Diatom taxonomic composition of streams and small rivers in the Strouma basin, SW Bulgaria

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Received: May 09, 2007 ▹ Accepted: September 25, 2007

Abstract. One hundred and twenty-two diatom taxa were identified from twenty-nine sites on small rivers and streams in low-inhabited mountain regions in the river Strouma basin (SW Bulgaria). Almost all sites were studied for the first time. Thirty-tree of the taxa were reported for the first time for the Strouma basin, and six taxa (*Achnanthidium atomus, A. minutissimum* var. *affinis, Cymbella excisa* var. *procera, Encyonopsis naviculoides, Gomphonema bohemicum* and *Nitzschia* aff. *sociabilis*) were newly found for Bulgaria. Raphid pennate species predominated. The species-richest genera were: *Nitzschia*, *Navicula*, *Gomphonema* and *Achnanthidium*. The most common and abundant taxon found at almost all sites was *A. minutissimum*. Similarity between diatom compositions of sites was not high (total similarity was below 25 %, maximum similarity between pairs was below 60 %). According to the physicochemical characteristics and diatom composition, high mountain sites differentiate from the lower sites. Thirty-nine taxa are presented in 158 microphotographs.

Key words: Bulgaria, diatoms, mountains, rivers, streams, Strouma

Introduction

Intensive studies of Bulgarian river diatoms have been carried out in the last several years. The rivers in the south and west part of the country were mainly subject to these researches. The diatom taxonomic composition of river Strouma is known from a score of sampling sites situated on its main watercourse (Ivanov & al. 2006). The river flows mainly trough urbanized territories from its springs to the national border. Its tributaries in the surrounding mountainous regions have not been studied, except for some springs in Mt Ograzhden (Temniskova-Topalova & Misaleva 1982). Research on the diatom flora of the main tributaries – small rivers and streams in non- to low-urbanized parts of the Strouma basin -began in 2004. Almost all sites were studied for the first time for diatoms. The goal of this study is to increase the taxonomic knowledge of diatoms in the Strouma basin as a basis for estimation of the ecological status of water with diatom-based methods.

Materials and methods

Twenty-nine epilithic diatom samples were collected from different sites in the Strouma basin (SW Bulgaria) during the period April–May 2005 (Fig. 1, Table 1). Almost all sites were located on small to medium-sized streams and rivers in the mountain regions, before the main towns and villages in the Strouma basin. Sites no. 1 to 6 belong to Mt Vitosha region, 1320–800 m. Site no. 6 is located in the highly anthropogenically affected stretch of river Strouma, after Stoudena dam. Sites no. 7 to 12 fall into Mt Kraishte region, 760–510 m. Site no. 11 is situated at a high-

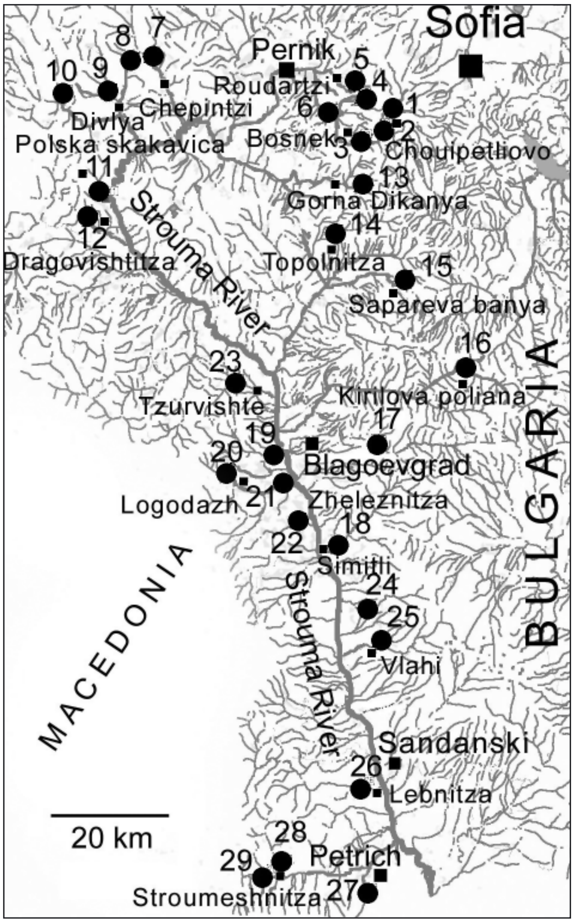


Fig. 1. Study area and sample sites – numbered black circles. Towns and villages are marked with black squares. For details see also Table 1.

ly carbonated stream Golemi Dol, starting from the nearest karst spring and forming a huge travertine terrace. Sites no. 13 and 14 belong to Mt Verila region, 730–660 m. Sites no. 15 to 18 are included in the Rila Mts region, at altitudes 1470–330 m. Sites no. 19 to 23 belong to Mt Vlahinska, at altitudes 670–410 m. Sites no. 24 and 25 belong to the Pirin Mts region, 650–570 m. Sites no. 26, 28 and 29 belong to Mt Ograzhden, 240–160 m. Site no. 29 is situated on a trans-border river, coming from Macedonia. Site no. 27 falls into Mt Belasitsa region, 340 m. Twenty-eight of the sites (excluding no. 3) and 25 out of all 27 water bodies (excluding the rivers Strouma and Lebnishka) were studied for the first time.

Sampling and laboratory pretreatment of the diatoms were carried out according to EN 13946/2003 (*Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*). Benthic (epilithic) diatoms were sampled from cobbles, pebbles or other available stone substrata and fixed with 4 % formaldehyde. Approximately 10 cm^2 of each cobble (or other substrate) were scraped. Pretreatment of diatom samples was made according to A.5.2 method of EN 13946/2003 – with cold sulfuric acid (H_2S0_4) and potassium permanganate ($KMnO_4$).

Table 1. Studied sites, rivers/streams, position and measured physicochemical factors.

