# Epilithic Diatom Diversity in Golestan Waterfall

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## Abstract

Waterfalls are considered as aquatic ecosystems, of which limited study was conducted on their biodiversity. Golestan waterfall is one of the outstanding waterfalls in eastern edge of Golestan National Park. Since no research was performed on algal flora in the area, the present study aimed to identify the epilithic diatoms of the waterfall, as a component of this ecosystem biodiversity. The samples were collected from stony substrates at each season. The results represented 24 genera including 47 species, which all taxa belonged to Bacillariophyceae. In addition, Cymbopleura with five species and *Gomphonema*, and *Navicula* with four species had more species, respectively. Further, *Achnanthidium minutissimum*, *Amphipleura pellucida*, *Cymbella affinis*, *Cymbopleura kuelbsii*, and *Gomphonema pumilum* were determined as the most abundant taxa. Furthermore, *Delicatophycus verenae*, *Stauroneis separanda* and *Tryblionella brunoi* were found in the diatom flora of Iran for the first time. Due to the few diatom studies in Iran, conducting detailed and local studies can improve data on diatom flora of Iran.

**Keywords**: Bacillariophyceae, *Cymbopleura*, Diatom Flora, Golestan Province, Iran

## Introduction

Biodiversity plays a major role in ecosystem function and stability and is the life support system (Rawat and Agarwal, 2015). Diatoms are considered as an extremly diverse group of algae occurring in almost all aquatic systems. Additionally, they are a systematic group characterized by their siliceous wall. Taxonomic diversity in diatoms is important, which reflects the biodiversity and stability of an aquatic ecosystem (Andrejic et al., 2012). Further, their rapid response to environmental changes can represent the ecological conditions of the living environment, which makes them useful in water quality assessments (Delgado et al., 2012; Kelly et al., 2007; Martin et al., 2010; Noga et al., 2013).

Since 2016, some studies focused in Golestan procince regarding diatoms in Zarringol

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River (Dadgar, 2016), Chehal Chay River in Minudasht (Lakzaie et al., 2018), Gharah Chay River in Ramian (Bayani, 2019), three springs in Ramian (Ahmadi et al., 2019), and Khorrmarud River (Aghatabay et al., 2018). However, few studies have conducted on algal flora and diatom so far. Thus, the Further studies can enhance the knowledge on diatom flora in local and country scales. Waterfalls are interesting ecosystems, which are considered as vertical wetlands, and kept cool in summer and mild in winter. Many outstanding waterfalls exist in Golestan province, the algae of which have not been assessed floristically so far.

## Material and methods

Golestan province with the area of 22033 km$^2$ is located in northeast Iran, southeast Caspian Sea, which has diverse climate and aquatic ecosystems due to its geographical position. Golestan waterfall is placed 47 km away from Galikesh city at the eastern edge of Golestan National Park (Fig. 1). The present study sought to recognize the diatoms of the waterfall as a basic study. The diatoms were sampled from stony substrates by using toothbrush seasonally in three different sites and transferred to laboratory after fixation with 4% formalin (Bellinger and Sigee, 2010). In addition, the samples were cleaned through acid digestion method (Taylor et al., 2007). Further, permanent slides were prepared using Canada Balsam and diatoms were identified by using a light microscope and considering available flora (Bahls, 2006, 2012, 2013; Krammer and Lange-Bertalot, 1986, 1988, 1991a, b; Krammer, 2002, 2003; Lange-Bertalot, 2001). Taxa names were checked in www.algaebase.org. In each slide, 300-400 valves were counted and the relative abundance of each taxon was calculated.

Along with algal samples, water ones were added into 1L containers and transferred to laboratory in order to analyze nitrate, am-

