New records for Freshwater Benthic Diatom Flora of Iran, from Damavand River basin (Central Alborz), Iran

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Abstract

Diatoms are a key group of organisms of the periphyton and have a significant contribution to primary production in rivers. Despite their importance, little information exists about the taxonomy and distribution of periphytic diatoms in Iran. Central Alborz is one of the hotspots known globally for its fauna and flora and the Damavand River is one of the permanent rivers flowing in the region. Epilithic diatom samples were taken along the Damavand River between the summer of 2017 and the spring of 2019 to determine the benthic diatom flora. As a result, five species within the genera *Gomphonema*, *Cymbella*, *Cymbopleura*, and *Sellaphora* were found as new records from the Central Alborz. These species include *Cymbella affinis* var. *angusta* (Krammer) W. Silva, *Cymbopleura similiformis* Krammer, *Gomphonema innocens* Reichardt, *Sellaphora capitata* D.G. Mann & S.M. McDonald and *Sellaphora lanceolata* D.G. Mann & S.J.M. Droop. The morphology of these taxa, comparisons to similar taxa, and geographic distributions are discussed. These results expand the knowledge of diatom diversity in the Central Alborz Region.

Keywords: Diatoms, New record, Hotspot, Taxonomy, Ecology

Introduction

The Central Alborz Region includes the high mountains of Alborz ranges in northern Iran, along the southern coast of the Caspian Sea. The region is a globally important biodiversity hotspot for its fauna and flora (Noroozi et al., 2018). The mountainous area is the source of many rivers and streams Yet, its diatom flora has been minimally surveyed (Jamaloo et al., 2006; Soltanpour-Gargari et al., 2011; Kheiri et al., 2018a; Kheiri 2019; Naseri et al., 2022). Among these studies, Kheiri et al. (2018a) presented a study on the diatom diversity of the Karaj River, one of the long rivers of the Central Alborz, reporting 128 species out of which 42 taxa were new records, with high-quality LM illustrations and description of diagnostic features that clarify the identity of uncertain species (characterized as “cf.”), to depict

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the novelty of the diatom flora in the region. Continuing the research on diatom diversity in the Central Alborz region, we studied the diatom flora of another permanent river, Damavand River, and its tributaries in the region and found many species to be the members of species groups that need further investigation. Of those groups, five species from four complex groups of *Cymbella affinis* Kützing, *Cymbopleura amphicephala* (Nägeli ex Kützing) Krammer, *Gomphonema parvulum* (Kützing) Kützing and *Sellaphora pupula* (Kützing) Mereschkovsky are identified and reported as new records in this study.

The species complex of *Cymbella affinis*/ *tumidula*/ *turgidula* was analyzed by Krammer (2002), Tuji (2007) and Da Silva et al. (2015). Based on the latest taxonomy, the species belonging to *Cymbella excisa* Kützing and the varieties described by Krammer were transferred to *C. affinis*. Furthermore, re-evaluation of type specimens of *C. affinis* Kützing sensu Krammer, and *C. affinis* var. *procera* Krammer showed that these species present the characteristics of *Cymbella tumidula*. Thus, they were renamed as *C. tumidula* and *C. tumidula* var. *procera* (Krammer) W.Silva (Da Silva et al. 2015).

The *Cymbopleura amphicephala* group was defined by fifteen species that have small valves and rostrate to capitate ends with variable outlines. The species possess such a small central area that the central area can be considered lacking. Within the group, the most similar species to *C. amphicephala* (Nägeli ex Kützing) Krammer are *C. similis* (Krasske) Krammer, *C. similiformis* Krammer and *C. monticola* (Hustedt) Krammer (Krammer 2003).

Gomphonema parvulum is a species complex presenting considerable morphological variations and its taxonomy has been the subject of study and interest (Rose 2008, Kermarrec et al. 2013, Abarca et al. 2014). The long-term morphometric and culture studies of this species complex by Rose (2008) suggested that some varieties of *G. parvulum* may actually belong with other *Gomphonema* species. Kermarrec et al. (2013) investigated the diatom diversity of *Gomphonema parvulum* in a tropical Island (Mayotte) and mainland Europe using a combination of molecular and morphological data and found that this species complex contains some semi-cryptic species that are difficult to distinguish based on morphology alone.

They proposed two morphological characters including the shape of the central area and the distance between the central raphe ending and the stigma position, which should be included in identification keys to delimit species in this group.