No. of site	River/stream	Locality	Position, Lat/Lon grad min sec	Elevation, m	T, °C	pH	Cond., µS/cm	Phosph., mg/l
1	stream	before Choupetlovo	N42 31 29.1 E23 14 55.8	1320	8.0	7.42	52	0.18
2	Strouma	Choupetlovo	N42 30 50.1 E23 14 25.0	1200	7.9	7.52	48	0.04
3	Strouma	before Bosnek	N42 29 50.4 E23 11 57.2	1050	13.4	7.73	159	0.15
4	Mutnitsa	before Kladnitsa	N42 33 36.2 E23 11 46.1	1230	7.6	6.57	56	0.14
5	Roudarska	before Roudartsi	N42 34 38.0 E23 10 56.1	970	8.6	7.62	82	0.09
6	Strouma	Stoudena	N42 32 04.1 E23 08 01.9	800	12.7	8.20	201	0.89
7	Svetlya	before Chepintsi	N42 37 17.5 E22 46 03.0	720	6.1	8.15	482	2.15
8	stream	before Gorna Vrabcha	N42 36 45.3 E22 43 34.0	760	10.0	8.36	442	2.75
9	Penkyovska	before Divlya	N42 33 57.2 E22 40 52.6	680	7.3	8.34	447	0.27
10	Treklyanska	before Treklyano	N42 33 53.6 E22 35 25.5	760	7.2	7.69	111	0.64
11	Golemi Dol	after Polska Skakavitsa	N42 24 45.5 E22 41 08.8	510	–	–	–	–
12	Dragovishtitsa	Dragovishtitsa	N42 22 29.1 E22 38 34.0	530	8.7	8.13	199	2.08
13	stream	before Gorna Dikanya	N42 25 38.6 E23 11 42.0	730	15.3	7.46	202	0.09
14	Topolnitsa	Topolnitsa	N42 21 14.2 E23 08 26.6	660	12.7	7.67	192	0.11
15	Dzherman	Sapareva Banya	N42 17 07.0 E23 16 55.2	790	10.3	7.82	89	0.06
16	Rilska	Kirilova Polyana	N42 09 09.3 E23 24 18.0	1470	4.4	7.46	24	0.00
17	Bistritsa	before Bistritsa	N42 02 15.4 E23 13 39.6	750	8.1	7.10	25	0.02
18	Gradevska	beforer Simitli	N41 53 01.2 E23 08 52.2	330	11.0	8.33	135	0.00
19	Lisiiska	Bulgarchevo	N42 01 16.7 E23 01 12.7	410	14.1	8.28	400	0.07
20	stream	before Logodazh	N41 59 40.9 E22 55 19.2	670	9.5	7.98	416	0.11
21	Leshnishka	Pokrovnik	N41 58 41.3 E23 02 15.9	410	17.4	8.54	634	0.05
22	Stara	Zheleznitsa	N41 55 15.6 E23 04 01.6	480	16.7	8.55	324	0.01
23	Koprivlen	before Tzurvishte	N42 07 46.5 E22 56 23.5	550	21.2	8.49	386	0.12
24	stream	Oshtavska Banya	N41 47 17.3 E23 12 26.2	650	15.0	7.85	188	0.00
25	Kosovska	Vlahi	N41 44 33.4 E23 13 57.7	570	14.0	8.08	192	0.00
26	Lebnitsa	before Lebnitsa	N41 31 39.1 E23 12 25.1	160	19.0	8.35	157	0.21
27	Petrichka	before Petrich	N41 22 50.0 E23 12 26.9	340	11.8	8.35	299	0.01
28	stream	Stroumeshnitsa	N41 24 26.3 E23 02 25.5	240	22.0	8.38	278	0.12
29	Stroumeshnitsa	before Stroumeshnitsa	N41 23 35.5 E23 00 41.7	180	17.0	7.79	316	0.95

Light microscopy and identification were carried out according to EN 14407/2004 (*Water quality. Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from rivers*). The cleaned material was mounted on permanent slides with Naphrax. Light microscopy was performed with Olympus BX51, Amplival Carl Zeiss Jena and Boeco microscopes equipped with 100× oil-immersion objectives and digital camera for light microphotographs. At least 300 valves per sample were determined and counted. The material was stored in the algal collection of the Department of Botany of the St Kliment Ohridski Sofia University. The diatoms were determined mainly according to Krammer & Lange-Bertalot (1986–1991), Lange-Bertalot & Krammer (1989), Krammer (1997a, b, 2000, 2002, 2003), Lange-Bertalot (2001). The taxonomy of diatoms was according to Round & al. (1990), with some additions according to Bukhtiyarova & Round (1996) and Krammer (2003).

Conductivity, pH, temperature and phosphate phosphorus of the water were measured with Hanna instruments, respectively Primo 5, pHep3 and multiphotometer C200.

To compare physicochemical conditions at the different sites, a principal component analysis (PCA) and complete linkage hierarchical cluster analysis were performed. To characterize the diatom distributional patterns, a complete linkage hierarchical cluster analysis and non-metric multidimensional scaling (MDS) were used. The cluster analysis and MDS included all diatoms and their relative abundance and Bray-Curtis similarity was used, with square root data transformation. Computer programme PRIMER v.5 (Clarke & Warwick 2001) was applied for the calculations.

Results and discussion

Physicochemical characteristics

The measured physicochemical parameters are presented in Table 1. The temperature varied in a wide range: from 4.4 °C at site no. 16 (river Rilska at Kirilova Polyana), to 22 °C at site no. 28 (stream at Stroumeshnitsa). The pH ranged within 2 units: from slightly acid to slightly alkaline – 6.57 at site no. 4 (stream Mutnitsa before Kladnitsa) to 8.55 at site no. 22 (river Stara at Zheleznitsa). Conductivity varied within 24 mS/cm at site no. 16 (river Rilska at Kirilova Polyana) to 634 mS/cm at site no. 21 (stream Leshnishka at Pokrovnik). The concentration of phosphate phosphorus ranged from 0.0 mg/l at sites no. 16 (river Rilska at Kirilova Polyana), no. 18 (river Gradevska before Simitli), no. 24 (stream at Oshtavska Banya), and no. 25 (river Kosovska at Vlahi), to 2.75 mg/l at site no. 8 (the stream before Gorna Vrabcha). Fig. 2 represents the PCA plot of the sites, according to their physicochemical characteristics and elevation. The first two axes, PC1 and PC2, explain 80.7 % of the cumulative variation. The first axis PC1 (estimation by sight and percent variation respectively: 2.76 and 55.1) has a positive correlation with temperature, pH and conductivity, and a negative correlation with elevation (linear coefficients respectively: 0.441, 0.538, 0.478, and -0.526). The second axis PC2 (estimation by sight and percent variation respectively: 1.28 and 25.6) has the highest positive correlation with phosphorus, and a negative correlation with temperature (linear coefficients respectively: 0.803 and -0.475).

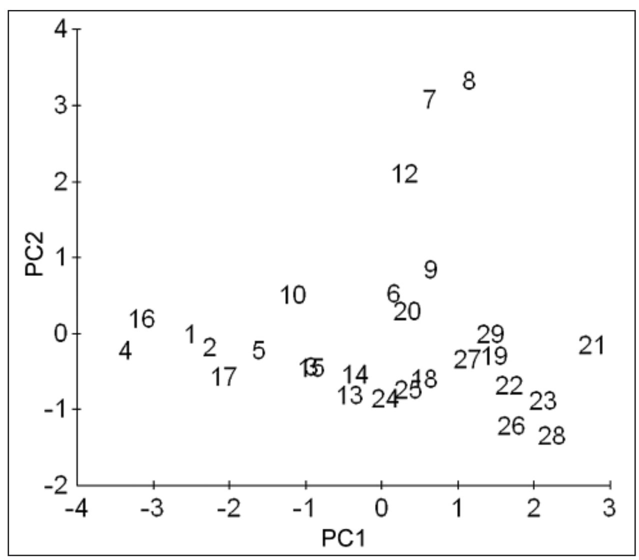


Fig. 2. PCA plot of the sites according to their physicochemical characteristic and elevation.

