# **RESEARCH ARTICLE**

ARAŞTIRMA MAKALESİ

# *Entomoneis parikhani* sp.nov., a new diatom (Entomoneidaceae, Bacillariophyta) from Aras River (Iran)

*Entomoneis parikhani* sp.nov., Aras Nehri (İran)'nden yeni bir diatom (Entomoneidaceae, Bacillariophyta)

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Abstract: The diatom *Entomoneis* has a series of unique features such as connecting lines with different shapes, sigmoid raphe on the bilobate keel, different curtains, and multiple girdle bands. Most species belonging to this genus are found in marine and saline habitats. In fact, the genus *Entomoneis* is an epipelic diatom that is found in streams with high salinity and high electrolyte concentration. To date, no specific type of freshwater has been identified for this species. In this study, a new species of freshwater diatom, *Entomoneis parikhani*, is described based on detailed morphological observations using a scanning electron microscope. The samples were collected the Aras River near Oltan village of Parsabad city in the northwest Iran. A special feature of *Entomoneis parikhani* is its triple valve. The genus *Entomoneis* comprises of diatoms with sigmoidal raphe canals and girdle bands. This taxon is similar to *Entomoneis parikhani*. This new species had very small cells, delicate frustules without valve lines, and wide lanceolate valves. In this study, the wide distribution of this taxon in the Aras River has been evaluated.

Keywords: Entomoneis, new taxon, Aras River

Öz: Çift taraflı simetrik bir lobut görümünündeki diyatom türü *Entomoneis*, vücut yapısı üzerinde sigma yapan rafesi, farklı şekillerde görülen bağlantı hatlarıyla diğer diyatom türlerinden şekil olarak ayrılır. Bu cinse ait türlerin çoğu denizel ve tuzlu habitatlarda bulunur. Aslında, *Entomoneis* cinsi yüksek tuzluluk ve yüksek elektrolit konsantrasyonuna sahip akarsularda bulunan bir epipelik diyatomdur. Bugüne kadar, bu tür için belirli bir tatlı su tipi tanımlanmamıştır. Bu çalışmada, taramalı elektron mikroskobu kullanılarak yapılan ayrıntılı morfolojik gözlemlere dayanarak yeni bir tatlı su diyatomu türü olan *Entomoneis parikhani* tanımlanmıştır. Örnekler, İran'ın kuzeybatısındaki Parsabad şehrinin Oltan köyü yakınlarındaki Aras Nehri'nden toplanmıştır. Yeni bir diyatom türü olan *Entomoneis parikhani*'nin özel bir yapısı üçlü valfidir. *Entomoneis cinsi*, sigma yapan rafe kanallarına ve kuşak bantlarına sahip olan bir cinstir. Bu takson, LM ve SEM gözlemleriyle gösterildiği üzere *Entomoneis annagodhei* ve *Entomoneis tenera*'ya benzerdir ve *Entomoneis parikhani*'ni yi ayırmak için yeterli morfolojik farklılıklar vardır. Bu yeni türün çok küçük hücreleri mevcuttur, früstülleri çok ince yapıda olup kapak çizgileri ise mevcut değildir ve kapakları da geniştir. Bu çalışmada, bu taksonun Aras Nehri'ndeki geniş dağılımı değerlendirilmiştir.

Anahtar kelimeler: Entomoneis, yeni takson, Aras Nehri

# INTRODUCTION

Diatoms belong to the branch Heterokontophyta and class Bacillariophyceae. They are known for their siliceous cell walls and bivalve structure. Entomoneis is one of the most important species of diatom that lives in saline environments. The genus Entomoneis is mainly marine, and its representatives are found in saline water (Liu et al., 2018). Entomoneis have single rectangular guitar-shaped cells. It is twisted along the longitudinal axis. Entomoneis contain one or two chloroplasts. The inserted rims have multiple holes. In Entomoneis, the seam was S-shaped. In Entomoneis, the shells are thick and have a girdle outline (Mejdandžić et al., 2018). Species belonging to the genus Entomoneis have attracted the attention of scientists because of their importance in water quality assessment and biological monitoring (Mejdandžić et al., 2018). The genus Entomoneis includes species with a unique morphology. Most species belonging to this genus are salt-loving and live in places with

