshwater (e.g., Round *et al.*, 1990). As far as we are aware, no exclusively freshwater species of *Entomoneis* has been clearly described. Thus, *Entomoneis triundulata* is the first strictly freshwater species of *Entomoneis* to be fully described. *Entomoneis ornata*



Table 1. Valve outline and types of winged keel in twelve *Entomoneis* taxa

No.	Taxon	Valve outline and illustration type	Keel outline and illustration type	Reference
1	<i>E. triundulata</i> sp. nov.	Triundulate, both LM and SEM	2-shaped, both LM and SEM	This paper
2	<i>E. aequabilis</i> K. Osada & H. Kobayasi	Linear, both LM and SEM	2-shaped, both LM and SEM	Osada & Kobayasi 1991, p. 159, fig. 3, p. 165, fig. 28
3	<i>E. japonica</i> (Cleve) K. Osada	Panduriform, both LM and SEM	2-shaped, both LM and SEM	Osada & Kobayasi 1985, p. 218, fig. 8, p. 219, fig. 10
4	<i>E. paludosa</i> (W. Smith) Reimer	Linear-lanceolate, SEM	2-shaped, SEM	Dalu <i>et al.</i> 2015, p. 22, fig. 39
5	<i>E. pseudoduplex</i> K. Osada & H. Kobayasi	Linear-lanceolate, LM	2-shaped, LM	Osada & Kobayasi 1990a, p. 169, fig. 5
6	<i>E. pulchra</i> (J.W. Bailey) Reimer	Linear-lanceolate, drawing	2-shaped, drawing	Patrick & Reimer 1975, p. 5-6, pl. 2, fig. 2
7	<i>E. punctulata</i> (Grunow) K. Osada & H. Kobayasi	Linear-lanceolate, LM	2-shaped, LM	Osada & Kobayasi 1990a, p. 169, fig. 7
8	<i>E. reimeri</i> D.C. Reinke & Wujek	Lanceolate, SEM	2-shaped, SEM	Reinke & Wujek 2013, p. 115, fig. 2
9	<i>E. alata</i> (Ehrenberg) Ehrenberg	Lanceolate, SEM	S-shaped, SEM	Poulin <i>et al.</i> 1987, p.71, fig. 7
10	<i>E. centrospinosa</i> K. Osada & H. Kobayasi	Lanceolate, LM	S-shaped, LM	Osada & Kobayasi 1990b, p. 389, fig. 2
11	<i>E. decussata</i> (Grunow) K. Osada & H. Kobayasi	Linear-lanceolate, LM	S-shaped, LM	Osada & Kobayasi 1990c, p. 255, fig. 2
12	<i>E. tenera</i> Mejdandžić & Bosak	Lanceolate, both LM and SEM	S-shaped, SEM	Mejdandžić <i>et al.</i> 2017, p. 5, fig. 8, p. 9, fig. 15

has been found frequently in lakes and fresh waters of Montana with a mean pH of 8.1 and a mean specific conductance of 2188  $\mu\text{S}/\text{cm}$ , which is considerably lower than the conductivity preferred by many other species of *Entomoneis* (Bahls, 2012).

Even though specimens of *Entomoneis triundulata* were found in profusion, the living cell has not yet been studied nor were transmission electron microscope (TEM) images or molecular data gained. Further study will yield these data.

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