The cluster dendrogram of the sites according to their physicochemical characteristics and altitude is presented in Fig. 3. Two main groups occur differentiating the sites of the Vitosha and the Rila Mts (subcluster A1 on the dendrogram) from the others (subcluster B1 on the dendrogram). Also, some pairs (and groups) of samples with high similarity based on geographical proximity were observed (1, 2 and 4; 3 and 5; 7 and 8; 19, 21, 22, and 23; 26, 28 and 29). The results from PCA and cluster analysis represent the natural gradient caused by the physical processes along the stream water flows and differentiate the high mountain sites of Mt Vitosha and Rila Mts from the other lower sites.

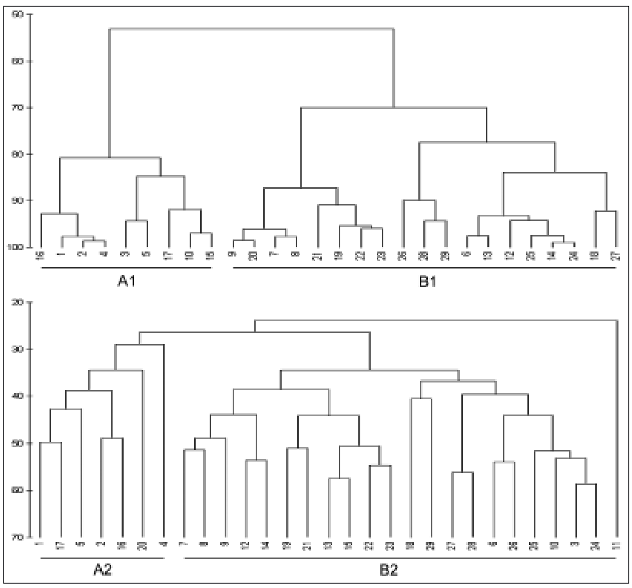


Fig. 3. Cluster dendrograms of the similarity sites: up – based on the physicochemical characteristic; down – based on the diatom composition of the sites. A1, B1, A2 and B2 – subclusters. On the abscissa – similarity, %.

Diatom composition and distribution

Epilithic diatom taxonomic composition of the studied sites was presented by 122 species, varieties and forms from 38 genera. Thirty-nine of the taxa are presented in 158 microphotographs (Plates I & II). Thirty-three of the taxa (27 species and 6 varieties) were reported for the first time for the Strouma basin and 6 taxa (4 species and 2 varieties) were reported for the first time for Bulgaria (Table 2). Raphid pennate species (class *Bacillariophyceae*) predominated: 108 species, varieties and forms (89 % of all) from 33 genera (87 % of all). The most abundant genera of the class *Bacillariophyceae* were: *Nitzschia* Hassal – 18 taxa, *Navicula* Bory de St. Vincent – 17 taxa, *Gomphonema* Ehrenb. – 13 taxa, *Achnanthidium* Kütz. – 8 taxa, *Cocconeis* Ehrenb. – 5 taxa, *Cymbella* C. Agardh – 4 taxa. Araphid pennate species (class *Fragilariophyceae*) were presented by 14 species, varieties, forms and morphotypes (11 % of all) from 5 genera (13 % of all). The most abundant genera of class *Fragilariophyceae* were: *Fragilaria* Lyngb. – 6 taxa and *Diatoma* Bory – 5 taxa, the other 3 taxa of the class were presented by a single species. Centric diatoms (class *Coscinodiscophyceae*) were not observed in the samples.

There were two unidentified species (one of them presented in Plate II, 16-21 – *Encyonopsis* sp.), and three taxa were probably determined up to species level (aff.) – Table 2, Plates I & II. All valves of *Diatoma moniliformis* Kütz. found in the samples no. 8 and 26 (respectively 4.7 % and 0.8 % of the valves) were teratological (curved in valve view at one or both distal ends). This phenomenon was also observed in two samples from the river Strouma at Ruzhdavitsa and Nevestino in March 2003 (Ivanov unpubl.).

Fifteen diatom taxa were found in more than half of the sampling sites (Table 2). The most common diatoms were recorded with percentage of the sampling sites in which they exist: *Achnanthidium minutissimum* (Kütz.) Czarnecki (97 %), *Encyonema minutum* (Hilse) Mann (76 %), *Amphora pediculus* (Kütz.) Grunow (76 %), *Gomphonema rosenstockianum* Lange-Bert. & Reichardt (72 %), *Reimeria sinuata* (Gregory) Kociolek & Stoermer (69 %), *Nitzschia dissipata var. dissipata* (Kütz.) Grunow (69 %), *G. pumilum* var. *elegans* Reichardt & Lange-Bert. (66 %), *Planothidium frequentissimum* (Lange-Bert.) Lange-Bert. (62 %), *P. lanceolatum* (Bréb.) Round & Bukhtiyarova (59 %), *Navicula lanceolata* (C. Agardh) Ehrenb. (59 %), *N. reichardtiana* Lange-Bert. (55 %), *N. tripunctata* (O.F. Müller) Bory (55 %), *G. olivaceum var. olivaceum* (Hornemann) Bréb. (52 %), *N. gregaria* Donkin (52 %) and *N. cryptotenella* Lange-Bert. (52 %). Forty-one taxa were found in only one site.

Achnanthidium lineare W.M. Smith (Plate I, 15-27) found in two of the sampling sites is probably the same as *Achnanthidium* sp. found earlier in the Rhodopes, in river Trigradska (Ivanov & al. 2006, Plate I, 1-3). Except in these regions, the species was also reported for Bulgaria from river Iskur, above Samokov, approximately at 1000 m (Kawecka 1974). According to the restricted locations (only four known), the taxon seems to be rare for Bulgaria and appears in streams and small rivers in low-inhabited mountain regions, at low temperatures, conductivity and phosphorus concentration. The potential difficulties in determining its small valves could also affect its finding.

The diatom flora of the studied regions was presented by a different number of taxa (Table 3). The maximum number of taxa (72) was found in the sites (6) from Vitosha region, followed by 62 taxa in the sites (3) from Ograzhden region. The minimum number of taxa (19) was found in the site from Belasitsa region, and 21 taxa were found in the sites (2) from Verila. The maximum number of taxa per sample (41 taxa) were found in site no. 26 from Ograzhden (river Lebnitsa before Lebnitsa) and the minimum number of taxa per sample (9 taxa) in site no. 13 from Verila (the stream before Gorna Dikanya). The number of observed taxa is lower than that reported earlier for the Strouma basin: 203 taxa found in 90 samples from 11 sites (mostly from the main watercourse of Strouma) during the period 2000–2005, but some new taxa were found for the Strouma basin and for Bulgaria.