# high salinity (Round et al., 1990).

The diatom genus *Entomoneis* Ehrenberg (1846) has species that can be identified by light microscopy because of a series of features, such as multiple girdle bands, sigmoidal raphe, and pandori-shaped cells (Patrick and Reimer, 1975). In most *Entomoneis* species, the basal fibula is visible under an electron microscope (Round et al., 1990). Patrick and Reimer (1975) classified the genus *Entomoneis* as a species that has pandori-shaped cells, sigmoid raphe, multiple striae, and multiple intercardiac bands (Majewska et al., 2019). These results were obtained based on detailed morphological observations and electron microscopy (Ross et al., 1979).

Currently, Entomoneidaceae has two genera: *Entomoneis Ehrenberg* and *Platichthys* (Lange-Bertalot et al., 2015), which these two genera have a series of common morphological characteristics such as the raphe canal and the

number and number of valve surfaces. *Entomoneis* belongs to the raphe canal diatoms and is a non-monophyletic group in which the raphe canal evolved independently at two separate locations. According to the phylogenetic analyses and data of three chloroplast genes (SSU, rbcL, and psbC) and the theory of (Ruck et al., 2016) *Entomoneis* has monophyletic origin. *Platichthys* genus, which is morphologically similar to the *Entomoneis*. However, it has unknown phylogenetic position and is monophyletic. During the last two decades, only four new *Entomoneis* species have been identified based on their morphological characteristics.

A number of biomonitoring and floristics studies have been performed over the past few decades to document diversity and ecology of Iranian algae including: Algues des dèserts d'Iran (Compère, 1981), Anzali Lagoon (Nejadsattari, 2005), Caspian Sea (Fatemi et al., 2005), Gharasou River (Atazadeh et al., 2007), Lake Neure (Nejadsattari, 2005), streams in Ramsar (Soltanpour-Gargari et al., 2011), Balikhli River (Panahy Mirzahasanlou et al., 2020) and Karaj River (Kheiri, 2018). In recent studies, the total number of algal species in Iran has reached 1304. These algae species are divided into 8 divisions, 15 classes, 37 orders, 96 families and 262 genera. Among these divisions, Bacillariophyta forms the largest group and is located after Chlorophyta, Cyanophyta and Euglenophyta (Zarei-Darki, 2009). In all investigated waters of Iran, diatoms (Bacillariophyta) were the dominant algae. During recent studies, more than 90% of species and subspecies (i.e. 479 species and 612 infra-specic taxa) have been revealed as new species. Entomoneis is a relatively large genus of diatoms, and there is great morphological diversity among the species in the genus Entomoneis. So far, different species of Entomoneis genus have been reported in the rivers of Iran, and these rivers have salty water. A species of the Entomoneis genus was reported in the Etrak River of Boinord City, which has salty water (Nejadsattari, 2005). 2 species of the Entomoneis genus were reported in the Qarasu River (Atazadeh et al., 2007). 3 species of Entomoneis genus have been reported in Urmia River (Mehrjuyan and Atazadeh, 2022). So far, no species of Entomoneis genus has been reported in Iranian rivers that have fresh water.

In this study, *Entomoneis parikhani* sp. nov, a small and silicified taxon in Ultan village located in Parsabad City of the Aras River in northwestern Iran, is described as a new species, and these descriptions are based on morphological characteristics. In the present study, has tried to compare the similarities and differences *Entomoneis parikhani* with other species. Genus is also discussed.