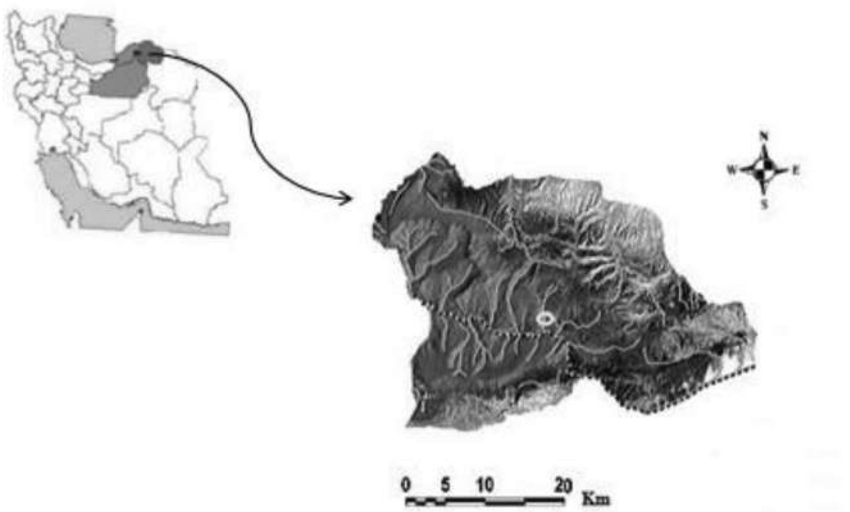



**Fig. 1.** Location of Golestan waterfall

monium, silicate, and phosphate (Clesceri et al., 1999.( Furthermore, dissolved oxygen (DO), temperature, EC, salinity, pH, and TDS were measured in situ by using a HQ40d portable device.

**Results**

Table 1 summarizes the physicochemical results related to water in different seasons. A total of 47 taxa belonging to 24 genera were identified in Golestan waterfall (Table 2, Plates I and II), all of which were related to the class Bacillariophyceae. Based on the results, the most species-rich genus included *Cymbopleura* with five species and *Gomphonema* and *Navicula* each with four species, followed by *Diploneis*, *Surirella*, and *Tryblionella* with three species (Fig. 2). Additionally, *A. minutissimum*, *Amphipleura pellucida, Cymbopleura kuelbsii, Gomphonema pumilum,* and *Cymbella affinis* were charecterized as the most abundant taxa.

Based on the morphological groups, the identified taxa were classified into six groups, the highest number of which belonged to asymmetrical biraphid with 18 species and 8 genera, symmetrical biraphid with 13 species and 7 genera. Nitzschioid with 7 species and 3 genera, and Monoraphid with 3 species and 2 genera, respectively. Further, the least number was observed in Surirelloid with 3 species and 1 genera, and Araphids with 3 species in 3 genera, respectively.

In the present study, three species were determined as new for the diatom flora of Iran, which are provided as follows.

**Table 1.** Results related to water physicochemical parameters in Golestan waterfall during different seasons

| Parameteres | Spring | Summer | Fall | Winter |
|---|---|---|---|---|
| $PO_4$ (mg.l$^{-1}$) | 0.02 | 0.016 | 0.015 | 0.02 |
| $SiO_2$ (mg.l$^{-1}$) | 0.57 | 0.74 | 0.95 | 0.39 |
| $NO_3$ (mg.l$^{-1}$) | 0.61 | 2.33 | 1.42 | 0.55 |
| $NH_3$ (mg.l$^{-1}$) | 0.011 | 0.015 | 0.011 | 0.016 |
| T (ºC) | 21.3 | 14.8 | 6.8 | 7 |
| DO (mg.l$^{-1}$) | 7.97 | 8.89 | 11.53 | 11.01 |
| EC (µs.cm$^{-2}$) | 444 | 476 | 497 | 447 |
| TDS (mg.l$^{-1}$) | 216 | 230 | 239 | 216 |
| Air T (ºC) | 31.4 | 23.8 | 11.4 | 9.7 |
| pH | 7.75 | 7.67 | 7.81 | 7.75 |
| Salinity | 0.21 | 0.23 | 0.24 | 0.22 |

**Table 2.** Taxa identified in Golestan waterfall during seasons of 2019 (L: Length, W: Weight, S: Striae, C: Costae, F: Fibula)