The cluster dendrogram based on the similarities between the diatom composition (taxonomic composition and abundance) of samples is presented in Fig. 3. Generally, the similarity between the diatom compositions of samples was not high and there was not any high number of similar samples. Total similarity between samples is less than 25 % and maximum similarity between some pairs of samples is below 60 %. Two main groups of samples plus one separated sample (no. 11 – Skakavitsa stream) were observed. The smaller group (subcluster A2 on the dendrogram) combines seven samples (four from Vitosha, two from Rila and one from Mt Vlahinska). The bigger group (subcluster B2 on the dendrogram) combines the other 21 samples. Some pairs (and groups) of samples based on geographical proximity were observed (7, 8 and 9; 19 and 21; 22 and 23; 27 and 28). Similarity of sample grouping was observed between the two dendrograms: taxonomic and physicochemical. Six out of seven samples of the taxonomic subcluster A2 were presented in the physicochemical subcluster A1 forming the clusters of higher-altitude samples. The coincidence between the results from physicochemical and taxonomic analyses confirms the connection between physicochemical parameters and diatom taxonomic composition.

The MDS plot (stress = 0.2) of the samples with bubble values of some of the most abundant and frequent taxa is presented in Fig. 4. The MDS showed similar tendencies as the cluster analysis. The sites were situated in a 2-dimensional plot regarding the similarities between them. On the left side of the plot, most of the sites from Vitosha and Rila Mts are located (no. 1, 2, 4, 5, 16 and 17). On the upper and on the right sides of the plot, site no. 11 (stream Golemi Dol) showed the same dissimilarity with the others in the cluster dendrogram. *Achnanthidium minutissimum* was the most abundant taxon. The ecological preferences of the species are well known regarding pH, oxygen and saprobity: respectively neutrophilous, continuously high oxygen and beta-mesosaprobic (Van Dam & al. 1994). This characterized the ecological conditions in most sites as close to the natural status.

Ceratoneis arcus was most abundant in samples no. 2 (Strouma at Choupetlovo) and no. 16 (Rilska at Kirilova Polyana) differentiating these sites from the others. The taxon is rheophilous (nitrogen-autotrophic sensible, oligo-mesotraphentic, beta-mesosaprobic, with continuously high oxygen demand according to Van Dam & al. (1994)). *Cymbella excisa* (alcaliphilous, nitrogen-autotrophic sensible, beta-mesosaprobic, with continuously high oxygen demand) and *Eolimna subminuscula* (alcaliphilous, obligate nitrogen-heterotroph, alfa-meso-polysaprobic, with low oxygen demand) were the dominants at the isolated sites no. 11 and no. 29 respectively. The latter site was one of the lowest sites, situated on a trans-border river (from Macedonia) with the highest impact of all studied sites. Unfortunately, there were no data about the physicochemical conditions of the water at site no. 11 which is also differentiated on the cluster dendrogram and is situated at a highly carbonated stream Golemi Dol. *Gomphonema olivaceum* (alcalibiont obligate nitrogen-autotrophic tolerant, beta-mesosaprobic, with fairly high oxygen demand) was most abundant at most of the sites from Kraishte and Verila.

Generally, no high similarities of the taxonomic composition and abundance of the diatoms between different sites were observed. The studied samples showed diverse taxonomic composition of the tributaries in the different regions of the Strouma basin. As compared to an earlier study of different habitats in Mt Ograzhden (Temniskova-Topalova & Misaleva 1982), great differences were found in the taxonomic composition of diatoms. No centric diatoms, *Campylodiscus* and *Epithemia,* were found in our samples and the genera *Eunotia, Stauroneis, Pinnularia,* and *Surirella* were presented with very few species, as compared to the earlier reports. On the contrary, the order *Achnanthales* was very rich in taxa and abundant in most of the studied sites than the earlier reported. Different types of habitats in both studies caused these differences. The established diatom composition rather corresponds to some of the earlier reported similar habitats in the Rila Mts (Kawecka 1974) and the Mesta basin (Passy-Tolar & al. 1999; Ivanov & al. 2006).

Table 2. Taxonomic composition of the diatoms found on the sites and frequency of occurrence of taxa F, %

Taxa / Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	F. %
Achnanthes petersenii Hust.*					4.4	0.7																								6.9
Achnanthidium atomus (Hust.) O. Monnier, Lange-Bert. & Ector**																2.7		58.5							1.2					10.3
A. eutrophilum (Lange-Bert.) Lange-Bert.									1.8																					3.4
A. lineare W.M. Smith*				19.5												7.5		0.0												6.9
A. minutissimum (Kütz.) Czarn.	41.8	8.6	32.6	9.1	49.8	4.4	13.2	25.9	7.0	14.5	3.6	0.7	41.2	5.2	59.8	15.9	44.3	2.7	42.8	47.6	23.1	22.5	30.3	34.3	5.8	3.7	61.2	59.9		96.6
A. minutissimum var. *affinis* (Grunow) Bukhtiyarova**	6.4										1.8																			6.9
A. minutissimum var. *jackii* (Kütz.) Czarn.*					20.4																									3.4
A. pyrenaicum (Hust.) Kobayasi			0.8	1.0		13.8		6.5	1.8		8.4	73.1						4.3					3.4		3.7	5.3	7.3	4.2		44.8
A. subatomus (Hust.) Lange-Bert.	5.9		2.1	8.1											2.3	0.9	10.8	1.1	0.9				1.3			1.2		0.6		37.9
Adlafia minuscula (Grunow) Lange-Bert.			0.8			3.6	0.4	0.4		0.7			2.6	0.4					2.6	7.9	0.6		2.6			0.4				41.4
A. minuscula var. *muralis* (Grunow) Lange-Bert.*																											2.6	1.4		6.9
Amphora copulata (Kütz.) Schoemann & R.E.M. Archibald																												0.6		3.4
A. pediculus (Kütz.) Grunow	1.8		0.4			2.2	7.8	2.9	1.8	3.6	0.6	1.0		4.8			1.0	1.6	2.2	4.3	1.6	0.3		1.9	5.0	2.0	2.2	0.6	18.4	75.9
Caloneis bacillum (Grunow) Cleve	0.5	0.4				0.4	1.2	0.7			0.6													1.1	0.4	0.4				31.0
Ceratoneis arcus (Ehrenb.) Kütz.	3.2	59.6	2.1			0.4				0.7					1.9	46.0	2.1						0.4					0.6		34.5
Cocconeis pediculus Ehrenb.																		0.5										0.6		6.9
C. placentula var. *euglypta* (Ehrenb.) Grunow			1.2	1.0		0.7				1.4		1.0		0.9		0.9			5.7					2.2	3.7		6.2	5.9	5.4	44.8
C. placentula var. *lineata* (Ehrenb.) Van Heurck	0.5	0.4														0.9												0.6	0.3	17.2
C. placentula var. *placentula* Ehrenb.	0.9		0.4				0.4			0.7	0.6			0.4			1.5		4.4					0.4	3.3	2.0	3.7	3.1	8.4	48.3
C. placentula var. *pseudolineata* Geitler	0.5	0.4			0.2				0.6	1.4							0.5								1.2		1.5	0.6		31.0
Cyclotella ocellata Pant.					0.2																									3.4
Cymbella compacta Østrup							2.5				1.2										1.0	2.0	0.9		1.7			0.8		24.1
C. excisa Kütz.											47.9								7.4		10.9	7.0	1.3							17.2
C. excisa var. *procera* Krammer**											7.2																			3.4
C. helvetica Kütz.							0.4				2.4																			6.9
Cymbopleura naviculiformis var. *naviculiformis* (Auersw.) Krammer*	1.4																													3.4
Diadesmis perpusilla (Grunow) D.G. Mann		0.4			0.2											0.9	1.0													13.8
Diatoma ehrenbergii Kütz.																						0.3								3.4
D. hyemalis (Roth) Heib.		1.1			0.2													0.5												10.3
D. mesodon (Ehrenb.) Kütz.	2.7	6.4	2.1	0.3	1.4											9.7	6.7	0.5		8.3					0.8		0.7	0.3	0.3	44.8
D. moniliformis Kütz.							1.2				0.6							0.5		0.8	0.3							0.8	3.6	24.1

