

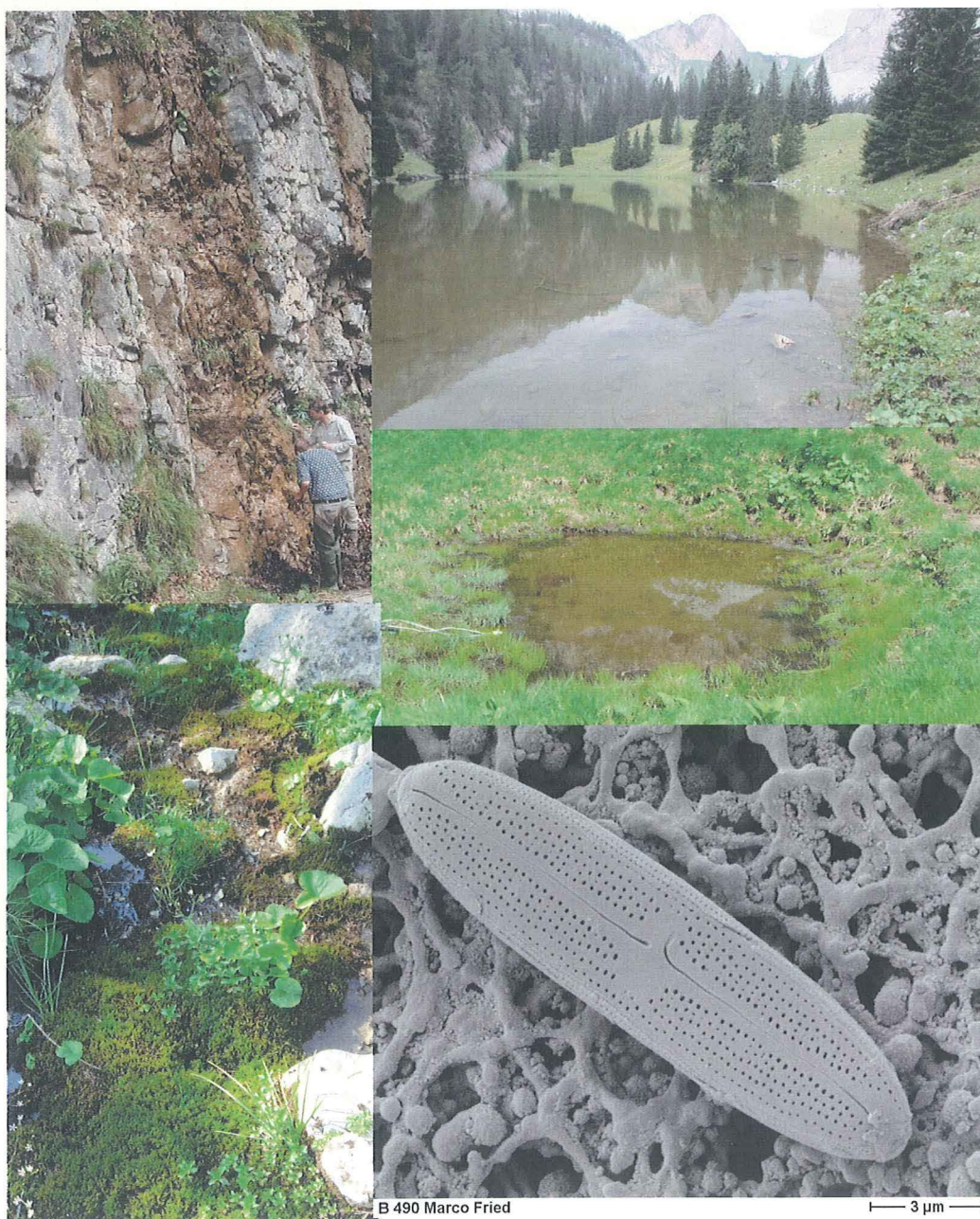
Diatoms from springs and other aquatic habitats of the Gesäuse National Park (Austria)

Final report (End of December 2010)

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Front page images

- Left, upper part: Spring HAGL (hygropetric rheocrene)
Left, lower part: Small rheohelocrenic spring KAMS
Right, upper part: Sulzkarsee (from: Jersabek *et al.* 2004, Photo E. Weigand)
Right, middle: Temporary pool FRIED
Right, lower part: *Neidium alpinum* Hustedt at the Scanning Electron Mic.

Premise

The following final report includes two main parts: The report *sensu stricto*, and two Appendices. The Report describes all technical details of the work performed, and also includes Tables with the results (row data: Number of valves counted) of the identification and quantification work. The Appendices present some iconographic documentation of the diatom taxa found in two representative environments: the hygropetric rheocrene HAGL and the Lake Sulzkarsee.

Note for the German speaking readers: Spring code ARSCH is referred to the name of the mountain pass close to which this spring comes to daylight (ARSCH = "*Quelle unter Teufelsarsch*").

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PART II

Appendix 1 – Iconography of the diatom microflora of the hygropetric rheocrenic spring HAGL.

Appendix 2 - Iconography of the littoral diatom microflora of the Lake Sulzkarsee (material sampled from stones, -0.2 m).

PART I

Introduction

Diatoms are unicellular algae characterized by a silica frustule –with a structure often likened to a pill box- that encloses the cell. They are widespread in fresh, brackish and salty waters, and in all terrestrial habitats where some moisture is at least sporadically available. Diatoms have a prominent role among primary producers in the biosphere: About one fifth of the oxygen available in the atmosphere has been produced by diatoms (abundant also in the plankton of the oceans). Diatoms are a very diverse group of organisms: According to current estimates, as many as about 200.000 species may exist (Mann & Droop 1996). Many species have very specific environmental requirements, and diatom ecology is being thoroughly investigated since some decades: This makes diatoms powerful environmental indicators that have found applications in a range of subjects spanning from environmental assessments and ecological characterization to industrial patents and forensic sciences (Stoermer & Smol 2010).

Oligotrophic freshwater systems, in spite of having been comparatively less-intensively investigated than more impacted systems, at least as regards diatoms (Kociolek & Stoermer 2009), can present a remarkable diatom-taxa richness (e.g., Lange-Bertalot & Metzeltin 1996), host high amounts of endangered and Red-List (Lange-Bertalot 1996) taxa (e.g., Cantonati *et al.* 2009), may provide important clues for the identification of pristine sites to favour their conservation and for the restoration of impacted environments. Strictly oligotrophic freshwaters are the ones where the highest number of endemic diatom taxa can be expected (Moser *et al.* 1998). However, due to direct and indirect (diffuse) human impacts these types of habitats are becoming rarer and rarer. Headwater streams account for the majority stream-channel length in most areas, are relevant building blocks in the construction of protected-area networks, act for refugia for riverine species during specific life-history stages and critical periods of the year, are characterized by close terrestrial-aquatic linkage, are very sensitive to natural or anthropogenic disturbance of surrounding lands, present a combination of accessibility and compelling natural history that confer them great education value, and are critical habitat for rare and endangered freshwater species (Lowe & Likens 2005). In recognition of their relevance, headwaters and oligotrophic aquatic habitats in general are often included in Nature Parks to favour their conservation.

Springs are probably the most typical oligotrophic aquatic habitats and the origin of many headwater streams. They are habitats with peculiar features, such as: high diversity of environmental situations (different conditions as regards e.g. ecomorphology, lithology, isolation, shading, permanence of flow, current velocity etc.) determining a high degree of heterogeneity, a multiple

ecotone nature (groundwater – surface water, aquatic – terrestrial-, spring mouth – running water system), a pronounced microhabitat-mosaic structure (Cantonati *et al.* 2006), and the possibility to act as *refugia* for the most sensitive species in densely populated and highly exploited areas.

Investigations performed in the last fifteen years [frequently in natural preserves of the Alps, e.g.: Adamello-Brenta (Cantonati 1998) and Prealpi Giulie (Cantonati 2003) Nature Parks, Dolomiti Bellunesi (Cantonati & Spitale 2009) and Berchtesgaden (Cantonati & Lange-Bertalot 2010) National Parks] have shown that springs host diatom assemblages especially suitable to investigate relationships with aquifer lithology (Werum & Lange-Bertalot 2004, Cantonati & Lange-Bertalot 2006), geogenic-variables –pH, alkalinity-gradients (Cantonati 1998), nitrate concentrations (diffuse airborne pollution, Cantonati 1998), shading (Cantonati & Lange-Bertalot 2009), alterations of the morphology and nitrate pollution (Angeli *et al.* 2010). Moreover, the diatom flora of springs was found to be very species rich, to frequently include a high proportion of Red List species, and even taxa new to science (Cantonati & Lange-Bertalot 2006, 2009, 2010; Cantonati *et al.* 2009, 2010).

Diatoms can be very useful also for the characterization of spring types (Cantonati 2004, Cantonati *et al.* in prep. 2012). The traditional hydrobiological classification of springs (Steinmann 1915, Thienemann 1924) distinguishes three main spring types: rheocrenic (flowing springs), helocrenic (seepages, water emerges in a diffuse way originating a swampy area), limnocrenic (pool springs). Some frequently encountered types are also rheohelocrenic springs (seepages on steep slopes), and hygropetric rheocrenic springs (sources in which the water flows over the rock forming a thin film).

Diatoms are increasingly used for the assessment of the trophic status of the littoral zone of lakes (e.g., Schönfelder *et al.* 2002, Poulíčková *et al.*, 2004), due to their species diversity and the well documented environmental requirements of many species. Epilithic diatoms are most commonly used, since stones and boulders are among the most ubiquitous substrates, particularly in oligotrophic systems (e.g., King *et al.* 2006). The shallow eulittoral is usually the only zone sampled, because it can be easily reached. This part of the lake littoral zone is, however, the most dramatically affected by water-level fluctuations, which are modifying and altering its ecological function in an increasing number of lakes (e.g., Wantzen *et al.* 2008). Hansson (1988, 1992) found that phytoplankton abundance was amongst the most relevant factors limiting phyto**ben**thos quantities due to competition for light (i.e. shading effects), rather than for nutrients. In Lake Tovel (Cantonati *et al.* 2009), pronounced seasonal changes in epilithic diatom bio**volum**es were mainly due to increased phytoplankton biomass which reduced light transparency. Lowe (1996) stated that the unique species-rich community of the deep littoral is a distinct subset of lentic phyto**ben**thos. These special diatom assemblages are however menaced by eutrophication, that would cause an increase of phytoplankton biomass capable of eliminating most of the microphyto**ben**thos of the infralittoral by means of heavy shading.