| Scientific name | Dimensions |
|---|---|
| **Bacillariophyceae** | |
| **Achnanthidaceae** | |
| *Achnanthidium grasillimum* (F.Meister) Lange-Bertalot | L: 17.5-21 µm W:3-4 µm S:14-16 |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | L:7.5-13 µm W:2.5- 3 µm S:27-30 |
| **Amphipleuraceae** | |
| *Amphipleura pellucida* (Kützing) Kützing | L:60-93 µm W:9-11 µm |
| *Frustulia vulgaris* (Thwaites) De Toni | L:48-49 µm W: 9-10 µm |
| **Basillariaceae** | |
| *Hantzschia amphioxys* (Ehrenberg) Grunow | L: 35 µm W:6 µm F:6 |
| *Nitzschia commutatoides* Lange-Bertalot | L: 112 µm W: 15 µm F:9-11 |
| *Nitzschia dissipata* (Kützing) Rabenhorst | L:20-42 µm W:3-4 µm F:7-11 |
| *Nitzschia* sp. | L: 58-120 µmW:3-6 µm F:10-11 |
| *Tryblionella angustata* W.Smith | L: 44-52 µm W: 6-7µm S:17-18 |
| *Tryblionella apiculate* W.Gregory | L:44-47.5 µm W:5 µm S:16-17 |
| *Tryblionella brunoi* (Lange-Bertalot) Cantonati & Lange-Bertalot | L:79-83 µm W:10 µm S:13 |
| **Catenulaceae** | |
| *Amphora inariensis* Krammer | L:12-16 µm W:2.5-4 µm S:15-17 |
| *Amphora pediculus* (Kutzing) Grunow | L:10-12.5 µm W:2.5 µm S:18 |
| **Cocconeidaceae** | |
| *Cocconeis pediculus* Ehrenberg | L:27.5 µm W:19-21 µm S:16 |
| **Cymbellaceae** | |
| *Cymbella affinis* Kutzing | L:27-28.5 µmW:7.5-8 µm S:9-11 |
| *Cymbella compacta* Østrup | L: 44 µm W:14 µm S:11 |
| *Cymbopleura amphicephala* (Nageli) Krammer | L:30-32.5 µm W:10-11µm S:8-10 |
| *Cymbopleura citrus* (J.R.carter & Bailey-Watts) Krammer | L: 30 µm W:10 µm S: 10 |
| *Cymbopleura kuelbsii* Krammer | L:38-32 µm W:6-7.5 µm S:11-13 |
| *Cymbopleura* sp. | L:30-35 µm W:7-7.5 µm S:8-10 |
| *Cymbopleura* cf. *vrana* Krammer | L: 36 µm W:10.5 µm S: 9 |
| **Diploneidaceae** | |
| *Diploneis calcilacustris* Lange-Bertlot & A.Fuhramann | L:17-20 µm W:10-12 µm S:11-13 |
| *Diploneis krammeri* Lange-Beterlot & E.Reichardt | L: 45 µm W:17-17.5 µm S: 12 |
| *Diploneis separanda* Lange-Beterlot | L:25 µm W:12 µm S:12 |
| **Fragilariaceae** | |
| *Fragilaria recapitellata* Lange-Bertalot & Metzeltin | L:17-18 µm W:5 µm S:13-14 |
| **Gomphonemataceae** | |
| *Delicatophycus sinensis* M.J.Wynne | L:27.5 µm W:5 µm S:16 |
| *Delicatophycus verenae* M.J.Wynne | L:33-42 µm W:6-7.5 µm S:12-14 |
| *Encyonopsis minuta* Krammer & E. Reichardt | L:12.5 µm W:2.5-3 µm |
| *Gomphonema micropus* Kützing | L: 25 µm W: 7 µm S: 11 |
| *Gomphonema parvulum* (Kützing) Kützing | L:12-23 µm W:5-6 µm S:12-13 |
| *Gomphonema pumilim* (Grunow) E.Reichardt & Lange- | L:16-30 µm W:3-5 µm S:10-13 |

