Study of the Genus *Pinnularia* (Naviculales, Bacillariophyta) in the Taleghan River and a New Record for Diatoms Flora of Iran

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Abstract

The diatoms are widespread organisms that occurred in various environments like soil, marine, freshwater, and even in extreme conditions. Taleqhan River is one of the main tributaries of Sepid Roud, the second longest river in Iran with approximately 670 km length. During the sampling from eight stations in the Taleghan River and Dam, *Pinnularia lundii* var. *linearis* Krammer was identified as a new record for diatoms flora of Iran. Given the importance of *Pinnularia* genus as an indicator of pollution, it was discussed together with *Pinnularia brebissonii* (Kütz.) Krammer in this paper. This study was performed during November 2017 to October 2018 on the sediments monthly in the North of Iran. The most characteristic morphological features were the valve outline, density of striae, hexagonal-shaped central area, non-deflected proximal raphe endings and capitated apices. The most similar species to this taxon are *P. subanglica* Petersen and *Pinnularia* biceps Gregory. They have deflected proximal raphe ending, unlike *Pinnularia lundii* var. *linearis*.

Keywords: Diatom, Epipelic, GLM Model, *Pinnularia lundii* var. *linearis* Krammer, Taleghan River.

Introduction

The ecological process is one of the main prominent fields in the biological community which reveals the functional evolutionary and the stability of the ecosystems. One of the common algal groups found in most ecosystems and habitats is diatoms. They can be considered as a systematic group and are applied to evaluate the water quality (Noga et al., 2014; Atazadeh et al., 2007). Environmental factors can uniquely influence the taxa and historically each effect may be unknown. The taxa responses to the environmental and chemical water variations (Winter and Duthie, 2000). The diatoms often exist in freshwater and brackish water bodies. Diatoms tolerate extensive range of pH gradient, in aquatic bodies with high nutrient concentration, organic and inorganic pollutions, and various temperatures (Wehr and Sheath, 2004).

Pinnularia is a diatom, more specifically a type of Bacillariophyta that exists in freshwa-

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ter. This predominantly fresh-water diatom is usually found in lagoons and damp soil. It can also be found in springs, estuaries (sea tidal inlets), sediments and oceans. Members of this genus are most commonly found at depth 40 cm in aquatic ecosystems, at 5 °C. Like most diatoms, *Pinnularia* can reproduce by simple cell division.

At present, there are 1385 species among the species, 747 have been identified as accepted ones taxonomically based on the recorded literature under the species name (Guiry and Guiry, 2020). The typus generis *Pinnularia* Ehrenberg 1843 nom. Cons is *Bacillaria viridis* Nitzsch 1817 (Typ. cons.); *Pinnularia viridis* (Nitzsch) Ehrenberg 1843. The genus has three isopolar axes with Navicoloid valves however, genus do not have septa and intercalary bands. The size of the most species varies from very small to large. Two structures in the diatom body make it stabilize; one the costae system and another may be a double wall system which it has united by transapical costae. There is a closed alveolus on the costae system in which broad openings have connected them to the interior valve. There is some row of small areolae on each alveolus in the quincunx pattern (Krammer, 2000). Generally, they have a groove and tongue raphe (Krammer, 1992a), however, they have changed in some large species which has made the outer fissure as the irregular course (complex raphe).

Taleghan River or Taleghan Rud starts in the Kahar and Kandovan mountains located in the western part of the Alborz slope. Further, the Taleghan Dam is located 120 km northwest of Alborz province. Taleghan River is one of the longest rivers in the center of Iran with 180 km length; that form ShahRud River after joining to Alamut River. Its height varies from 1080 to 4260 m, with an average annual rainfall of 558 mm and a temperature range of -5.2°C -20.7°C. There are 72 permanent and 25 seasonal rivers in Taleghan area. Its water is used for drinking by Karaj and Tehran residents; it also provides agriculture water for Qazvin plain.

This study aimed to investigate the *Pinnularia* genus of Taleghan River and Dam, also, evaluated the effect of physicochemical elements in distribution of *Pinnularia* species. This study was conducted to evaluate the relationship between *P. lundii* var. *linearis* and *P. brebissonii* in relation to physicochemical changes in water.

