



## Diatom diversity in a mountain lake in southern European Alps (Italy)

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### Abstract

Mountain lakes, often regarded as sentinels of environmental degradation and climate change, are extreme environments. Diatoms are widely considered good indicators in freshwater ecosystems. Littoral diatoms in lakes are highly effective in detecting trophic and thermal conditions and point-source pollution and are considered early warning of potential changes in lake ecosystems. An extensive study of littoral diatoms of Upper Lake Balma (southern European Alps, Italy) was conducted in 2021. Sampling was carried out at seven littoral stations over two periods (July and October). The results revealed a high diversity of taxa, with a total of 116 identified. Plates with figures for 103 taxa and measures (length, width and striae/fibulae number) for 104 taxa are given. Iconographic studies significantly enhance the description of diatom biodiversity in these environments.

**Key words:** diatom Red List, high-elevation lake, shallow lake, Upper Balma Lake

### Introduction

Mountain lakes are extreme environments, typically ice-covered for much of the year. They usually display low nutrients, high radiation, low temperatures and oligotrophic status (Catalan *et al.* 2006) and are considered sentinels of environmental degradation and global environmental changes (Rogora *et al.* 2018, Moser *et al.* 2019).

Diatoms serve as effective indicators in freshwater ecosystems and littoral diatoms in lakes are indicative of trophic and thermal conditions (Rivera-Rondón & Catalan 2020), as well as efficient in detecting point-source pollution, serving as early indicators of ecosystem changes (Cantonati & Lowe 2014, Rimet *et al.*, 2016).

In Europe there are studies on littoral diatom in a large number of mountain lakes (Levkov *et al.* 2005, Štefková 2006, Buczkó 2016, Feret *et al.* 2017, Rivera-Rondón & Catalan 2017, Ossyssek *et al.* 2023, Solak *et al.* 2023a). Renewed interest in diatom research has led to the discovery of new species, such as *Psammothidium toroi* Blanco, Pla-Rabes, Wetzel & Granados 2017 (Blanco *et al.* 2017), *Punctastriata subalpina* C.E.Wetzel & Ector 2020 and *Punctastriata catenata* C.E.Wetzel & Ector 2020 (Wetzel & Ector 2020), *Sellaphora lucectoriana* Solak, S.Blanco, P.B.Hamilton, Peszek 2023 (Solak *et al.* 2023b), *Achnantheidium pavense* V.Vassal, Heudre, C.E.Wetzel & Tudesque 2023 and *Achnantheidium ecrinense* V.Vassal, Heudre, C.F.Wetzel & Tudesque 2023 (Vassal *et al.* 2023) and *Orthoseira helvetica* Peszek, C.T.Robinson, M.Rybak & Kawecka 2023 (Peszek *et al.* 2023).

Diatom diversity is particularly high in oligotrophic environments including mountain lakes and it is still underestimated (Lange-Bertalot & Metzeltin 1996, Rivera-Rondón & Catalan 2017). Besides the high diversity of littoral

diatom community in these environments, few iconographic publications are available: Romania (Buczko 2016), Austria, Germany and Finland (Lange-Bertalot & Metzeltin 1996) and Pyrénées (Rivera-Rondón & Catalan 2017).

For Italy investigations of benthic diatoms in mountain lakes both for the Alps and the Apennines are quite limited (Tolotti 2001, Marchetto *et al.* 2004, Angeli & Cantonati 2005, Cantonati *et al.*, 2021, Marchetto *et al.* 2021, Padula *et al.* 2021, Lepori & Tolotti 2023). Aim of the study is to improve the knowledge of littoral diatom assemblages of mountain lakes in southern European Alps.

## Study area

Upper Balma Lake (Elevation: 2214 m a.s.l.; Surface: 1.82 ha; Maximum depth: 2.77 m; perimeter: 774 m) is a high elevation, small and shallow lake in the Cottian Alps (Piedmont, north-western Alps, Italy). The lake catchment is mainly composed of ophiolite metamorphic bedrocks. The lake is included in the Special Area of Conservation (SAC) and Special Protection Area (SPA) IT1110006, called “Orsiera Rocciavré” and in the Orsiera Rocciavré Nature Park.

General overview of the study area, including physicochemical water parameters and sampling sites short description, is reported in Bertoli *et al.* (2023). High P concentrations (mean value: 46.7 ug/L in July and 54.8 ug/L in October) and low transparency (Secchi disk  $1.1 \pm 0.1$  m) are the main feature of the lake, with levels which are typical of calcareous catchment (Boggero *et al.* 2005, Fjellheim *et al.* 2009). Anthropogenic impacts include fish introduction for recreational fishing in the 1970s (*Salvelinus fontinalis*) and livestock grazing.

## Methods

Benthic diatoms were studied during the ice-free period, in July and October 2021. Diatoms were collected in seven littoral areas from hard substrates (boulders, cobbles) and processed in the laboratory to obtain permanent slides by cold and hot oxidation with 37% H<sub>2</sub>O<sub>2</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, and addition of HCL 1N. Cleaned frustules were mounted in Naphrax (refraction index 1.65) for LM. For morphological analysis, 500 valves per sample were counted and identified to species level using Olympus BX51 microscope at 1000x magnification and imaging system Leica DMC4500 and LAS Version 4.12.0. For each taxon a subsample of specimens was measured for length and width ( $\mu\text{m}$ ) and striae/fibulae number.

Taxonomic identification relied on monographic references for high-altitude lakes (Lange-Bertalot & Metzeltin 1996, Buczko 2016, Rivera-Rondón & Catalan 2017), as well as Lange-Bertalot *et al.* (2017) and national floras of Poland and Russia (Bał et al. 2012, Kulikovskiy *et al.* 2016). Other books used for identification were those of Süßwasserflora von Mitteleuropa (Krammer & Lange-Bertalot 1991a, 1991b, 1997a, 1997b), Diatoms of Europe (Krammer 2000, Lange-Bertalot 2001, Krammer 2002, Krammer 2003, Lange-Bertalot *et al.* 2011, Levkov *et al.*, 2016), Bibliotheca Diatomologica (Lange-Bertalot & Krammer 1989, Krammer 1997a, b, Lange-Bertalot 1993, Kociolet *et al.* 2014) and Iconographia Diatomologica (Lange-Bertalot & Genkal 1999, Werum & Lange-Bertalot 2004, Siver *et al.* 2005, Zidarova *et al.* 2016)

Research papers for Monoraphid (Bukhtiyarova 1996, Monnier *et al.* 2007, Buczkó *et al.* 2013, Blanco *et al.* 2017), Brachyraphid (Pavlov & Levkov 2013), Biraphid (Cantonati *et al.* 2009, Van de Vijver *et al.* 2012, Novais *et al.* 2013, Wojtal *et al.* 2014, Buczkó *et al.* 2015, Wetzel *et al.* 2015, Van de Vijver *et al.* 2021a, 2021b, 2022a, Alibert *et al.* 2023) and Araphid diatoms (Flower 2005, Jüttner *et al.* 2015, García *et al.* 2021, Heudre *et al.* 2021, Van de Vijver *et al.* 2022b) were also used.

Taxa were cross-referenced with the Diatom Red List (Hofmann *et al.* 2018) using OMNIDIA software (Ver. 6.1.8, upgrade May 2024) (Lecointe *et al.* 1993).

## Results

Surveys conducted in July and October 2021 across seven littoral stations identified 116 diatom taxa, spanning 9 orders, 24 families, and 37 genera. Table 1 lists all taxa, with references to plates and figures. The number of specimens measured for length, width and striae/fibule number in 10  $\mu\text{m}$  is indicated ( $n = x$ ).

**TABLE 1.** Taxon list of species found in Upper Balma Lake, with relative abundance, reference to Diatom Red List and measures. Abundance in July (Jul) and October (Oct): >50%=dominant (d), 10–50%= abundant (a), 5–10%= frequent (f), 1–5%=occasional (o), <1%= rare (r). Diatom Red List (RL) (Hofmann *et al.* 2018): a) Highly endangered, b) Threatened of extinction, c) Risk not estimated, d) Early alert, e) Not endangered, f) Not enough data. Number of measured specimens (n) for length (L) and width (W) (µm) and striae/fibule number in 10 µm (Str/fib).