| | |
|---|---|
| Bertalot | |
| *Gomphonema_subclavatum_*(Grunow) Grunow | L:37-52 µm W:7-10 µm S:10-12 |
| **Naviculaceae** | |
| *Gyrosigma_*sp. | L:120 µm W:24 µm S:13 |
| *Navicula_capitatoradiata_*H.Germain ex Gasse | L: 37 W:7.5 S:15-17 |
| *Navicula_cryptotenella_*Lange-Bertalot | L:16-22 µm W:5-6 µm S:12-14 |
| *Navicula_rostellata_*Kützing | L: 38 µm W: 9 µm S:12 |
| *Navicula_tripunctata* (O.F.Müller) Bory | L:46-52 µm W:8-9 µm S:11-12 |
| **Rhoicospheniaceae** | |
| *Rhoicosphenai_abbreviata_*(C.Agardh) Lange-Bertalot | L:27.5-40 µm W:4-5 µm S:12- 14 |
| **Stauroneidaceae** | |
| *Craticula_buderi_*(Hustedt) Lange-Bertalot | L:20-26 µm W:5-6.5 µm S:18-19 |
| *Stauroneis_separanda_*Lange-Bertalot & Werum | L: 15-16 µm W: 3.5-4.5 µm |
| *Stauroneis_smithii_*Grunow | L:27 µm W:7.5 µm |
| **Surirellaceae** | |
| *Surirella_angusta_*Kützing | L:23 W:7.5 S:25 |
| *Surirella* sp. | L:36 W:24 F: 5-6 C:18 |
| *Surirella_librile* (Ehrenberg) Ehrenberg | L:60-88 µm W:20 µm |
| **Tabellariaceae** | |
| *Diatoma_moniliformis_*(Kützing) D.M.Williams | L:12.5-16 µm W:3.5-4 µm C:7 |
| *Halamphora veneta* | L:19-22 µm W:4-5 µm S:16-17 |
| **Ulnariaceae** | |
| *Ulnaria ulna* (Nitzsch) Compere | L:102-140 µm W: 6 µm S:10 |

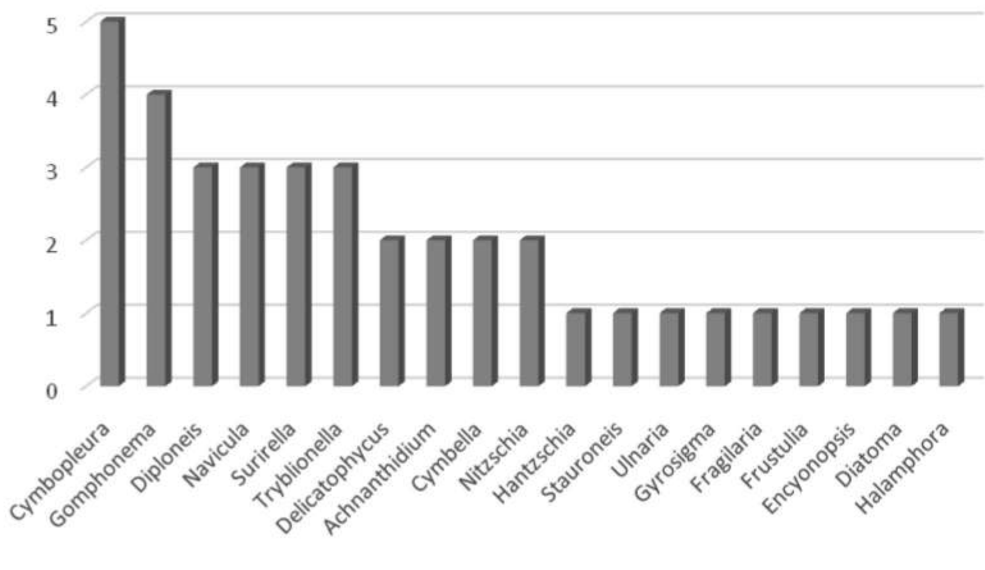



**Fig. 2.** Number of taxa in each of the genus recognized in the study

**Order:** Cymbellales

**Family:** Gomphonemataceae

**Genus:** *Delicatophycus*

*Delicatophycus verenae* M.J.Wynne

**Synonyms:** *Delicata verenae* Lange-Bertalot & Krammer

**Reference:** as *Delicata verenae* in Krammer, 2003, Plate 137, Figs. 1-15.

**Description:** Valves slightly dorsiventral, elliptic-lanceolate with round not protracted apices, with 33-42.5 µm length and 6-7.5 µm width; axial area widening towards the middle of valve; central area almost absent; raphe lateral, narrowing towards the distal ends; striae radiate, 12-14 /10 µm in middle of valve.

**Order: Bacillariales**

**Family:** Bacillariaceae

**Genus:** *Tryblionella* W. Smith

*Tryblionella brunoi* (Lange-Bertalot) Cantonati & Lange-Bertalot

**Synonyms:** *Nitzschia brunoi* Lange-Bertalot (Powers, 2018; as *Nitzschia brunoi* in Lange-Bertalot and Metzeltin, 1996, Plate 101, Figs. 11-15)

Description: Valves linear with wedge-shaped ends with 78-82.5 µm length and 10-11 µm width; striae are parallel distinctly punctate 13/10 µm.