Materials and Methods

Study location and Sampling method

Taleghan River is one of the main tributaries of SefidRud, the second-longest river in Iran SefidRud river length is 670 km approximately. It originates from the Alborz Mountains in the northwest and flows into the Caspian Sea. It also is one of the main headers of SefidRud.

Epipelic samples were collected from eight stations from November 2017 to October 2018 in three replicates, along the Taleghan River and Dam (Fig. 1 and Table1).

To collect epipelic samples, a plastic syringe was inserted into the substrate and 160 ml to 170 ml of epipelic samples were collected from each station in three replicates and transferred to a plastic bottle and labeled. A total of 288

sediment samples were collected monthly. The collected samples were fixed with 4% formaldehyde and transferred to the laboratory of Islamic Azad University (Iran). A suspension of 10 ml of each sample was prepared. Samples were treated with 25 to 30 ml of hydrogen peroxide (H_2O_2) (1.5 h at 100 °C) and then 10 ml hydrochloric acid (HCl) (2 h at 120 °C) to remove organic matters (Van der Werff, 1995; Battarbee, 1986). A single drop of ammonium chloride (NH_4Cl, 10%) was added for every 10 ml of diluted diatom suspension (Mcbride, 1988). Coverslips were prepared with diatom suspension and installed on slides in Naphrax and prepared according to protocols Karthik et al. (2010).

To provide Light Microscopy images, slides were investigated using an Olympus microscope (Model CX31) under 100x oil immersion objective with differential interference contrast (DIC). The images were captured with Canon camera (Model EOS 5D Mark III). To identify diatoms species, used the Algaebase (Guiry and Guiry, 2020), and Diatoms of Europe (Krammer, 2000), Fig. 45: 1-17, and pl. 75, Figs 1-3, 5-6.

Physiochemical parameters

The water samples were collected from sampling sites per month and were analyzed for physicochemical parameters at Mabna and Alborz Environmental laboratory (Karaj, Iran). The electrical conductivity (EC), temperature (T°C), oxidation-reduction potential (ORP), and dissolved oxygen (DO) were measured in situ, and other parameters included SiO_2, salinity, chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), turbidity (nephelometric turbidity unit (NTU), PO_4^{2-}, NO_3^{2-}, Fe^{2+} and Mg^{2+} were examined according to American

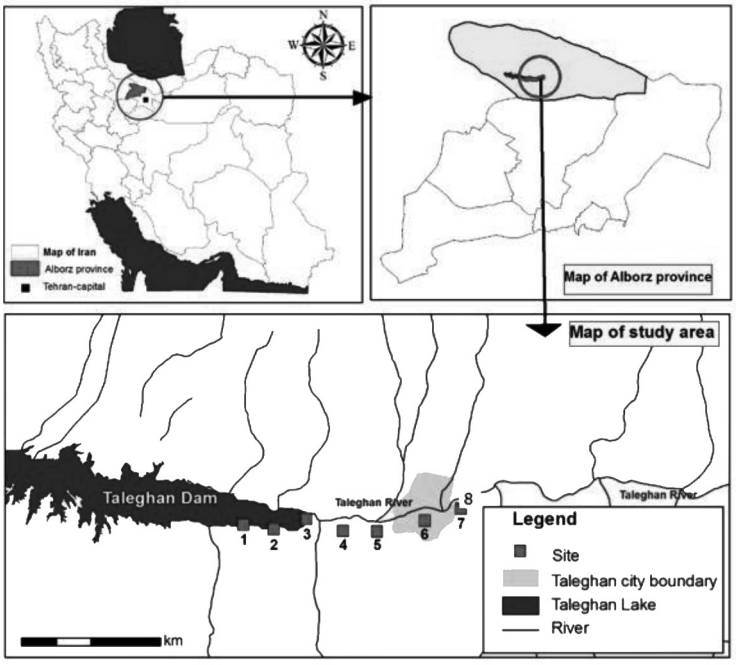

Fig. 1. Map of Iran and the sampling location.