Table 2. Continuation

Taxa / Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	F, %
D. moniliformis Kütz. - teratological								4.7																		0.8				6.9
D. vulgaris Bory																							2.1					0.3		6.9
Diploneis oblongella (Nдgeli) A. Cleve*																		0.5		0.4										6.9
D. ovalis (Hilse) Cleve*																		0.5												3.4
Encyonema minutum (Hilse ex Rebenh.) D.G. Mann	5.5	8.2	9.9	0.7	11.8	0.4	0.4	0.7			3.6	0.3		0.4	1.5	1.3	8.8	1.1	3.1			1.4	0.9	6.7	0.8	2.4		2.8		75.9
E. reichardtii (Krammer) D.G. Mann *						1.5				0.7								0.5								0.4				13.8
E. silesiacum (Bleisch) D.G. Mann		0.7	1.2		0.5					0.7							3.1								3.7	3.7		0.3	0.3	31.0
E. ventricosum (C. Agardh) Grunow								0.7											0.9		43.3	0.3								13.8
Encyonopsis microcephala (Grunow) Krammer*																					0.3									3.4
E. naviculoides Hust.**											2.4																			3.4
Encyonopsis sp.*											1.8																			3.4
Eolimna minima (Grunow) Lange-Bert.				0.7								0.3		0.9			1.0	1.1						2.6		0.4		0.6	5.1	31.0
E. subminuscula (Manguin) G. Moser, Lange-Bert. & Metzeltin						0.4		0.4			0.6							2.1								2.0			27.4	20.7
Eucocconeis laevis (Østrup) Lange-Bert.		0.4															0.5													6.9
Eunotia exigua (Bréb.) Rabenh.*	5.5															0.9	2.1													10.3
Fragilaria capucina var. *capitellata* (Grunow) Lange-Bert.			0.4								0.6							0.5					0.4					0.8	0.9	20.7
F. capucina var. *capucina* Desm.			0.4				0.4	0.7		2.9			6.2		1.2		1.0							0.7			0.4			31.0
F. capucina var. *rumpens* (Kütz.) Lange-Bert. ex Bukhtiyarova	5.9	3.6			0.7	0.4																			0.8					17.2
F. capucina var. *vaucheriae* (Kütz.) Lange-Bert.		1.1				0.7		1.4												0.8				0.7	2.1	1.2				24.1
F. gracilis Østrup	0.5															0.9													0.3	10.3
F. ulna (Nitzsch) Lange-Bert.																0.4	0.5					0.3			0.4	0.4		0.6		20.7
Frustulia vulgaris (Thwaites) De Toni	1.8				0.2																									6.9
Geissleria decussis (Østrup) Lange-Bert. & Mitzeltin*																										0.4		0.3		6.9
Gomphonema acuminatum Ehrenb.*	1.8																													3.4
G. angustatum (Kütz.) Rabenh.	0.9																													3.4
G. bohemicum Reichelt & Fricke**							0.8																							3.4
G. calcifugum Lange-Bert. & Reichardt		1.1	2.5		0.2				27.5								4.1													17.2
G. micropus Kütz.		0.7	2.5	3.7	0.9	0.7		1.4		0.7	0.6					1.8				0.4			0.4	0.7						41.4
G. minutum (C. Agardh) C. Agardh																		0.5												3.4
G. olivaceoides Hust.*				5.4	0.2																	0.6					2.9		0.3	17.2
G. olivaceum var. *olivaceum* (Hornem.) Bréb.	0.9	0.4				15.3	8.6	31.7	11.1		0.6	8.0		21.2			1.5	1.1	3.1	0.8		0.8	5.6							51.7
G. parvulum (Kütz.) Kütz.										0.7																				3.4