# MATERIAL AND METHODS

#### Study area

The Aras River originates in the Arpa Yay area of Anatolia, Türkiye (Parikhani et al., 2023). The river has passed through the border of Türkiye, Nakhchivan, Jolfa and Armenian, and after crossing the Iranian border enters the Republic of Azerbaijan and flows into the Kura River. Aras River, is one of the largest rivers in northern Iran and the watershed of the Caspian Sea and supplies an important part of the region's water. In Aras River is one of the longest and water most Border Rivers in Iran. According to Figure 1 the study area is part of the Aras River in the village of Oltan in the city of Pars Abad. Pars Abad Moghan City in the northwest of the country, northeast of Azerbaijan Iran and north of Ardebil province and in the Moghan area. This city covers a vast part of the northern region of the Moghan Plain. Table 1 shows the geographic coordinates of the studied point in Ares River (Parikhani et al., 2023).

Table 1. Location of of the studied point along the Aras River with site GIS coordinates

Site Name	Latitude	Longitude	Altitude (m)	
Oltan	47.76384033	39.60737133	63	



Figure 1. Map of the studied point in Aras River, Northwestern Iran

Table 2 shows the pH, electrical conductivity (EC) and total dissolved solids (TDS) values measured in the sampling point. pH, EC and TDS values were recorded by pH meter, EC meter and TDS meter belonging to Tabriz University Ecology and Environment Laboratory. The method of measurement was in such a way that the sensor belonging to each device was placed in the flowing water of the river. After about twenty seconds, the fixed number was recorded on the device's monitor (Carranzo, 2012).

Table 1	<b>2.</b> pH,	EC and	TDS of	Aras	River i	in the	sampling site
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Station	рН	EC (µS/cm)	TDS (mg/L)
Oltan	9.0	1681	1075

Samples were collected from the Aras River at sampling station in northwestern Iran during or period. Diatom samples were collected from cobbles, pebbles, or rocks in a collection bottle (200 mL), and then Lugol's solution (2 mL) was added for preservation (Williams et al., 2016). Temporary slides were prepared for live diatoms to identify the species composition. For diatom species identification and enumeration, the samples were prepared following the method of Battarbee et al. (2001). The samples were digested with 10% hydrogen peroxide in a beaker at 90°C on a hotplate for 2 h, after which two drops of 10% hydrochloric acid were added. The beakers were filled with distilled water and left to settle overnight, after which the supernatants were discarded. This process was repeated four times. The subsamples (400 µl were air-dried on coverslips and mounted using Naphrax (Battarbee et al., 2001). All slides were identified and counted per slide using a Zeiss microscope with differential interference contrast at ×1000 magnification. For scanning electron microscopy (SEM), the rinsed samples were resuspended in a solution of distilled water for 30 min and then rinsed three times in distilled water. Frustule suspensions were dried directly on 22 mm diameter aluminum stubs and gold coated with a Dynavac Xenosput sputter coater. Frustules were imaged using a TESCAN fieldemission scanning electron microscope with a working voltage of 2.0 kV and a spot size of 2. The morphology of the new species was compared with that of several described taxa (Flower et al. 1996; Hamsher and Saunders, 2014).

# RESULTS

The taxonomic classification of the *Entomoneis* as follows:

Division: Bacillariophyta

Class: Bacillariophyceae (Medlin and Kaczmarska, 2004)

Order: Surirellales (Mejdandzic et al., 2017)

Family: Entomoneidaceae (Round et al., 1990) (Patrick and Reimer, 1975)

Genus: Entomoneis (Mejdandzic et al., 2018) Entomoneis parikhani sp.nov. (Figure 2-6)