Abarca et al. (2014) used molecular techniques to analyze the genetic diversity of different populations of *G. parvulum* sensu lato from different regions including Mexico, Europe and Korea and found that species from this complex group including *G. parvulum* f. *saprophilum* Lange-Bertalot & Reichardt, *G. parvulum* var. *lagenula* (Kützing) Frenguelli

and *G. parvulum* var. *exilissimum* Grunow were different from *G. parvulum* var. *parvulum* and were elevated to species rank. The seminal work of Mann et al. (2004) showed that the *Sellaphora* species complex represented true biological species. He investigated specimens of *Sellaphora pupula* (Kützing) Mereschkovsky from Edinburgh, UK, and described five new species using a combination of morphometric analysis, ultrastructure, and mating experiments. These species were named *S. auldreekie* D.G. Mann & S.M. McDonald, *S. blackfordensis* D.M. Mann & S. Droop, *S. capitata* D.G. Mann & S.M. McDonald, *S. lanceolata* D.G.Mann & S.M. McDonald and *S. obesa* D.G. Mann & M.M. Bayer. They were distinguished from each other based on morphology, but most importantly, by mating type which demonstrated true biological species.

In this paper, we report the species *Cymbella affinis* var. *angusta* (Krammer) W.Silva, *Cymbopleura similiformis* Krammer, *Gomphonema* innocens Reichardt, *Sellaphora capitata* D.G. Mann & S.M. McDonald and *Sellaphora lanceolata* D.G. Mann & S.J.M. Droop from these species complexes for the first time from the Central Alborz region. The morphological and distributional data for the taxa are also discussed.

Material and methods

The Damavand River basin with a catchment area of 778 km^2 includes the Damavand River as its principal river flowing in the direction of north-east to south-west with a length of approximately 30 km. Its main tributary is the Tar River which originates from the Tar Lake from an elevation of 3000 km and on its way toward Damavand County, northeast of Tehran Province, joins the Bar River to form the Damavand River (at the elevation of 1890 m). This River passes different villages in its path and finally merges with the Jajrud River in the village of Mamloo southeast of Tehran. The river is the source of irrigation for agricultural lands in the basin (Alimohammadi, 2009) (Figure 1).

Epilithic samples were collected by brushing the surfaces of rocks in six stations of the Damavand River basin in 2017-2019. Samples were prepared by boiling in 30% hydrogen peroxide (H_2O_2) (4-8 h at 100 °C) for the removal of organic matter. A few drops of hydrochloric acid (HCl) were added to the suspension to dissolve carbonates. The samples were repeatedly washed with distilled water to eliminate the byproducts and diatom cells were mounted with Naphrax (Renberg, 1999). Microscopic slides were examined using an Olympus BX53 microscope equipped with an Olympus DP72 camera. Diatoms were identified according to Mann et al. (2004), Krammer (2002, 2003), Lange-Bertalot et al. (2017), Da Silva et al. (2015), and Reichardt (1999). Physicochemical parameters were measured in the laboratory and results are summarized in Table 1.

Results

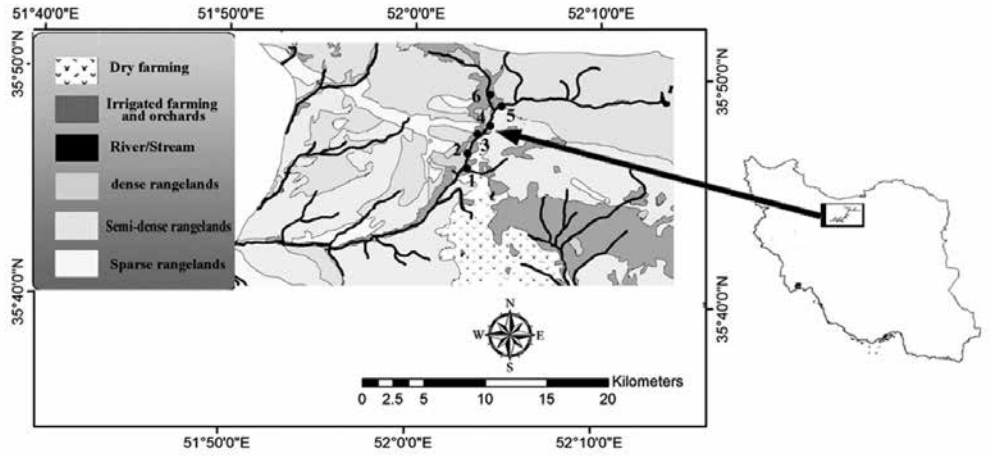


Fig. 1. Map of land use in the Damavand Region showing sampling sites (1-6) along the Damavand River basin