Mires are very selective environments, characterized by high water temperatures in summer with marked fluctuations, low pHs, low nitrogen availability (e.g., Krivograd Klemenčič & Vrhovšek 2003). The colonisation by specifically adapted organisms makes them very important environments for nature conservation. In spite of the fact that high altitude and high latitude aquatic ecosystems have been identified as being especially sensitive to environmental changes (Kilroy *et al.* 2006), literature on high-mountain and mountain mires is particularly scarce (e.g., Nováková 2002, Gesierich & Rott 2004). According to Watanabe *et al.* (2000) algae are negligible as primary producers in mires compared with bryophytes, grasses and sedges, but algal species diversity is high, and there are many algal species endemic to mires. Nevertheless mires have been, and sometimes still are, menaced by different types of direct impacts (land reclamation, peat extraction, wetlands' drainage, organic pollution and eutrophication etc.) that cause their fragmentation or even complete destruction (Minelli 2004). Amongst the organism colonizing mires, algae and cyanoprokaryotes play an important role, even if scarcely visible (e.g., Poulícková *et al.* 2003, Kulikovskiy 2009). These organisms are indeed mostly represented by microscopic forms, that only occasionally develop macroscopic covers or colourings. Taxa numbers can however be high, and the contribution to overall mire biodiversity is thus relevant. Diatoms (e.g., Sherer 1988), even if still understudied, are also usually relevant in these environments (e.g., Watanabe *et al.* 2000). The Project LIFE04 NAT/IT/000177, devoted to conservation and sustainable touristic use of the mires of Danta (Cadore, Veneto, south-eastern Alps), presented by the Municipality of Danta, was approved by the European Union in 2004 and included also investigations on fauna, vegetation, fungi, lichens, bryophytes, and algae, considered introductory for a special management plan. Cantonati *et al.* (2011) contributed with a taxonomic and ecological characterization of the diatom assemblages of shallow pools of the Site of Community Importance "Danta Mires", with the specific objective to show the potential contribution of these habitats to diatom biodiversity conservation. In particular they could show that the mire pools studied hosted a very high proportion (72%) of rare and Red List taxa (including also species of the categories "Threatened with extinction", "Severely endangered", and "Endangered").

Since diatom assemblages in springs are supposed to an ideal component of the biota to study the aquatic biodiversity of a geographic area (e.g. Nature Park), the goal of the present work was to produce a characterization of spring diatom assemblages of the Gesäuse National Park (GNP, Northern Alps, Germany). Further goals were to start exploring the diatom microflora of some other aquatic habitats (mire and temporary pools), and to use littoral diatom assemblages for an assessment of the environmental quality of the human-impacts affected (Pavuzá & Stummer 2003-2004, Jersabek *et al.* 2004) Lake Sulzkarsee.

Methods

Diatom sampling, identification, and quantification. Description of new species

To investigate diatom assemblages, different substrata (stones, bryophytes, surface sediment, and –in some cases–, the macroscopic xanthophyte *Vaucheria*) were collected where available. Epilithic diatoms were collected by brushing seven to ten stones following the European standard for sampling diatoms in running water (EN 13946, 2003). Kelly *et al.* (1998) report that a minimum of five is needed for a representative sample of epilithic diatoms present at a site. For epibryon dominant bryophyte species, either submerged or closest to the water, were collected. Identification of the bryophyte species collected for the epibryon investigations is underway. Surface sediment (upper few mm) was sampled by aspiration using a large bore syringe. The collected materials, including the bryophytes, which were cut into small pieces, were digested using hydrogen peroxide (EN 13946 2003). The cleaned material was mounted in Naphrax (refractive index of 1.74). For each sample, suspensions were dried on and mounted on three cover-slips on one permanent slide. A total of about 450 valves were identified by counting for more or less the same number of valves on each cover-slip using a Zeiss Axioskop 2 (Zeiss, Jena, Germany) and 1000x magnification. All slides were then scanned for taxa with low relative abundances for several hours and in at least two occasions. For identification and nomenclature we followed Krammer & Lange-Bertalot (1986-1991), Round *et al.* (1990), Lange-Bertalot & Metzeltin (1996), Krammer (1997a,b), Lange-Bertalot (2001), Krammer (2002-2003), and Werum & Lange-Bertalot (2004). To confirm identifications and document taxa with poorly-observed ultrastructure, several taxa were examined with SEM (Zeiss-EVO40XVP, Carl Zeiss SMT Ltd., Cambridge, UK) at the Museo Tridentino di Scienze Naturali.

For species new to science and of special interest SEM observations were made primarily at the University of Frankfurt using a Hitachi S-4500 (Hitachi Ltd., Tokyo, Japan) and some SEM observations at the Museo Tridentino di Scienze Naturali using the Zeiss-EVO40XVP mentioned above at high vacuum on gold coated stubs.

Materials (slides, prepared material, and aliquots of the original samples) are held at the diatom collection of the Museo Tridentino di Scienze Naturali of Trento (Northern Italy), Accession numbers: cLIM DIAT 1512-1543, 1860-1882, 1883-1885, and 1887-1890 (Fig. 1). For possible new species, isotype slides and aliquots of prepared material from the same locality and substratum will be sent to the Curators of internationally recognized diatom collections.

To perform the ecological characterization of potential new species and to reconstruct their distribution, samples of the diatom collection of the Museo Tridentino di Scienze Naturali of Trento will be scanned: In particular, samples collected in the DBNP (Cantonati & Spitale 2009) and in the Autonomous Province of Trento (south-eastern Alps, Italy) during the CRENODAT Project (Biodiversity assessment and integrity evaluation of springs of Trentino –

Italian Alps – and long-term ecological research, 2004-2007; Cantonati *et al.* 2012 in prep.).

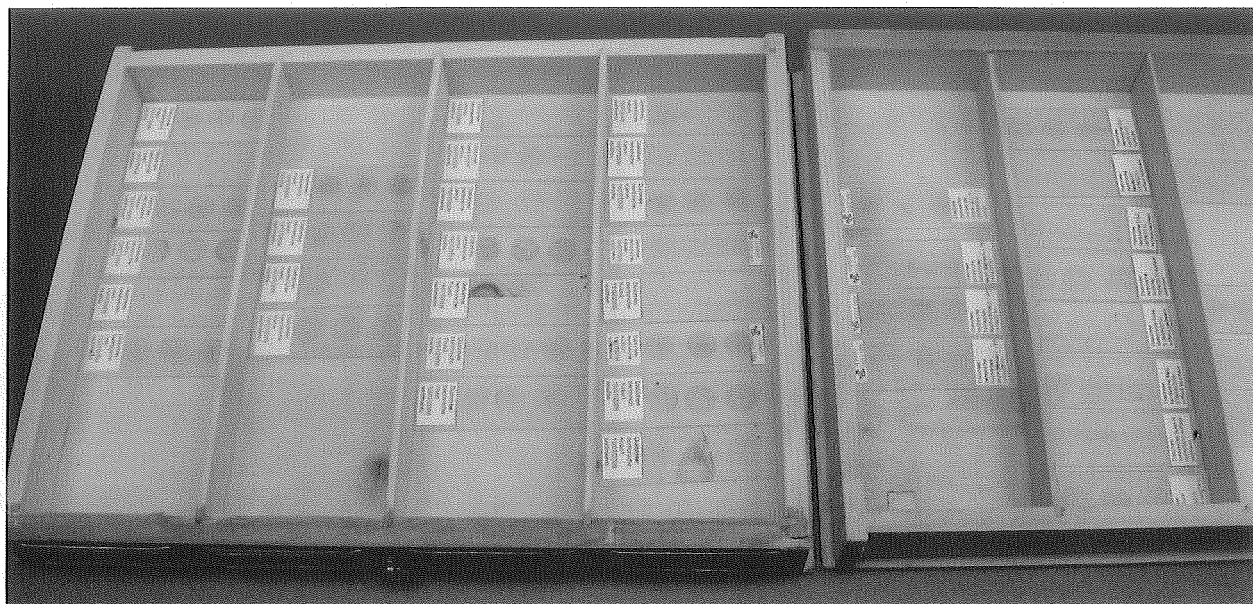


Fig. 1. Some of the diatom permanent mounts obtained from samples collected in the Gesäuse National Park in the diatom collection of the Museo Tridentino di Scienze Naturali.

Data processing

To investigate diatom ecology, preferences of the individual taxa with respect to moisture, trophic status, and pH were determined consulting van Dam *et al.* (1994). To assess rarity, the German Red List was used (Lange-Bertalot 1996).

Selection of sites

The samples used for the present work were collected in the frame of two Spring-Habitat Sampling Workshops (*Quellwochen*) in July 2009 and June 2010 respectively. In 2009 the Author had the possibility to participate directly into the workshop and collect the materials. In 2010 diatom samples were kindly collected by DI Elmar Pröll. The materials were collected in springs, mountain temporary pools, mire pools, in the spring-stream Johnsbach, and in the small mountain lake Sulzkarsee. The following samples were prepared (digested) to produce permanent mounts:

Sampling site Code	Sampling date	Substratum sampled
Leuc	13/07/2009	cobbles&boulders
Leuc	13/07/2009	bryophytes
Leuc	13/07/2009	surface sediment
Arsch	13/07/2009	epilithon

Arsch	13/07/2009	bryophytes
Arsch	13/07/2009	surface sediment
Fried	13/07/2009	surface sediment
Titue2	14/07/2009	surface sediment
Titue2	14/07/2009	bryophytes
Kams	14/07/2009	bryophytes
Kams	14/07/2009	surface sediment
Kobo2	15/07/2009	cobbles&boulders
Kobo2	15/07/2009	bryophytes
Kobo2	15/07/2009	surface sediment
Kobo1	15/07/2009	bryophytes
Kobo1	15/07/2009	surface sediment
KOBO1	15/07/2009	<i>Vaucheria</i>
Zach	15/07/2009	cobbles&boulders
Zach	15/07/2009	surface sediment
Huepf	16/07/2009	cobbles&boulders
Huepf	16/07/2009	bryophytes
Huepf	16/07/2009	surface sediment
Suech	16/07/2009	cobbles&boulders
Suech	16/07/2009	bryophytes
Suech	16/07/2009	surface sediment
Jamoq	16/07/2009	cobbles&boulders
Jamoq	16/07/2009	bryophytes
Jamoq	16/07/2009	surface sediment
Jamot	16/07/2009	surface sediment
Jamot	16/07/2009	bryophytes
Kamm	16/07/2009	cobbles&boulders
Kamm	16/07/2009	bryophytes
Kamm	16/07/2009	surface sediment
KAMM	16/07/2009	<i>Vaucheria</i>
Jotra1	07/06/2010	cobbles&boulders
Jotra2	07/06/2010	cobbles&boulders
Jotra3	08/06/2010	cobbles&boulders
Jotra4	08/06/2010	cobbles&boulders
Jotra5	11/06/2010	cobbles&boulders
Brutz	10/06/2010	cobbles&boulders
SeeMa	10/06/2010	bryophytes
Zaun	10/06/2010	bryophytes
Gitü	08/06/2010	bryophytes
Hagl	11/06/2010	rocky surface
SuHüMo	09/06/2010	surface sediment
Suse-N	09/06/2010	surface sediment
NeuMo-W	07/06/2010	surface sediment
Suse-S	09/06/2010	surface sediment
Hatü-S	09/06/2010	surface sediment