Table 1. Sampling sites along the Taleghan River.

Station	Elevation (m)	Location Site	Latitude and Longitude
1	1788	Near the downstream of Zidasht town	36°10.1′N, 50°42.65′ E
2	1791	Near the Zidasht town	36°10.9′N, 50°42.8′ E
3	1794	-	36°10.1′N, 50°8 E
4	1795	Near the Zidasht town	36°10′N, 50° 44.5′ E
5	1797	Near the Glienake Bridge,	36°10′N, 50°44′ E
6	1817	Near Taleghan city	36°10.2′N, 50°46′ E
7*	1832	Near the Jazzan Bridge	36°10.3′N, 50°46.6′ E
8*	1832	Near the Jazzan Bridge	36°10.3′N, 50°46.6′ E

* There is 3 meters distance between station 7 and 8 however, the physicochemical parameters are different which make them as separate habitats.

public health association (APHA) (Clesceri et al., 1990). The pH, DO, ORP, and T were measured using the Hach HQ30 analyzer; Turbidity was measured by Wagtech turbidity detector; nitrate and phosphate parameters were measured using the HachDr 2800 spectrophotometer.

Cell counting and statistical analysis

Slides were investigated along random transects to estimate the percent abundance of species based on 300 valves for each site. Each month, three permanent slides were prepared collected from each station. To estimate the abundance of *Pinnularia*, at least, 300 valves for each station were counted. Also, *Pinnularia* abundance was estimated following Delgado et al. (2013), (F) frequent (1.5–5%), (R) rare (<1.5%) and (A) abundant (>5%) were observed. The mean and standard deviation of physicochemical parameters, cell abundance of *Pinnularia* species for each station, and mounts were analyzed. Correlation coefficient and explanatory variables were also carried on data. Pearson's correlation coefficient between *P. lundii* var. *linearis* and *P. brebissonii* was calculated.

Different parameters have been used to investigate their relation with *P. lundii* var. *Linearis*, however, dissolved oxygen has the highest correlation. A general linear model (GLM) was used to examine a relationship between the abundance percentage of *P. lundii* var. *linearis* (the dependent variable) and dissolved oxygen concentration (the independent variable). The data of three-time intervals were used, November-January, February-April and August-October. All calculations were performed using the stats package of R 3.5.1.

Results

The morphological results

The valve of *Pinnularia* species is asymmetric and includes central pores, terminal fissure, and raphe fissure. In the most spe-

cies, the proximal raphe or the central pore are bent to the reverse side of the terminal fissure. Two species of *Pinnularia* have been identified in this study including *P. lundii* var. *linearis* and *P. brebissoni*. *P. lundii* var. *linearis* is a new record for diatom flora of Iran. It has valve length 35-38 µm, valve breadth 8-9 µm, and non-striae central valves. The density of striae is 11–13 in 10 µm, apices are head-shaped, proximal raphe endings are spherical, and terminal fissures are semi sickle. *P. brebissonii* (Kütz.) Krammer has the following specifications: striae density is 9-11 in 10 µm, parallel in small forms in the middle in average forms, radiate weakly but convergent at the ends with fusiform valves; 42 µm length, 9 µm breadth, broadly apiculate apices; rhombic fascia, spherical and deflected proximal raphe endings. The morphological characteristics of our findings have been compared with two similar species, *P. subanglica* and *P. biceps* (Fig. 2a-h).

Physicochemical data

Pinnularia lundii var. *linearis* in our samples, it occurred in rather be found in habitats with moderate to high electricity (300-1100 µs.cm), alkaline pH (8.2 - 8.86), temperature (-4.3 - 20.7°C), TDS (221 -364 $mg.l^{-1}$), ORP (-80- to -102), COD (<3 mg.l-