Table 1. Geographical distribution of the Damavand River basin and physicochemical ranges of parameters (elevation, specific conductance (EC), and pH) at the six stations, collected 2017-2019

	S1	S2	S3	S4	S5	S6
Latitude	35.65027	35.66138	35.7	35.7008	35.7275	35.7463
Longitude	52.0175	52.0288	52.0566	52.06	52.0844	52.05916
Elevation (m)	1729	1785	1892	1967	2007	2147
EC (µS/cm)	400-600	800	400	300-400	100-500	400-500
pH	7.1-8.1	8.1	6.9	7.1-7.8	7.4-8.3	6.8-7.7

Five taxa are reported as new diatom records in Iran including *Cymbopleura similiformis*, *Cymbella affinis* var. *angusta*, *Gomphonema innocens*, *Sellaphora capitata* and *Sellaphora lanceolata*.

Description of the Taxa

Cymbellaceae

Cymbella C.A. Agardh, 1830

Cymbella affinis* var. *angusta (Krammer) W. Silva (Figs 7-11)

Basionym: *Cymbella excisa* var. *angusta* Krammer

References: Da Silva et al. (2015) (p. 11, Figs: 29-35)

Dimensions: Valve length 24.92-34.31, Valve width 6.05-8.03, striae number in 10 µm 10-12 in the middle of the valve and 11-13 at the end of the valve, L/B: 3.9-4.6

Taxonomic note: *Cymbella affinis* var. *angusta* is a member of the species complex of *Cymbella affinis/tumidula/turgidula.* Da Silva et al. (2015) studied the holotype and lectotypes from different countries in Europe including Croatia, Italy, Hungary, France, and Serbia. Through his observations, he concluded that the species and the related varieties belonging to *Cymbella excisa* should be transferred to *Cymbella affinis.* However, he did not define the criteria of delimitation of varieties sharply for the three taxa of *C. affinis* var. *angusta*, *C. subcapitata* (Krammer) W. Silva, and *C. neoprocera* W.

Silva. All these taxa share an overlapping range of close striae density range (9-15, 8-12, 11-14 respectively), size (L: 20-35, 21-31.7, 27-40.3; w: 6-8.5, 7.4-9, 8-11), and all have straight ventral side and one stigma at the end of central striae on the ventral valve. Da Silva et al. (2015) also concluded that these species can be conspecific. Based on the current taxonomy, the characteristics of valves in our specimens including size range, striae density, and presence of a straight ventral side match the descriptions of species in Da Silva et al. (2015).

Within the complex, the species of *C. subturgidula* Krammer, *C. turgidula* Grunow, and *C. tropica* Krammer can be compared with *C. affinis* var. *angusta*. The mentioned species share similar stria density and valve shape. However, regarding the size, the valves in these taxa are bigger. Among these three species, *C. subturgidula* has a straight or slightly convex ventral valve that makes it most similar to *C. affinis* var. *angusta* unlike the two other species (*C. turgidula* and *C. tropica*) that have convex ventral areas. Stigmata in the central part of the valve has a range of 1-3 in *C. subturgidula* Krammer and *C. turgidula* Grunow. Only *C. tropica* has one central pore in the center of the valve like that of *C. affinis* var. *angusta*.

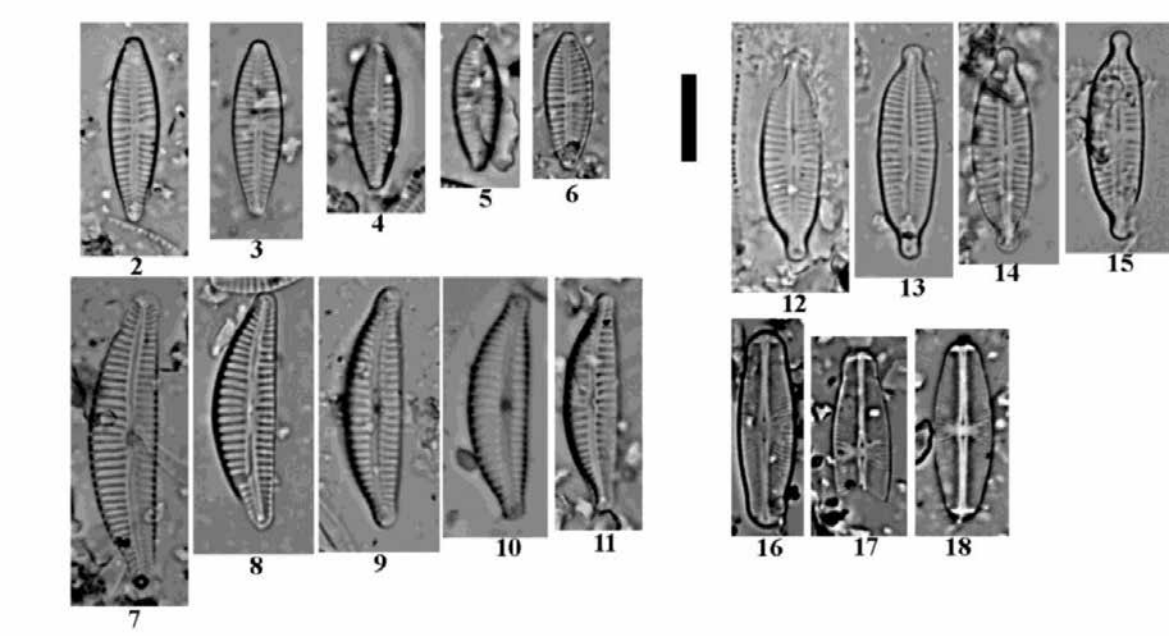
Distribution: Europe (oligotrophic Lakes in Croatia and Italy) (Guiry & Guiry, 2023, Krammer 2002, Da Silva et al. 2015).