Taxon	Jul	Oct	RL	Plate & Figure	n	L	W	Str/Fib
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki 1994 sensu lato	f	o		Pl.3 - Figs 2–25				
<i>Adlafia</i> sp.		r		Pl.5 - Figs 30–31	3	14.8–18.0	3.3–3.6	24–27
<i>Aulacoseira alpigena</i> (Grunow) Krammer 1991	r		c	Pl.1 - Figs 1–4				
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen 1979		r	e					
<i>Aulacoseira nivalis</i> (W.Smith) J.English & Potapova 2009	r		e	Pl.1 - Figs 5–6				
<i>Brachysira calcicola</i> Lange-Bertalot 1994	r		a	Pl.5 - Figs 19–20				
<i>Brachysira</i> aff. <i>neoexilis</i> Lange-Bertalot 1994	r			Pl.5 - Figs 16–18	5	22.9–27.5	4.4–4.9	–
<i>Brachysira confusa</i> Van de Vijver, R.L.Albert, B.Kennedy & Kusber 2021	r	r		Pl.5 - Figs 11–15	6	18.8–27.4	5.7–6.5	25–28
<i>Brachysira</i> sp.	r			Pl.5 - Figs 21–22				
<i>Caloneis</i> sp.	r			Pl.4 - Fig. 6				
<i>Caloneis bacillum</i> (Grunow) Cleve 1894 sensu lato	r		f	Pl.4 - Figs 1–3	5	28.6–38.6	6.7–7.2	20–22
<i>Caloneis lauta</i> J.R.Carter 1981		r	c	Pl.4 - Figs 4–5	3	28.4–35.9	5.5–6.2	16–17
<i>Cavinula pseudoscutiformis</i> (Hustedt) D.G.Mann & Stickle 1990	r		b	Pl.6 - Figs 41–44				
<i>Chamaepinnularia</i> cf. <i>mediocris</i> (Krasske) Lange-Bertalot 1996	r		d	Pl.6 - Fig. 28	1	9.0	3.0	19
<i>Chemopinnularia</i> sp.	r			Pl.6 - Figs 24–27	4	6.6–13.0	2.3–2.6	18
<i>Cymbopleura</i> sp.	r			Pl.6 - Fig. 57	1	41.9	10.1	14
<i>Encyonema brevicapitatum</i> Krammer 1997	o	o		Pl.6 - Figs 58–62	8	11.9–19.3	4.2–5.0	15–18
<i>Encyonema kalbei</i> Krammer 1997	r		f	Pl.6 - Figs 65–67	1	16.3	4.3	15
<i>Encyonema lunatum</i> (W.Smith) Van Heurck 1880	r	r	f	Pl.7 - Fig. 2	3	37.7–40.6	6.0–6.5	11–13
<i>Encyonema lunatum</i> var. <i>boreale</i> Krammer 1997	r			Pl.7 - Fig. 1	1	42.0	5.4	9
<i>Encyonema minutum</i> (Hilse) D.G.Mann 1990	o	o	e	Pl.6 - Figs 50–54	6	9.6–13.8	4.1–4.6	15–18
<i>Encyonema neogracile</i> Krammer 1997	r		b	Pl.7 - Figs 3–4	3	28.1–39.3	5.7–6.5	12–13
<i>Encyonema perlangebertalotii</i> Kulikovskiy & Metzeltin 2012	r	r		Pl.6 - Figs 63–64	3	15.1–16.8	5.0–5.2	17–18
<i>Encyonema perpusillum</i> (A.Cleve) D.G.Mann 1990	r		b	Pl.6 - Figs 55–56	2	21.9–22.0	3.9–4.0	9–11
<i>Encyonema rostratum</i> Krammer 1997	r			Pl.6 - Figs 68–71	3	15.9–16.1	3.6–3.9	14–15
<i>Encyonema silesiacum</i> (Bleisch) D.G.Mann 1990	r	r	e	Pl.6 - Figs 46–49	3	23.1–25.2	7.1–7.3	13–14
<i>Encyonema</i> sp. 1	r	r		Pl.7 - Figs 5–6	3	32.3–36.0	5.6–5.8	11–12
<i>Encyonema</i> sp.2		r		Pl.7 - Figs 7–8	5	19.5–22.5	4.6–5.4	16–17
<i>Encyonema</i> sp.3	r			Pl.7 - Fig. 9	1	35.0	7.6	13
<i>Encyonopsis</i> aff. <i>neerlandica</i> Van de Vijver, Verweij, van der Wal & A.Mertens 2012	r	r		Pl.7 - Figs 21–23	4	19.0–22.3	3.7–3.9	25–26
<i>Eunotia boreoalpina</i> Lange-Bertalot & Nörpel-Schempp 1998	r		c	Pl.2 - Figs 1–5	2	19.8–24.2	4.5	16–17
<i>Eunotia boreotenuis</i> Nörpel-Schempp & Lange-Bertalot 1996	r		c	Pl.2 - Fig. 16	2	12–17.4	2.9–3.8	17
<i>Eunotia botuliformis</i> F.Wild, Nörpel & Lange-Bertalot 1993	r			Pl.2 - Fig. 15	1	9.3	3.2	19
<i>Eunotia exigua</i> (Brébisson ex Kützing) Rabenhorst 1864	r	r	e	Pl.2 - Figs 9–12	8	13.3–19.2	2.4–3.1	20–24
<i>Eunotia groenlandica</i> Nörpel-Schempp & Lange-Bertalot 1996	r		c	Pl.2 - Fig. 32	1	27.8	3.0	14
<i>Eunotia islandica</i> Østrup 1918	r		f	Pl.2 - Figs 13–14	2	27.5–27.9	6.8–7.0	13–14,5

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**TABLE 1.** (Continued)

Taxon	Jul	Oct	RL	Plate & Figure	n	L	W	Str/Fib
<i>Eunotia juettnerae</i> Lange-Bertalot 2011	r		c	Pl.2 - Fig. 30–31	3	60–77.6	3.3–3.5	15–16
<i>Eunotia minor</i> (Kützing) Grunow 1881		r		Pl.2 - Fig. 17	1	35.8	7.0	13
<i>Eunotia neocompacta</i> var. <i>vixcompacta</i> Lange-Bertalot 2011	r		c	Pl.2 - Figs 6–8	3	15.0–20.1	2.9–3.2	19–22
<i>Eunotia paludosa</i> Grunow 1862	r		d	Pl.2 - Fig. 33	1	18.1	2.8	22
<i>Eunotia praerupta</i> Ehrenberg 1843	r	r	a		2	42.2–83.9	13.5–14	13–16
<i>Eunotia pseudogroenlandica</i> Lange-Bertalot & Tagliaventi 2011	r	r	c	Pl.2 - Fig. 18–21	4	12.1–25.6	2.9–3.2	15–18
<i>Eunotia</i> aff. <i>pseudogroenlandica</i> Lange-Bertalot & Tagliaventi 2011	r	r		Pl.2 - Fig. 22–26	8	10.2–23.4	3.3–3.8	13–16
<i>Eunotia valida</i> Hustedt 1930	r	r	c	Pl.2 - Fig. 27–29	5	44.9–72.0	4.3–5.1	11–13
<i>Eunotia</i> sp.1	r				1	22.5	4.0	14
<i>Eunotia</i> sp.2	r							
<i>Fragilaria</i> cf. <i>campyla</i> (Hilse) Van de Vijver, Kusber & D.M.Williams 2022	r				5	47.2–50.2	2.6–3.6	21–22
<i>Fragilaria</i> cf. <i>nanana</i> Lange-Bertalot 1993	a	d		Pl.1 - Figs 19–23	12	24.2–48.5	2.0–2.3	21–23
<i>Fragilaria radians</i> (Kützing) D.M.Williams & Round 1988		r	e	Pl.1 - Figs 15–18	5	17.9–22.0	2.6–3.0	20–22
<i>Fragilaria</i> sp.	r	r						
<i>Frustulia crassinervia</i> (Brébisson ex W.Smith) Lange-Bertalot & Krammer 1996	r	r	d	Pl.5 - Figs 32–33	3	35.5–41.6	10.1–11.5	–
<i>Geissleria</i> aff. <i>moseri</i> Metzeltin, Witkowski & Lange-Bertalot 1996	r	r		Pl.5 - Figs 23–25	4	11.0–19.6	5.7–5.9	15–17
<i>Genkalia boreoalpina</i> Wojtal, C.E.Wetzel, Ector, Ognjanova-Rumenova & Buczkó 2014	r	r	c	Pl.5 - Figs 34–38	4	13.4–15.0	3.8–4.1	–
<i>Genkalia digitulus</i> Lange-Bertalot & Kulikovskiy 2012	r	r	b	Pl.5 - Figs 39–42	4	9.4–11.8	3.9–4.1	26–30
<i>Gomphonema hebridense</i> W.Gregory 1854	r		d	Pl.7 - Figs 12–15	9	30.3–34.9	5.5–6.1	14–17
<i>Gomphonema subclavatum</i> (Grunow) Grunow 1884	r	r	e	Pl.7 - Figs 10–11	7	34.0–50.6	7.4–8.8	10–11
<i>Gomphonema varioeruduncum</i> Jüttner, Ector, E. Reichardt, Van de Vijver & E.J.Cox 2013	o	r	f	Pl.7 - Figs 16–20	15	15.2–26.8	4.0–5.0	12–14
<i>Humidophila schmassmannii</i> (Hustedt) Buczkó & Wojtal 2015	o	o	c	Pl.6 - Figs 33–36	9	7.2–9.6	2.6–3.1	
<i>Humidophila</i> sp.	r	r		Pl.6 - Figs 29–32	7	6.4–10.3	1.9–2.6	
<i>Kobayasiella parasubtilissima</i> (H.Kobayasi & T.Nagumo) Lange-Bertalot 1999	r	r	d	Pl.5 - Figs 26–29	3	19.2–20.6	3.7–3.9	
<i>Mayamaea</i> cf. <i>fossalis</i> (Krasske) Lange-Bertalot 1997	r		e	Pl.6 - Fig. 45	1	3.5	2.7	22
<i>Meridion constrictum</i> Ralfs 1843	r	r	e	Pl.1 - Figs 7–9	4	12.5–18.6	5.5–6.4	20–24
<i>Microcostatus maceria</i> (Schimanski) Lange-Bertalot 1999	r	r	b	Pl.6 - Figs 37–39	2	13.8–14.5	4.2–4.3	
<i>Microcostatus krasskei</i> (Hustedt) J.R.Johansen & Sray 1998		r	d	Pl.6 - Fig. 40	1	8.6	3.4	
<i>Microfissurata paludosa</i> Cantonati & Lange-Bertalot 2009		r		Pl.6 - Fig. 1	2	16.5–17.9	4.6	
<i>Navicula exilis</i> Kützing 1884		r	b	Pl.5 - Figs 6–8	4	28.4–36.0	5.9–6.2	14–15
<i>Navicula</i> sp.	r			Pl.5 - Fig. 9	2	18.8–20.0	5.8–6.0	18–19
<i>Neidium</i> aff. <i>levanderi</i> (Hustedt) Lange-Bertalot & Metzeltin 1996	r			Pl.5 - Figs 4–5	4	22.4–30.7	4.6–6.4	32–33
<i>Neidium alpinum</i> Hustedt 1943	r	r	a		3	20.1–26.6	4.2–5.1	
<i>Neidium longiceps</i> (W.Gregory) R.Ross 1947	r	r	c	Pl.5 - Figs 1–3	4	25.3–32.2	5.6–7.3	28–30
<i>Nitzschia acidoclinata</i> Lange-Bertalot 1976	r	r	d	Pl.7 - Figs 32–33	3	19.0–21.2	3.0–3.1	26–28 (8–10)
<i>Nitzschia alpina</i> Hustedt 1943		r	b	Pl.7 - Fig. 34	1	20.5	3.2	11 (30–31)
<i>Nitzschia bryophila</i> (Hustedt) Hustedt 1943	r	r	c	Pl.7 - Figs 24–27	6	15.5–29	4.1–40.6	