**Order: Naviculales**

**Family:** Stauroneidaceae

**Genus:** *Stauroneis* Ehrenberg

*Stauroneis separanda* Lange-Bertalot & Werum

No Synonym

**Reference:** Bahls, 2012; Levkov et al., 2016, Figs. 48-63.

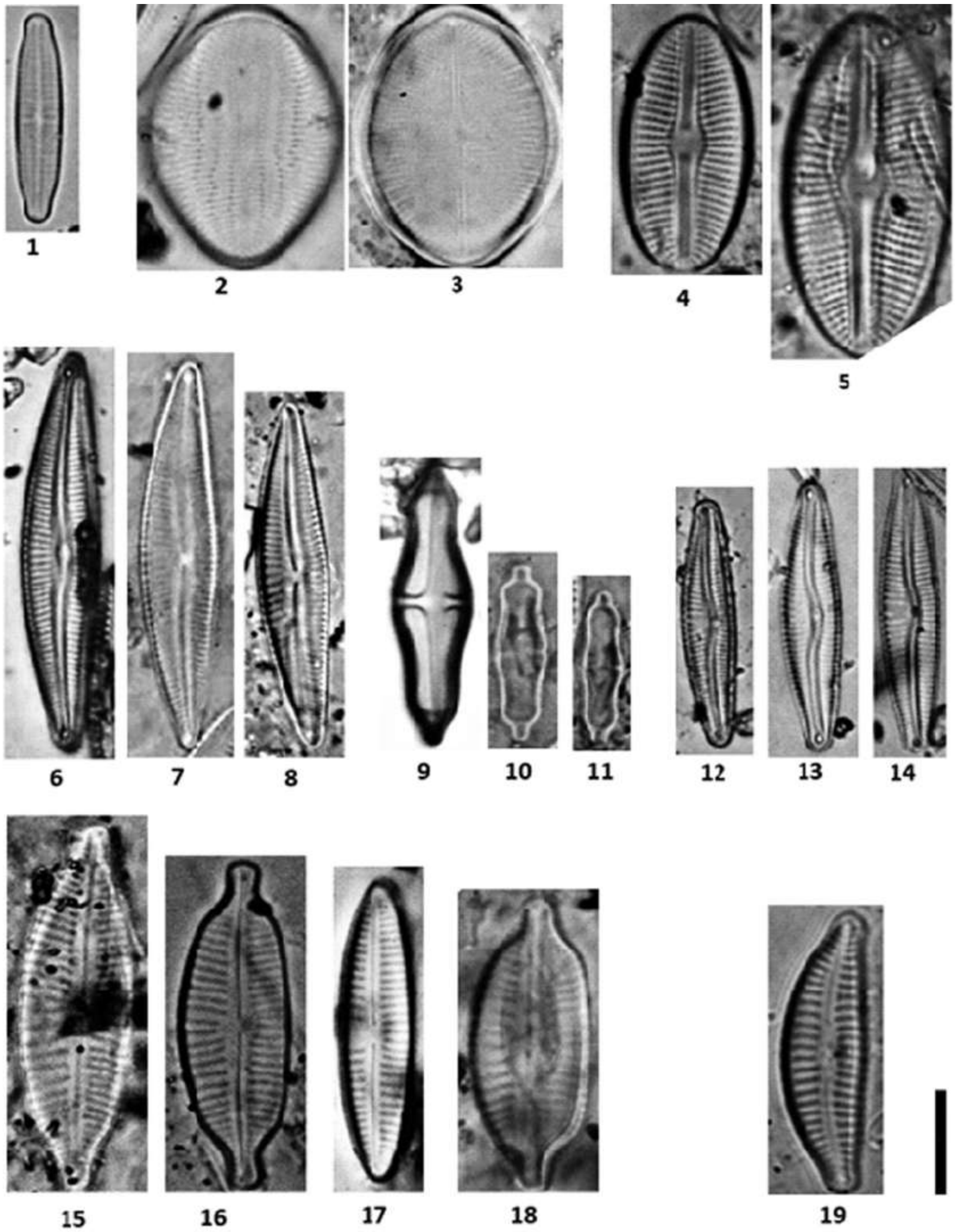
**Description:** Valves linear- lanceolate, wider at the center, with triundulate margins, apices rostrate, with the length of 15-16 µm and 3.5-4.5 µm width; central stauros is narrow and linear; pseudoseptum present at the apices; striae fine.