Cymbellaceae

Cymbopleura (Krammer) Krammer 1997

Cymbopleura similiformis Krammer (Figs 12-15)

References: Krammer (2003), (p. 69, pl. 90:



Figs 2-18. Light micrographs, with 10 µm scale bar. Figs 2-6. *Gomphonema innocens,* Figs 7-11. *Cymbella affinis* var. *angusta*, Figs 12-15. *Cymbopleura similiformis,* Fig. 16. *Sellaphora capitata,* Fig. 17. *Sellaphora lanceolata,* Fig. 18. *Sellaphora pupula*

1-21)

Dimensions: Valve length 23.6-24-9, width 6.6-7.1, striae number in 10 µm 12-13 in the middle of the valve

Distribution: Europe (Germany), North America, Western Canada (Guiry & Guiry 2023)

Taxonomic note: Krammer (2003) described *Cymbopleura similiformis* as a linear to elliptical species characterized by having "shoulders", or valves that are broader near the apices. This attribute separates it from its similar taxon, *Cymbopleura amphicephala (*Nägeli ex Kützing) Krammer which has an elliptical outline. Both species share similar striae density, length range, and central area shape. Regarding the difference in outline, *Cymbopleura amphicephala* has wider valves compared to *Cymbopleura similiformis* (7.2-8.7 vs 6.6-8.5 µm) (Krammer 2003).

Gomphonemataceae

Gomphonema Ehrenberg 1832

Gomphonema innocens Reichardt (Figs 2-6)

References: Reichardt (1999) (p.32-pl.33:1-12, 24-29, 32-34); Lange-Bertalot et al. (2017) (p.307-pl.99:51-55)

Dimensions: Valve length 15.6-21, valve width 5.2-5.7, striae number in 10 µm 14-15

Distribution: Europe and United States (Guiry & Guiry 2023)

Taxonomic note: *Gomphonema parvulum* (Kützing) Kützing is the most similar species to

G. innocens in terms of overall shape, central area, stria density**,** and size. Due to these similarities, *G. innocens* is often mistakenly identified as *G. parvulum* in biomonitoring programs. The difference between the species is that *G. innocens* has a drawn-out and flat rounded head pole and narrowly rounded foot pole in the case that *G. parvulum* has subrostrate or rostrate ends. Furthermore, *G. innocens*'s valve shape is mostly ovate to clavate. But, *G. parvulum* has been observed with a more variable shape even seen as lanceolate (Cantonati et al. 2016). *Gomphonema saprophilum* (Lange-Bertalot & Reichardt) Abraca, R. Jahn, J. Zimmermann & Enke is another species that can be mistaken by *G. innocens.* It has been identified as a variety of *G. parvulum* previously (Lange-Bertalot 1993). Abarca et al (2014) elevated it to species rank based on morphological characters defined as a rather rhomboid valve shape, short rostrate head pole, subcapitate foot pole that has wider ends compared to *G. parvulum* with rostrate, narrowly rounded poles, and the valve wider than those of *G. parvulum* (6-8 vs. 5-7.5 µm). Also, the molecular data in their research supported the delimitation of taxa based on morphology.