Gitü2	08/06/2010	surface sediment
GLAU	08/06/2010	surface sediment
Sulzkarsee littoral - 0.2 m	07/06/2010	cobbles&boulders
Sulzk. depth transect: - 1 m	07/06/2010	cobbles&boulders
Sulzk. depth transect: - 3 m	07/06/2010	cobbles&boulders
Sulzk. depth transect: - 7 m	08/06/2010	cobbles&boulders

The following were received but have not yet been selected for diatom digestion to produce permanent mounts:

Glau 08/06/2010 surface sediment
 SuHü 09/06/2010 cobbles&boulders
 Hawa 08/06/2010 cobbles&boulders
 Saug 12/06/2010 cobbles&boulders
 Zwanz 10/06/2010 cobbles&boulders
 Esch1 08/07/2010 cobbles&boulders
 NeuMo-W 07/06/2010 cobbles&boulders

During preliminary observations on the prepared material or during first scans of the permanent mounts, diatoms were found to be virtually absent from the following samples: Arsch 13/07/2009 surface sediment, Suech 16/07/2009 epilithon, Jamoq 16/07/2009 bryophytes, Arsch 13/07/2009 epilithon, Leuc 13/07/2009 epilithon, Kobo2 15/07/2009 surface sediment, Zach 15/07/2009 cobbles&boulders and surface sediment, Suech 16/07/2009 surface sediment.

The following permanent mounts were found to be extremely diatom-poor: KOBO1 15/07/2009 *Vaucheria*, SeeMa 10/06/2010 bryophytes, Gitü2 08/06/2010 surface sediment, LEUC 13/07/2009 rocky surface&cobbles, Arsch 13/07/2009 surface sediment, Arsch 13/07/2009 cobbles&boulders, Kobo1 15/07/2009 surface sediment, Huepf 16/07/2009 bryophytes, Huepf 16/07/2009 surface sediment, Kamm 16/07/2009 surface sediment.

The following were found to document relatively poor diatom populations making quantitative analyses lengthy and difficult: Glau 08/06/2010 surface sediment, KAMM 16/07/2009 *Vaucheria*, Jotra5 11/06/2010 cobbles&boulders, Hatü-S 09/06/2010 surface sediment, Suse-S 09/06/2010 surface sediment, SuHüMo 09/06/2010 surficial sediment, Suse-N 09/06/2010 surface sediment, Jamoq 16/07/2009 sediment, Titue2 14/07/2009 bryophytes.

Benthic diatoms were almost missing in the following lake samples: Sulzkarsee 07/06/2010 depth transect: - 1 m, -3 m, -7 m. In this (and in several other cases) the virtual absence of benthic diatoms from the material was confirmed observing smear slides of the original (unprepared) raw material.

The following samples could be counted (450 valves): LEUC bryophytes, KAMS bryophytes, KOBO1 bryophytes, HUEPF stones, KAMM bryophytes, HAGL rocky surface.

The following samples were analysed assessing the relative abundances in a semi-quantitative way: LEUC surface sediment, ARSCH bryophytes, KOBO2 cobbles&boulders, KOBO1 surface sediment, HUEPF bryophytes, SUECH bryophytes, JAMOQ cobbles&boulders, JAMOQ surface sediment. The 1-to-5 descriptor scale for abundance estimates of microscopic growth forms scale reported in EN 15708 (2009) adapted to work on diatoms in permanent mounts was as follows: 1. *Rare* – one or very few valves are observed, 2. *Occasional* – the taxon is seen several times during scans of permanent mounts of a particular sample but never in great numbers, 3. *Frequent* – a representative of this taxon is present in most fields of view observed during scans of permanent mounts, 4. *Abundant* – more than one valve of the taxon is present in most fields of view observed during scans of permanent mounts, 5. *Dominant* – the most common organism(s) observed in all scans of the sample.

Methods for conducting ecological research in springs were recently reviewed in a monographic volume (Cantonati *et al.* 2007a), that includes also a contribution dealing specifically with algae and cyanoprokaryota (Cantonati *et al.* 2007b).

Results and Discussion

Overall, four main habitat types (springs, mire pool, ephemeral mountain pools, mountain lake), and 14 sampling sites (22 samples analysed since in several cases different substrata were considered + depth transect in the lake) were studied by using samples collected in 2009 and 2010: - 10 springs (LEUC, ARSCH, KAMS, KOBO2, KOBO1, HUEPF, SUECH, JAMOQ, KAMM, HAGL; - 1 mire pool (JAMOT), - 2 temporary pools (FRIED, TITUE2); - Sulzkarsee, the only mountain lake located within the Gesäuse National Park.

Several of these habitats proved to be rather harsh habitats (probably due to only seasonal or temporary presence of water and / or scarce nutrient availability), since several samples were found to contain very low diatom amounts making them unsuitable for quantitative or semi-quantitative, and sometimes even for qualitative, analysis.

The spring types that could be considered are the following: Rheocrenic springs (KOBO2, KOBO1, HUEPF, SUECH, KAMM), small rheocrenic spring feeding a bordering mire (JAMOQ), very-low discharge rheocrenic spring (substrata in the spring-mouth are represented by bryophytes and debris + surface sediment: tendency to rheohelocrenic morphology; KAMS), hygropetric rheocrenic spring (HAGL), rheocrene spring with a small limnocrenic spring head (ARSCH), small rheocrenic spring with hygropetric parts (LEUC).

Tables 1-4 list all the diatom taxa found in 4 habitat types. Altogether, 181 diatom taxa were identified. As many as 100 diatom taxa were found in the 10 springs studied. They belong to 38 genera. The high number of genera is in

part due to the fact that the most updated high-resolution taxonomy was adopted for the analyses of the samples. This figure is almost identical to results obtained during a recent study of 9 springs in the Berchtesgaden National Park, where 104 diatom taxa belonging to 39 genera were found (Cantonati & Lange-Bertalot 2010). The total numbers of diatom taxa found in the in the other habitat types are as follows: - mire pool: 27 (14 genera), - ephemeral pools: 40 (16 genera), - mountain lake: 37 (19 genera).

In springs the number of diatom taxa observed in the permanent mounts ranged from 10 to 30, and the number of taxa encountered during counts from 10 to 26 (Table 1). The highest number of taxa was found in a very-low-discharge rheocrenic spring with only bryophytes and surface sediment in the spring-mouth (KAMS), and the lowest in a stony and very-shaded rheocrenic spring (KOB02). In the mire pool 27 taxa were observed and 23 counted (Table 2). In the ephemeral pools the number of taxa observed ranged from 22 to 36 and that of taxa counted from 17 to 30. FRIED was by far the richest site of this habitat category (Table 3). In the lake 37 taxa were observed and 34 counted (Table 4).

Each habitat type appears to have its peculiar diatom microflora as shown by the fact that only one taxon [*Achnantheidium minutissimum* (Kützinger) Czarnecki] was found in all habitat types, and even this is a partial artefact, since what is commonly called *Achnantheidium minutissimum* is meanwhile known to be a group of species difficult to identify and keep separated at the microscope. The most characteristic taxa of the different habitat types might be listed (in order of quantitative relevance and frequency) as follows:

- Rheocrenic springs (Table 1): *A. minutissimum* species group, *Planothidium lanceolatum*, *Psammothidium grischunum*, *Achnantheidium strictum*, *Navicula cataracta-rheni*, *Meridion circulare*, *Diatoma mesodon*, *Achnantheidium pfisteri*, *Encyonema sublangebertalotii*;
- Hygropetric rheocrenic spring (Table 1): *Fragilaria distans*, *Gomphonema lateripunctatum*, *Delicata minuta*, *Achnanthes trinodis* (micrographs of these species are available in Appendix 1);
- Mire pool (Table 2): *Frustulia saxonica*, *Eunotia paludosa*, *Kobayasiella micropunctata*, *Frustulia crassinervia*, *Eunotia neocompacta*;
- Ephemeral pools (Table 3): species of the genera *Nitzschia*, *Naviculadicta*, *Pinnularia*, *Craticula*, *Stauroneis*, *Sellaphora*, *Mayamaea*, *Neidium*, *Eunotia*;
- Meso-eutrophic mountain lake (Table 4): *Staurosirella pinnata*, *A. minutissimum* species group, *Cymbella excisa* var. *angusta*, *Encyonema caespitosum*, *Denticula tenuis*, *Achnantheidium inconspicuum*, *Pseudostaurosira robusta*, *Encyonopsis krammeri*, *Navicula cryptotenelloides*, *N. radiosa* (micrographs of most of these species are available in Appendix 2).

Table 1. List of all the diatom taxa found in springs of the Gesäuse National Park with indications on the numbers of valves counted or of the abundance assessed following the 1-to-5 descriptor scale for abundance estimates (EN 15708 2009; numbers in *Italics*), on their distribution and autecology, and a comparison with the diatom microflora found in springs of the Dolomiti Bellunesi (DBNP) and Berchtesgaden (BNP) National Parks. SAB = Standard Abbreviations for diatom genera proposed by Simkhada (2006); those in brackets were made *ex novo* since not available in that publication. St = Stones; Br = Bryophytes; De = Debris + surface sediment; RS = rocky surface. o = observed while scanning the permanent mounts but not encountered during the counts.