## Discussion

In general, most of the previous studies on waterfalls have primarily focused on tourism, geology, and hydrology, while limited ones have highlighted biodiversity in the ecosystem (Offem and Ikpi, 2012). Therefore, waterfalls are sometimes considered as lifeless zones (Chernicoff et al., 1997). The present study aimed to identify epilithic diatoms in Golestan waterfall.

Reviewing the studies conducted on diatom in Golestan province demonstrated 80% similarity between the results of the present study with those of Ahmadi et al. (2019) regarding the diatoms of three springs. In addition, the taxa of *Amphipleura pellucida*, *Diploneis calcilacustris*, *D. krammeri, D. separanda*, *Fragilaria recapitellata*, *Frustulia vulgaris*, *Gomphonema subclavatum, Stauroneis smithii*, and *Surirella angusta* were found only in these two studies. However, the others except for new records were reported in the previous research.

Among the most abundant taxa, *A. minutissimum*, and *C. affinis* were abundant in most ecosystems of the province (Aghatabay, 2018; Bayani, 2019; Dadgar, 2016; Lakzaie, 2016). Further, *A. minutissimum* is considered as one of the most frequently recorded taxa worldwide (Falasco et al., 2012, 2016; Kelly et al., 2007; Kheiri et al., 2019; Krammer and Lange-Bertalot, 1991b;) from oligo- to hypertrophic in alkaline to acidic waters (Potapova and Hamilton, 2004). Although Van Dam et al. (1994) classified *C. affinis* as eutrophic taxon, BCG classification indicated human disturbance (Davies and Jackson,




**'late I.** (1- 20). (1) *Achnanthidium gracillimum*, (2,3) *Cocconeis pediculus*, (4) *Diploneis eparanda*, (5) *Diploneis krammeri*, (6-8) *Delicatophycus verenae*, (9) *Stauroneis smithii*, 10, 11) *Stauroneis separanda*, (12-14) *Delicatophycus sinensis*, (15) *Cymbopleura* cf. *rana*, (16) *Cymbopleura amphicephala*, (17) *Cymbopleura kuelbsi*, (18) *Cymbopleura itrus*, (19) *Cymbella affinis*. Bar: 10 µm.

