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Distribution and ecology of *Didymosphenia geminata* (Lyngbye) M. Schmidt (Bacillariophyta) in Trentino watercourses (Northern Italy)

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Résumé – Distribution et écologie de *Didymosphenia geminata* (Lyngbye) M. Schmidt (Bacillariophyta) dans les cours d'eau du Trentino (Nord de l'Italie). Depuis 2000, le Parlement Européen et le Conseil de l'Union Européenne recommandent aux pays membres d'évaluer la qualité de l'eau en utilisant les diatomées, en tant qu'élément du phytobenthos. En Italie, cette recommandation a conduit à l'étude des communautés de diatomées et à l'application d'indices biotiques. Pendant l'été 2004, 11 rivières du Trentino (Nord de l'Italie) ont été échantillonnées et ont révélé la présence, en faible abondance, de la diatomée *Didymosphenia geminata* (Lyngbye) M. Schmidt. Les sites où l'espèce est présente sont caractérisés par un faible impact de pollution. Répartis sur des géologies différentes, ils sont situés à l'aval des lacs ou bien possèdent un régime hydrologique régulé. Les traits morphologiques des frustules de *D. geminata* ont été analysés en MO et MEB et les spécimens observés peuvent être rattachés au morphotype « geminata ». Dans de nombreux pays cette espèce voit sa distribution s'étendre et en Nouvelle Zélande elle est considérée comme une algue invasive. Ce travail fournit les premières données sur la présence de *D. geminata* dans la province de Trento et contribue à augmenter la connaissance de sa distribution en Italie et de son écologie dans les Alpes.

***Didymosphenia geminata* / diatomées / indicateurs biologiques / espèces invasives / Italie**

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Abstract – In 2000 the European Parliament and The European Union Council recommended that member countries assess water quality using diatoms, as part of the phytobenthos. In Italy this recommendation has given new impetus to the study of diatom communities and the application of biotic indices. During the summer of 2004, a total of 11 rivers of the Trentino province (Northern Italy) were sampled, revealing the presence in low abundances of the diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt. The sites where this diatom occurred were characterised by a low pollution impact; they were also lake fed or had a regulated flow regime, although with different geologies. Morphological features of *D. geminata* frustules were analysed using LM and SEM and specimens could be referred to the morphotype "geminata". In many countries this species is expanding its distribution and in New Zealand it is considered an invasive alga. This work provides an initial report on the presence of *D. geminata* in the Trento province, and contributes to increasing the knowledge on its distribution in Italy and its ecology in the Alps.

***Didymosphenia geminata* / diatoms / biological indicators / invasive species / Italy**

INTRODUCTION

Directive 2000/60/EC (WFD) (European Parliament, 2000) establishes a framework for Community action in the field of water policy and introduces an holistic vision of aquatic ecosystems, considering both biotic and abiotic elements involved in the definition of the quality of the ecosystem. Among biotic parameters, diatoms (as part of the phytobenthos) are one of the most studied groups. In Italy the Water Framework Directive has given new impetus to the study of diatom communities and the applicability of biotic indices, such as the Eutrophication and Pollution Index with Diatoms (EPI-D) (Dell'Uomo, 2004).

During the summer of 2004, 11 rivers of the Trento province in the Trentino-Alto Adige region (Northern Italy) were sampled and the presence of *Didymosphenia geminata* (Lyngbye) M. Schmidt in A. Schmidt *et al.* (Bacillariophyta) was recorded.

Due to its relatively large size and characteristic shape, *Didymosphenia geminata* is one of the earliest described diatom species. It was first described in 1819 as *Echinella geminata* Lyngbye and in 1899 the genus *Didymosphenia* M. Schmidt in A. Schmidt *et al.* was established. Frustules are heteropolar both in valvar and girdle views, and are usually capitate at the poles (Krammer & Lange-Bertalot, 1997a). The central area is characterized by the presence of one or more stigmata. Several studies have been conducted on its morphological variability: Dawson (1973) gave a detailed description of the frustule structure and Antoine & Benson-Evans (1984) found greater variability in size, number, and distribution of stigmata in the central area for populations from England, Scotland, and Wales compared to those indicated in the literature they used for comparison. Stoermer *et al.* (1986) carried out a quantitative morphological investigation, revealing the presence of several geographically differentiated morphotypes. Metzeltin & Lange-Bertalot (1995) reviewed the genus *Didymosphenia* as a whole and distinguished five species and three morphotypes of *D. geminata*. Kociolek *et al.* (2000) described the ultrastructure of *D. dentata* (Dorogostaisky) Skvortzow & Meyer from Lake Baikal, suggesting a taxonomic differentiation from *D. geminata*. In 2006 a new species, *D. tatrensis* Mrozińska, Czerwik-Marcinkowska & Gradziński, was described from streams of the Western Carpathians of Poland and Slovakia (Mrozińska *et al.*, 2006).

Didymosphenia geminata is an epilithic and epiphytic diatom, attached to the substratum *via* a mucilage stalk secreted from small, unoccluded pores at the base pole of the valves (Round *et al.*, 1990).

Didymosphenia geminata is autochthonous in northern Europe and North America. In Europe it has been recorded in the Czech Republic (Gágyorová & Marvan, 2002), Finland (Kawecka & Eloranta, 1987), Iceland (Jonsson *et al.*, 2000), Ireland (Ellwood & Whitton, 2007), Norway (Skulberg & Lillehammer, 1984), Poland (Kawecka & Sanecki, 2003; Noga, 2003), Serbia (Subakov-Simić & Cvijan, 2004), Spain (Blanco & Ector, 2008), Sweden (Johansson, 1982), Switzerland (Robinson & Kawecka, 2005), and the United Kingdom (Ellwood & Whitton, 2007). Its presence has been mentioned also in Turkey (Kolayli & Şahin, 1998; Şahin, 2001), China (Li *et al.*, 2003) and even in the southern hemisphere (Chile: Asprey *et al.*, 1964; Rivera & Gebauer, 1989; New Zealand: Kilroy, 2004). Although described as typical of the upper mountain reaches of oligotrophic rivers, recent studies have revealed its presence in a wider range of ecological conditions such as mesotrophic or sporadically eutrophic waters of middle river reaches (Krammer & Lange-Bertalot, 1997a; Kawecka & Sanecki, 2003). Figure 1 shows its fossil and recent distributions in Europe as far as is known based on the existing literature. In Italy its presence has been reported in northern regions such as Alto Adige (Cappelletti *et al.*, 2007; Beltrami *et al.*, 2008), Friuli Venezia Giulia (Zorza *et al.*, 2006), Lazio (Giaj-Levra & Abate, 1994), Lombardy (Bonardi, 1888), Piedmont (Battegazzore *et al.*, 2007), and Val d'Aosta (Brun, 1880).