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**TABLE 1.** (Continued)

Taxon	Jul	Oct	RL	Plate & Figure	n	L	W	Str/Fib
<i>Nitzschia gracilis</i> Hantzsch 1860	r		e		3	–	2.7–3.0	– (13–16)
<i>Nitzschia palea</i> var. <i>debilis</i> (Kützing) Grunow 1880		r	e	Pl.7 - Figs 35–37	4	19.8–29.1	2.7–3.0	– (12–13)
<i>Nitzschia perminuta</i> Grunow 1881	o	o	e	Pl.7 - Figs 28–31	11	18.6–29.0	2.6–3.1	29–32 (10–13)
<i>Odontidium mesodon</i> (Ehrenberg) Kützing 1849	r	r	e	Pl.1 - Figs 28–32	15	11.3–19.2	6.2–7.2	24–26
<i>Pinnularia ammerensis</i> Kulikovskiy, Lange-Bertalot & Metzeltin 2010	r		f	Pl.4 - Figs 14–15	7	22.6–36.3	5.4–6.5	11–14
<i>Pinnularia borealis</i> Ehrenberg 1843	r		e		2	37.0–45.0	8.7–10.8	4–5
<i>Pinnularia</i> cf. <i>lindanedbalovae</i> B.van de Vijver & A.Moravcová 2013	r	r		Pl.4 - Figs 12–13	5	22.0–28.5	4.7–5.0	12–14
<i>Pinnularia microstauron</i> (Ehrenberg) Cleve 1891	r		d	Pl.4 - Figs 18–19	7	50.7–63.0	9.1–11.2	9–10
<i>Pinnularia</i> aff. <i>microstauron</i> var. <i>rostrata</i> Krammer 2000	r	r		Pl.4 - Figs 20–23	6	25.2–27.5	5.0–5.7	12
<i>Pinnularia</i> cf. <i>notabilis</i> Krammer 1985	r	r	c	Pl.4 - Figs 16–17	13	55.7–95.0	11.7–18.0	10–11
<i>Pinnularia silvatica</i> J.B.Petersen 1935	r		f		3	17.2–20.6	3.7–3.8	20–22
<i>Pinnularia sinistra</i> Krammer 1992	r	r	e	Pl.4 - Figs 7–11	14	24.0–41.2	4.0–5.1	11–12
<i>Planothidium distinctum</i> (Messikommer) Lange-Bertalot 1999	r	r	b	Pl.3 - Fig. 1				
<i>Psammothidium</i> aff. <i>acidoclinatum</i> (Lange-Bertalot) Lange-Bertalot 1999	r	r		Pl.3 - Figs 51–55	10	10.3–14.3	4.9–5.9	25–26
<i>Psammothidium bioretii</i> (H.Germain) Bukhtiyarova & Round 1996	r		e	Pl.3 - Fig. 35				
<i>Psammothidium bristolicum</i> Bukhtiyarova 1996	o	r	f	Pl.3 - Figs 41–45	12	10.2–14.2	4.4–5.0	25–27
<i>Psammothidium chlidanos</i> (M.H.Hohn & Hellerman) Lange-Bertalot 1999	r	r	b	Pl.3 - Figs 31–34	4	10.0–16.4	4.9–5.7	
<i>Psammothidium helveticum</i> (Hustedt) Bukhtiyarova & Round 1996	r	r	e	Pl.3 - Figs 26–30	6	12.4–18.6	5.0–6.7	22–24
<i>Psammothidium helveticum</i> var. <i>minus</i> (Flower & Jones) Buczkó 2016	r		e	Pl.3 - Figs 76–79	5	8.2–10.6	4.8–6.0	22–27
<i>Psammothidium levanderi</i> (Hustedt) Bukhtiyarova & Round 1996		r	c	Pl.3 - Figs 80–82	3	8.5–8.7	4.2–5.0	23–26
<i>Psammothidium marginulatum</i> (Grunow) Bukhtiyarova & Round 1996	r		f	Pl.3 - Figs 36–40	6	11.3–12.7	4.5–4.8	25–27
<i>Psammothidium microscopicum</i> (Cholnoky) S.Blanco 2016	o	r	f	Pl.3 - Figs 71–75	5	4.0–4.8	3.1–3.5	28
<i>Psammothidium rechtense</i> (Leclercq) Lange-Bertalot 1999	r	r	a	Pl.3 - Figs 46–50	4	11.2–12.5	4.4–4.8	28
<i>Psammothidium scoticum</i> (R.J.Flower & V.J.Jones) Bukhtiyarova & Round 1996	o	o	a	Pl.3 - Figs 61–65	11	8.5–10.5	3.6–4.4	26–31
<i>Psammothidium subatomoides</i> (Hustedt) Bukhtiyarova & Round 1996	a	o	d	Pl.3 - Figs 56–60	12	6.1–9.1	3.6–4.7	
<i>Psammothidium toroi</i> Blanco, Pla-Rabes, Wetzel & Granados 2017	r	r		Pl.3 - Figs 66–70	11	6.6–10.7	3.4–4.1	22–26
<i>Psammothidium</i> sp.	r			Pl.3 - Figs 83–85	2	8.0–8.5	4.4–4.5	25
<i>Pseudostaurosira parvissima</i> Kociolek 2014	r	r		Pl.1 - Figs 33–37	8	6.7–12.0	1.9–2.5	18–19
<i>Pseudostaurosira</i> sp.	f	f			30	8.6–16.4	3.0–5.2	12–14
<i>Sellaphora</i> aff. <i>medioconvexa</i> (Hustedt) C.E.Wetzel 2015	r	r		Pl.6 - Figs 7–10	1	11.8	3.7	
<i>Sellaphora</i> aff. <i>multiconfusa</i> (Lange-Bertalot) C.E.Wetzel, Ector, B.Van de Vijver, Compère & D.G.Mann 2015	r	r		Pl.6 - Figs 11–14	5	9.6–13.9	4.1–4.8	24–26
<i>Sellaphora pseudopupula</i> (Krasske) Lange-Bertalot 1996	r	r	c	Pl.6 - Figs 2–6	4	17.8–20.3	5.4–6.0	22

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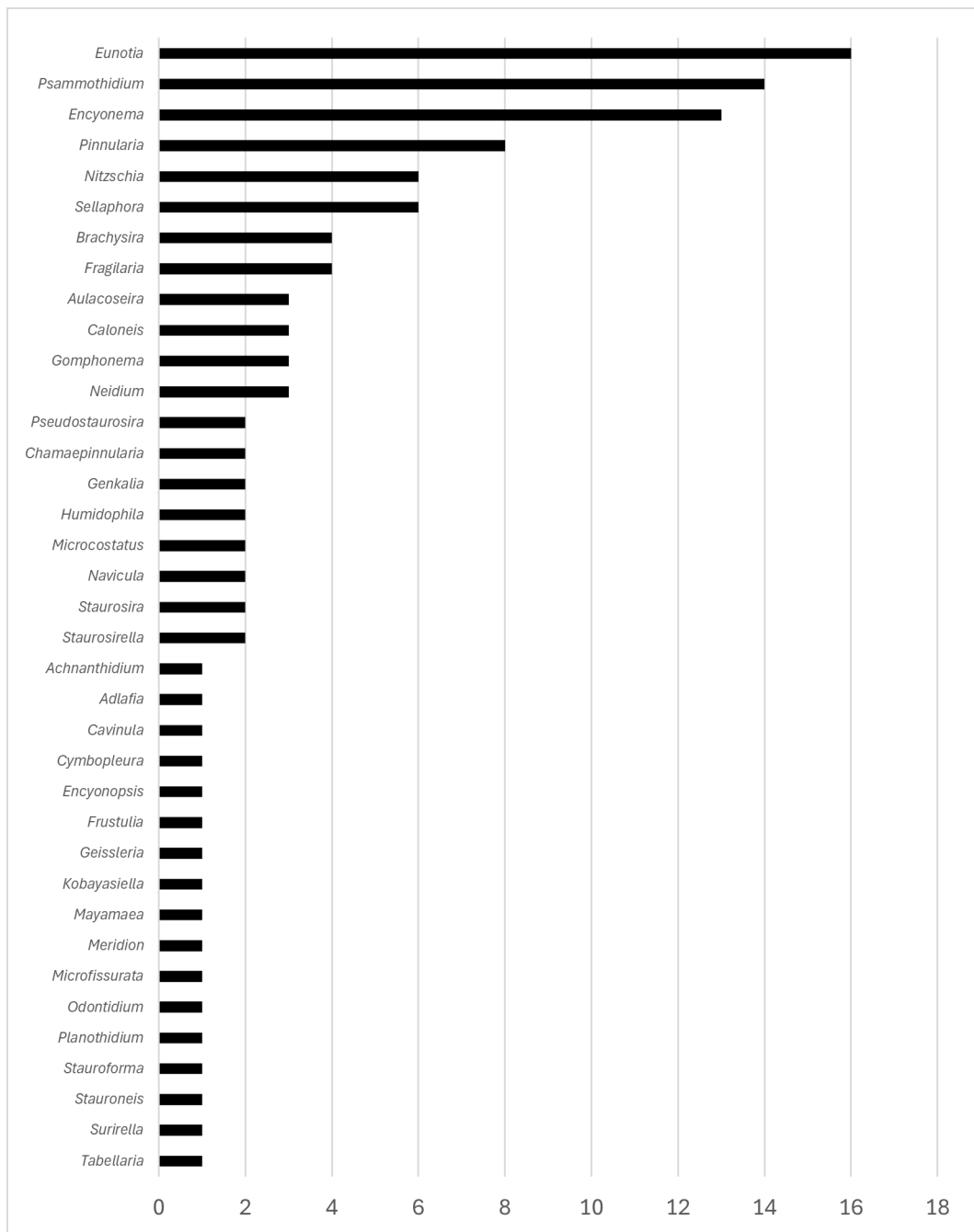