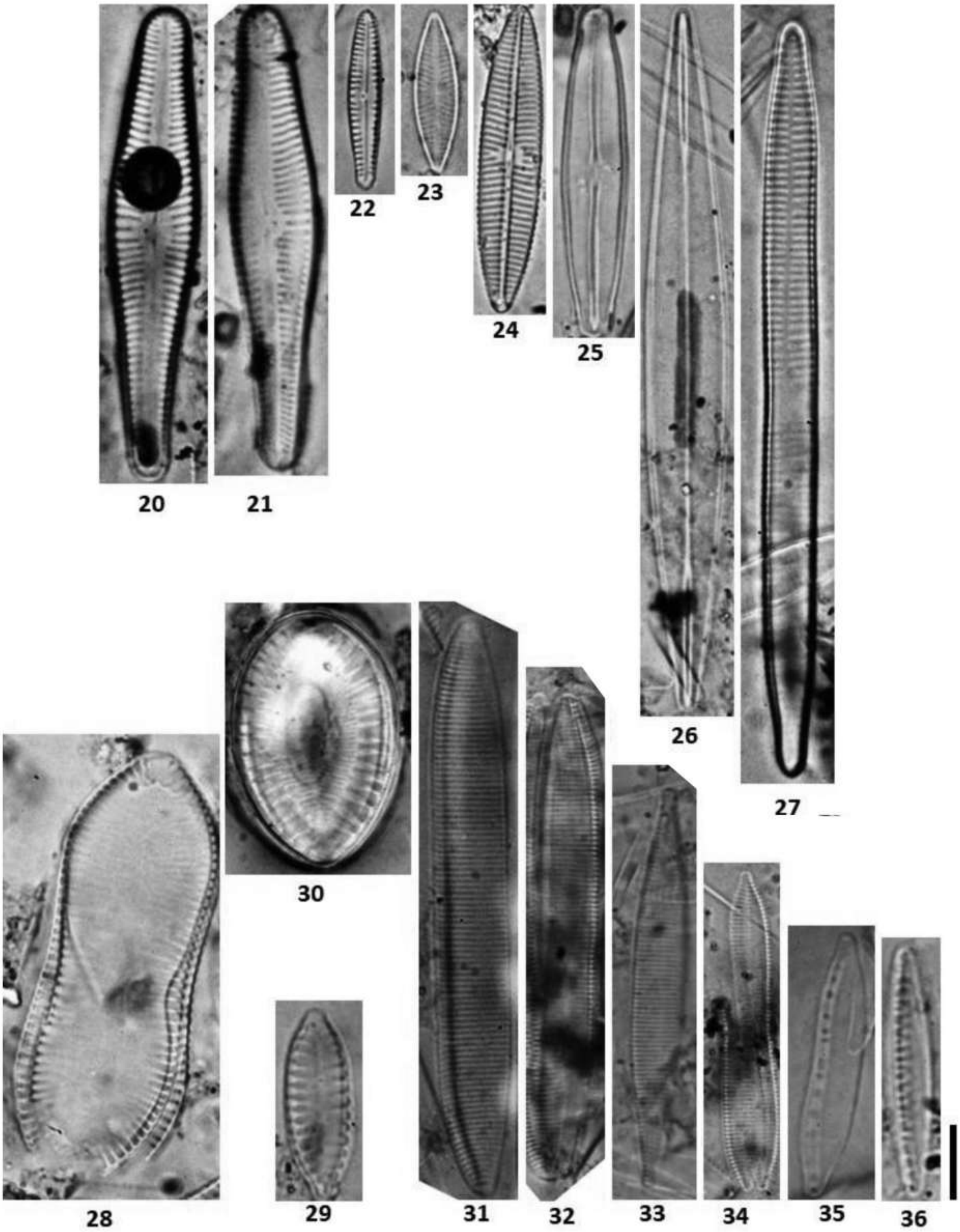


**Plate II.** (20-36). (20, 21) *Gomphonema subclavatum*, *(*22) *Gomphonema pumilum, (*23) *Navicula cryptotenella*, (24): *Navicula tripunctata,*(25) *Frustulia vulgaris*, (26). *Amphipleura pellucida* (27) *Ulnaria uln*, *(*28) *Surirella libril*, (29) *Surirella angusta*, (30) *Surirella* sp., (31,32) *Tryblionella brunoi* (33) *Tryblionella angustata*, (34) *Tryblionella apiculata*, (35) *Hantzschia amphyoxys* (36) *Nitzschia dissipata.* Bar: 10 µm.

2006) characterized *C. affinis* as highly sensitive species (Potapova, 2011) in California State. *C. affinis*, a common taxon both in rivers and lakes (Krammer and Lange-Bertalot, 1986; Patrick and Reimer, 1975), is abundant in oligotrophic and mineralized systems (Tornes, et al., 2007) and neighbor countries (Khazal et al., 2018). Further, *A. pellucida* is an oligo-mesotrophic species (Van Dam et al., 1994), as well as a cosmopolitan alkaliphilous taxon, which is found in pH 6.2-8 (Lowe, 1974). Additionally, the species can be observed as planktonic despite its existence as benthic taxon in most cases (Krizmanic et al. 2008). Although *A. pellucida* is a widely-distributed taxon, it was only reported from Kashkan River (Safiallah et al., 2020) and Gole-Ramian spring with low abundances in Iran (Ahmadi et al., 2019). Furthermore, *Cymbopleura kuelbsii* firstly recorded by Kheiri et al (2019), this taxon is newly initiated species (Krammer, 2003) and there is not more data on its ecology, however in our study *C. kuelbsii* was abundant only in winter. The water was well oxygenated with low nutrient and moderate conductivity.