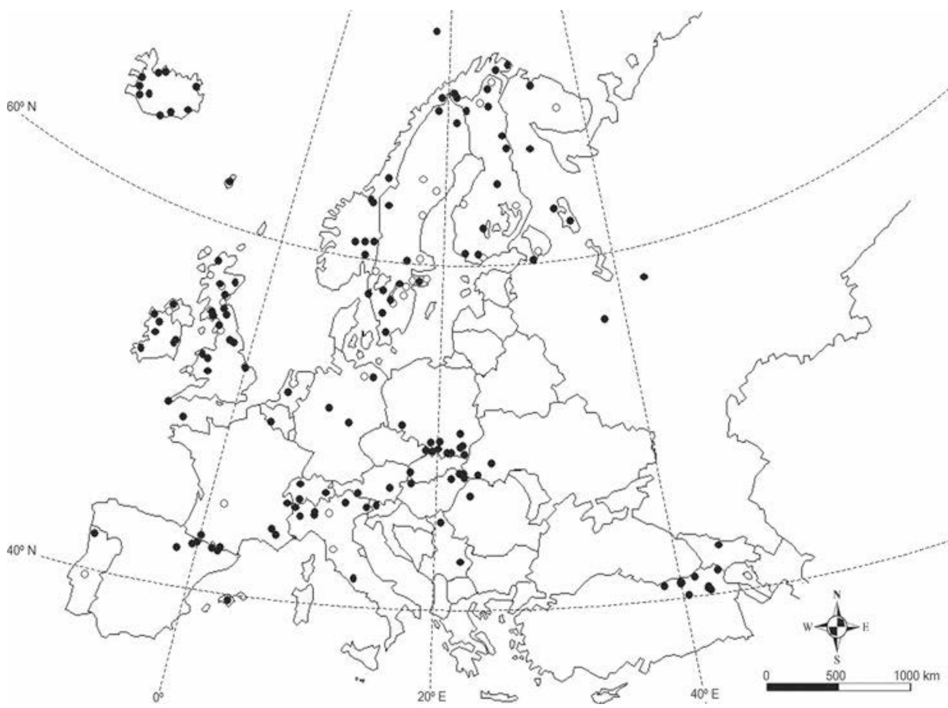


Fig. 1. European distribution of *Didymosphenia geminata*. Black dots: recent records; white dots: fossil or subfossil records.

Massive proliferations of *Didymosphenia geminata* can create benthic mats mainly made up by the long stalks, composed of sulphated xylogalactan, uronic acid and proteins (Gretz, 2007). These events, which are characteristic of this species, are often seasonal and can disappear rapidly (Kawecka & Sanecki, 2003; Ellwood & Whitton, 2007). These "nuisance blooms" (Spaulding & Elwell, 2007) appear to arise under oligotrophic conditions (Jonsson *et al.*, 2000; Sherbot & Bothwell, 1993; Kirkwood *et al.*, 2007); a high phosphatase activity, localized in the stalk, may give a competitive advantage to the species (Ellwood & Whitton, 2007).

In New Zealand, where it was observed for the first time in the Waiau River in 2004 (Kilroy, 2004), *Didymosphenia geminata* is considered as an invasive alga. Since then it colonized 14 river and lake systems in the South Island (Vieglais, 2007), forming "invasive blooms" in many sites (Spaulding & Elwell, 2007). Several actions have been undertaken by Biosecurity to understand the ecology of the species and prevent the spreading in the North Island (Kilroy *et al.*, 2005; Vieglais, 2007). Such blooms seem to have a high impact on the ecosystem: the streambed can be totally covered by *D. geminata* mats, whose massive growth alters macroinvertebrate communities with a dominance of Diptera and a reduction of Ephemeroptera, Plecoptera and Trichoptera taxa (Kilroy *et al.*, 2006; Larned *et al.*, 2006). However, no apparent impact has been noticed on fish communities, neither in Europe, North America or New Zealand (Bothwell *et al.*, 2007; Jonsson *et al.*, 2007; Lindstrøm & Skulberg, 2007; Shearer *et al.*, 2007). Only Larson & Carreiro (2007) found a significant decline in trout adult population in sites where nuisance blooms where observed. According to Mundie & Crabtree (1997) *D. geminata* can cause gill troubles in fry and can limit spawning of trout adults. Furthermore, massive growths can have an economic impact, plugging water filters and limiting the utilization of stream water (Kawecka & Sanecki, 2003; Spaulding & Elwell, 2007).

In Italy, massive benthic mat formations were noticed in September 2006 along the Brusago stream, a small oligotrophic siliceous watercourse in the northeastern area of the Trento province (Beltrami *et al.*, 2007), and in the Erro stream in the Alessandria province (Lombardy) in June 2007 (G. Bona, pers. comm.).

The objectives of this work are to provide a first report on the presence of *Didymosphenia geminata* in the Trento province, to give new data on its distribution in Italy, and to improve our knowledge on its ecological preferences in the Southern Alps.

MATERIAL AND METHODS

River surveys were conducted from June to October 2004 in 11 rivers in Trentino (Northern Italy), for a total of 30 sampling sites (Fig. 2). Physical and chemical characteristics of sites are shown in Table 1, geodata are reported in Gauss-Boaga (W Fuse) on Rome 40 datum (Italian datum).

Chemical analyses were conducted monthly (APAT-IRSA/CNR, 2003).

Standard procedures for diatom sampling were followed (Kelly *et al.*, 1998; European Committee for Standardization, 2003). Diatoms were cleaned using 30% hydrogen peroxide and hydrochloric acid. Cleaned diatom frustules were mounted in permanent slides using Naphrax as mounting medium. Slides were observed using light microscopy (LM) at 1000 magnifications, and