Table 2. Continuation

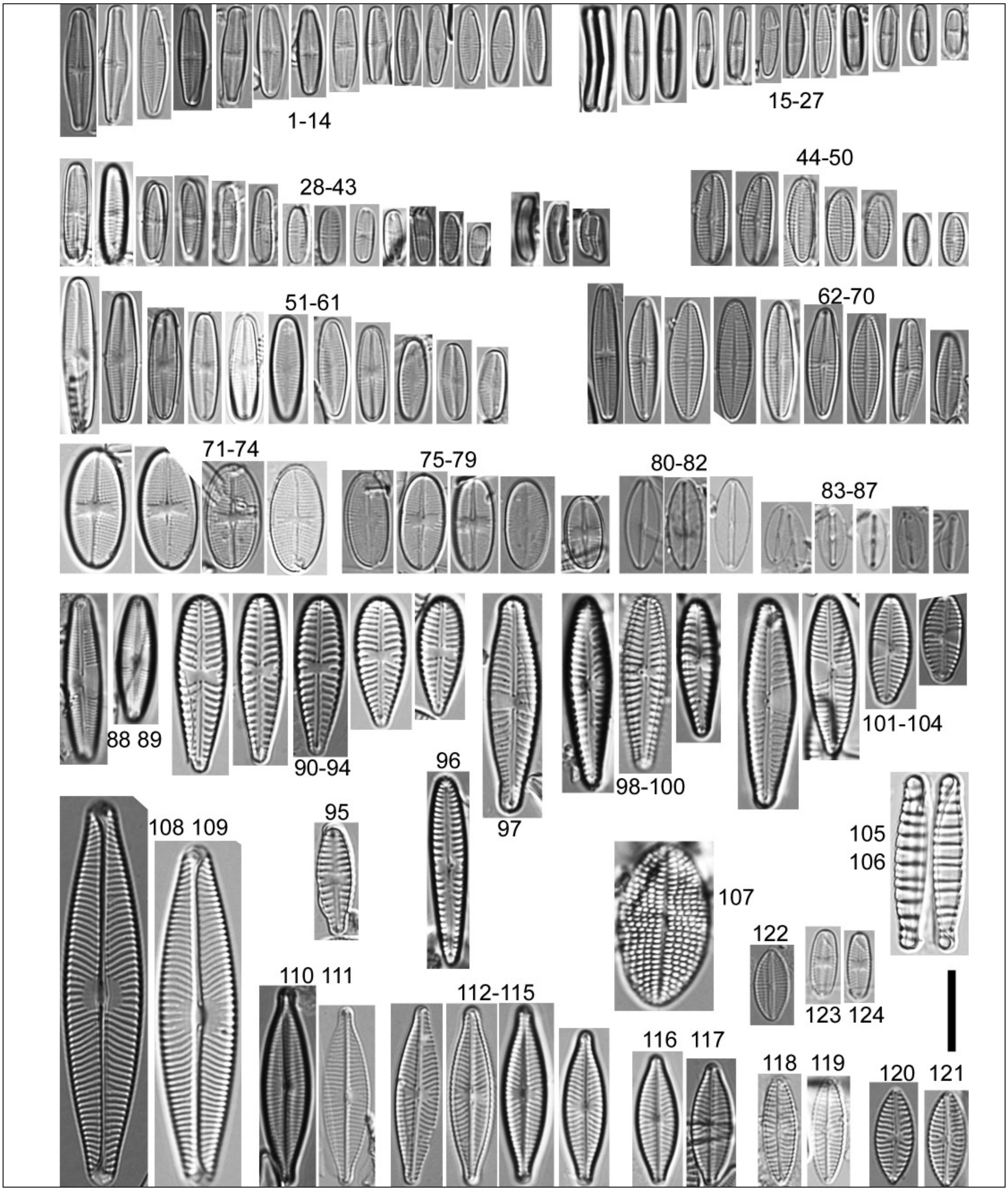
Taxa / Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	F, %
G. pumilum var. *elegans* Reichardt & Lange-Bert.			1.7	21.2	0.2			0.7	0.6	6.5	0.6	1.0		1.7	4.6	0.4		2.7				2.8	3.8	0.4	3.7	0.4	1.1	0.3		65.5
G. rhombicum Fricke*	0.9															7.5														6.9
G. rosenstockianum Lange-Bert. & Reichardt			14.5			3.6	23.0	4.0	40.4	10.1		10.1	38.7	43.7	26.3		1.0	2.7	10.5	1.6	11.9	60.3	34.2		12.4	2.9		1.7	0.6	72.4
G. tergestinum Fricke								0.7										0.5												6.9
Hantzschia amphioxys (Ehrenb.) Grunow																								0.7						3.4
Karayevia laterostrata (Hust.) Kingston*																	0.5													3.4
Lemnicola hungarica (Grunow) Round & Basson																													0.3	3.4
Luticola goeppertiana (Bleisch) D.G. Mann																													0.6	3.4
L. mutica (Kütz.) Mann					0.2																									3.4
Mayamaea atomus (Kütz.) Lange-Bert.*						2.2				1.4														1.1		2.0				13.8
M. atomus var. *permitis* (Hust.) Lange-Bert.						4.7							3.1					1.1	1.7							4.9	0.4		3.3	24.1
Meridion circulare var. *circulare* (Grev.) C. Agardh	0.5	3.2	1.2	3.0	0.7		0.4	0.4	1.2	4.3	1.2		2.1							2.4				1.1						44.8
Navicula aff. *exilis* Kütz.*				1.0																										3.4
N. aff. *harderi* Hust.																						0.3								3.0
N. antonii Lange-Bert.																		0.5		0.4	1.0			2.2	1.7			0.3	0.6	24.1
N. capitatoradiata H. Germ.																										0.4				3.4
N. cryptocephala Kütz.			0.4	2.0		0.7																								10.3
N. cryptotenella Lange-Bert.							10.7	3.2	2.3		1.2	0.3	1.0	0.4				2.1					1.3	2.6	2.5	1.6	2.9	0.8	6.6	51.7
N. erifuga Lange-Bert.*																										0.8				3.4
N. exilis Kütz.*	0.9																													3.4
N. gregaria Donkin	0.5		0.4	0.7	0.2	4.4				2.9				0.9	0.8			1.1					0.9	5.2	1.7	3.7		0.8	0.9	51.7
N. hambergii Hust.																								0.7						3.4
N. lanceolata (C. Agardh) Ehrenb.			1.7		0.2	1.8	1.2			15.9		0.3	3.1	3.0	0.8	0.4		2.1				0.3		3.0	16.9	3.3		0.3	3.3	58.6
N. radiosa Kütz.																			0.4											3.4
N. recens (Lange-Bert.) Lange-Bert.																											0.4			3.4
N. reichardtiana Lange-Bert.	0.9		0.8			4.4	2.5	3.2	0.6									0.5	2.2	0.8	1.0		0.9	1.9	2.1	0.4	1.8	0.6		55.2
N. tripunctata (O.F. Müll.) Bory			0.4				0.8	1.1	0.6		0.6	0.3						1.1	2.2		2.2	0.3	1.3	0.4	1.7		1.8	0.6	0.6	55.2
N. trivialis var. *trivialis* Lange-Bert.*					0.5																									3.4
N. viridula (Kütz.) Ehrenb.																							0.9		0.4			0.3		10.3
Nitzschia acicularis (Kütz.) W.M. Smith																										0.4				3.4
N. acidoclinata Lange-Bert.										1.4		0.3														1.6				10.3
N. aff. *sociabilis* Hust.**							0.8																							3.4
N. capitellata Hust.						1.8																	0.4							6.9
N. debilis (Arn.) Grunow						2.9																								3.4