Spring code		LEUC		ARSCH	KAMS	KOBO2	KOB01		HUEPF		SUECH	JAMOQ		KAMM	HAGL	RL	M	T	pH	DB
Substratum (support)	SAb	Br	De	Br	Br	St	Br	De	St	Br	Br	St	De	Br	RS					
<i>Achnanthes trinodis</i> (W. Smith) Grunow															18	3	-	-	-	
<i>Achnanthidium affine</i> (Kützing) Czarnecki	<i>Achd.</i>														23	*	-	-	4	=
<i>Achd. dolomiticum</i> Cantonati & Lange-Bertalot				23												(G)	-	-	-	=
<i>Achd. inconspicuum</i> Østrup		49	3			2						3	3			(*)	-	-	-	
<i>Achd. minutissimum</i> (Kützing) Czarnecki & species group		119	5	5	170	3	345	3	24	3	4	3		48	164	**	3	7	3	=
<i>Achd. neomicrocephalum</i> Lange-Bertalot & F. Staab											1				10	(R)	-	-	-	=
<i>Achd. pfisteri</i> Lange-Bertalot			1	1		1	7		13			1				(R)	-	-	-	
<i>Achd. pyrenaicum</i> (Hustedt) Kobayasi		134	1				12	1		1		2			25	**	-	3	4	=
<i>Achd. strictum</i> Reichardt		7	2		8	2	1	1	119			4			11	(G)	-	-	-	=
<i>Adlafia bryophila</i> (Petersen) Lange-Bertalot	<i>Adal.</i>														1	V	5	3	3	=
<i>Adal. minuscula</i> (Grunow) Lange-Bertalot var. <i>minuscula</i>		7		1			3									*	4	1	4	=
<i>Amphora copulata</i> (Kützing) Schoeman & Archibald	<i>Amph.</i>											1				**	1	5	4	
<i>Amph. inariensis</i> Krammer														4		3	-	1	-	
<i>Amph. lange-bertalotii</i> Levkov & Metzeltin				2												(G)	-	-	-	
<i>Amph. ovalis</i> (Kützing) Kützing							1									**	1	5	4	
<i>Amph. pediculus</i> (Kützing) Grunow		1	2			1		1	12			2	2	24		**	3	5	4	=
<i>Amph. sp.</i> "long&slender"														59		(R)	-	-	-	
<i>Brachysira calcicola</i> ssp. <i>pfisteri</i> Lange-Bertalot & Werum															8	2	-	-	-	
<i>Caloneis alpestris</i> (Grunow) Cleve	<i>Calo.</i>			5												G	3	3	4	=
<i>Calo. fontinalis</i> Lange-Bertalot & Reichardt				o					1					11		(*)	-	-	-	=
<i>Calo. tenuis</i> (Gregory) Krammer				2												G	4	3	3	=
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehrenberg) Grunow	<i>Coco.</i>		2			1		1	3	1				22		**	2	5	4	
<i>Coco. pseudolineata</i> (Geitler) Lange-Bertalot									2							D	-	-	-	=
<i>Cymbella excisa</i> var. <i>angusta</i> Krammer							1								6	(*)	-	-	-	=
<i>Cymb. hantzschiana</i> Krammer				16											o	(R)	-	-	-	B
<i>Cymb. vulgata</i> var. <i>plitvicensis</i> Krammer				o												(R)	-	-	-	
<i>Cymboppleura austriaca</i> Grunow var. <i>austriaca</i>				6												V	-	-	-	=
<i>Cymp. diminuta</i> (Grunow) Krammer														4		(D)	-	-	-	B
<i>Cymp. subaequalis</i> (Grunow) Krammer														o		G	3	2	3	B
<i>Cymp. subaustriaca</i> Krammer				9												(V)	-	-	-	
<i>Delicata delicatula</i> (Kützing) Krammer															1	G	3	1	4	
<i>Delicata minuta</i> Krammer	<i>(Deli.)</i>														26	(G)	4	1	4	=
<i>Denticula kuetzingii</i> Grunow															4	*	3	3	4	
<i>Denticula tenuis</i> Kützing	<i>Dent.</i>	3	4	o		17	2							1	19	*	3	3	4	=
<i>Diadesmis contenta</i> (Grunow) D.G. Mann	<i>Diad.</i>		1						57							**	4	7	4	=
<i>Diad. paracontenta</i> Lange-Bert. & Werum ssp. <i>paracontenta</i>		2	1						2							(G)	-	-	-	=
<i>Diad. perpusilla</i> (Grunow) D.G. Mann			1						110							**	5	1	3	=
<i>Diatoma hyemalis</i> (Roth.) Heiberg	<i>Diat.</i>						o									*	2	-	4	=
<i>Diat. mesodon</i> (Ehrenberg) Kützing		6	2	2				2	9	2	1			52	3	*	2	3	3	=
<i>Diploneis krammeri</i> Lange-Bertalot & Reichardt	<i>Dipl.</i>		1		1	1										(G)	-	-	-	=
<i>Diploneis oculata</i> (Brébisson) Cleve												1				*	3	-	3	
<i>Encyonema alpiniforme</i> Krammer				5										o		(R)	-	-	-	
<i>Ency. alpinum</i> (Grunow) D.G. Mann in Round <i>et al.</i>	<i>Ency.</i>														1	G	5	1	4	=
<i>Ency. minutum</i> (Hilse ex Rab.) D.G. Mann						1		3	1	1				o		*	-	-	3	
<i>Ency. sublangbertalotii</i> Lange-Bertalot & Cantonati		5	2				1		20	1	2			5		(G)	-	-	-	=
<i>Ency. ventricosum</i> (Agardh) Grunow in A. Schmidt			2			1						1	1	2		(*)	-	-	-	=
<i>Encyonopsis cesatii</i> (Rabenhorst) Krammer	<i>Encs.</i>			3												*	3	1	3	=
<i>Encs. falaisensis</i> (Grunow) Krammer			1	1	18											G	3	2	-	=
<i>Encs. microcephala</i> (Grunow) Krammer		1														*	3	4	4	
<i>Encs. minuta</i> Krammer & E. Reichardt															30	(*)	-	-	-	
<i>Encs. "sagittata" pheridomene</i>				1	5											(R)	-	-	-	B

RL = Red List species (Lange-Bertalot 1996), 1 = threatened with extinction, 2 = severely endangered, 3 = endangered, V = decreasing, G = presumed endangered, R = extremely rare, D = data scarce, * = at present not considered threatened, ** = surely not threatened; (V, G, R) = taxa supposed to be rare by the Authors (H.L.-B. & M.C.) on the basis of experience and / or the literature. Ecological preferences according to van Dam *et al.* (1994). M = Moisture preferences. 1 = almost never occurring outside water bodies, 2 = mainly occurring in water bodies, 3 = mainly occurring in water bodies & regularly on wet places, 4 = mainly occurring on wet places, 5 = nearly exclusively occurring outside water bodies. T = trophic preferences. 1 = oligotraphentic, 2 = oligo-mesotraph., 3 = mesotraphentic, 4 = meso-eutraphentic, 5 = eutraphentic, 7 = oligo. to eutraphentic. pH = pH preferences: 1 = acidobiontic (<5.5), 2 = acidophilous (<7), 3 = circumneutral (about 7), 4 = alkaliphilous (>7). DB = comparison with the taxa found in springs in the DBNP and BNP. "B" = means sampled as well in the BNP; "=" means sampled as well in the BNP and in the DBNP.

Table 2. List of all diatom taxa found in the mire pool studied in the Gesäuse National Park with indications on their distribution and autecology. Br = Bryophytes; De = Debris + surface sediment. o = observed while scanning the permanent mounts but not encountered during the counts.

Taxon	JAMOT		RL	M	T	pH
	De	Br				
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki & species group	1	3	**	3	7	3
<i>Aulacoseira alpigena</i> (Grunow) Krammer		4	G	1	1	2
<i>Brachysira intermedia</i> (Østrup) Lange-Bertalot	2		(G)	-	-	-
<i>Caloneis</i> sp.		o	-	-	-	-
<i>Encyonema lunatum</i> (W. Smith) Van Heurck	1		3	3	2	2
<i>Encyonema neogracile</i> Krammer	1	o	(G)	-	-	-
<i>Encyonema perpusillum</i> (Cleve-Euler) D.G. Mann	7	2	3	4	1	2
<i>Eunotia kruegeri</i> Lange-Bertalot	6	1	(R)	-	-	-
<i>Eunotia neocompacta</i> Mayama	10	3	3	-	1	2
<i>Eunotia paludosa</i> Grunow var. <i>paludosa</i>	24	204	V	4	1	1
<i>Eunotia paratridentula</i> Lange-Bertalot & Kulikovskiy	2	1	*	-	-	-
<i>Eunotia subarcuatoides</i> Alles <i>et al.</i>	1		**	3	1	1
<i>Eunotia tenella</i> (Grunow) Hustedt	1		V	3	1	2
<i>Eunotia superpaludosa</i> Lange-Bertalot nom. prov.		1	(D)	-	-	-
<i>Frustulia crassinervia</i> (Brebisson) Lange-Bertalot	30	19	V	3	1	1
<i>Frustulia saxonica</i> Rabenhorst	318	157	V	3	1	1
<i>Gomphonema utae</i> Lange-Bertalot & Reichardt	o		D	-	-	-
<i>Kobayasiella micropunctata</i> (Germain) Lange-Bertalot	17	192	(R)	-	-	1
<i>Kobayasiella parasubtilissima</i> (Kobayasi & Nagumo) Lange-Bertalot	4		V	3	1	1
<i>Kobayasiella subtilissima</i> (Cleve) Lange-Bertalot		2	3	3	1	1
<i>Naviculadicta</i> cf. <i>difficillima</i> Hustedt	o		G	3	2	2
<i>Neidium</i> sp.	o		-	-	-	-
<i>Nitzschia acidoclinata</i> Lange-Bertalot	o		*	3	3	3
<i>Nitzschia perminuta</i> (Grunow) M. Perag.	5		*	-	-	2
<i>Pinnularia microstauron</i> (Ehrenberg) Cleve	1	1	V	3	7	3
<i>Pinnularia rupestris</i> Hantzsch	3	9	G	3	1	3
<i>Tabellaria flocculosa</i> (Roth) Kützing	1		**	3	3	2
Total Number of valves counted	435	599				
Total Number of taxa counted	19	14				
Total Number of taxa observed	23	16				

RL = Red List species (Lange-Bertalot 1996), 1 = threatened with extinction, 2 = severely endangered, 3 = endangered, V = decreasing, G = presumed endangered, R = extremely rare, D = data scarce, * = at present not considered threatened, ** = surely not threatened; (V, G, R) = taxa supposed to be rare by the Authors (H.L.-B. & M.C.) on the basis of experience and / or the literature. Ecological preferences according to van Damet *et al.* (1994). M = Moisture preferences. 1 = almost never occurring outside water bodies, 2 = mainly occurring in water bodies, 3 = mainly occurring in water bodies + regularly on wet places, 4 = mainly occurring on wet places, 5 = nearly exclusively occurring outside water bodies. T = trophic preferences. 1 = oligotraphentic, 2 = oligo.-mesotraph., 3 = mesotraphentic, 4 = meso.-eutraphentic, 5 = eutraphentic, 7 = oligo. to eutraphentic. pH = pH preferences: 1 = acidobiontic (<5.5), 2 = acidophilous (<7), 3 = circumneutral (about 7), 4 = alkaliphilous (>7).