# Description

Morphological, ultrastructural, and ecophysiological characteristics of the species were investigated under the light micrograph (LM). In the belt view, the frustules were two blobs. The frustules are deeply contracted and visible, with a connecting line near the central node. The edges of the wings are thin. Some of these features appear at longer irregular intervals. Finally, it ends at a small point (Figure 2). Scanning electron microscopy revealed membranous valves without ornamentation and a heavily silicified fibular keel. *Entomoneis* 

parikhani is distinguished from other Entomoneis species by the number of distinct striae and the micro morphometric structure of the valve surface. In Entomoneis parikhani, the abdomens are rectangular in the girdle view. The valves are lanceolate, linear, and have parallel margins. The raphe canal was unusual. The transapical striae are parallel. Areoles small, fbulae short, delicate, and unevenly distributed. Fascia is shortened due to irregular central striae. The raphe florem expanded with the external central pores. The axial region of the cell was very narrow and linear and hardly extended near the central region. In Entomoneis parikhani nov sp, single cells with frustules are well silicified. The valves have a lanceolate line and slightly concave edges in the middle. Two plate-like plastids are arranged parallel to each other in each cell. Pandora-shaped frustules are located in the girdle view. According to Figure 3 the transapical striae are parallel in the middle and slightly radiate towards the apex. Areoles in the raphe canal are distinct and longer than the surface of the valve. Tran's epic is long. Each transapical row Striae corresponds to an elongated halo in the raphe canal. The fibulae are irregular and distributed along the length of the valve.

The external valve view shows a series of fine lines as dense transapical lines according to the SEM. The small indentations near the valve tip correspond to the transition between the valve body and the top. The raphe keel is narrow and high and sigmoid-shaped with a gap like a spear in the middle. The central fissures of the raphe are simple, next to a transapical fascia, and are clear in the inner view of the valve. The end slits of the raphe are hooked.

According to Figure 4 valve apices are slightly elongated in valve view, and it sharpens in the view of the belt. Rafted keel is narrow and sigmoid. The central region of the raphe forms a canal that has a lance-shaped cleft opening, and visible when the transapical fascia is out of focus. The striae are very fine and can be seen under the light microscope. According to Figure 4 the external view of the valve shows a deep channel with a lanceolate slit. The marginal end of the fascia is separated by a small number of areoles. In *Entomoneis parikhani*, the end of the external central raphe is weakly expanded and elongated apically.



Figure 2. Entomoneis parikhani under LM. The central region of the raphe canal shows spear-like slits



Figure 3. Entomoneis parikhani under SEM. a and c) central part of the valve with central node and simple central raphe endings, b and d) show the view of the valve. The sigmoid raphe canal, dense transapical striae, and a spear-like slit opening can be seen in the central raphe canal region



Figure 4. Entomoneis parikhani under SEM. a) shows the tip of the valve with the end of the raphe, b and d) show the view of the valve belt indentation in the transition between the valve body and the keel, c) shows the tip of the sharp valve where the terminal raphe slit is hooked

In *Entomoneis parikhani* the valves are highly irregular. The raphe canal is slightly above the level of the valve. Raphe is florum. At the apex, the terminal raphe of the end of the valve is strongly hooked at the same level as the valve, and ends with the mantle (Figure 5). The valve plate is flat. The proximal valve mantle is shallow. A very narrow band forms the distal valve mantle.



Figure. 5. SEM microghraphs of Entomoneis parikhani sp. nov.

According to Figure 6 striae are composed of linoleum. Lineole pores are round and prominent. The spaces between the fbulae are variable and tend to become larger towards the apical valve. The fibulae are narrow and similar in shape. At the apex, the raphe ends in smaller helitoglossae. In the inner part, the transapical striae are composed of smaller transapical ones. The areolas are elongated and placed at the same level. The ends of the external central raphe are elongated abically. Valve epic never lasts long. Transapical striae radiate in the middle part of the valves and abruptly converge.



Figure 6. Entomoneis parikhani, SEM. Girdle view of the top of the cell showing the end of the apical raphe

The new species *Entomoneis parikhani* has unique shaped valves which are lanceolate, linear, and wide lanceolate. A distinctive feature of *Entomoneis parikhani* is its very delicate appearance, which is because the cells have very little silica.

Other species of *Entomoneis* have very many silicified frustules, which makes these species generally appear stronger. Different species of *Entomoneis* are different from *Entomoneis parikhani* in terms of morphology and shape, as shown in Table 3.