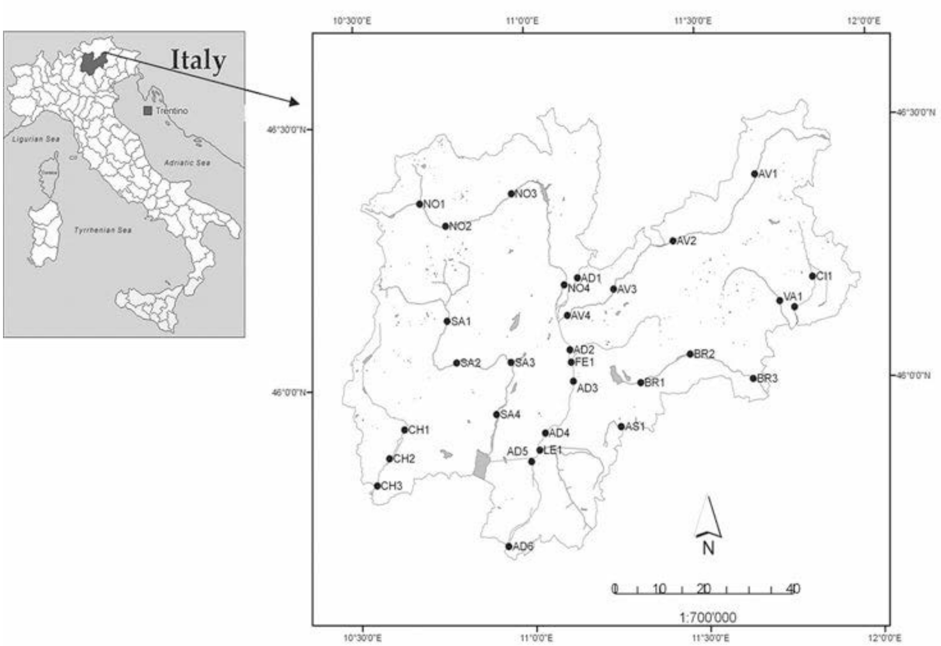


Fig. 2. Sampled sites location in Trento province of Trentino-Alto Adige region (Northern Italy). Site codes refer to Table 1.

species identified according to Krammer & Lange-Bertalot (1991a, b, 1997a, b). At least 400 valves were counted on each slide (European Committee for Standardization, 2004). The OMNIDIA version 4.2 software (Lecointe *et al.*, 1993) was used to calculate the Specific Pollution Sensitivity Index (IPS) (Coste in Cemagref, 1982) and the Eutrophication and Pollution Index with Diatoms (EPI-D) (Dell'Uomo, 2004) for water quality evaluation.

A total of 155 valves of *Didymosphenia geminata,* 100 from the Adige river (site San Michele - AD1) and 55 from the Noce stream (site Mezzolombardo - NO4), were observed using LM and photographed using a Leica DC 300F digital camera. Image analysis software (IM 1000) was used to measure valve length, width, number of striae/10 µm, and number of puncta/10 µm. Scanning electron microscopy (SEM), performed using a Leica Stereoscan 430 operating at 20 kV, was used to analyse valve structures.

In order to assess the ecological preferences of *Didymosphenia geminata* along physical and chemical gradients, weighted averages were calculated for each parameter.

RESULTS

Table 1 shows yearly average values of physical and chemical parameters for all sampling sites: the trophic state of sites ranges from oligotrophic to eutrophic (TP: 0.01-0.08 mg l^{-1}; N-NO_3: 0.5-2.74 mg l^{-1}), with low to medium mineral content and neutral to alkaline pH.

Table 1. Mean annual values of physical and chemical characteristics of sampling sites and their geo-referenced location (Gauss-Boaga, W Fuse on Rome 40 datum) .

Site code	*Watercourse*	*Station*	*Sampling date*	*Latitude (N)*	*Longitude (E)*	*Altitude m*	*Distance from source km*	*Conductivity* $\mu S\ cm^{-1}$	*pH*	$\%O_2$	BOD_5 $mg\ l^{-1}$	*COD* $mg\ l^{-1}$	Cl^- $mg\ l^{-1}$	SO_4^{2-} $mg\ l^{-1}$	*TP* $mg\ l^{-1}$	$N\text{-}NH_4^+$ $mg\ l^{-1}$	$N\text{-}NO_2^-$ $mg\ l^{-1}$	$N\text{-}NO_3^-$ $mg\ l^{-1}$
AD1	Adige	San Michele	26/09/04	1665262	5119199	207	145,9	236	8,0	103	1,9	5,1	4,6	40	0,04	0,04	0,008	0,70
AD2	Adige	Trento	26/09/04	1663600	5104000	193	164,4	238	8,0	102	2,0	5,3	4,9	33	0,05	0,04	0,009	0,82
AD3	Adige	Mattarello	26/09/04	1664385	5097323	188	171,4	243	8,0	99	2,0	5,2	5,6	37	0,06	0,05	0,010	0,84
AD4	Adige	Villa Lagarina	26/09/04	1658081	5086375	173	185,6	249	8,2	96	1,6	6,3	5,3	37	0,04	0,04	0,008	0,81
AD5	Adige	Mori	26/09/04	1655044	5080319	162	193,1	250	8,1	95	1,7	5,7	6,3	34	0,03	0,03	0,010	0,86
AD6	Adige	Avio	26/09/04	1649949	5062387	120	214,0	260	8,1	97	2,6	5,8	5,3	34	0,04	0,06	0,010	1,02
NO1	Noce V. del Monte	Pejo	24/06/04	1629915	5134805	1156	14,7	123	7,8	103	1,5	5,9	1,2	23	0,05	0,02	0,003	0,67
NO2	Noce	Pellizzano	14/06/04	1635700	5130156	924	23,3	95	7,7	103	1,7	5	0,7	23	0,01	0,02	0,003	0,50
NO3	Noce	Cavizzana	24/09/04	1650433	5137001	620	41,7	135	8,0	105	2,2	5,0	2,3	17	0,04	0,06	0,005	0,67
NO4	Noce	Mezzolombardo	26/09/04	1662340	5117761	212	73,9	209	8,2	104	1,8	5,2	3,0	17	0,03	0,02	0,007	0,94
AV1	Avisio	Soraga	24/09/04	1705030	5141225	1208	23,8	346	8,5	105	1,7	9,3	2,7	72	0,08	0,02	0,006	0,80
AV2	Avisio	Castello di Fiemme	24/09/04	1686727	5127050	810	51,9	314	8,5	104	1,8	5,2	4,5	64	0,05	0,02	0,006	0,88
AV3	Avisio	Faver	27/09/04	1673416	5116828	450	73,5	264	8,4	103	1,8	5,0	5,03	38	0,02	0,02	0,003	0,78
AV4	Avisio	Lavis	27/09/04	1663015	5111260	230	88,3	282	8,4	108	2,4	6,1	6,3	38	0,03	0,02	0,006	1,01
FE1	Fersina	Trento	27/09/04	1663901	5101102	194	29,2	261	8,4	104	2,3	7	6,5	36	0,05	0,03	0,008	1,90

Table 1. Mean annual values of physical and chemical characteristics of sampling sites and their geo-referenced location (Gauss-Boaga, W Fuse on Rome 40 datum) *(continued)*.