**TABLE 1.** (Continued)

Taxon	Jul	Oct	RL	Plate & Figure	n	L	W	Str/Fib
<i>Sellaphora sorella</i> (M.H.Hohn & Hellerman) C.E.Wetzel, Ector, B.Van de Vijver Compère & D.G.Mann 2015	r	r		Pl.6 - Figs 15–19	11	5.8–10.0	3.0–3.5	17–20
<i>Sellaphora vanlandinghamii</i> (Kociolek) C.E.Wetzel 2015		r		Pl.6 - Figs 20–23	8	5.7–7.0	3.4–4.0	28
<i>Sellaphora</i> sp.	r				1	12.7	3.7	24
<i>Stauroforma exiguiformis</i> (Lange-Bertalot) R.J.Flower, V.J.Jones & Round 1996	r	r	b	Pl.1 - Figs 24–27	3	26.8–28.2	3.3–3.6	17–18
<i>Stauroneis</i> aff. <i>acidoclinata</i> Lange-Bertalot & Werum 2004	r		e	Pl.5 - Fig. 10	2	35.3–38.0	7.5	23–24
<i>Staurosira</i> aff. <i>aventralis</i> Lange-Bertalot & Rumrich 2000	o	o		Pl.1 - Figs 38–42	21	8.0–12.7	3.1–3.9	14–16
<i>Staurosira</i> cf. <i>venter</i> (Ehrenberg) Cleve & J.D.Möller 1879	r	o	e	Pl.1 - Figs 43–47	20	5.8–9.6	3.4–4.2	14–15
<i>Staurosirella ansata</i> (M.H.Hohn & J.Hellerman) J.C.Kingston 2000	r	r		Pl.1 - Figs 48–52	7	8.3–15.8	2.8–3.7	12
<i>Staurosirella</i> sp.		r		Pl.1 - Figs 53–56	2	4.3–5.1	2.62.7	12–13
<i>Surirella roba</i> Leclercq 1983	r		c					
<i>Tabellaria flocculosa</i> (Roth) Kützing 1884	o	r	e	Pl.1 - Figs 10–14	9	14.8–25.2	5.6–7.2	14–18

Genera with a higher number of species were *Eunotia* (16 species), *Psammothidium* (14), *Encyonema* (13) and *Pinnularia* (8) (Figure 1). The most abundant genera (>1% relative abundance) included *Achnantheidium*, *Encyonema*, *Fragilaria*, *Humidophila*, *Nitzschia*, *Psammothidium*, *Pseudostaurosira* and *Staurosira* in July and October and *Brachysira*, *Eunotia*, *Gomphonema* and *Tabellaria* in July. For 27 taxa identification was possible to genus level, both for low number of valves found (i.e. *Adlafia* sp., *Cymbopleura* sp.) and an unclear correspondence with known species (i.e. *Psammothidium* aff. *acidoclinatum* (Lange-Bertalot) Lange-Bertalot). For the latter, further SEM investigations are needed. The needle-shaped *Fragilaria* cf. *nanana* Lange-Bertalot 1993 was the dominant species, with a mean percentage of 37.0 and 68.7 respectively in July and October. Other abundant species (>1%) were *Achnantheidium minutissimum* s.l., *Encyonema brevicapitatum* Krammer 1997, *Encyonema minutum* (Hilse) D.G.Mann 1990, *Humidophila schmassmannii* (Hustedt) Buczkó & Wojtal 2015, *Nitzschia perminuta* Grunow 1881, *Psammothidium scoticum* (R.J.Flower & V.J.Jones) Bukhtiyarova & Round 1996, *Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round 1996, *Pseudostaurosira* sp., *Staurosira* aff. *aventralis* Lange-Bertalot & Rumrich 2000 and *Staurosira* cf. *venter* (Ehrenberg) Cleve & J.D.Möller 1879 in July and October, and *Gomphonema varioeduncum* Jüttner, Ector, E. Reichardt, Van de Vijver & E.J.Cox 2013, *Psammothidium bristolicum* Bukhtiyarova 1996, *Psammothidium microscopicum* (Cholnoky) S.Blanco 2016 and *Tabellaria flocculosa* (Roth) Kützing 1844 in July. Most of taxa were found with low abundance (<1%).

The diatom Red List for protection (Hofmann *et al.* 2018) includes 71 out of 116 species found (Figure 2). Among them, 24 species are classified as worth of protection as highly endangered (5) [*Brachysira calcicola* Lange-Bertalot 1994, *Eunotia praerupta* Ehrenberg 1843, *Neidium alpinum* Hustedt 1943, *Psammothidium rechtense* (Leclercq) Lange-Bertalot 1999 and *Psammothidium scoticum* (R.J.Flower & V.J.Jones) Bukhtiyarova & Round 1996], threatened of extinction (10) [*Cavinula pseudoscutiformis* (Hustedt) D.G.Mann & Stickle 1990, *Encyonema neogracile* Krammer 1997, *Encyonema perpusillum* (A.Cleve) D.G.Mann 1990, *Genkalia digitulus* Lange-Bertalot & Kulikovskiy 2012, *Microcostatus maceria* (Schimanski) Lange-Bertalot 1999, *Navicula exilis* Kützing 1884, *Nitzschia alpina* Hustedt 1943, *Planothidium distinctum* (Messikommer) Lange-Bertalot 1999, *Psammothidium chlidanos* (M.H.Hohn & Hellerman) Lange-Bertalot 1999 and *Stauroforma exiguiformis* (Lange-Bertalot) R.J.Flower, V.J.Jones & Round 1996] and early alert (9) [*Chamaepinnularia* cf. *mediocris* (Krasske) Lange-Bertalot 1996, *Eunotia paludosa* Grunow 1862, *Frustulia crassinervia* (Brébisson ex W.Smith) Lange-Bertalot & Krammer 1996, *Gomphonema hebridense* Gregory 1854, *Kobayasiella parasubtilissima* (H.Kobayasi & T.Nagumo) Lange-Bertalot 1999, *Microcostatus krasskei* (Hustedt) J.R.Johansen & Sray 1998, *Nitzschia acidoclinata* Lange-Bertalot 1976, *Pinnularia microstauron* (Ehrenberg) Cleve 1891, *P. subatomoides* (Hustedt) Bukhtiyarova & Round 1996].



**FIGURE 1.** Number of species observed per genera in Upper Balma Lake.

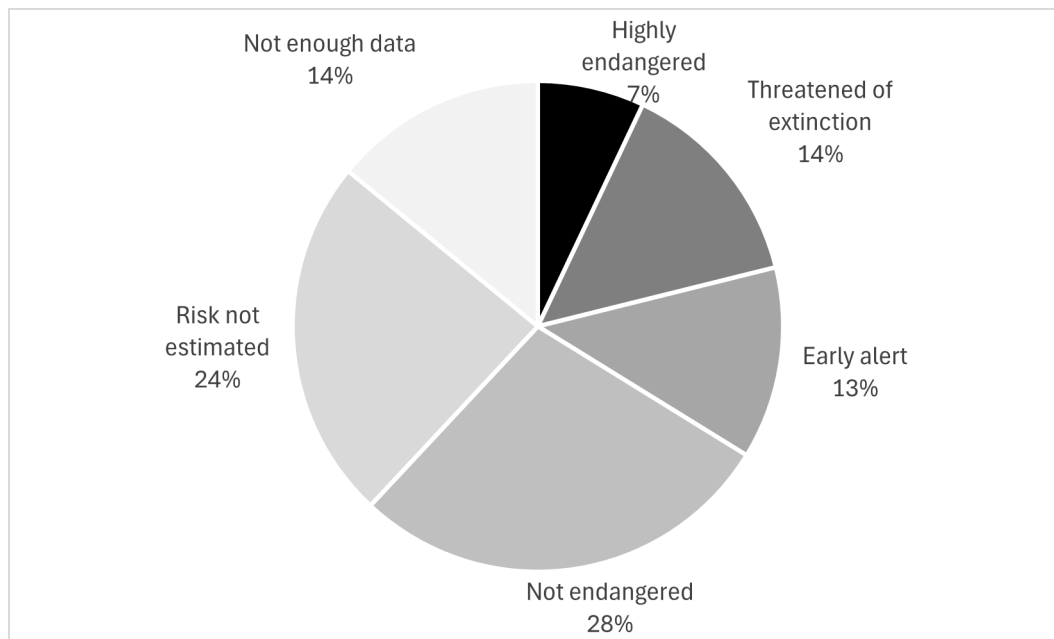


FIGURE 2. Species included in the Diatom Red List (Hofmann *et al.* 2018).

## Discussion

Upper Balma Lake is a circumneutral (pH  $7.6 \pm 0.07$ ), low-mineralized lake (conductivity:  $12.81 \pm 1.34 \mu\text{S cm}^{-1}$ ) situated on siliceous substrate in the Alps. Elevated total phosphorus levels ( $46.7 \pm 1.31 \mu\text{g L}^{-1}$  in July) likely result from nutrient enrichment due to fish stocking and livestock grazing (Bertoli *et al.* 2023).