Table 2. Continuation

Taxa / Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	F, %
N. dissipata var. *dissipata* (Kütz.) Grunow		0.4	0.4	0.3	0.2	5.8	21.4	2.2	0.6	1.4		1.0		1.3			0.5	1.6			1.9		0.9	1.5	0.8	1.6		0.6	1.5	69.0
N. fonticola Grunow					0.2	0.4		1.8			10.8								0.4			0.3	0.9			0.4	0.7			31.0
N. frustulum var. *frustulum* (Kütz.) Grunow*																									2.1					3.4
N. hantzschiana Rabenh.		0.4	0.4																											6.9
N. inconspicua Grunow																		1.6						0.7		22.4	0.7		4.2	17.2
N. linearis var. *linearis* (C. Agardh) W. Smith																							0.9						0.6	6.9
N. palea (Kütz.) W.M. Smith			0.8			4.7		1.4		5.8											0.6		0.4	0.7	2.5			0.3	0.6	34.5
N. palea var. *debilis* (Kütz.) Grunow																										0.8				3.4
N. paleacea (Grunow) Grunow						2.2													1.7							0.8				10.3
N. perminuta (Grunow) M. Peragallo		1.1			0.2	0.7							2.1	0.9					0.9	1.6					3.3	0.8			0.3	34.5
N. recta Hantzsch										0.7																				3.4
N. sinuata var. *tabellaria* (Grunow) Grunow					0.2																									3.4
N. supralitorea Lange-Bert.																										0.4				3.4
Nupela lapidosa var. *lapidosa* (Lange-Bert.) Lange-Bert.*																		0.5												3.4
Pinnularia sp.*	0.9																													3.4
Planothidium frequentissimum (Lange-Bert.) Lange-Bert.	0.5	0.7	0.8	0.3	0.7	0.4		1.8		4.3	0.6	0.7		0.9			1.0		0.4	0.8				4.9	0.4	0.4			0.3	62.1
P. lanceolatum (Bréb.) Round & Bukhtiyarova		0.4	4.1	0.3	0.9	0.4		1.1	0.6	2.9							0.5		1.3	20.9			0.9	2.2	0.4	0.4	1.5		0.6	58.6
Psammothidium bioretii (Germain) Bukhtiyarova & Round	5.0			2.4	1.6		0.4									0.4														17.2
P. grischunum f. *daonensis* (Lange-Bert.) Bukhtiyarova & Round			0.4	1.7	1.9												3.6													13.8
Reimeria sinuata (W. Greg.) Kociolek & Stoermer		0.4	12.4	17.2	0.5	11.3				3.6		1.0		12.6	0.8		2.1	3.2	5.2		0.3	0.3	2.1	19.0	12.8	17.1		7.3	3.6	69.0
R. uniseriata S.E. Sala, J.M. Guerrero & M.E. Ferrario				0.3			0.4											0.5												10.3
Rhoicosphenia abbreviata (C. Agardh) Lange-Bert.									1.2	9.4																0.8			0.6	13.8
Rossithidium pusillum (Grunow) Round & Bukhtiyarova*							0.4																			4.1				6.9
Staurosira pinnata Ehrenb.	1.8	0.4												0.4		0.4														13.8
Surirella brebissonii Krammer & Lange-Bert.						2.2	0.4	0.4	0.6											0.4			0.9			0.4				24.1
S. minuta Bréb.																												0.3		3.4
number of taxa in the sites	28	24	30	22	30	33	24	26	17	26	24	16	9	18	10	19	24	33	21	17	15	17	27	27	31	41	19	35	30	

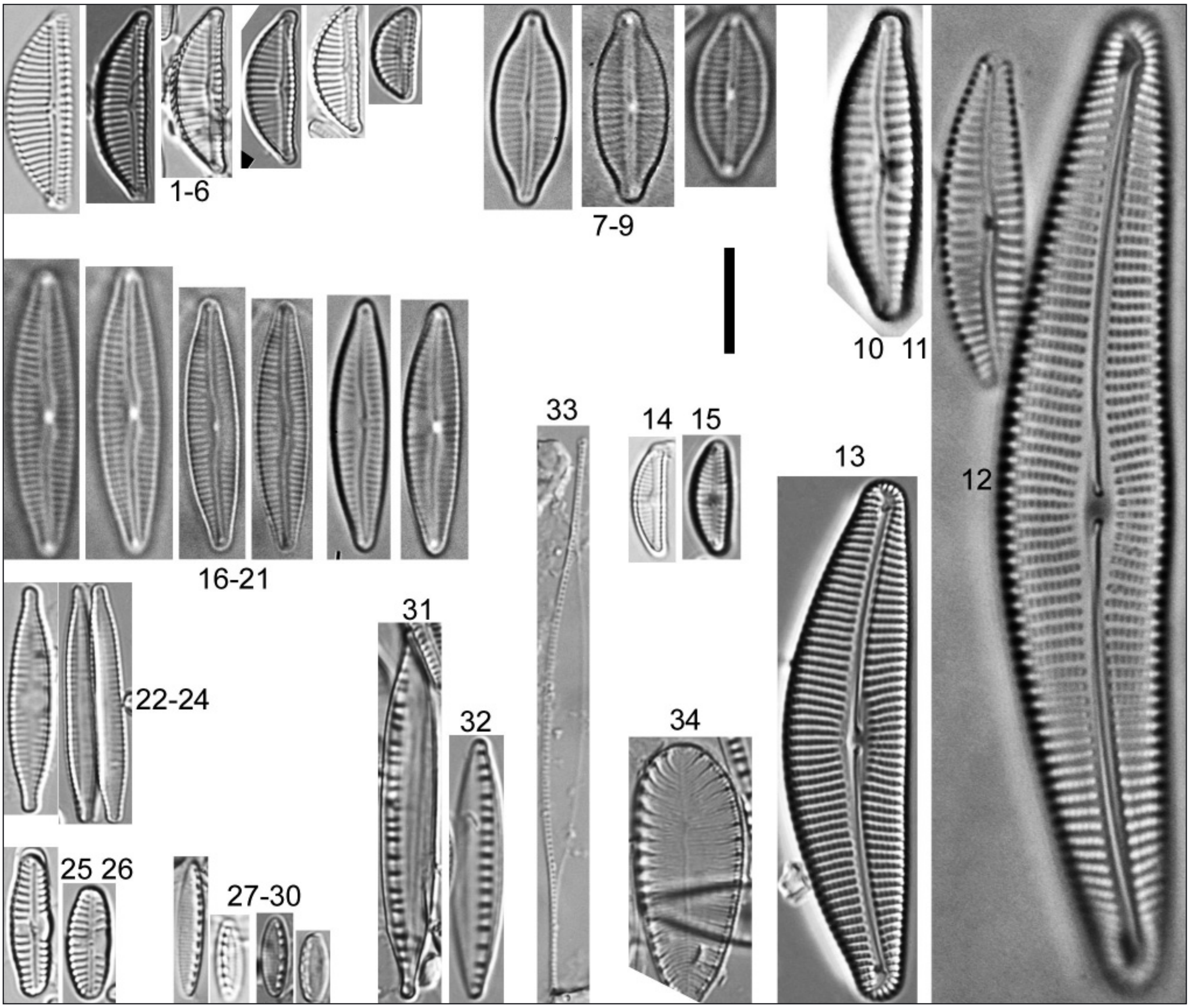
Note: The new taxa are marked with: asterisk (*) for the Strouma basin and with double asterisk (**) – for Bulgaria.