Site code	*Watercourse*	*Station*	*Sampling date*	*Latitude (N)*	*Longitude (E)*	*Altitude m*	*Distance from source km*	*Conductivity* $\mu S\ cm^{-1}$	*pH*	$\%O_2$	BOD_5 $mg\ l^{-1}$	*COD* $mg\ l^{-1}$	Cl^- $mg\ l^{-1}$	SO_4^{2-} $mg\ l^{-1}$	*TP* $mg\ l^{-1}$	$N\text{-}NH_4^+$ $mg\ l^{-1}$	$N\text{-}NO_2^-$ $mg\ l^{-1}$	$N\text{-}NO_3^-$ $mg\ l^{-1}$
LE1	Leno	Rovereto	4/10/04	1656881	5082793	175	24,9	269	8,6	106	2,0	5,0	3,25	9	0,02	0,02	0,004	0,75
BR1	Brenta	Levico	27/09/04	1679517	5097057	436	4,2	355	7,8	97	1,7	7,6	6,5	30	0,04	0,08	0,013	1,25
BR2	Brenta	Borgo Valsugana	27/09/04	1690592	5103116	353	17,2	372	8,2	104	2,6	6,3	7,1	42	0,06	0,03	0,025	1,75
BR3	Brenta	Grigno	28/09/04	1704769	5097941	243	33,5	260	8,2	103	1,8	5,5	4,2	23	0,03	0,02	0,008	1,30
AS1	Astico	Folgaria	4/10/04	1675132	5087743	615	9,0	318	8,4	102	1,3	5,6	9,25	10	0,07	0,02	0,003	2,74
CH1	Chiese	Pieve di Bono	24/09/04	1626539	5087048	491	31,3	343	8,4	103	1,8	6,6	4,0	47	0,06	0,02	0,010	1,97
CH2	Chiese	Condino	27/09/04	1623164	5080963	410	36,5	325	8,6	111	3,2	5,8	4,2	51	0,02	0,04	0,005	1,10
CH3	Chiese	Storo	13/09/04	1620395	5075191	374	43,4	200	8,1	106	1,7	5	1,8	18	0,02	0,03	0,009	1,04
SA1	Sarca	Caderzone	20/09/04	1636066	5110011	715	23,8	124	8,1	102	1,3	5	2,2	6	0,05	0,02	0,003	0,94
SA2	Sarca	Ragoli	20/09/04	1638183	5101207	480	38,0	203	8,0	102	1,9	5,3	3,3	9	0,06	0,12	0,018	1,05
SA3	Sarca	Calavino	29/09/04	1650378	5101304	257	44,7	238	8,1	104	1,5	5,0	2,7	6	0,03	0,03	0,003	1,38
SA4	Sarca	Dro	27/10/04	1647134	5090300	105	58,5	244	8,6	107	1,0	5	3,3	7	0,03	0,02	0,003	1,33
CI1	Cismon	Siror	23/08/04	1718025	5119592	785	13,0	310	8,4	103	1,1	5	2,5	78	0,01	0,02	0,003	0,67
CI2	Cismon	Imer	23/08/04	1714019	5113088	577	22,1	298	8,5	104	1,9	5	2,0	57	0,02	0,05	0,005	0,69
VA1	Vanoi	Canal S.Bovo	23/08/04	1710660	5114420	632	19,4	65	7,7	103	1,6	5,4	1,4	7	0,01	0,02	0,003	0,62

Analysis of the diatom community and application of IPS and EPI-D suggested that sites had a good water quality. They were classified either as unpolluted or slightly polluted, *i.e.* I or II quality class (Table 2).

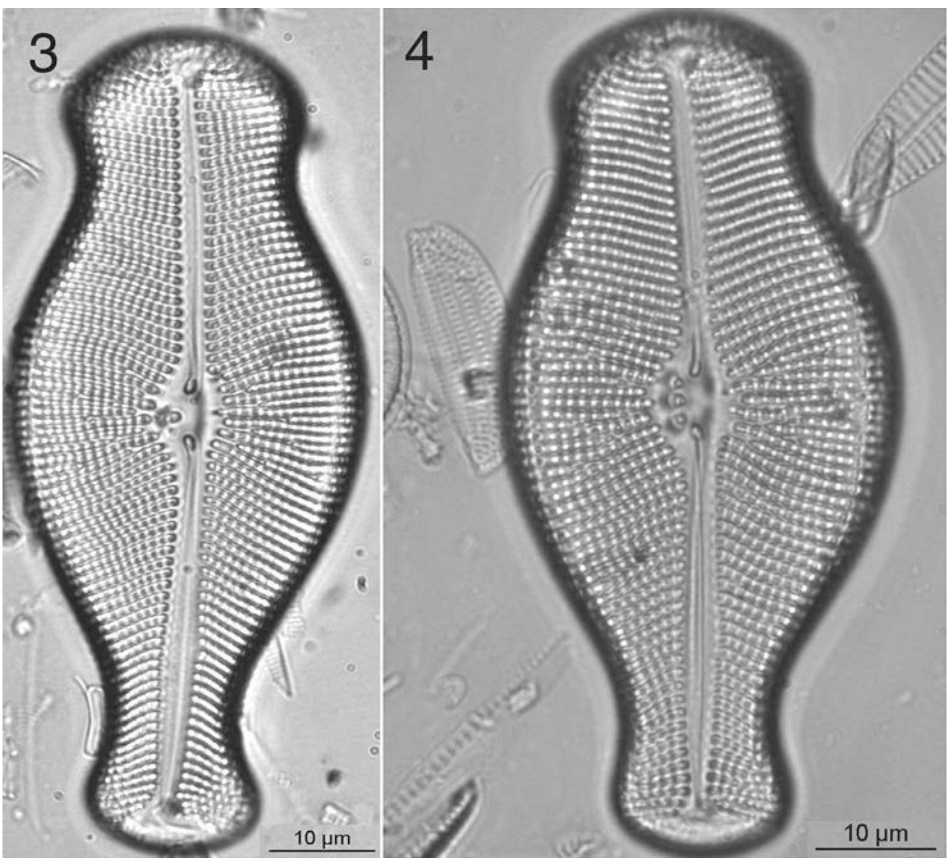
Table 2. Water quality assessed applying IPS and EPI-D indices and *Didymosphenia geminata* occurrence in monitored sites of Trentino Province.