The periphytic diatom community comprises 116 taxa, 27 identified to genus level, with dominant genera including *Achnantheidium*, *Encyonema*, *Fragilaria*, *Humidophila*, *Nitzschia*, *Psammothidium*, *Pseudostaurosira* and *Staurosira*.

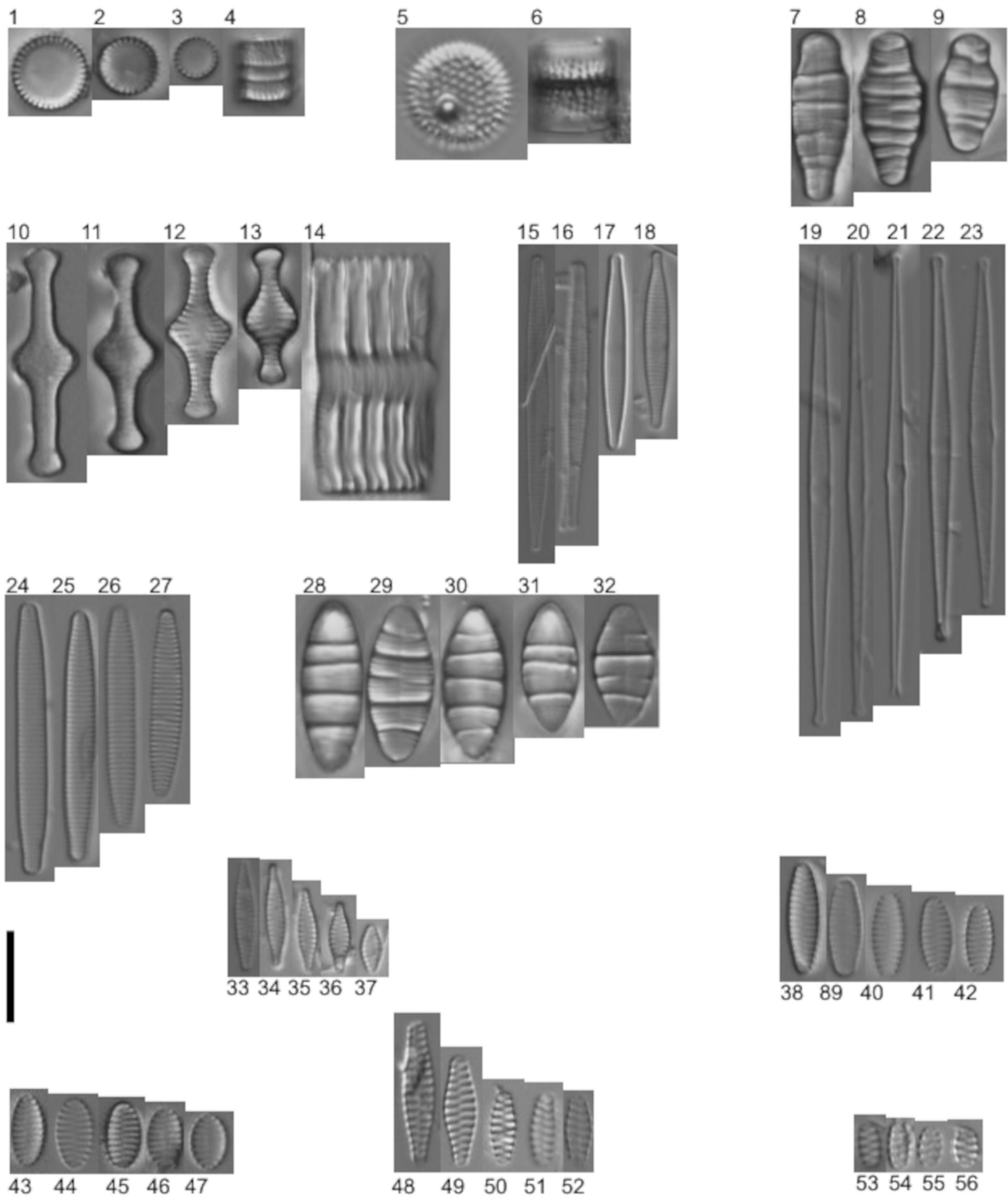
Most of known taxa are typical of oligo to dystrophic, electrolyte poor and weakly acid or circumneutral habitats, on siliceous substrate, i.e. *G. hebridense* W.Gregory 1854, *N. perminuta* Grunow 1881 *P. subatomoides* (Hustedt) Bukhtiyarova & Round 1996 and *Psammothidium helveticum* (Hustedt) Bukhtiyarova & Round 1996 (Lange-Bertalot *et al.* 2017, Van Dam *et al.* 1994).

Among the most represented genera, *Eunotia* is typical of naturally acid, oligotrophic to dystrophic freshwater habitats (i.e. *Eunotia botuliformis* F.Wild, Nörpel & Lange-Bertalot 1993; *Eunotia minor* (Kützing) Grunow 1881) and of acid peat bogs and fens which often neighbor mountain lakes (i.e. *E. paludosa* Grunow 1862) (Cantonati *et al.*, 2011, Lange-Bertalot *et al.* 2017, Rimet *et al.* 2023).

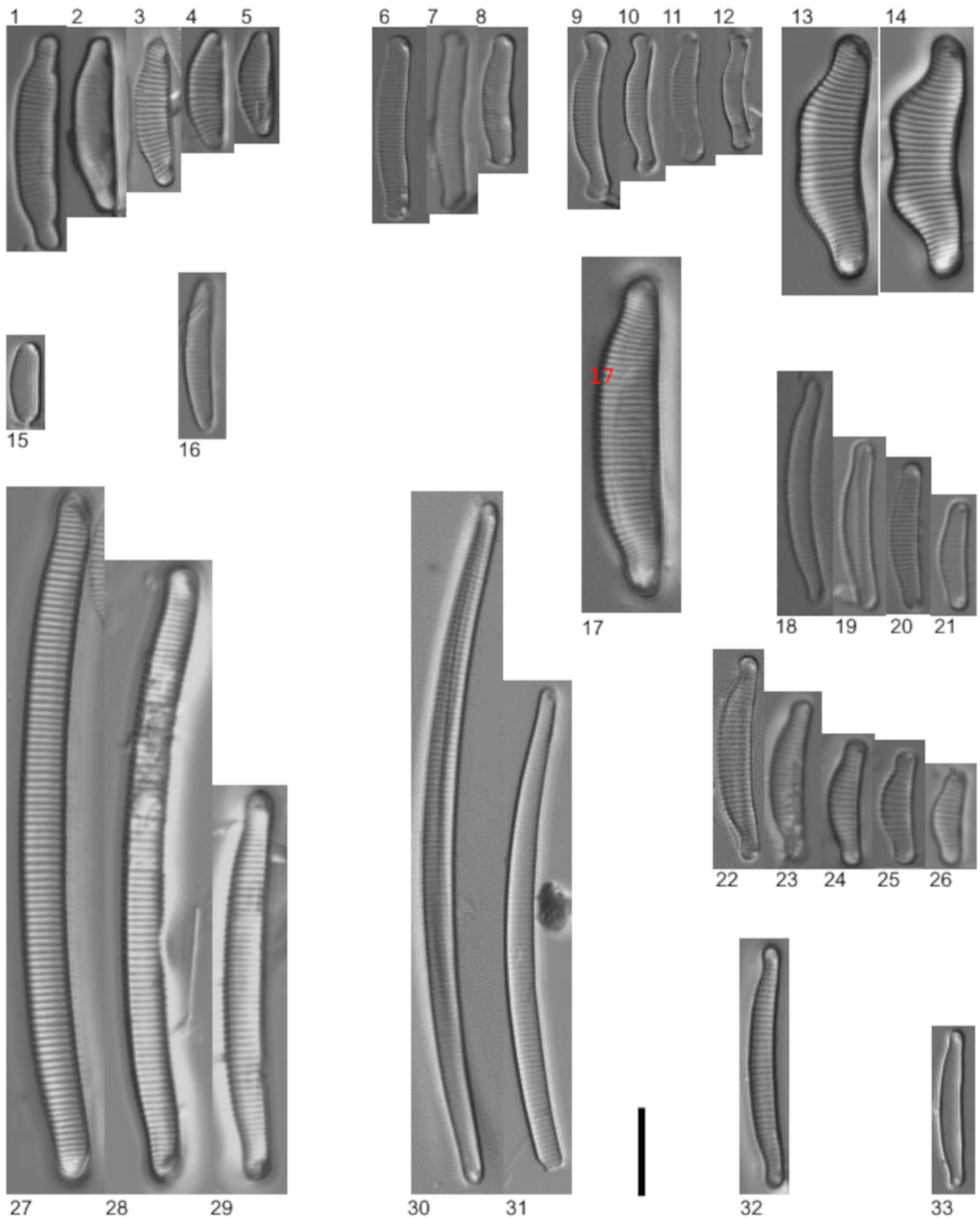
The genus *Psammothidium* is frequently observed in lentic and lotic freshwater habitats and includes typical cold stenothermic species (Blanco *et al.* 2017, Cantonati *et al.* 2021). Among the 14 species found in the lake, *P. microscopicum* (Cholnoky) S.Blanco 2016 is a cryophilic species of oligotrophic lakes (Cantonati *et al.*, 2021) and *Psammothidium levanderi* (Hustedt) Bukhtiyarova & Round 1996 and *Psammothidium toroi* Blanco, Pla-Rabes, Wetzel & Granados 2017 are typical of oligotrophic and very low mineralized lakes (Kopalová *et al.* 2016, Blanco *et al.*, 2017, Feret *et al.*, 2017).