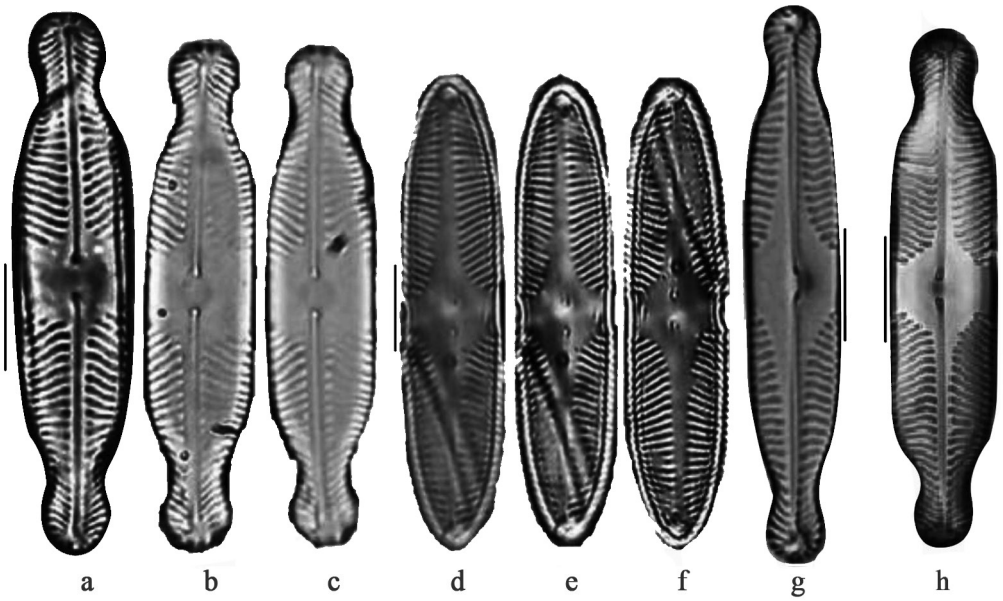

Fig. 2. a-h. a-c: *P. lundii* var. *linearis* is a new record for Iran; valve length is 35-38 µm, valve breadth 8-9 µm, non-deflected proximal raphe endings, the central area is hexagonal and apices are capitated; d- f: *P. brebissoni.* Images are taken by Olympus optical microscope with a magnification of 100 (10µm scale bar); g: *P. subanglica* (Souffreau et al., 2011, p. 2, Fig.1, h) and h: *P. biceps* (Kihra et al., 2015, Fig. 185) are the most similar species to *P. lundii* var. *linearis*. They have deflected proximal raphe ending, unlike *P. lundii* var. *linearis.*

^{1}), PO_4^{2-} (0.1 - 4.5 mg.l^{-1}), NO_3^{2-} (1 - 2.2 mg.l^{-1}), Si (2.9 - 8.57 mg.l^{-1}), Mg^{2+} (12 - 22.4 mg.l^{-1}) and high oxygen levels (up to 9 mg.l^{-1}).

P. brebissonii found in stations 2, 3, 4, 5, 7, and 8 during September to December and January to February. This species was found living in similar condition as *P. lundii* var. *linearis* Krammer, but its live is in deep water, lower flow rate and EC up to 500 µs.cm^{-1}.

Cells abundancy

The maximum cell number of *Pinnularia lundii* var. *linearis* and *P. brebissonii* was observed in December and November, respectively. Tables 2 and 3 indicate that the highest number of cells belongs to *P. lundii* var. *linearis*, with an average of 6.11/cells cm^2 in December, among eight stations. Moreover, in *P. brebissonii* the average number of cells in station 8 is the highest

Table 2. *Pinnularia* cells abundance in eight sampling stations (Mean ± Standard Deviation).

Station	*P. brebissonii*	*P. lundii* var. *linearis*
1	.00±000	5.50.097
2	2.55±4.588	.00±000
3	2.41±5.352	3.41±6.132
4	2.38±4.512	3.41±6.138
5	2.08±3.297	4.36±5.555
6	.00±000	2.97±5.526
7	3.47±5.500	5.69±7.641
8	4.11±5.147	6.11±6.332

Table 3. *Pinnularia* cells abundance based on sampling months (Mean ± Standard Deviation).