Site code	D. geminata *presence*	*IPS values*	*Quality class*	*EPI-D values*	*Quality class*
AD1	YES	16,4	II	13,7	II
AD2	YES	18,9	I	15,4	I-II
AD3	YES	19,1	I	14,8	I-II
AD4	YES	17,5	I	14,7	I-II
AD5	YES	17,9	I	15,8	I
AD6	YES	18,7	I	16,3	I
NO1	NO	19,3	I	17,0	I
NO2	NO	19,4	I	17,2	I
NO3	YES	19,9	I	17,5	I
NO4	YES	18,1	I	15,6	I
AV1	NO	17,6	I	16,2	I
AV2	YES	18,4	I	16,4	I
AV3	YES	19,0	I	16,6	I
AV4	YES	19,0	I	16,7	I
FE1	NO	16,7	II	14,7	II-I
LE1	YES	19,0	I	17,1	I
BR1	NO	17,6	I	16,5	I
BR2	YES	16,8	II	15,5	I-II
BR3	YES	18,3	I	16,4	I
AS1	NO	17,6	I	16,5	I
CH1	NO	19,6	I	17,3	I
CH2	YES	16,1	II	14,3	II
CH3	YES	19,4	I	17,0	I
SA1	NO	17,1	I	15,3	I-II
SA2	NO	15,4	II	14,1	II
SA3	YES	18,0	I	15,1	I-II
SA4	YES	18,0	I	16,2	I
CI1	YES	19,5	I	17,4	I
CI2	YES	18,2	I	15,8	I
VA1	YES	18,1	I	16,6	I

Didymosphenia geminata (Figs 3 and 4) was found in 21 of the 30 sites monitored (Tab. 2), always with low relative abundance (less than 1%, Appendix 1); during sampling no periphytic mats were observed. It was less frequent in the upper reaches of the sampled streams, being absent in sites with altitude > 900 m and present in less than 50% of sites with distance from source < 40 km (Figs 5, 6). In theTrentino province *D. geminata* had a preference (Table 3) for moderate conductivity, alkaline and oxygen-saturated waters, and was tolerant to medium pollution levels (TP: 0.01-0.06 mg l-1; BOD_5: 1-3.2 mg l-1), with a preference for mesotrophic waters (TP: 0.03 mg l-1).

The LM-based analysis of valve morphological features (Table 4) was compared to data available in the literature. Morphometric ranges were: length 60-106 µm, width 28-40 µm, 8-11 striae/10 µm, 9-12.5 puncta/10 µm; 1 to 4 stigmata.

In some individuals a marginal ridge ending in two spines at the head pole was seen (Fig. 7). SEM images showed that striae were uniseriate and bore large poroids. Areolae were located deeply in the siliceous wall, surrounded by inclined walls, and their openings were circular on the internal valve face (Fig. 11),



Figs 3-4. *Didymosphenia geminata*. LM images from Noce Mezzolombardo site (NO4). Heteropolar valves, capitate at poles, central raphe, one to three central stigmata, the base pole appears translucid for the presence of fine unoccluded pores, striae are uniseriate, slightly radial.

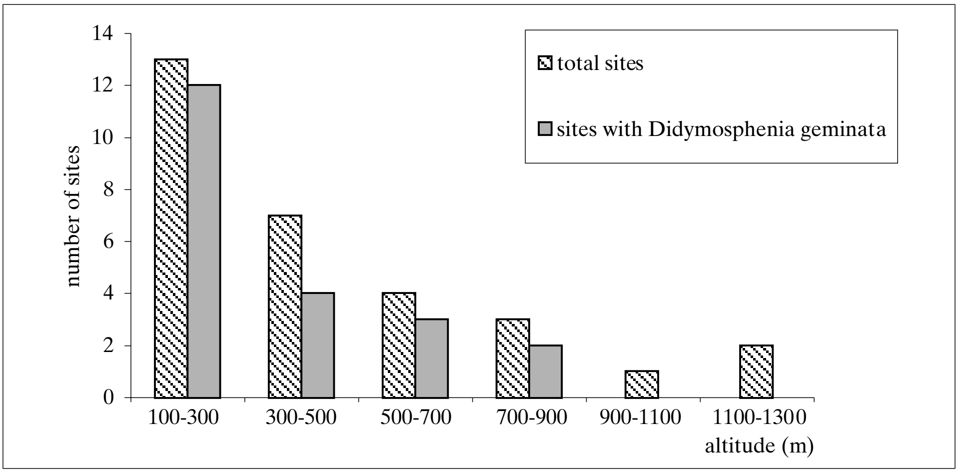


Fig. 5. *Didymosphenia geminata* occurrence in relation to site altitude: the number of sites where it is present is plotted against the total number of sites.

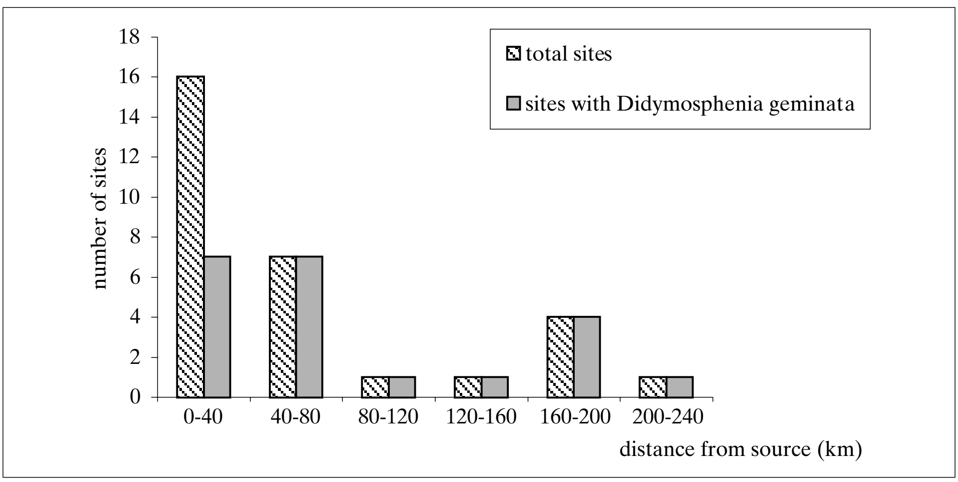


Fig. 6. *Didymosphenia geminata* occurrence in relation to distance from source: the number of sites where it is present is plotted against the total number of sites.

Table 3. Ecological preferences of *Didymosphenia geminata* in monitored sites: weighted averages and range of physical and chemical parameters where it occurred.

	Conductivity $\mu S\ cm^{-1}$	*pH*	$\%O_2$	BOD_5 $mg\ l^{-1}$	*COD* $mg\ l^{-1}$	Cl^- $mg\ l^{-1}$	SO_4^{2-} $mg\ l^{-1}$	*TP* $mg\ l^{-1}$	$N\text{-}NH_4^+$ $mg\ l^{-1}$	$N\text{-}NO_2^-$ $mg\ l^{-1}$	$N\text{-}NO_3^-$ $mg\ l^{-1}$
Minimum	65	7,7	95	1,0	5,0	1,4	6	0,01	0,02	0,003	0,62
Maximum	372	8,6	111	3,2	6,3	7,1	78	0,06	0,06	0,025	1,75
Weighted average	254	8,3	103,5	2,0	5,4	3,9	34,8	0,03	0,03	0,010	0,89

Table 4. Morphological features of *Didymosphenia geminata*: data from literature and from specimen of Trentino (100 individuals from the Adige river, site San Michele- AD1, and 55 from the Noce stream, site Mezzolombardo- NO4).