 Table 3. Comparison of taxa resembling Entomoneis parikhani (np: not present)

Species	Valve length (µ <m)< th=""><th>Valve width (µm)</th><th>Valve outline</th><th colspan="2">Striae density (in 10 μm) Plastids</th><th>References</th></m)<>	Valve width (µm)	Valve outline	Striae density (in 10 μm) Plastids		References
E.aequabilis	47-57 μm	7-9 µm	Linear, weakly sigmoid	32-37	Multi-lobed plastid	(Osada and Kobayasi, 1991)
E. annagodhei	50-82 µm	10-12 µm	Linear-lanceolate with slightly	52-58	Oblique transapical two, axial	(Al-Handal et al., 2020)
E.japonica	75-150 μm	20-40 µm	Linear-lanceolate	11-12	Multi-lobed plastid	(Osada, 1985)
E. parikhani	32-60 µm	8.84-8.61 µm	Linear-lanceolate	33-40	2	This study
E.paludosa	40-130 µm	20-50 µm	Broadly linear	21-26	Two, axial	(Osada, 1990a)
E.punctulata	18-99 µm	10-19 µm	Broadly linear	34-36	Multi-lobed plastid	(Osada, 1990b)
E.tenera	11-22 µm	3-7 µm	Broadly lanceolate	30-50 valve body: 18-42	One, lobed	(Mejdandžić et al., 2017)
E.vertebralis	33-58 µm	10-11 µm	Linear-lanceolate	The keel (SEM) np	Two, palate-like	(Clavero et al., 1999)

# DISCUSSION

Entomoneis parikhani Raphe is described based on its morphological characteristics. Of which we can refer to the Pandori frustules that grow on a winged keel. The new species of *Entomoneis parikhani* shows strange morphological characteristics that are very different from other *Entomoneis* species.

Unlike E. paludosa, Entomoneis parikhani has a multilobed plastid. There are two plastids in each cell. Some authors have reported that species belonging to the genus Entomoneis have one to two plastids. A characteristic of Entamonis parikhani is that the size of its cells is tiny, the cells are 32-60 µm long, while other species such as E. japonica, are 75-120 µm long or species such as E. paludosa are 40-130 µm long have. This reduction in cell size is due to the conditions of cell growth and life in fresh water. That maybe the cell has entered a ring in fresh water from living conditions in salt water and this has caused stress in the cells of Entomoneis parikhani. There is only one exception among Entomoneis species, Entomoneis vertebralis also has a delicate appearance and only the silicified part has a hyaline frustule with a raphe system. Entomoneis vertebralis is similar in general appearance to Entomoneis parikhani. Still, its difference from Entomoneis parikhani is that it is not visible in the cells of Entomoneis vertebralis in the vent line LM. In Entomoneis parikhani the valve has a very fine structure due to the recognizable ornamentation on the valve. The fine structure of the valve in Entomoneis parikhani consists of transapical parts and striae with round and circular holes. Entomoneis vertebralis no discernible vent ornamentation is seen during SEM examination. Entomoneis species have mostly single forms. They have never been seen as a colony in their natural habitat. But sometimes it is seen that these cells form chains in which the chain of cells are connected with their keels. These chain-like cells are mostly seen in

laboratory cultures. In any case, we cannot consider this form of life as a defining characteristic of these species, perhaps this chain of cells is formed because there was no separation between the cells after cell division, which caused the formation of a chain in these cells. The general morphology of *Entomoneis parikhani* includes pandora-shaped cells in girdle view, sigmoidal raphe canal and multiple median bands. Girdle view of the top of the cell showing the end of the apical raphe is shown in Figure 6.