Araphid diatoms *Staurosira* and *Pseudostaurosira* are well represented in the lake, with a mean percentage of 9.1 and 11.1 respectively in July and October. Unattached diatoms are typically present in lakes with long water renewal time, characterized by dominance of high-profile diatoms which compete for light and nutrients (Rimet *et al.* 2019). The needle-shaped *F. cf nanana* Lange-Bertalot 1993 is the dominant species found in the lake, and it has already been observed related to fish introduction (Sochuliaková *et al.* 2018, Cantonati *et al.* 2021).

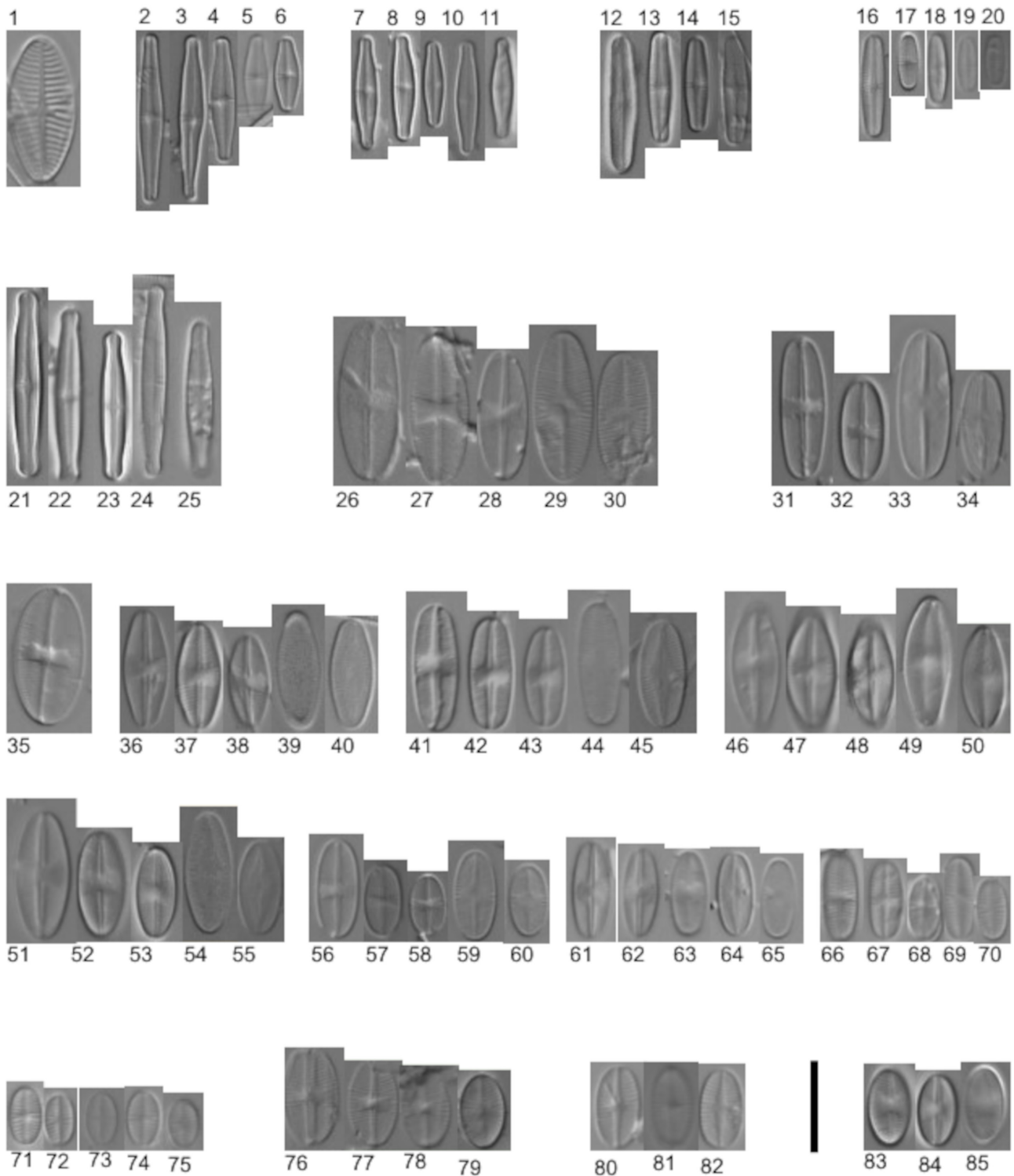




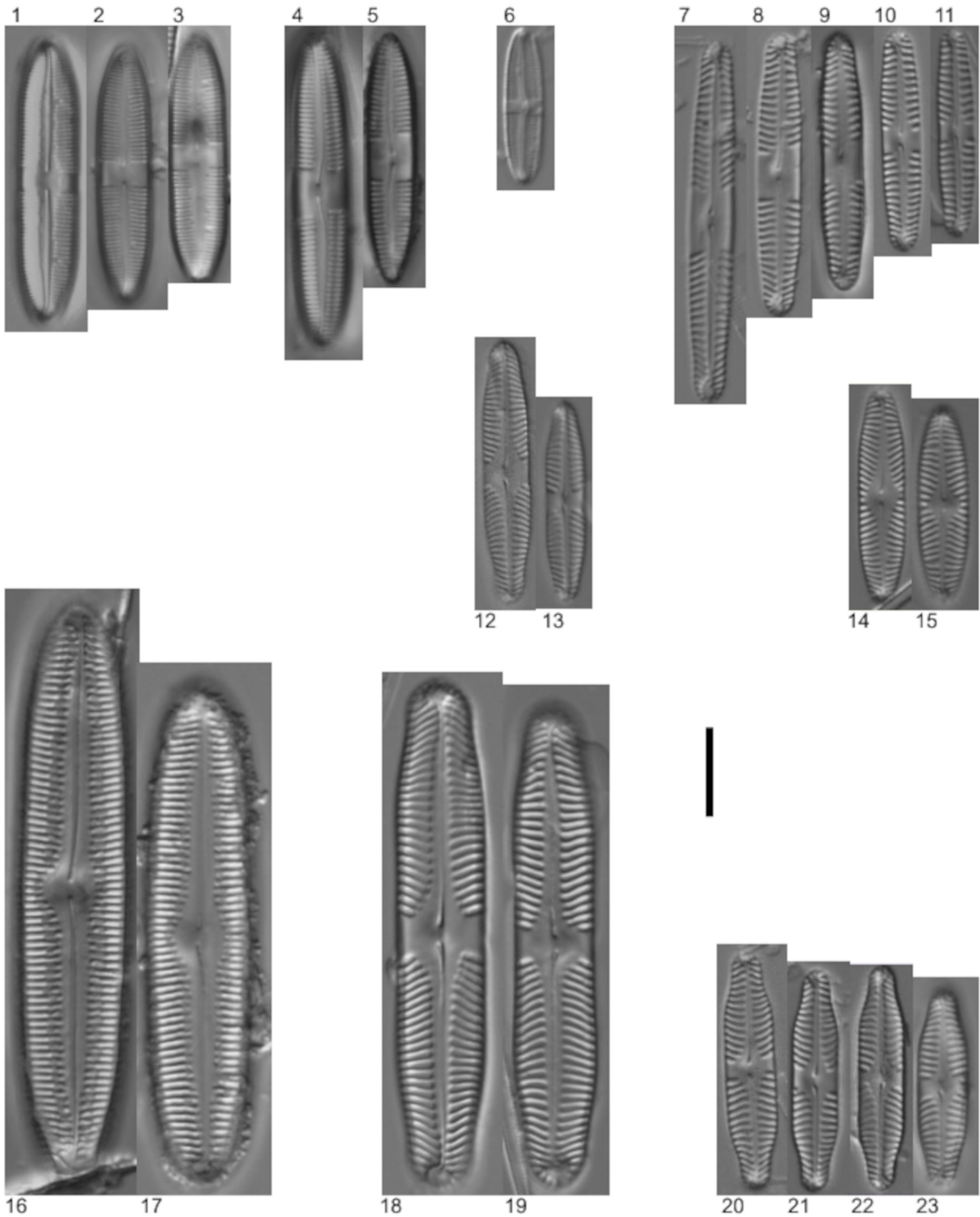
**PLATE 1.** Light micrographs of centric and araphid taxa. Figs 1–4. *Aulacoseira alpigena*. Figs 5–6. *A. nivalis*. Figs 7–9. *Meridion constrictum*. Figs 10–14. *Tabellaria flocculosa*. Figs 15–18. *Fragilaria radians*. Figs 19–23. *F.* cf. *nanana*. Figs 24–27. *Stauroforma exiguiformis*. Figs 28–32. *Odontidium mesodon*. Figs 33–37. *Pseudostaurosira parvissima*. Figs 38–42. *Staurosira* aff. *aventalis*. Figs 43–47. *S.* cf. *venter*. Figs 48–52. *Staurosirella ansata*. Figs 53–56. *Staurosirella* sp. Scale bar: 10  $\mu$ m.