Didymosphenia geminata morphotype geminata Reference:	*length (μm)*	*width (μm)*	*n. striae*	*n. stigmata*
Dawson, 1973	90-120	35-40	9-10	3-5
Antoine & Benson-Evans, 1984- Great Britain	58-151	25-47.5	9-14	0-9
Moffat, 1994- America	125-140	35-45		3-5
Metzeltin & Lange-Bertalot, 1995- Faroer-Insel	73-97	28-36	8-8.5	2-5
Metzeltin & Lange-Bertalot, 1995- Irland	82-105	31-37	8	2-5
Metzeltin & Lange-Bertalot, 1995- Sibirien	60-110	25-39	8.5-10	1-6
Metzeltin & Lange-Bertalot, 1995- Norway	48-97	26-35	9	2-5
Metzeltin & Lange-Bertalot, 1995- Scotland	85-132	35-42	8	2-6
Metzeltin & Lange-Bertalot, 1995-Onegasee	60-127	34-45	8-9	2-5
Kawecka & Sanecki, 2003- Poland	82.5-110.4	31.9-43.2	8-11	2-3
Noga, 2003- Poland	80-117	33-41	9-12	1-5
Subakov-Simić & Cvijan, 2004- Serbia	88-116	34-44	9-11	1-4
Trentino- Noce Rupe	60-101	30-40	8-11	1-4
Trentino- Adige S. Michele	75-106	20-40	8-10	1-3

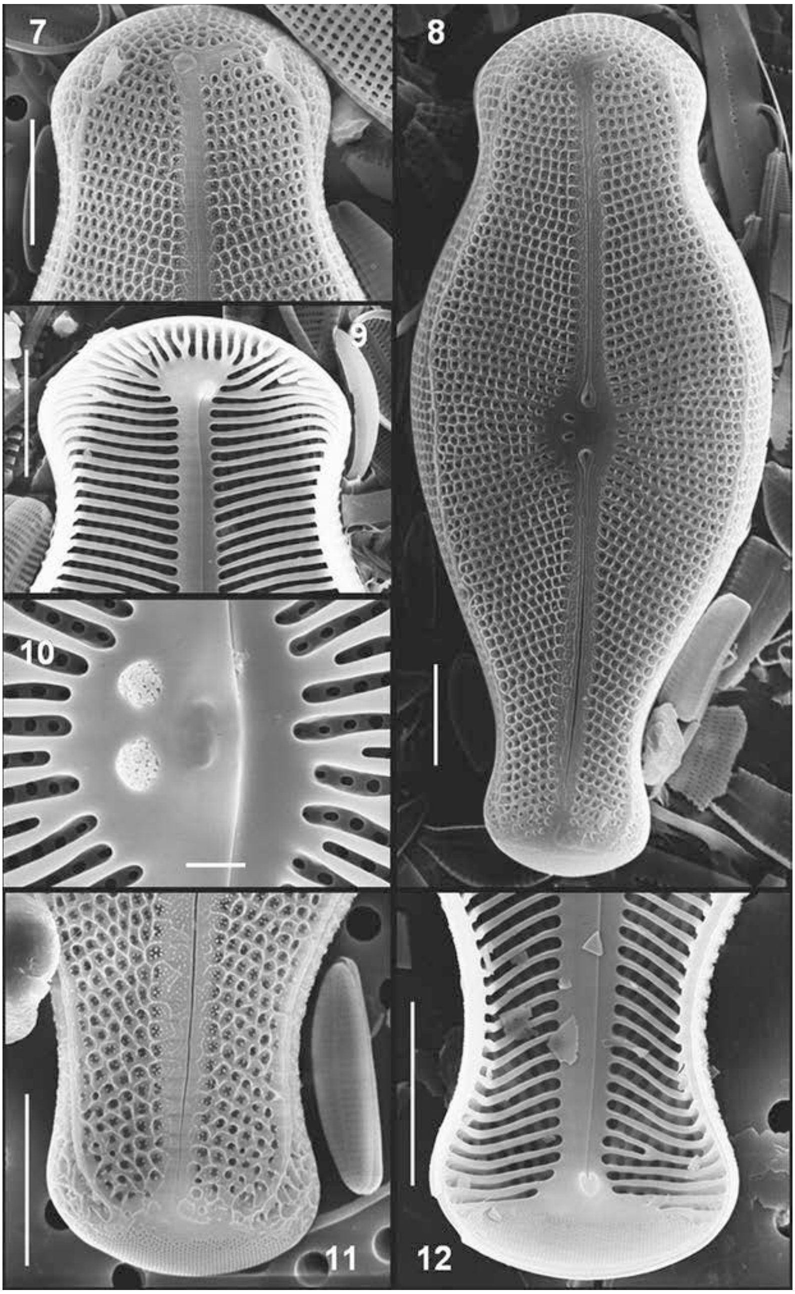
while externally the margin presented some spines or dendritic projections. Stigmata had open foramina towards the external valve face (Fig. 8), while on the internal face they appeared to be occluded by masses of silica (Fig. 10). The base pole (Figs 11, 12) was characterized by lines of fine unoccluded pores on both valve sides, through which the mucilage stalk was secreted (Fig. 13).

The raphe was central, with terminal raphe endings hook-shaped in external views, turning towards the side without stigmata in the central area. In internal views helictoglossa were visible (Figs 9, 12). The external central raphe endings were expanded as pores (Fig. 8), while internally the raphe fissure continued across the central nodule (Fig. 10).

In the 30 sites investigated, 91 diatom taxa were identified (Appendix 1). The most common species (mean relative abundance > 5 %) were *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, *A. minutissimum* (Kützing) Czarnecki, *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck, *C. placentula* var. *pseudolineata* Geitler, and *Nitzschia fonticola* Grunow.

DISCUSSION

Diatom analysis of 11 rivers of Trentino revealed the presence of *Didymosphenia geminata* in this Province of Northern Italy. By analysing LM and SEM images and comparing the size range in our samples with those found in the



Figs 7-12. *Didymosphenia geminata*. SEM images from Noce Mezzolombardo site (NO4), whole valve and details. **7.** Head pole with spines, terminal raphe ends hook-shaped. **8.** General morphology, heteropolar valve, central raphe, central stigmata. **9.** Inner face of the head pole with visible helictoglossa. **10.** Stigmata viewed from the inner face of the valve. **11.** External face: areolae occluded by typical dendritic projections. **12.** Internal face of base pole: helictoglossa and fine unoccluded pores are visible. Scale bars = 10 µm in Figs 7-9, 11, 12; scale bar = 2 µm in Fig. 10.