Plate I



Figs 1-124. SEM micrographs of:
1-14, *Achnanthidium minutissimum*; **15-27**, *A. lineare*; **28-43**, *A. atomus*; **44-50**, *A. subatomus*; **51-61**, *Achnanthes petersenii*; **62-70**, *Achnanthidium pyrenaicum*; **71-74**, *Psammothidium bioretii*; **75-79**, *P. grischunum* f. *daonensis*; **80-82**, *Adlafia minuscula*; **83-87,** *Mayamaea atomus* var. *permitis*; **88-89**, *Caloneis bacillum*; **90-94**, *Gomphonema olivaceum* var. *olivaceum*; **95**, *G. calcifugum*; **96**, *G. pumilum* var. *elegans*; **97**, *G. micropus*; **98-100**, *G. minutum*; **101-104**, *G. rosenstockianum*; **105-106**, *Diatoma moniliformis*; **107**, *Cocconeis placentula* var. *pseudolineata*; **108-109**, *Navicula lanceolata*; **110-111**, *N. gregaria*; **112-115**, *N. cryptocephala*; **116-117**, *N. gregaria*; **118-119**, *N. cryptotenella*; **120-121**, *N. antonii*; **122**, *Eolimna subminuscula*; **123-124**, *E. minima*. Scale bar = 10 µm.

Plate II



Figs 1-34. SEM micrographs of:
1-6, *Encyonema minutum*; **7-9**, *Encyonopsis naviculoides*; **10-11**, *Cymbella excisa*; **12**, *C. helvetica*; **13**, *C. compacta*; **14-15**, *Encyonema reichardtii*; **16-21**, *Encyonopsis* sp.; **22-24**, *Fragilaria capucina* var. *capucina*; **25-26**, *Reimeria sinuata*; **27-30**, *Nitzschia inconspicua*; **31-32**, *N. dissipata* var. *dissipata*; **33**, *N. acicularis*; **34**, *Surirella brebissnii*. Scale bar = 10 μm.

Table 3. Number of taxa (total, minimum, maximum and average) in the studied regions and average deviation.

Region	No. of sites	No. of taxa, total	No. of taxa in sites			
			min	mean	max	average deviation
Mt Vitosha	6	72	22	28	33	3,2
Kraishte	6	56	16	22	26	3,8
Mt Vlahina	5	48	15	19	27	3,7
Rila Mts	4	55	10	22	33	7,0
Mt Ograzhden	3	62	30	35	41	3,8
Mt Verila	2	21	9	14	18	4,5
Pirin Mts	2	39	27	29	31	2,0
Mt Belasitsa	1	19	19	19	19	0,0
Total	**29**	**122**	**9**	**24**	**41**	**6,0**

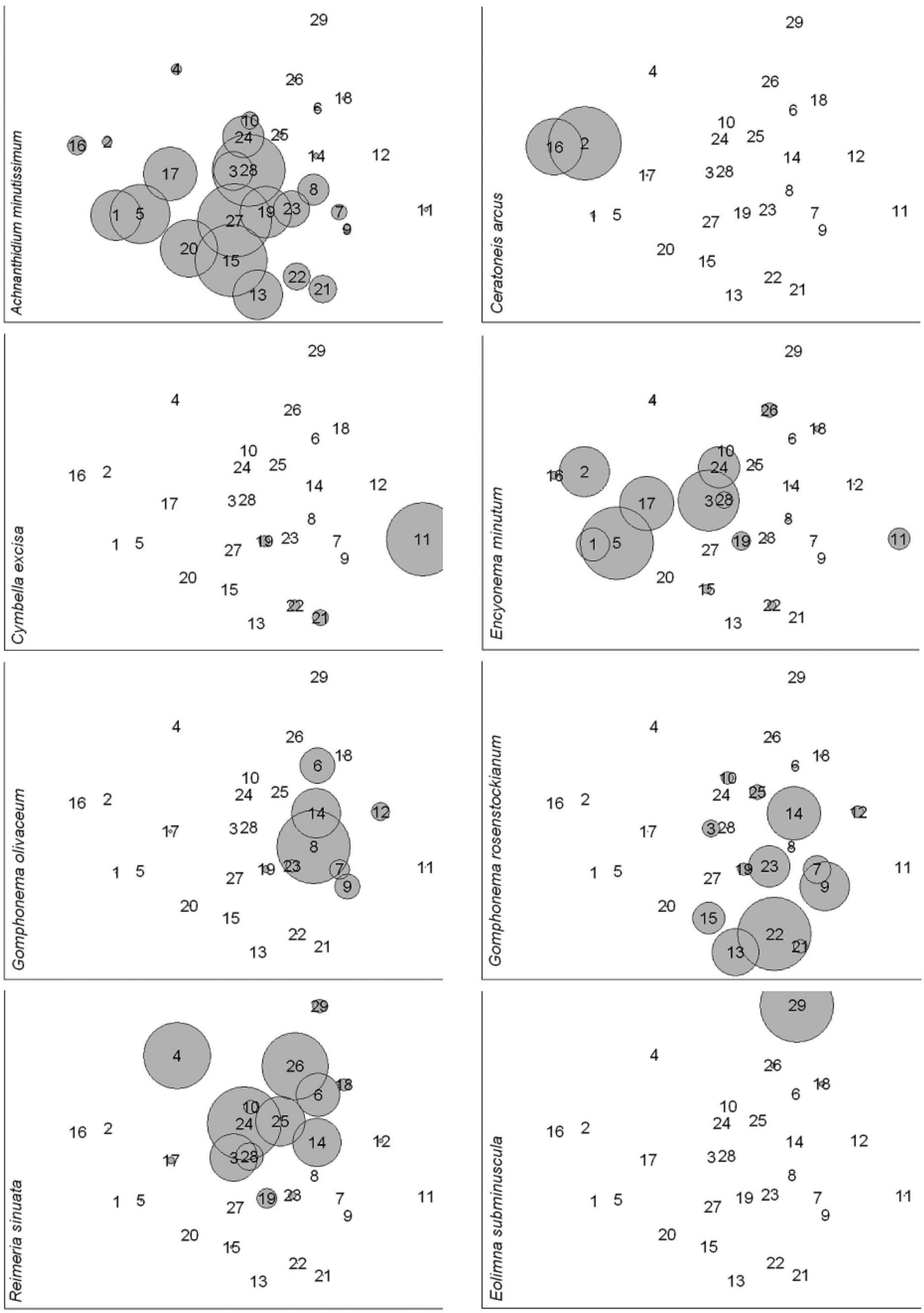


Fig. 4. MDS plot of the samples (stress=0.2), with bubble values of some of the most abundant and frequent taxa.

Acknowledgements. Funding of this research was supported by a scientific project of the Bulgarian Ministry of Education and Science, Contract No MU-B-1411/04.

Light microphotographs were made at the Central Laboratory of General Ecology, on Olympus BX 51 microscope bought under the BioCore project financed by the Bulgarian Ministry of Education and Science.

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