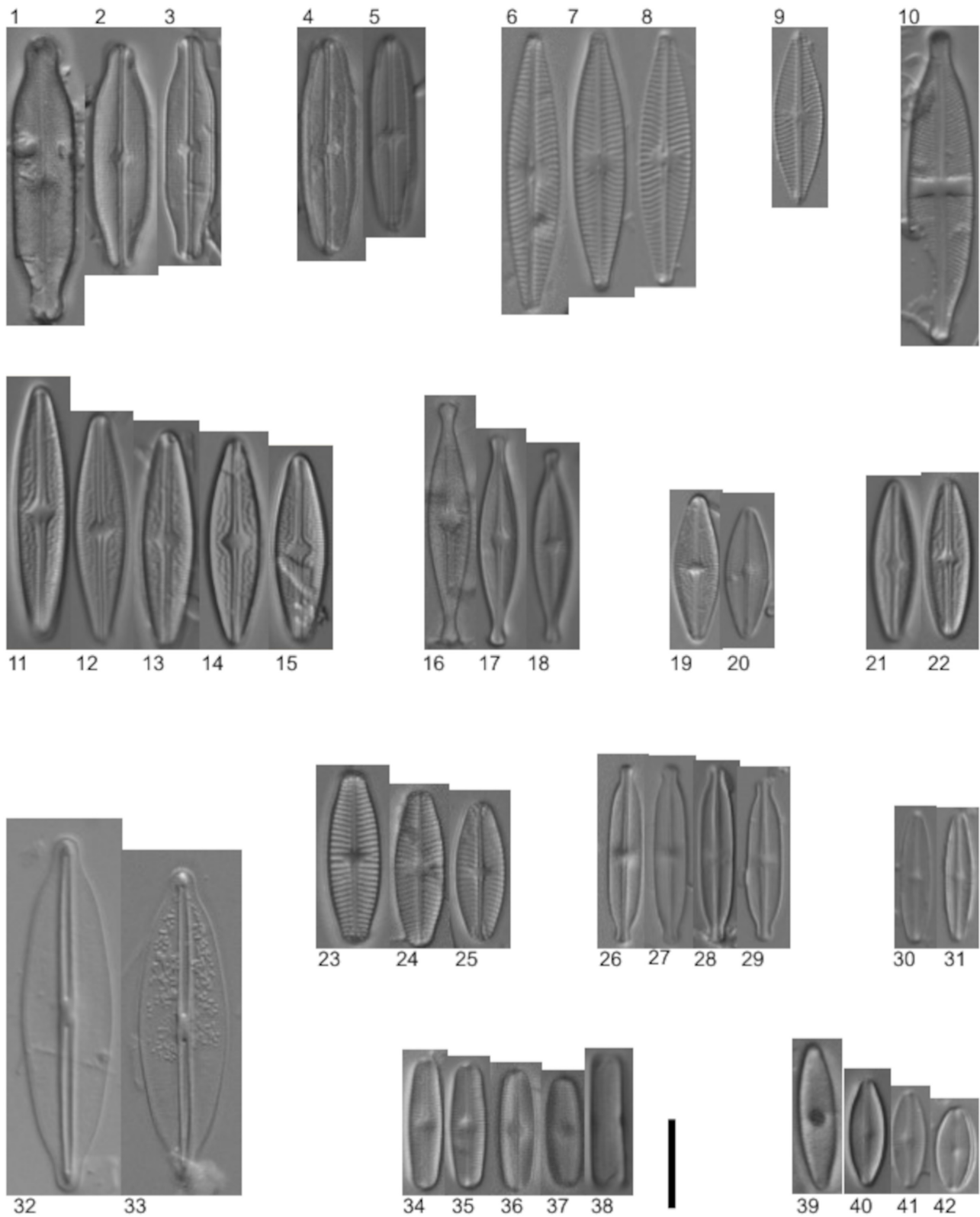
**PLATE 2.** Light micrographs of cunotioid taxa. Figs 1–5. *Eunotia borealpina*. Figs 6–8. *E. neocompacta* var. *vixcompacta*. Figs 9–12. *E. exigua*. Figs 13–14. *E. islandica*. Fig. 15. *E. botuliformis*. Figs 16. *E. boreotenuis*. Fig 17. *E. minor*. Figs 18–21. *E. pseudogroenlandica*. Figs 22–26. *E. aff. pseudogroenlandica*. Figs 27–29. *E. valida*. Figs 30–31. *E. juettnerae*. Fig. 32. *E. groenlandica*. Fig. 33. *E. paludosa*. Scale bar: 10  $\mu$ m.



**PLATE 3.** Light micrographs of monoraphid taxa. Fig. 1. *Planothidium distinctum*. Figs 2–6. *Achnantheidium minutissimum* s.l. forme A. Figs 7–11. *A. minutissimum* s.l. forme B. Figs 12–15. *A. minutissimum* s.l. forme C. Figs 16–20. *A. minutissimum* s.l. forme D. Figs 21–25. *A. minutissimum* s.l. forme E. Figs 26–30. *Psammothidium helveticum*. Figs 31–34. *P. chlidanos*. Fig. 35. *P. bioretii*. Figs 36–40. *P. marginulatum*. Figs 41–45. *P. bristolicum*. Figs 46–50. *P. rechtense*. Figs 51–55. *P. aff. acidoclinatum*. Figs 56–60. *P. subatomoides*. Figs 61–65. *P. scoticum*. Figs 66–70. *P. toroi*. Figs 71–75. *P. microscopicum*. Figs 76–79. *P. helveticum* var. *minus*. Figs 80–82. *P. levanderi*. Figs 83–85. *Psammothidium* sp. Scale bar: 10  $\mu$ m.

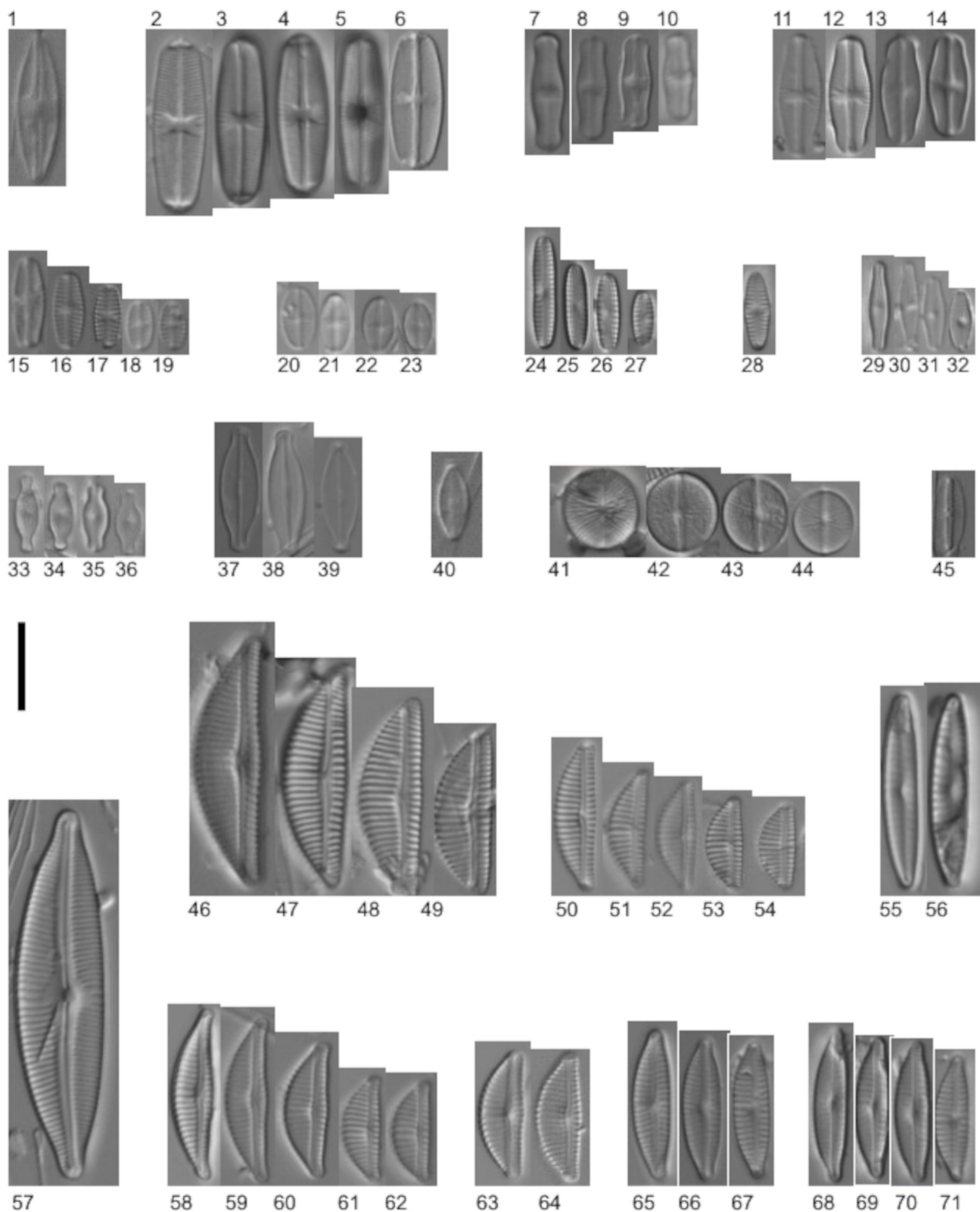


**PLATE 4.** Light micrographs of symmetric biraphid taxa. Figs 1–3. *Caloneis bacillum* s.l. Figs 4–5. *C. lauta*. Fig. 6. *Caloneis* sp. Figs 7–11. *Pinnularia sinistra*. Figs 12–13. *P. cf. lindanedbalovae*. Figs 14–15. *P. ammerenensis*. Figs 16–17. *P. cf. notabilis*. Figs 18–19. *P. microstauron*. Figs. 20–23 *P. aff. microstauron* var. *rostrata*. Scale bar: 10  $\mu$ m.