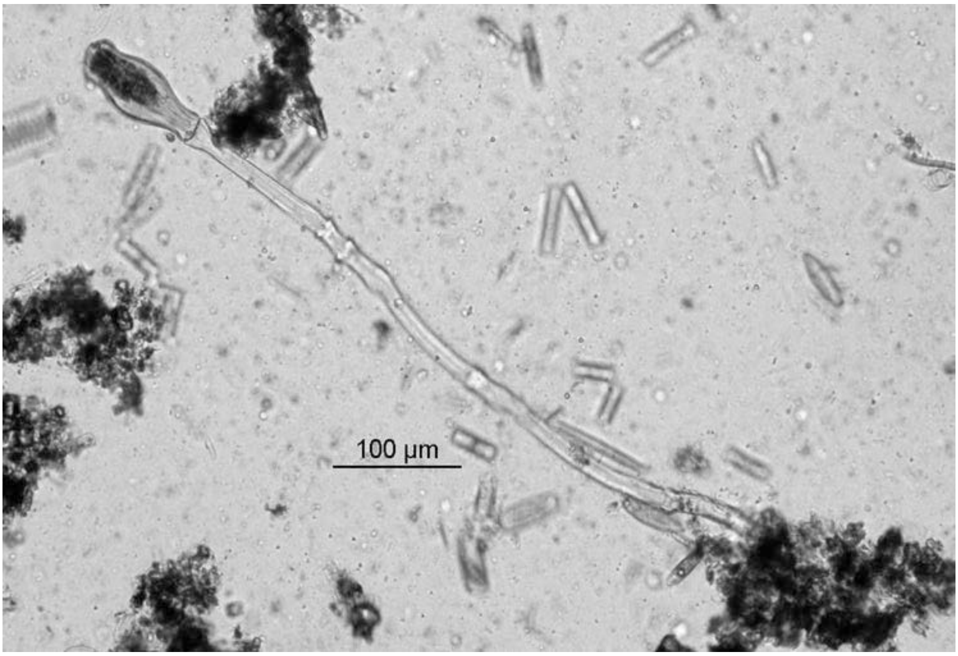


Fig. 13. Live material. From the base pole is secreted a long mucilage stalk by which the alga is attached to the substratum.

literature, we were able to refer the sampled populations to the morphotype "geminata" (Metzeltin & Lange-Bertalot, 1995). Stigmata were always found on one side of the central nodule only, which agrees with the general finding in the literature. Only Antoine & Benson-Evans (1984) described the presence of stigmata on both sides of the central area.

The areolae morphology is distinctive of the species: Mrozinska *et al.* (2006) compared their ultrastructure with that of *Didymosphenia tatrensis*, whose areolae are deeply seated in the wall (as in *D. geminata*), but are surrounded by vertical walls and lack spines.

Didymosphenia geminata was found in all the water courses investigated with the exception of Astico and Fersina streams. A survey conducted during 1999 in 6 sampling sites of the Fersina basin did not reveal the presence of *D. geminata* (Ciutti *et al.*, 2003). The possibility that it was overlooked was limited by the observation of both fresh material and several permanent slides.

During sampling no periphytic mats were observed and the presence of *Didymosphenia geminata* was discovered only during the slide analyses, which confirmed that this diatom was present in low abundance; the abundance of large-sized species is often known to be underestimated during counting (Jonsson *et al.*, 2000; Spaulding & Elwell, 2007), especially when communities are dominated by small-sized taxa.

Some authors suggested a possible relationship between the presence of *Didymosphenia geminata* and regulated or lake fed streams, probably due to regular flows (Skulberg, 1982; Kilroy *et al.*, 2005, 2006; Kawecka & Sanecki, 2003; Kirkwood *et al.*, 2007). This hypothesis should be tested in our region: *D. geminata* was found both in regulated stream reaches with low water discharge and downstream the water release point in reaches characterized by hydropeaking. The Fersina and Astico streams, where *D. geminata* is absent, have no reservoirs along their watercourse; on the other hand this diatom is also present in upstream basins, for instance in the site NO_3 which is located above the Santa Giustina reservoir. It could be asked if artificial lakes may constitute a starting-point for the diffusion of *D. geminata*, or if dams can represent a barrier to its diffusion to the upper reaches of watercourses. The hypothesis that waterfowl, for instance cormorants (Foged, 1953), could be local vectors for the diffusion of this species should be tested. In Trentino the population of *Phalacrocorax carbo sinensis* (Blumenbach, 1798) cormorants increased from a few individuals in 1993-1994 to 317 in 2002-2003 (Pedrini *et al.*, 2005). Their favourite feeding areas are lakes or wetlands, and they move from roosting areas to the feeding ones following the main Trentino watercourses with a pattern similar to the distribution of *D. geminata*.

From our data it is difficult to relate the presence or absence of *Didymosphenia geminata* only to physical and chemical characteristics of the water because it appears to be present in a wide range of environmental conditions. In Poland *D. geminata* has also been found in rivers classified as mesotrophic, suggesting the possible presence of different ecotypes or else that this diatom can live in wider ecological conditions (Kawecka & Sanecki, 2003). In Serbia *D. geminata* was first recorded in a glacial lake and was then found in 1997 in the Tisa river, which has moderately polluted water (Subakov-Simić & Cvijan, 2004). A literature review (Kilroy *et al.*, 2005) underlined the difficulties in correlating nutrient concentrations to *D. geminata* ecological preferences and distribution.

In this study *Didymosphenia geminata* was not very frequent in the upper reaches of watercourses, although the only massive proliferation observed in the province (Beltrami *et al.*, 2007) was in a site situated at 1100 m a.s.l. and 5 km away from the source.

From our data it cannot be established if *Didymosphenia geminata* is an invasive diatom in Trentino watercourses and there is very little historical data available for comparison. In any case, Largaiolli (1905) and Giaj-Levra & Abate (1994) did not mention the occurrence of *D. geminata* in the Trentino Province. In Italy this diatom probably has a wider distribution, and further investigations are necessary to better define its autecology and colonization patterns. A periodical control would be important in order to predict if this diatom should be considered as an invasive or nuisance species, which may cause a decrease in the quality status of the water bodies in Italy and neighbouring countries.

In our samples, *Didymosphenia geminata* was found with low relative abundance values, and in the Trentino province only a few blooms have been observed since 2006. The colonial growth behaviour of this species appeared similar to the one observed in the other European countries, and at the moment it should not create public alarm. However, sites where *D. geminata* occurred should be surveyed periodically in order to monitor the possible occurrence of nuisance blooms and their effects on other aquatic organisms, as well as monitoring the diffusion of this species to other rivers and water bodies in the Alps.