Sellaphoraceae

Sellaphora Mereschowsky1902

Sellaphora capitata D.G. Mann & S.M. McDonald Fig.16

Reference: Mann et al. (2004) (p.477-pl.4: j-l)

Dimensions: Valve length 22.9, valve width 6.4, striae number in 10 µm 23

Distribution: widespread in Europe, North America, Australia, South-western Asia

(India), Asia (China, Korea) (Guiry & Guiry 2023)

Taxonomic note: *Sellaphora capitata* belongs to the *S. pupula* complex that has been separated and described by Mann et al. (2004) based on the slight difference at the shape of the poles and higher stria density. *Sellaphora pupula* (Kützing) Mereschkovsky (refer to Fig. 18) has slightly rostrate ends compared to *S. capitata* which has subcapitate poles. Stria density is lower in *S. capitata* (16-22 vs. 21-26 in 10 µm). The most similar taxon to *S. capitata* from this group is *S. blackfordensis* D.G. Mann & S.Droop which has subcapitate poles, bow-tied central area, and similar stria density (18-22 vs, 16-22). However, *S. blackfordensis* is wider than *S. capitata* (8.1-9.3 vs. 7.2-8.2 µm) and has more robust valves.

Sellaphora lanceolata D.G. Mann & S. Droop Fig.17

Reference: Mann et al. (2004) (p.479-pl.4: p-r)

Dimensions: Valve length 23.4, valve width 6.7, stria number in 10 µm 22

Distribution: Europe (Britain, Germany), Asia (Japan), Russia (Guiry & Guiry, 2023)

Taxonomic note: *Sellaphora lanceolata* is a member of the *S. pupula* complex (Mann et al., 2004) and is distinguished from *S. pupula* based on the rostrate shape of the poles and convergent polar bars. In the case of *S. pupula* (Fig. 18) the poles are slightly rostrate and the orientation of polar bars is parallel or slightly convergent. Furthermore, the stria density is lower in *S. lanceolata* compared to *S. pupula* (18-22 vs. 21-26 in 10 µm). The most similar taxon to *S. lanceolata* is *S. auldreekie* D.G. Mann & S.M. McDonald which shares similar stria density (19-24 vs 18-22), and rostrate apices. It differs from *S. lanceolata* in the parallel orientation of polar bars and bow-tied shape of the central area compared to the elliptical-slightly oblong shape of the central area in *S. lanceolata.*

Discussion

In the present study, members of *Gomphonema*, *Cymbella*, *Cymbopleura,* and *Sellaphora* were highlighted as new records from the Damavand River basin in Central Alborz. This research is derived from a floristic study on diatoms of the Damavand River and its tributaries (Kheiri et al., 2019-2020) and is a part of the study on the biodiversity and ecology of diatoms in the Central Alborz region (Kheiri et al., 2020-2025). Here, we presented the morphological characteristics of the species, which were recently separated and distinguished within broad species complexes whose members have been separated recently (Da Silva et al., 2015; Mann et al., 2004; Krammer, 2003; Reichardt, 1999).

Members of the *Cymbella affinis* group are frequent in the other rivers flowing in the Central Alborz. Species such as *Cymbella excisa* and *Cymbella affinis* var. *neoprocera* W. Silva were recorded as common species in the Karaj and Taleghan Rivers (Kheiri et al., 2018a; Naseri et al., 2022) and in the rivers flowing in western Iran (Kheiri et al., 2018b; Safiallah et al., 2020). Cymbella

affinis var. *angusta* has a small geographic range, as it is reported only from Europe (Italy and Croatia) (Krammer 2002; Da Silva et al., 2015). *Cymbopleua similiformis* is considered a member of the *Cymbopleura amphicephala* group (Krammer, 2003). However, unlike *C. amphicephala,* it has a narrow distribution range - reported from Italy and Croatia (Guiry & Guiry, 2023). *Gomphonema innocens* is morphologically very similar to *Gomphonema parvulum* (Lange-Bertalot et al., 2017). Besides Europe, this species is reported from inland waters of the continental United States (Kociolek, 2015). *Sellaphora capitata* and *S. lanceolata* are separated from the *Sellaphora pupula* (Kützing) Mereschkovsky complex (Mann et al., 2004). *Sellaphora pupula* is reported from Central Alborz (Kheiri et al., 2018; Naseri et al., 2022). This taxon is also observed in our samples from the Damavand River. However, it appeared as a rare species like with two other taxa, *Sellaphora capitata,* and *Sellaphora lanceolata* that were observed as only one frustule in the samples. Therefore, we included the image of the *Sellaphora pupula* with the newly recorded species to have a clear comparison between these species.

Because of complex geology and phytogeography, the Central Alborz serves as a center of diversity of diatoms. The information in this research enhanced our understanding of the close taxa that have challenging taxonomy. Future studies will reveal more information about the diversity and novelties of diatoms in the area.

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