Table 3. List of all diatom taxa found in the ephemeral pools studied in the Gesäuse National Park with indications on their distribution and autecology. De = Debris + surface sediment, o = observed while scanning the permanent mounts but not encountered during the counts.

	FRIED	TITUE2	RL	M	T	pH
Taxon	De	De				
<i>Chamaepinnularia mediocris</i> (Krass.) Lange-Bert.	o	2	V	4	1	2
<i>Craticula</i> cf. <i>riparia</i> var. <i>mollenhaueri</i> Lange-Bertalot	49	4	R	-	-	-
<i>Craticula</i> cf. <i>submolesta</i> (Hustedt) Lange-Bertalot	10	4	3	-	-	-
<i>Diadesmis</i> cf. <i>brekkaensis</i> (Krasske) D.G.Mann	2	1	V	4	-	3
<i>Cymboplectura</i> cf. <i>citrus</i> (Carter & Bailey-Watts) Krammer	1		-	-	-	-
<i>Eolimna minima</i> (Grunow) Lange-Bertalot	2		**	3	5	4
<i>Eolimna tantula</i> (Hustedt) Lange-Bertalot	o		(*)	-	-	-
<i>Eunotia curtagrunowii</i> Nörpel-Schempp & Lange-Bertalot	o		(G)	-	-	-
<i>Eunotia exigua</i> (Bréb.) Rabenh.	1		**	3	7	1
<i>Eunotia</i> cf. <i>fallax</i> A. Cleve sensu auct.	1		D	4	1	2
<i>Eunotia nymanniana</i> Grunow lectotype	o	o	G	-	1	-
<i>Eunotia</i> sp. FRIED	4		-	-	-	-
<i>Eunotia</i> sp. TITUE2		2	-	-	-	-
<i>Eunotia neocompacta</i> var. <i>vixcompacta</i> Lange-Bert.	o	o	(*)	-	-	-
<i>Hantzschia abundans</i> Lange-Bert.	2		**	-	-	-
<i>Luticola</i> cf. <i>mutica</i> (Kützing) D.G. Mann	1		**	4	5	3
<i>Mayamaea</i> cf. <i>fossaloides</i> (Hustedt) Lange-Bertalot	20	o	D	4	-	-
<i>Mayamaea</i> sp. "köpfig"		o	-	-	-	-
<i>Naviculadicta</i> cf. <i>difficillima</i> Hustedt	75	5	G	3	2	2
<i>Naviculadicta</i> cf. <i>opportuna</i> Hustedt	41	3	2	-	-	-
<i>Neidium alpinum</i> Hustedt	15	3	3	3	1	2
<i>Neidium</i> cf. <i>ampliatum</i> (Ehr.) Krammer	1	1	G	1	3	3
<i>Neidium</i> cf. <i>Neidium</i> sp. pag. 202 Antoniadou <i>et al.</i>	1		(R)	-	-	-
<i>Neidium</i> cf. <i>dubium</i> (Ehr.) Cleve	3		*	1	4	3
<i>Neidium longiceps</i> (Gregory) R. Ross	19	o	G	3	1	2
<i>Nitzschia</i> cf. <i>gracilis</i> Grunow	101	4	*	3	2	4
<i>Nitzschia palea</i> var. <i>debilis</i> (Kützing) Grunow		3	*	3	1	3
<i>Pinnularia borealis</i> Ehrenberg	3	1	**	4	2	3
<i>Pinnularia</i> cf. <i>gibberula</i> Ehrenberg	61	4	1	-	-	-
<i>Pinnularia lata</i> (Brébisson) Rabenhorst	1		V	5	1	2
<i>Pinnularia microstauron</i> (Ehrenberg) Cleve	5		V	3	7	3
<i>Pinnularia perirrorata</i> Krammer	5	3	V	-	-	-
<i>Pinnularia sinistra</i> Krammer	2	1	*	-	-	-
<i>Planothidium lanceolatum</i> (Brébisson ex Kütz.) Lange-Bert.	o		**	3	5	4
<i>Sellaphora</i> sp. "no bars"	4		(D/R)	-	-	-
<i>Sellaphora</i> sp. "pupula" group "ganz zarte"	21		(D/R)	-	-	-
<i>Sellaphora</i> sp.			(D/R)	-	-	-
<i>Stauroneis</i> cf. <i>hassiac</i> "spitz vorgezogen"	30	3	(D/R)	-	-	-
<i>Stauroneis</i> cf. <i>hassiac</i> "stumpf vorgezogen"	3		(D/R)	-	-	-
<i>Stauroneis thermicola</i> var. <i>lanceolata</i> (Hust.) Hust.	2	1	(V)	-	-	-
Total Number of valves counted	486					
Total Number of taxa counted	30	17				
Total Number of taxa observed	36	22				

RL = Red List species (Lange-Bertalot 1996), 1 = threatened with extinction, 2 = severely endangered, 3 = endangered, V = decreasing, G = presumed endangered, R = extremely rare, D = data scarce, * = at present not considered threatened, ** = surely not threatened; (V, G, R) = taxa supposed to be rare by the Authors (H.L.-B. & M.C.) on the basis of experience and / or the literature. Ecological preferences according to van Dam *et al.* (1994). M = Moisture preferences. 1 = almost never occurring outside water bodies, 2 = mainly occurring in water bodies, 3 = mainly occurring in water bodies + regularly on wet places, 4 = mainly occurring on wet places, 5 = nearly exclusively occurring outside water bodies. T = trophic preferences. 1 = oligotraphentic, 2 = oligo.-mesotraph., 3 = mesotraphentic, 4 = meso.-eutraphentic, 5 = eutraphentic, 7 = oligo. to eutraphentic. pH = pH preferences: 1 = acidobiontic (<5.5), 2 = acidophilous (<7), 3 = circumneutral (about 7), 4 = alkaliphilous (>7).

Table 4. List of all diatom taxa found in Lake Sulzkarsee (stones, -0.2m) in the Gesäuse National Park with indications on their distribution and autecology. Genera are abbreviated using the standard abbreviations proposed by Simkhada (2006).

Taxa	Sulzkarsee - 0.2 m, epilithon	RL	M	T	pH	L	G
<i>Achnantheidium inconspicuum</i> Østrup	21	(*)	-	-	-	-	-
<i>Achd. minutissimum</i> (Kützing) Czarnecki & species group	56	**	3	7	3	-	-
<i>Adlafia bryophila</i> (Petersen) Lange-Bertalot	2	V	5	3	3	-	-
<i>Amphora pediculus</i> (Kützing) Grunow	4	**	3	5	4	-	-
<i>Amph. sp. 27x8µm cf. copulata</i> (Kützing) Schoeman & Archibald	10	**	1	5	4	-	-
<i>Cymbella compacta</i> Østrup	3	*	-	-	-	4.0	2
<i>Cymb. cymbiformis</i> Agardh	1	V	2	2	3		
<i>Cymb. lange-bertalotii</i> Krammer	3	(*)	-	-	-	-	-
<i>Cymb. excisa</i> var. <i>angusta</i> Krammer	32	(*)	-	-	-	2.4	1
<i>Cymboppleura cf. hercynica</i> (A. Schmidt) Krammer	1	-	-	-	-	-	-
<i>Cymp. cf. peranglica</i> Krammer	2	-	-	-	-	1.6	2
<i>Cymp. subaequalis</i> (Grunow) Krammer	2	G	3	2	3	-	-
<i>Denticula tenuis</i> Kützing	25	*	3	3	4	3.0	1
<i>Diadesmis contenta</i> (Grunow) D.G. Mann	2	**	4	7	4	-	-
<i>Diploneis krammeri</i> (Hustedt) Krammer & Lange-Bertalot	2	(G)	-	-	-	-	-
<i>Encyonema caespitosum</i> Kützing	30	**	-	7	-	3.7	1
<i>Ency. minutum</i> (Hilse ex Rab.) D.G. Mann	7	*	-	-	3	2.0	2
<i>Ency. silesiacum</i> var. <i>distinctepunctatum</i> Krammer	3	(R)	-	-	-	-	-
<i>Ency. ventricosum</i> (Agardh) Grunow in A. Schmidt	5	(*)	-	-	-	-	-
<i>Encyonopsis cesatii</i> (Rabenhorst) Krammer	1	*	3	1	3	1.5	3
<i>Encs. krammeri</i> Reichardt	10	*	-	-	-	-	-
<i>Encs. subminuta</i> Krammer & Reichardt	3	(G)	-	-	-	-	-
<i>Gomphonema angustum</i> Agardh	2	V	-	1	4	-	-
<i>Gomp. auritum</i> A. Braun	4	G	-	-	-	2.5	1
<i>Navicula cryptotenelloides</i> Lange-Bertalot	8	**	-	-	-	-	-
<i>Navi. radiosa</i> Kützing	8	**	3	4	3	-	-
<i>Neidiomorpha binodiformis</i> (Krammer) Cantonati et. al.	1	G	-	-	-	-	-
<i>Nitzschia angustata</i> Grunow	1	*	1	3	3	3.9	2
<i>Nitz. cf. bryophila</i> "schmal"	1	-	-	-	-	-	-
<i>Nitz. perminuta</i> (Grunow) Peragallo	0	*	3	2	2	-	-
<i>Nupela</i> sp.	0	-	-	-	-	-	-
<i>Pinnularia borealis</i> Ehrenberg	0	**	4	2	3	-	-
<i>Pseudostaurosira robusta</i> (Fusey) D.M. Williams et Round	10	*	2	7	4	2.5	1
<i>Sellaphora [bacillum K-LB] Φ 'button'</i> (Mann et al. 2008)	2	(R)	-	-	-	-	-
<i>Sela. cf. pseudopupula</i> (Krasske) Lange-Bertalot	3	G	-	-	-	-	-
<i>Sela. [laevissima K-LB] Φ 'normal'</i> (Mann et al. 2008)	1	(*)	-	-	-	2.5	1
<i>Staurosirella pinnata</i> (Ehrb.) Williams & Round	167	**	3	7	4	-	-
Total Number of valves counted	433						
Total Number of taxa counted	34						
Total Number of taxa observed	37						

RL = Red List species (Lange-Bertalot 1996), 1 = threatened with extinction, 2 = severely endangered, 3 = endangered, V = decreasing, G = presumed endangered, R = extremely rare, D = data scarce, * = at present not considered threatened, ** = surely not threatened; (*, V, G, R, D) = taxa assigned to Red List categories by the Authors (H.L.-B. & M.C.) on the basis of experience and / or the literature. Ecological preferences according to van Dam et al. (1994). M = Moisture preferences. 1 = almost never occurring outside water bodies, 2 = mainly occurring in water bodies, 3 = mainly occurring in water bodies + regularly on wet places, 4 = mainly occurring on wet places, 5 = nearly exclusively occurring outside water bodies. T = trophic preferences. 1 = oligotraphentic, 2 = oligo.-mesotraph., 3 = mesotraphentic, 4 = meso.-eutraphentic, 5 = eutraphentic, 7 = oligo. to eutraphentic. pH = pH preferences: 2 = acidophilous (<7), 3 = circumneutral (about 7), 4 = alkaliphilous (>7). Value of Hofmann Trophic Index (Hofmann 1999): L = trophic location (*Lokation*), G = weighting (*Gewichtung*).