Month	*P. brebissonii*	*P. lundii* var. *linearis*
January	3.45±2.653	6.87±4.972
February	1.08±1.558	1.58±2.448
Mar	.04±.204	.08±.282
April	00±.000	00±.000
May	.04±.204	.08±.282
Jun	.04±.204	.08±.282
July	04±204	.08±.282
August	.33±.761	.75±1.390
September	2.45±4.568	7.16±7.590
October	2.91±5.348	8.62±5.491
November	7.79±6.372	10.50±6.909
December	7.33±6.417	11.37±8.606

and 7.79/cm^2 in November.

Statistical analysis

Based on Pearson's correlation there was a significant positive correlation between *P. brebissonii* and *P. lundii* var. *linearis* (Table 4).

The GLM indicated that there was a significant positive relationship between dissolved oxygen and *P. lundii* var. *linearis* abundance. Also, the intercept of the model was statistically significant (Table 5). The data and the predicted values of the model are presented in Figure 3.

Discussion

There are over 1500 species of *Pinnularia*

Table 4. There was a significant positive correlation between *P. brebissonii* and *P. lundii* var. *linearis*.

r	t	P-value	df	Sig.
0.3392	3.4586	0.0008243	92	**

Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Table 5. The results of the GLM applied to examine a relationship between dissolved oxygen and *P. lundii* var. *linearis* abundancy.

	Estimate	Std. Error	t value	Pr(>|t|)	Sig.
(Intercept)	-5.2540	2.5654	-2.048	0.04552	*
DO	0.7797	0.2852	2.734	0.00849	**

Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

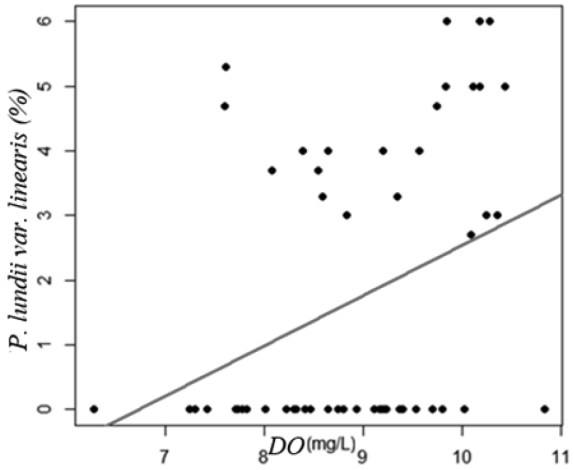

Fig. 3. Scatter plot of *P. lundii* var. *linearis* abundancy and dissolved oxygen concentration. The line indicated the predicted values of the GLM fitted on the data.

in the world, most of which have solitary life, except a few forming chains and most of them live in freshwater. *Pinnullaria* is a specified type belongs to Pinnulariaceae (Naviculales, Bacillariophyta). The Bacillariales or pennales live in freshwater or marine environments.