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APPENDIX 1

List of diatom taxa identified in the 30 sampling sites monitored.
Values of mean and maximum relative abundance (percentage) are reported for each taxa

Denomination	*Mean*	*Max*
Achnanthes minutissima Kützing var. *inconspicua* Oestrup	0.23	6.78
Achnanthidium atomoides Monnier, Lange-Bertalot et Ector	3.63	55.4
Achnanthidium minutissimum (Kützing) Czarnecki	22.1	69.7
Achnanthidium pyrenaicum (Hustedt) Kobayasi	32.1	91.1
Achnanthidium straubianum (Lange-Bertalot) Lange-Bertalot	0.02	0.73
Adlafia minuscula (Grunow) Lange-Bertalot	0.08	1.04
Adlafia minuscula var. *muralis* (Grunow) Lange-Bertalot	0.02	0.49
Amphora copulata (Kützing) Schoeman et Archibald	0.05	0.93
Amphora inariensis Krammer	0.3	2.16
Amphora pediculus (Kützing) Grunow	0.85	4.92
Caloneis silicula (Ehrenberg) Cleve	0.02	0.48
Cocconeis pediculus Ehrenberg	1.2	6.8
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	0.39	4.92
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	4.98	20.3
Cocconeis placentula var. *pseudolineata* Geitler	5.98	48.3
Cyclotella atomus Hustedt	0.02	0.25
Cyclotella cyclopuncta Håkansson et Carter	0.05	0.97
Cyclotella ocellata Pantocsek	0.05	0.95
Cymbella affinis Kützing	0.28	4.99
Cymbella helvetica Kützing	0.01	0.25
Denticula tenuis Kützing	0.38	2.36
Diatoma ehrenbergii Kützing	0.02	0.49
Diatoma mesodon (Ehrenberg) Kützing	0.05	0.72
Diatoma moniliformis Kützing	0.06	0.74
Diatoma vulgaris Bory	0.31	2.68
Didymosphenia geminata (Lyngbye) M. Schmidt	0.3	0.99
Ellerbeckia arenaria (Moore) Crawford	0.01	0.24
Encyonema minutum (Hilse) D.G. Mann	3.45	35.8
Encyonema silesiacum (Bleisch) D.G. Mann	1.38	7.18
Encyonopsis microcephala (Grunow) Krammer	0.37	5.24
Eolimna minima (Grunow) Lange-Bertalot	0.2	1.18
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot et Metzeltin	0.08	1.75

Denomination	*Mean*	*Max*
Fistulifera saprophila (Lange-Bertalot et Bonik) Lange-Bertalot	0.56	7.48
Fragilaria arcus (Ehrenberg) Cleve	0.46	6.17
Fragilaria capucina Desmazières var. *capucina*	0.06	0.72
Fragilaria capucina var. *capitellata* (Grunow) Lange-Bertalot	0.01	0.23
Fragilaria capucina var. *rumpens* (Kützing) Lange-Bertalot	0.05	0.7
Fragilaria capucina var. vaucheriae (Kützing) Lange-Bertalot	0.96	13.1
Frustulia vulgaris (Thwaites) De Toni	0.02	0.26
Geissleria decussis (Oestrup) Lange-Bertalot et Metzeltin	0.04	0.48
Geissleria schoenfeldii (Hustedt) Lange-Bertalot et Metzeltin	0.02	0.5
Gomphonema calcifugum Lange-Bertalot et Reichardt	0.25	6.28
Gomphonema minutum (C.A. Agardh) C.A. Agardh	0.16	1.49
Gomphonema olivaceum (Hornemann) Brébisson var. *olivaceum*	0.08	2.47
Gomphonema parvulum (Kützing) Kützing	0.07	1.5
Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot	1.07	10.5
Gomphonema tergestinum Fricke	0.02	0.48
Gomphonema truncatum Ehrenberg	0.01	0.23
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	0.02	0.25
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	0.73	4.95
Melosira varians C.A. Agardh	0.24	4.54
Meridion circulare (Greville) C.A. Agardh var. *circulare*	0.01	0.24
Navicula antonii Lange-Bertalot	0.18	0.73
Navicula capitatoradiata Germain	0.67	5.44
Navicula cryptocephala Kützing	0.06	0.91
Navicula cryptotenella Lange-Bertalot	1.75	16.6
Navicula exilis Kützing	0.04	0.5
Navicula gregaria Donkin	0.38	2.92
Navicula lanceolata (C.A. Agardh) Ehrenberg	0.1	0.91
Navicula reichardtiana Lange-Bertalot	0.35	1.71
Navicula rostellata Kützing	0.01	0.24
Navicula splendicula Van Landingham	0.01	0.25
Navicula tripunctata (O.F. Müller) Bory	1.99	11.2
Navicula viridula (Kützing) Ehrenberg	0.01	0.25
Nitzschia amphibia Grunow	0.02	0.47
Nitzschia archibaldii Lange-Bertalot	0.14	1.65

Denomination	*Mean*	*Max*
Nitzschia dissipata (Kützing) Grunow	0.31	1.81
Nitzschia fonticola Grunow	5.93	27
Nitzschia hantzschiana Rabenhorst	0.02	0.43
Nitzschia inconspicua Grunow	0.62	4.76
Nitzschia lacuum Lange-Bertalot	0.02	0.25
Nitzschia linearis (C.A. Agardh) W.M. Smith	0.05	0.91
Nitzschia palea (Kützing) W.M. Smith	0.35	2.07
Nitzschia palea var. *debilis* (Kützing) Grunow	0.04	1.22
Nitzschia perminuta (Grunow) M. Peragallo	0.03	0.5
Nitzschia pura Hustedt	0.02	0.25
Nitzschia pusilla (Kützing) Grunow	0.02	0.48
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.03	0.48
Planothidium lanceolatum (Brebisson ex Kützing) Lange-Bertalot	0.03	0.94
Psammothidium bioretii (Germain) Bukhtiyarova et Round	0.02	0.48
Pseudostaurosira brevistriata (Grunow) Williams et Round	0.01	0.24
Puncticulata radiosa (Lemmermann) Håkansson	0.01	0.24
Reimeria sinuata (Gregory) Kociolek et Stoermer	0.93	8.1
Rhoicosphenia abbreviata (C.A. Agardh) Lange-Bertalot	1.53	14.6
Sellaphora joubaudii (Germain) Aboal	0.02	0.48
Sellaphora pupula (Kützing) Mereschkowksy	0.01	0.25
Sellaphora seminulum (Grunow) D.G. Mann	0.02	0.48
Staurosira pinnata Ehrenberg	0.09	1.08
Surirella angusta Kützing	0.01	0.24
Surirella brebissonii Krammer et Lange-Bertalot	0.03	0.5
Ulnaria ulna (Nitzsch) Compère	0.09	1.46