Table 5. Different oligotrophic habitats (with a focus on springs, in bold) ranked by decreasing percentage of threatened Red List (Lange-Bertalot 1996) diatom taxa (from Cantonati 2010 modified). %RL = % diatom taxa belonging to the sum of all threatened Red List categories, N = N. of sampling stations, TNT = total number of taxa found, MNTc = maximum number of taxa counted in one sample. C.t.p. = close to pristine, org. pol. = organic pollution, str. = streams. ABNP = Adamello-Brenta Natural Park (Trentino, Italy), CRENODAT Project (Autonomous Province of Trento = Trentino, Italy), DBNP = Dolomiti Bellunesi National Park (Veneto Region, Italy), CESSPA Project (Province of Verona, Veneto Region, Italy), BNP = Berchtesgaden National Park (Bavaria, Germany), GNP = Gesäuse National Park (Styria, Austria), JPNP = Julian Prealps Natural Park (Friuli Venezia Giulia Autonomous Region, Italy).

Habitat type	Location	N	%RL	TNT	MNTc	Impacts	Reference
Mire pools	Danta di Cadore, south-eastern Alps	5	72	86	26	c.t.p.	Cantonati <i>et al.</i> 2010 <i>Nova Hedwig</i> . In press
Carbonate springs	BNP, north-eastern Alps	9	54	104	30	c.t.p.	Cant. & Lange-Bert. <i>Diat. Res.</i> In press Publ. 9
Carbonate springs	GNP, north-eastern Alps	10	52	100	26	c.t.p.	the present technical report
Crystalline high-mountain lakes	ABNP, south-eastern Alps		50			c.t.p.	Tolotti 2001 Lange-Bert. <i>Festschrift</i>
Carbonate and crystalline springs	ABNP, south-eastern Alps	30	48	254	54	c.t.p.	Cantonati 1998 <i>Diatom Res.</i>
Springs (all main lithologies)	Trentino, CRENODAT Project, s.-e. Alps	110	45			strict oligotrophy	Cantonati <i>et al.</i> <i>subm. a J-NABS</i>
Springs (all main lithologies)	Vicinities of Frankfurt		43				Werum & Lange-Bert. 2004 <i>Iconogr. Diat.</i> 13
Carbonate springs and streams	DBNP, south-eastern Alps	21	41	131	32	org. pol. (str.)	Cantonati & Spitale 2009 <i>FAL</i> Publ. 8
Low-altitude carbonate springs	JPNP, south-eastern Alps	3	40	60	36	org. pol.	Cantonati 2004 <i>Gortania</i>
Lake Tovel (epilithon euphotic z.)	ABNP, south-eastern Alps		40				Cantonati <i>et al.</i> 2009 <i>Eur. J. Phycol.</i>
Mountain springs	Springs in Vorarlberg, Austria, Alps	27	38	197			Gesierich & Kofler 2010b <i>Diatom Res.</i> In press
Helocrene springs (spring fens)	Western Carpathians		33				Fránková <i>et al.</i> 2009 <i>Fottea</i>
Lake Garda	South-eastern Alps	24	33	75	42	(NO ₃ ⁻), shore-morphology alteration	Spitale <i>et al.</i> 2010 <i>Hydrobiologia Subm.</i>
Pre-Alpine carbonate springs	Areas of Basel and Zürich, CH	17	30	118			Taxböck & Preisig 2007 <i>CEDIATOM1 Proc.</i>
Carbonate springs	Verona Province, pre-Alpine	25	24	138		NO ₃ ⁻ , capturing	Angeli <i>et al.</i> 2010 <i>Fottea</i> Publ. 18
Carbonate springs	Beauce region, Orléanais, N France	14	23	135		NO ₃ ⁻ ,	Bertrand <i>et al.</i> 1999 <i>Symbioses</i>
Carbonate springs	Bavaria		8			NO ₃ ⁻ ,	

Because of the practical relevance of a precise assessment of the trophic status of the lake that is affected by relevant impacts (part of the shores are pasture ground for cows, and fishes were stocked in the lake since the 1970s; Jersabek *et al.* 2004), the Trophic index based on the composition of epilithic littoral diatom assemblages was calculated following Hofmann (1994, 1999). The value was found to be 2.83, corresponding to mesotrophy since the value ranges for the trophic classes according to the mentioned index are as follows:

1.00÷1.99 (cl 1)	oligotrophic
2.00÷2.49 (cl 2)	oligo/mesotrophic
2.5÷3.49 (cl 3)	mesotrophic
3.5÷3.99 (cl 4)	meso/eutrophic
4.00÷5.00 (cl 5)	eutrophic.

However, the values of the trophic indices were available for 11 taxa accounting for only 26.8% of the diatom assemblage (Table 4). The values of the trophic index and location were not available for the dominant species *Staurosirella pinnata* (Ehrb.) Williams & Round (Appendix 2). This species is known to be meso-eutraphentic, if not eutraphentic: e.g., Rott *et al.* (1999) in their diatom-based quality assessment system for Austrian running waters classify it as meso-eutraphentic (*Trophiewert* = 2.2, 2.1-2.5 meso-eutraphentic; *Stickstoffzahl* = 2.3, 2.1-2.5 meso-eutraphentic; $TP_{OPT.} = 56 \mu g L^{-1}$; $NO_3^- - N_{OPT.} = 1166 \mu g L^{-1}$). Therefore the trophic status of Lake Sulzkarsee is likely to have been underestimated because of the many taxa that could not be used for index calculation following Hofmann (1994, 1999), i.e. one of the few trophic indices based on littoral diatoms developed in the Alpine area. Therefore the trophic status of Lake Sulzkarsee should more suitably be defined as meso-eutrophic, if not eutrophic. The same conclusion was reached by Jersabek *et al.* (2004) based on observations on total phosphorus concentration, and phytoplankton and zooplankton composition and biomass. Another important result of the benthic diatom analyses that further confirms these assessments was generated by the depth-distribution studies. Only the sample collected from stones taken from the shallowest depth (-0.2 m) presented benthic diatom populations sufficiently developed to be analysed. The samples collected at -1, -3, and -7m presented only very sparse diatoms, suitable for qualitative observations only. The -1m sample still presented some benthic species (*Staurosirella pinnata*, *Cymbella compacta*, *Encyonema* sp., *Eucocconeis laevis*, *Navicula antonii*, *Encyonopsis* sp.); the -3 and -7m samples were virtually devoid of benthic species, and included almost only plankton fallout (girdle bands of a planktic *Fragilaria* species, and a planktic centric diatom of the genus *Cyclotella*). This situation perfectly corresponds to the extinction of the infralittoral benthic assemblages predicted by Lowe (1996) in the case of lake eutrophication determining phytoplankton blooms and excessive shading of benthic substrata in deep waters. In Lake Sulzkarsee this situation was by sure further worsened by the fact that all hard substrata in deep waters were soon covered by silt and organic detritus (plankton fallout etc.). As repeatedly underlined by Jersabek *et al.* (2004) the siltation

(*Verschlammung*) problem extensively affects also stones and boulders in the shallow littoral zone. This is particularly relevant also for the benthic diatom assemblages, since many of the typical oligotraphentic taxa are mainly epilithic.

Red List status (Lange-Bertalot 1996) was available for relatively high percentages of the diatoms found in the different categories of habitats studied (Tables 1-4). The remaining taxa (about 30% of the total in the case of the springs) could be assigned to Red List categories by the author of the present report (in collaboration with Prof. Horst Lange-Bertalot) on the basis of experience and / or the literature (Tables 1). Overall, in the case of the springs (Tables 1), about 52% were found to belong to threatened categories of the Red List. One species, *Brachysira calcicola* ssp. *pfisteri* Lange-Bertalot & Werum (Appendix 1), belonged to category 2 ("severely endangered"), and four species to category 3 ("endangered"): *Achnanthes trinodis* (W. Smith) Grunow (Appendix 1), *Amphora inariensis* Krammer, *Platessa conspicua* (A. Mayer) Lange-Bertalot, and *Rossithidium petersenii* (Hust.) Round & Bukht. As regards trophic status, most species belonging to category 5 ("eutraphentic") are very widespread in carbonate springs, e.g.: *Amphora pediculus* (Kütz.) Grunow (Appendix 2), *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow, and *Planothidium lanceolatum* (compare e.g. Cantonati 1998, Cantonati & Spitale 2009). The occurrence of *Luticola mutica* (Kütz.) D.G. Mann in near-natural springs and streams is more likely due to desiccation periods rather than to higher trophic levels (Cantonati *et al.* 2001). Moreover, the complete absence of some typical indicators of nutrient enrichment in springs, such as *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck, *Fragilaria capucina* var. *vaucheriae* (Kütz.) Lange-Bert. and *Gomphonema parvulum* Kütz. (compare Cantonati & Spitale 2009 for the DBNP and Cantonati & Lange-Bertalot 2010 for the BNP), or their occasional occurrence with very low abundances (e.g., *Gomphonema micropus* Kütz.), appear to suggest a satisfactory situation of integrity as regards the trophic status of the springs of the GNP. As concerns pH (Van Dam *et al.* 1994, Table 1), the species found in the GNP springs studied are alkaliphilous (cat. 4), or circumneutral (cat. 4) coherently with the lithological substratum.