According to the research findings of algal percentage in Iranian aquatic ecosystems, diatoms account for 45% of the total number of species found in lakes (Zarei-Darki, 2009), and the Bacillariophyta account for 95% of the species diversity in Iran's algal flora. According to the results obtained 41 species of *Pinnularia* have been reported from two Karaj (Kheiri et al., 2018), and Balikhalai Rivers (Mirzahasanlou et al., 2018), Some of which are *P. acrosphaeria* Smith, *P. appendiculata* (C. Agardh) Schaarschmidt, *P. bogotensis* (Grunow) Cleve. *P. borealies* Ehr, *P. braunii* Cleve, *P. gibba* Eher, *P. divergens* Smith, P. globiceps Gregory, *P. intermedia* (Lagerstedt) Cleve, *P. major* (Kütz.) Rabenh, *P. microstauron* (Ehrenberg) Cleve and *P. brebissonii*. The species of *P. gibba* Ehrenberg and *P. viridis* (Nitzsch) Ehrenberg have always been reported in Iran's dams. *P. lundii* Krammer and its varieties are not among the species of diatoms reported in Iran. The morphological features of *P. lundii* var. *linearis* and *P. brebissonii* were measured every month. The morphological characteristics of our findings have been compared with two similar species, *P. subanglica* Petersen and *P. biceps* (Souffreau et al., 2011; Kihra et al., 2015). They have deflected proximal raphe ending, unlike *P. lundii* var. *linearis*. *P. subanglica* has linear valves, straight sides to very weak convex, capitateends, small central pores, rhombic central area, indistinct-shaped-terminal fissures, and narrow axial area. The length is 35-53 µm, breadth 7-8 µm, and striae 10.5-12 in 10 µm. Outline, size and area distinguish the taxon. Further, it prefers a very low electrolyte content waters with higher oxygen content (Krammer, 2000). Our results (about the new record) are according to Krammer's report of *P. subanglica*, both preferring water with higher oxygen content (Krammer, 2000). *P. biceps* has straight sides, or very slightly triundulate, narrower central inflation, capitates ends (clearly differentiated from the valve body by broad shoulders), slightly lateral raphe filiform, large central pores, and ?-shaped terminal fissures. The length is 48-85 µm, breadth 11-13 µm and striae 9-13/1 in 10 µm (Krammer, 2000). *P. lundii* var. *linearis* is distinguished from the other *Pinnularia* species by regular shape, hexagonal of the central area, non-deflected proximal raphe endings, and capitated apices (Fig. 2a-c). *P. lundii* var. *linearis* is mentioned in 98, 222, pl. 72: Fig. 1; pl. 75: Figs 1-6 by Krammer, 2000. In the study of diatom diversity of Karaj dam and river, *Pinnularia* had almost fewer species than other genera. On the other hand, *P. lundii* var. *linearis* was not reported in other parts of Iran. *P. lundii* var. *linearis* had a wider ecological range than *P. brebissonii*, which were presented in all stations.

Taleghan River and Dam have up to ten mines, activities of which impact on the physico-chemical parameters, subsequently increasing or decreasing diatom biodiversity.

P. lundii var. *linearis* found in EC 300-1100 µs.cm, based on BOD <2, O_2 deficit <15%,

it is an oligosaprobic species (Taylor et al., 2007). *P. brebissonii* found in stations 2, 3, 4, 5, 7, and 8 during September to December and January to February. The low temperature in February (4-9 °C) in 1 and 6 stations can be the reason for the absence of *P. brebissonii*. *P. brebissonii* was absent in the subsequent mounts until appropriate conditions were provided. It was observed in September which the temperature reached to 17.5 °C and also other necessary factors such as EC, SiO_2, NO_3^{2-} and dissolved oxygen were provided. This species was found living in similar condition as *P. lundii* var. *linearis*, but *P. brebissonii* live in deep water, lower flow rate and EC up to 500 µs.cm. *P. brebissonii* is found in EC up to 500 µs.cm and it can be considered as Eurtherm's species. The temperature under 6 °C has a decisive role in forming resting spores. It tolerates the temperature from 3 to 17.5 °C. Based on temperature, it is considered as Stenotherm (Husted, 1956). Some researchers believe that the significance of temperature in the community structure of plankton is not as important as other factors; however, it has been influential in our results (El-Karim, 2014). *P. brebissonii* prefers high phosphate level. Some periphytic species interested in phosphate or nitrate and may have influenced by phosphate more than other parameters (Mccormick et al., 2001; Noe et al., 2002). In this study, samples influenced by phosphate too. In the limnology of lakes, dissolved oxygen is the second important factor (Hallock and Hallock, 1993).

Dissolved oxygen is a vital distributing parameter affecting *Pinnularia* genus in the Taleghan River, and it is in line with the study of diatoms of Helle River in Bushehr province and Shahrood River in Semnan province, Iran (Farhadian et al., 2015; Sharifinia et al., 2016).

P. lundii var. *linearis* is a new record for diatom flora of Iran. Therefore, the necessity of identifying its distributing factors is considered. Our results show that *P. lundii* var. *linearis* is not a cosmopolitan species; however, it can be found in moderate to high electrical conductivity, alkaline and high oxygen content habitats. O_2 deficit <15%, *P. lundii* var. *linearis*, can be considered as oligosaprobic species based on BOD <2. This ecological assessment is performed without other organisms and it seems the impact of other organisms will be considered.

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