Assymetric biraphid was determined as the most diverse group, which is in line with the results of the prevoius research in Golestan province (Aghatabay, 2018; Bayani, 2019; Dadgar, 2016; Lakzaie, 2016), while they are inconsistent with those related to Kashkan River in Zagros (Safiallah et al., 2020). In the present study, the genera of the group included *Amphora*, *Cymbella*, *Cymbopleura*, *Delicatophycus*, *Encyonopsis*, *Gomphonema*, *Rhoicosphenia* and *Halamphora*. Further, symmetrical biraphid (*Amphipleura*, *Craticula*, *Diploneis*, *Frustulia*, *Stauroneis*, and *Navicula*) Araphids (*Diatoma*, *Fragilaria*, and *Ulnaria*), Nitzschioid (*Hantzschia*, *Nitzschia*, and *Tryblionella*), monoraphids (*Achnanthidium* and *Cocconeis*), and Surirelloid (*Surirella*) were present in the waterfall. Unlike the previous studies, centric group had no representative in our study along with Epithemioid and Eunotioid groups.

Among the diatom genera, *Nitzschia*, *Navicula*, and *Gomphonema*, as large genera with many species, have been usually reported as the most species-rich genera in the studies conducted in Iran (Ahmadi et al., 2019; Mehrani Adl, 2020; Panahy et al., 2018) and the world (Jakivljevic et al., 2016; Noga et al., 2013; Solak, 2011). Furthermore, *Cymbopleura* was obtained as the most species-rich genus, which is not in line with the results of other studies in Golestan province, and above-mentioned ones. Among the species of this genus identified in our study *C. citrus* for the first time introduced by Aghataby (2018) and Ahmadi et al (2019) to diatom flora of Iran from Golestan Province. Other species of *Cymbopleura* including *C.* cf. *vrana* have not been reported from Golestan Province yet, even though were recorded from Karaj River by Kheiri et al (2019). *Gomphonema* and *Navicula* were among the major genera too, however unlike their studies, *Nitzschia* was less important in our work.

In this research, three taxa were recorded for the first time for diatom flora of Iran. *Delicatophycus* established as a new genus, for substituting genus *Delicata* which was declared invalid by Wynee (2019). Indeed, *Delicatophycus* sinensis that were recorded by Kheirei et al (2019) as *Delicata sinensis* from Karaj River as new record for Iran, and *Delicatophycus* in which there is no record in diatom flora of Iran. Additionally, *D. verenae* was reported as a new species only by Krammer (2003) and no data are available on the ecology of the species. The results of the present study demonstrated the above-mentioned species of the genus in alkaline water with low conductivity, and nutrients, both of which were abundant in samples, especially *D. Sinensis*. *D. verenae* was introduced for the first time.

Further, genus *Tryblionella* was determined as another new record, which was described by Smith (1853), and many of its species were derived from the large genus *Nitzschia* by Round et al. (1990). Regarding *Tryblionella*, raphe is eccentric, similar to that of *Nitzschia*, while *Tryblionella* has longitudinal undulations on valve face. Furthermore, *Tryblionella brunoi* (T. brunoi) was reported in meso-to oligotrophic waters, as well as eutrophic ones in a few cases (Powers, 2018).

*Stauroneis* is a genus from symmetrical biraphid group with naviculoid cells. Further, the main characteristics of the genus are presence of stauros in the valve center and punctate striae. Additionally, Most species of this genus are characteristic of oligotrophic waters in the tepmerate zone (Lange-Bertalot and Metzeltin, 1996). However *S. separanda* is distinguished by smaller valves and low undulation in outline, is similar to *S. smithii*. Furthermore, this taxon was recorded firstly by Werum and Lange-Bertalot (2004), as widely distributed in calcareous springs with high conductivity in Europe. While Levkov et al. (2016) recorded this species from mesotrophic calcareous waters from Macedonia. Moreover, this taxon was observed in co-occurrence with *S. smithii* that is in accordance with present study.

Given that Iran is considered as a vast country with diverse aquatic systems, such local and detailed studies can result in improving the knowledge on diatom flora in Iran. In fact, the present study is the first research on the diatom diversity of waterfall in Iran. In general, the diatom flora of Golestan Waterfall included some cosmopolitan taxa found in various habitats, along with taxa with narrow distribution. The presence of taxa with limited distribution worldwide indicated the importance of conserving waterfalls. Accordingly, further studies should be conducted in such ecosystems. Due to the tourism value of waterfalls, they are subjected to human impacts and environmental disturbances. Thus, the protection of waterfall habitats is important for conserving aquatic diversity.

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