As regards the mire pool studied (Table 2), the number of sites considered in the GNP is too low to allow any general consideration. In any case, the percentage of species belonging to threat categories is very high (almost $\frac{3}{4}$ of the taxa found belong RL threat categories!), and well comparable to figures highlighted by Cantonati *et al.* (2011) for similar habitats in the south-eastern Alps. Almost no widely distributed opportunistic taxa seem to colonize the studied mire pool. The specialized taxa are almost all classified as endangered to various degrees since these habitats (mires and bogs) are highly menaced, especially in central Europe. Four of the threatened Red List species found belong to category 3 ("endangered"): *Encyonema lunatum* (W. Smith) Van Heurck, *Encyonema perpusillum* (Cleve-Euler) D.G. Mann, *Eunotia neocompacta* Mayama, and *Kobayasiella subtilissima* (Cleve) Lange-Bertalot. As concerns pH preferences according to Van Dam *et al.* (1994), the acidic

conditions in the mire determined the occurrence of acidobiontic, acidophilous, and circumneutral species in spite of the carbonate lithology of the area (Table 2).

The diatom microflora found in the ephemeral pools (Table 3) was surprisingly rich of poorly known and interesting taxa, as is clearly shown also by the many "cf." and working IDs in the table. Also in this case the percentage of threatened Red List species is very high (66%). One species belonged to category 1 ("threatened with extinction"), one to category 2 ("severely endangered") and two to category 3 ("endangered"). However, with the exception of *Neidium alpinum* Hustedt (cat. 3; see SEM micrograph on the front page of the present report) more observations are necessary to confirm these identifications. The somewhat unexpected complexity of the diatoms of these vernal pools explains why most of the work on the diatoms found in these special habitats in the GNP is still in progress. The main reason for the great interest of these microfloras is likely to be the fact that in-depth diatom studies on this habitat type have been very rare, with few exceptions (Lange-Bertalot *et al.* 2003). The unstable conditions typical of the temporary pools determine the fact that all species identified with certainty belong to moisture preference category (Van Dam *et al.* 1994) 3 or higher (3 = "mainly occurring in water bodies + regularly on wet places", 4 = "mainly occurring on wet places", 5 = "nearly exclusively occurring outside water bodies").

With reference to Lake Sulzkarsee (Table 4), the percentage of species belonging to threat categories of the Red List (33%) is not as low as expected because of the impacts (stocking with fishes, cow pasture) affecting the lake since several decades. To gain a reference, it might be compared with the value (40%, Cantonati *et al.* 2009) found in an oligotrophic carbonate lake with similar conductivity, but much larger and deeper (Lake Tovel, Brenta Dolomites). The occurrence of several species belonging to moisture preference category 3 (Van Dam *et al.* 1994, Table 4), and even of some belonging to categories 4 or 5 might be related to water-level fluctuations that used to be even more important in the lake before the spring-stream water was diverted to enter the lake to favour the rearing of salmonid fishes (Jersabek *et al.* 2004). Of the desiccation tolerant species mentioned the numerically most relevant, after the dominant *Staurosirella pinnata* is *Denticula tenuis*. During a study focussing on the depth distribution of benthic diatoms in a carbonate mountain lake characterized by marked seasonal water-level fluctuations (Cantonati *et al.* 2009), this species was found to be one of those with a statistically significant preference for the upper shallow zone. Trophic preferences according to Van Dam *et al.* (1994) were available for 46% of the taxa only (Table 4): significantly, almost one third of these taxa were "tolerant", i.e. known to occur from oligo- to eutrophic conditions.

The percentages of taxa belonging to threat categories of the Red List (Lange-Bertalot 1996) can be compared to the values found in several similar habitats of the Alps in Table 5 (from Cantonati 2010, mod.). The comparison shows that the values found in the GNP are consistent with those reported for oligotrophic habitats of the Alps of the same categories. For what concerns

ephemeral pools, the data produced provide first useful elements for the quantification of the presence of rare and endangered diatom species in this strongly-understudied habitat type.

From the taxonomic point of view the most relevant observation is that taxa that are likely to turn out to be new to science were identified in all habitat types. However, in the case of the mire pool the taxon most likely to be new was present with such a low number of specimens to make its characterization impossible. Much work is still necessary to complete the morphological and ecological characterization of these taxa. This will be done in the coming months, and the results will be published in international journals.

There are several other taxa the occurrence or absence of which in the GNP is of special interest. They will be shortly commented in the following.

Achnanthes trinodis (W. Smith) Grunow (Appendix 1) is a relatively rare oligotraphentic, alkaliphilous species. It was found to be relatively abundant in a carbonate oligotrophic mountain lake with abundant epilithic substratum (Cantonati *et al.* 2009) and in one out of 16 springs studied in the Dolomiti Bellunesi National Park (Cantonati & Spitale 2009). In the GNP its occurrence appears to be related to hygropetric microhabitats.

Achnanthidium dolomiticum Cantonati et Lange-Bertalot was discovered in springs of the Dolomiti Bellunesi National Park, and found to be epiphytic, typical of unstable environments that undergo seasonal desiccation, and apparently bound to drainage basins dominated by dolomite rocks (Cantonati & Lange-Bertalot 2006). This peculiar ecological trait could be confirmed thanks to the investigation in the Berchtesgaden springs where *A. dolomiticum* was found only in three springs, the main substratum of which was the Ramsau dolomite. In the limestone dominated GNP, an *Achnanthidium* population displaying the typical morphological characters of this species was found to be epiphytic on mosses in just one very small spring (KAMS).

Achnanthidium pfisteri Lange-Bertalot is a recently described taxon (Werum & Lange-Bertalot 2004). It was frequent and sometimes abundant in the DBNP (Cantonati & Spitale 2009) whilst it was not found in the BNP (Cantonati & Lange-Bertalot 2010).

Achnanthidium strictum Reichardt is a recently described taxon (Reichardt 2004). It was sampled in the BNP and in the DBNP (Cantonati & Lange-Bertalot 2010). It was also frequent and sometimes abundant in several springs considered for the CRENODAT Project in the Autonomous Province of Trento (Cantonati *et al.* 2012 in prep.). It appeared to show a clear preference for the epilithic substratum, and the findings in the GNP confirm these observations.

Brachysira calcicola ssp. *pfisteri* Lange-Bertalot & Werum (Appendix 1) was not found in the BNP (Cantonati & Lange-Bertalot 2010) and collected in just one spring and with very low abundances in the DBNP (Cantonati & Spitale 2009). During the CRENODAT Project it was observed in a spring with spring

associated limestones and hygropetric microhabitats (Cantonati *et al.* 2012 in prep.).

Caloneis constans was described by Erwin Reichardt in 1994 (Reichardt 1994) but only very rarely reported in the literature (Fránková *et al.* 2009). It appears to be characteristic of carbonate, rheohelocene springs. Since this typology could not be considered during this investigation, this could explain its absence from the GNP, while it was found in the BNP where two rheohelocene springs were considered (Cantonati & Lange-Bertalot 2010).

Cymbella tridentina Lange-Bertalot, Cantonati et Scalfi is a species discovered in the southeastern Alps (Brenta Dolomites; Cantonati *et al.* 2010). It appears to be characteristic of the uppermost part of spring-fed, carbonate streams. This might explain its absence in the GNP where material was collected from spring streams but could not yet be considered for analysis. In the BNP springs it was extremely rare (only one specimen observed during SEM work; Cantonati & Lange-Bertalot 2010).

Encyonema sublangebertalotii Lange-Bertalot et Cantonati, very recently described on the basis of materials collected in the BNP springs (Cantonati & Lange-Bertalot 2010) resembles taxa that have a widespread distribution and are very relevant also for running waters quality evaluation systems (*Encyonema lange-bertalotii*, *E. minutum*, *E. ventricosum*, *E. silesiacum*), but can be distinguished by the combination of its morphological characteristics including in particular outline, dimensions, raphe course etc. In the GNP it is relatively common showing ecological preferences that confirm the observations made in the BNP and DBNP.

The phenodeme provisionally called *Encyonopsis "sagitta"* refers to populations found in Berchtesgaden in a couple of springs and again in the GNP, and that might turn out to be a species new to science. However, on the basis of the information available at the present moment it is not possible to exclude that these morphotypes just belong to the morphological variability of *Encyonopsis cesatii* (Rabenhorst) Krammer. It is therefore preferred to designate this phenodeme only in a taxonomically informal way. The individuals are distinguished especially by the outline and in particular by the markedly pointed apices.

Eunotia glacialispinosa Lange-Bertalot et Cantonati, very recently described on the basis of materials collected in the BNP springs (Cantonati & Lange-Bertalot 2010), is very characteristic from the morphological point of view, since it resembles *Eunotia glacialifalsa* Lange-Bertalot from which it is however clearly distinguished by four evident (by careful focusing) spines. Also the distribution and ecology already appear to be of special interest. In the BNP it was frequently associated with *Eunotia arcubus* Nörpel-Schempp & Lange-Bertalot. In spite of the fact that the latter species was relatively frequent in the DBNP (6 springs), *E. glacialispinosa* was not found in this natural preserve. A similar situation occurred in the GNP where *E. arcubus* was found in two springs (very abundant in one case, Table 1) but *E. glacialispinosa* was not observed.

Gomphonema lateripunctatum Reichardt & Lange-Bertalot (Appendix 1) was found both in the BNP and in the DBNP, even if it was less important from the quantitative point of view in the former nature preserve. In the GNP it was found to be one of the most characteristic species of the hygropetric rheocrenic spring. This is in excellent agreement with typical assemblages of the different spring types identified by means of fuzzy clustering on the data obtained studying more than 100 springs for the CRENODAT Project (Cantonati *et al.* 2012 in prep.).

Surirella spiralis Kützing is a rare species, considered crenophilous and bound to mountain carbonate springs with above-average mineral content. It was found in the DBNP (Cantonati & Spitale 2009), in the BNP (Cantonati & Lange-Bertalot 2010), and in the GNP always in just one spring: In the case of the GNP this was the remarkable KAMS spring.