In Entomoneis parikhani plate-like plastids with other Entomoneis species such as E. alata (Liu et al., 2018). Cell twisting was not observed in Entomoneis parikhani, while it was observed in some species such as E. alata and E. paludosa (Osada, 1990a). When comparing the shape of the valve, Entomoneis parikhani is more similar to E. alata. E. punctulata has lanceolate linear valves. The apex of the valve is similar in Entomoneis parikhani and E. alata. However, the species differ in valve length, which is given in Table 2. E. alata and E. punctulata both have wide bilobate keels with prominent and very wide lobes (Cox, 2001). Which are located on the edge of the valve. While Entomoneis parikhani has narrow bilobate keels with relatively long wings that do not exceed the valve margin and valve face (Lobban et al., 2019). The valves of Entomoneis parikhani appear hyaline due to the very fine veins in the LM, as do several other species such as E. vertebralis, which have hyaline valves. Entomoneis parikhani has dense striae with rounded areoles. Which is more similar to the ultrastructure of E. paludosa (Mejdandzic et al., 2018).

Recent research on *Entomoneis* has combined morphological observations with phylogenetic observations (Al-Handal et al., 2019). However, the research of (Al-Handal et al., 2019) did not combine these morphological studies with phylogenic. Because the morphological features were specific and unusual enough to define the definite limits of Entomoneis annagodhei. However, this does not mean that future research should not focus on molecular data collection. Because molecular data are always useful in diatom research. There is still much to do and study (Barkia et al., 2019). In the cells of this diatom, the caps were seen as linear-sharp and rarely swollen in the central part of the caps. In all the observed samples, two blastocysts were seen in the cell. This species was separated based on the length and size of the cells, the shape of the end of the cell (round or sharp tip), and the location of the chloroplasts. The cells in the members of this genus had bilateral symmetry. Rafe is straight. The length of cells in this genus is variable according to its species, and most of the time, their length was measured between 32-60 and their width between 8-9 micrometers. This genus has wavy chloroplasts, and the position and shape of chloroplasts, the length of cells and the position of furrows are important in distinguishing the species of this genus. Entomoneis parikhani differs from other Entomoneis species in terms of wing shape in girdle view and having central spines. The length of the valves of E. gigunteu species is reported to be between 160 and 190, while in Entomoneis parikhani it is almost half of this size. Entomoneis parikhani has smaller valves and closer veins on the valve body. The new species has narrower wings and lacks a central spine. One of the strange features in the new species is the spines on each side of the central nodule, it is also recognizable in LM, and the spine is limited to only one valve. In Entomoneis parikhani, the number of spines on both sides of the central nodule is unequal. In Entomoneis parikhani, the transapical costae are completely continuous from the margin of the valve to the raphe canal at the top of the valve. The wings of the body are longer and wider than the inner wings. And the wings seem motionless. When viewed using LM, the girdle of Entomoneis parikhani shows the actual structure as if a diagonally striated transparent layer is folded in two on top of the wing. In LM observations, it can be seen that the fibulas are located as small dots on the wings. These wings connect the costae to the opposite walls. Finally, Entomoneis parikhani was identified as a new species based on the measurement of size (length and height), linear density of fibula, stria, areola, presence or absence of fracture in the

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raphe gap, central nodule, and the distance between the two middle fibulae, and the following characteristics Among the distinctive features of *Entomoneis parikhani* are considered: an arched connecting line, the valve areolae are separated from each other, and the areolae of the girdle band are large, some of these characteristics, such as the length, width, density of the fibula, and striae, are affected. Physical and chemical parameters such as salinity, nutrients, temperature, and water movement are important for the species and it was concluded that *Entomoneis parikhani*, pH, TDS, and the fresh water of the Aras River have greatly affected these characteristics.

# CONCLUSION

To date, all reported species of *Entomoneis* are specific to salt water, and no *Entomoneis* species live in freshwater has been reported (Lobban et al., 2019). The present study is related to a new species of *Entomoneis parikhani*, which is specific to freshwater in the Aras River.

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# AUTHORSHIP CONTRIBUTIONS

Fatemeh Parikhani performed the experiments and wrote the draft.

# CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

#### ETHICAL APPROVAL

This article does not contain any human or animal studies performed by any authors.

# DATA AVAILABILITY

The data for the assessment included in the study is given in the text.

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