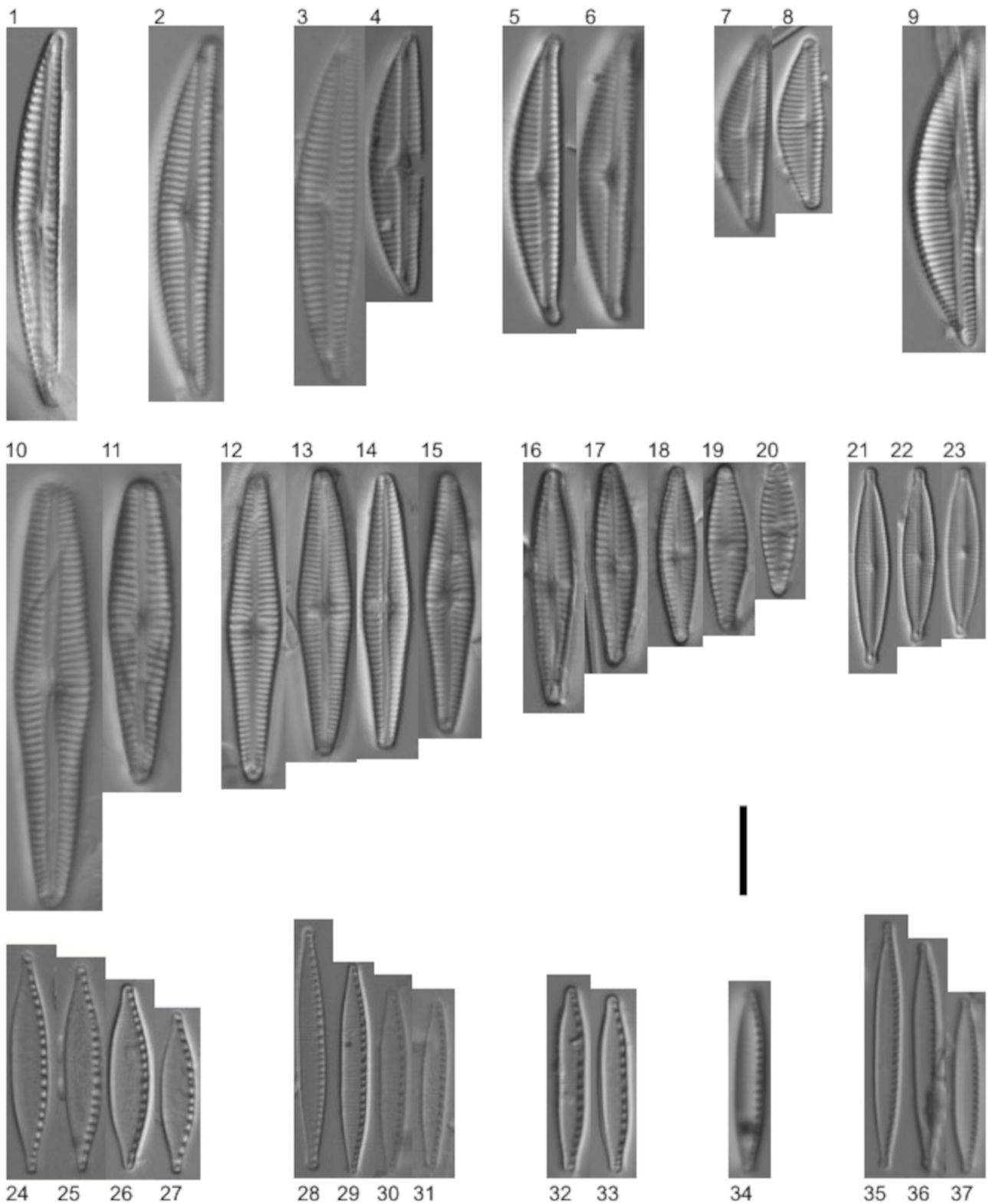
**PLATE 5.** Light micrographs of symmetric biraphid taxa. Figs 1–3. *Neidium longiceps*. Figs 4–5. *Neidium* aff. *levanderi*. Figs 6–8. *Navicula exilis*. Fig. 9. *Navicula* sp. Fig. 10. *Stauroneis* aff. *acidoclinata*. Figs 11–15. *Brachysira confusa*. Figs 16–18. *B.* aff. *neoexilis*. Figs 19–20. *B. calcicola*. Figs 21–22. *Brachysira* sp. Figs 23–25. *Geissleria* aff. *moseri*. Figs 26–29. *Kobayasiella parasubtilissima*. Figs 30–31. *Adlafia* sp. Figs 32–33 *Frustulia crassinervia*. Figs 34–38. *Genkalia boreoalpina*. Figs 39–42. *G. digitulus*. Scale bar: 10 µm.





**PLATE 6.** Light micrographs of symmetric and asymmetric biraphid taxa. Fig. 1. *Microfissurata paludosa*. Figs 2–6. *Sellaphora pseudopupula*. Figs 7–10. *S.* aff. *mediiconvexa*. Figs 11–14. *S.* aff. *multiconfusa*. Figs 15–19. *S. sorella*. Figs 20–23. *S. vanlandinghamii*. Figs 24–27. *Chamaepinnularia* sp. Fig. 28. *Chamaepinnularia* cf. *mediocris*. Figs 29–32. *Humidophila* sp. Figs 33–36. *Humidophila schmassmannii*. Figs 37–39. *Microcostatus maceria*. Fig. 40. *M. krasskei*. Figs 41–44. *Cavinula pseudoscutiformis*. Fig. 45. *Mayamaea* cf. *fossalis*. Figs 46–49. *Encyonema silesiacum*. Figs 50–54. *E. minutum*. Figs 55–56. *E. perpusillum*. Fig. 57. *Cymbopleura* sp. Figs 58–62. *E. brevicapitatum*. Figs 63–64. *E. perlangebertalotii*. Figs 65–67. *E. kalbei*. Figs 68–71. *E. rostratum*. Scale bar: 10  $\mu$ m.





**PLATE 7.** Light micrographs of asymmetric biraphid and nitzschioid taxa. Fig. 1. *Encyonema lunatum* var. *boreale*. Fig. 2. *E. lunatum*. Figs 3–4. *E. neogracile*. Figs 5–6. *Encyonema* sp. 1. Figs 7–8. *Encyonema* sp. 2. Fig. 9. *Encyonema* sp. 3. Figs 10–11. *Gomphonema subclavatum*. Figs 12–15. *G. hebridense*. Figs 16–20. *G. varioreduncum*. Figs 21–23. *Encyonopsis* aff. *neerlandica*. Figs 24–27. *Nitzschia bryophila*. Figs 28–31. *N. perminuta*. Figs 32–33. *N. acidoclinata*. Fig. 34. *N. alpina*. Figs 35–37. *N. palea* var. *debilis*. Scale bar: 10  $\mu\text{m}$ .

Among the 70 species listed in the Diatom Red List (Hofmann *et al.* 2018), 24 taxa (34.3%) are recognized as conservation priorities.

High elevation freshwater ecosystems comprise the least impacted, pristine and extreme environments (Zaharescu *et al.* 2016). In the Arctic and Antarctic regions, limnological and paleolimnological studies have shown that diatoms are informative of environmental changes in the short and long term. These ecosystems are alarm bells, since they show signs related to climate change first and to the highest degree (Douglas & Smol 2010). Global warming is now affecting temperate zones as well, but as expected, lakes in these areas show a temporal shift in changes (in some cases up to a century) (Lotter *et al.* 2010, Spaulding & McKnight 2010). The risk of extirpation of a large number of cold-stenothermal diatom species, in particular from southern refugia has been hypothesized by Bahls (2017) as a consequence of climate change.

Extensive studies on the littoral diatoms of high elevation lakes, conducted through multiple sampling stations and campaigns, can reveal the hidden biodiversity of these environments (Cantonati *et al.* 2021). Single-sample surveys in other regions revealed the presence of 355 species in 83 lakes of the Pyrenees (Rivera-Rondón & Catalan 2017), 127 species in 34 lakes from the territory of the Tatra Mountain (Štefková 2006) and 326 species in 62 natural lakes above 1300 m a.s.l. in the French Alps (Feret *et al.* 2017).

Even if cosmopolitan species play a fundamental role in diatom assemblages, geological and geographical peculiarities have been observed, and diatoms may frequently develop local forms (Cantonati *et al.* 2001) and presence of endemic species and sub-species has been observed (Bahls 2017, Rimet *et al.* 2023), together with the high number of threatened taxa (Cantonati *et al.* 2022).

In the Upper Balma Lake we found a high number of taxa (116), most of which with low abundances (<1%). Light microscopy (LM) observations allow identification to genus level for 27 taxa (24%). The observation in high elevation freshwater ecosystems of species or varieties not yet described has been underlined by other authors (Buczko 2016; Rivera-Rondón & Catalan 2017; Safiallah *et al.* 2020) and recent studies on high elevation, lentic and lotic environments led to description of new species, e.g. *Sellaphora lucectoriana* Solak, S.Blanco, P.B.Hamilton, Peszek 2023 (Solak *et al.* 2023b) and *Orthoseira helvetica* Peszek, C.T.Robinson, M.Rybak & Kawecka 2023 (Peszek *et al.* 2023) or new records for local floras (Kheiri *et al.* 2018, Singh Rana *et al.* 2022).

Traditional morphological analysis of diatoms will be increasingly supported by genetic investigations (DNA metabarcoding), which thus will provide valuable contribution in detecting hidden biodiversity (e.g. Kang *et al.* 2021; Rimet *et al.* 2023). Iconographic publications can play a valuable role in enhancing the description of diatom diversity within these extreme environments (Lange-Bertalot & Metzeltin 1996, Buczko 2016, Rivera-Rondón & Catalan 2017, Kheiri *et al.* 2018).

## Conclusions

High elevation lentic and lotic freshwater ecosystems are characterized by harsh environmental conditions, supporting highly specific aquatic communities, rich in endemic and threatened and yet undescribed taxa. Both direct (e.g., eutrophication, fish introduction) and indirect (e.g., climate change, acidification, microplastics) anthropogenic impacts can affect these pristine ecosystems. Research on biodiversity is critical for understanding ecosystem alteration mechanisms.

Together with genetic studies, iconographic publications can play a valuable role in enhancing the description of diatom diversity within these unique environments.

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