Conclusions:

- In spite of being sometimes relatively harsh environments (small, probably mostly not-perennial springs), the springs studied host a rich diatom microflora, well comparable with what is known from other carbonate nature preserves of the Alps (e.g., Dolomiti Bellunesi, Cantonati & Spitale 2009; Berchtesgaden, Cantonati & Lange-Bertalot 2010) but notwithstanding with its own peculiar traits.
- The high percentage (about 50%) of Red List taxa found in the springs of the Gesäuse National Park is consistent with similar figures found in spring habitats of other Nature Parks of the Alps (e.g., Adamello-Brenta, Cantonati 1998; Dolomiti Bellunesi, Cantonati & Spitale 2009; Berchtesgaden, Cantonati & Lange-Bertalot 2010). This high proportion testifies that these springs still possess a relatively high ecological integrity because the number of Red List (Lange-Bertalot 1996) species tends to reduce with increasing impacts (compare Table 5).
- The diatom microflora of the two temporary pools sampled in 2009 turned out to be extremely interesting (rare, and putative new species). This is probably mainly due to the fact that in-depth studies with high taxonomic resolution of the diatom assemblages of this type of habitats are still very rare.
- The diatom microflora of the mire pool studied appeared to be characteristic and interesting. However, it would be necessary to study more sites with similar characteristics to get a reasonably complete figure of the potential contribution of this category of habitat to the diatom biodiversity of the Gesäuse National Park.
- The study of the benthic diatom assemblages of the Sulzkarsee allows to classify this small mountain lake as eutrophic with silted ("verschlammt") hard substrata. The siltation of the hard substrata by

debris and plankton fallout, and the shading due to the important development of planktic populations impede the development of an epilithic diatom flora at depths >0.5 m. The development of a "deep-water" benthic diatom flora, shown to include the most interesting and rare species also in a carbonate lake of the south-eastern Alps (Cantonati *et al.* 2009), is thus at present not possible in Lake Sulzkarsee due to the enhanced trophic status. The conclusions of previous studies (Jersabek *et al.* 2004) are thus completely confirmed.

- Cantonati *et al.* (2009) showed for an oligotrophic carbonate lake of the south-eastern Alps that the deep-water zone (infralittoral) is a particularly relevant habitat for benthic algae in lakes that are affected by marked natural or man-induced water-level fluctuations. This was the case of Lake Sulzkarsee prior to the diversion of spring-stream water into the lake, and it might be the situation of the lake again after restoration actions, including also the cessation of spring-stream water inflow (compare Jersabek *et al.* 2004), will have been undertaken.

- Several diatom taxa (1-2 in the springs, 2-3 in the temporary pools, 1-2 in the lake) were found that deserve further investigations to complete their morphological and ecological characterization. These taxa will probably turn out to be new to science.

Outlook:

- If restoration actions, such as those proposed in Jersabek *et al.* (2004), are undertaken, benthic diatom assemblages in Lake Sulzkarsee should be able to recover. In fact, characteristic oligotraphentic and oligo.-mesotraphentic species typically keep being present in assemblages of worse trophic categories even if in very small amounts.

- A fundamental pre-requisite for the re-establishment of characteristic oligo- / oligo.-mesotraphentic communities is the restoration of the epilithic habitat. This might be achieved as proposed by Jersabek *et al.* (2004). If the lake would be emptied to carry out restoration actions, it would be very useful to remove fine sediments not only from the shores but also from part of the stony substrata in deeper water to try to favour the re-colonization by the highly specialized and characteristic deep-water (infralittoral) assemblages.

- Diatoms should have the potential both to offer a reference condition of pre-disturbance assemblages if sediment cores are studied and to reflect changes induced by restoration actions if cobbles and boulders in the eulittoral zone are sampled during the restoration and at regular intervals for some years after the actions have been carried out.

To continue the present diatom study the following is planned or proposed:

- To present part of the results at international Congresses, and publish part of the findings (springs, lake) in international journals.
- To complete the ecological and morphological characterization of the most promising interesting taxa, and publish the descriptions of a part of the species new to science.
- If further funding would be available, it would be advisable to complete the study of the overall diatom biodiversity of the Gesäuse National Park (i.e., the study of the diatom diversity of all main types of aquatic habitats occurring in the nature preserve). To do this, the following work would be especially necessary: to analyse the five samples collected in a representative stream of the National Park, to analyse at least two other mire pools and at least one other temporary pool, to analyse other 2-3 springs focussing in particular on spring types that could not yet be considered (rheohelocrenes, helocrenes).

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Appendix 1 – Iconography of the diatom microflora of the hygropetric rheocrenic spring HAGL.

R valve

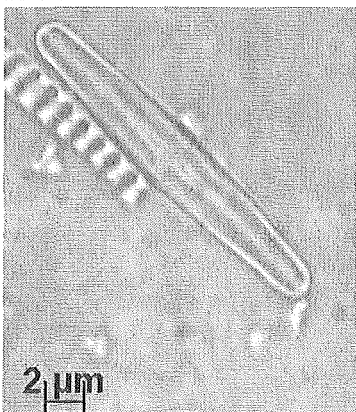
RL valve



Achnanthes trinodis (Red List species category 3 = endangered)

R valve

RL valve



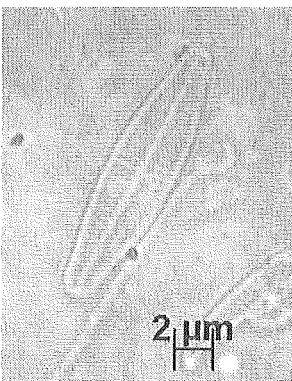
Achnanthidium affine



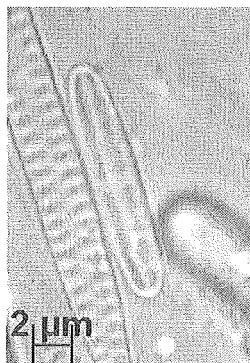
Achnanthidium neomicrocephalum

RL valve

R valve



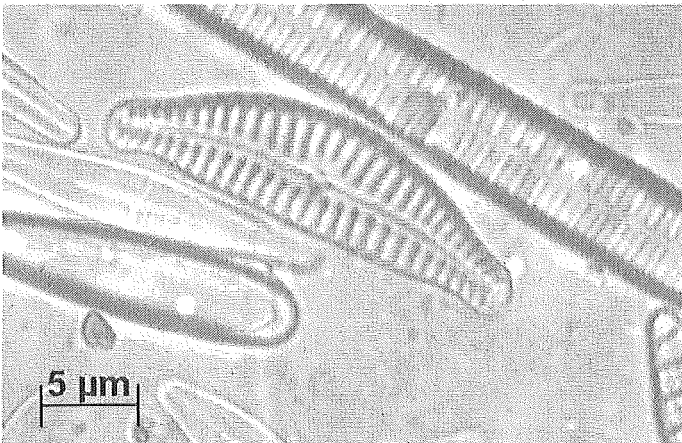
Achnanthidium pyrenaicum



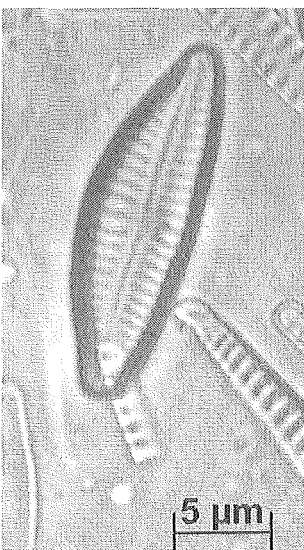
Achnanthidium strictum



Brachysira calcicola ssp. *pfisteri* (Red List species category 2 = severely endangered)



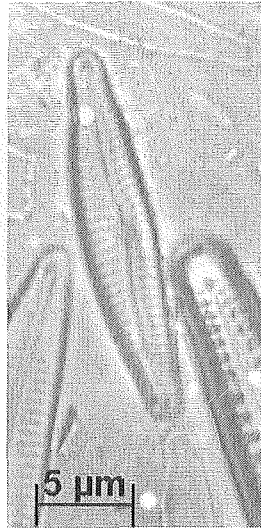
Cymbella excisa var. *angusta*



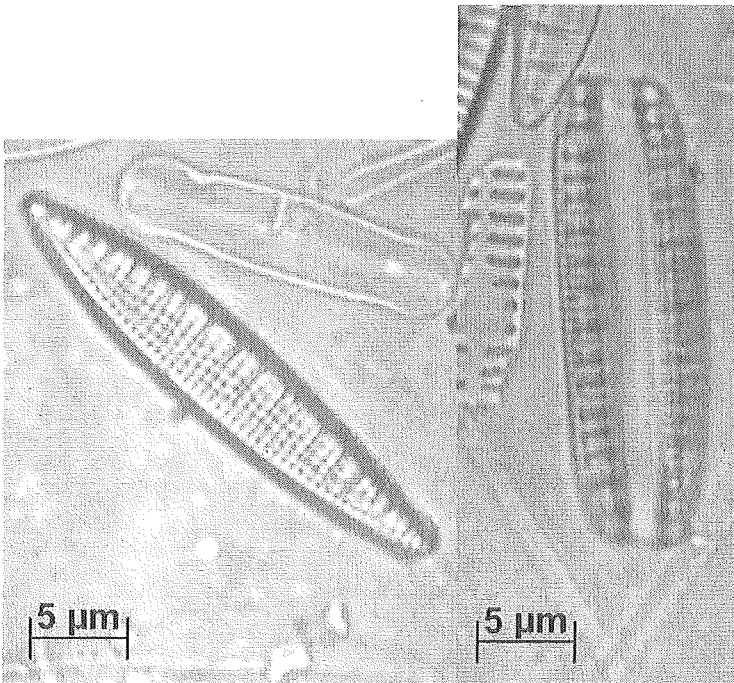
Cymboplectura diminuta



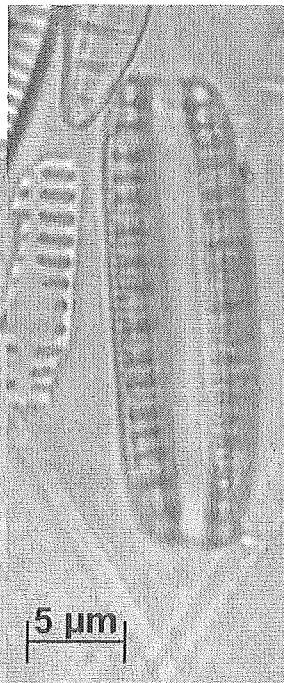
Delicata delicatula



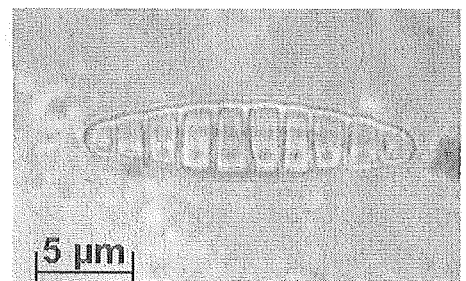
Delicata minuta



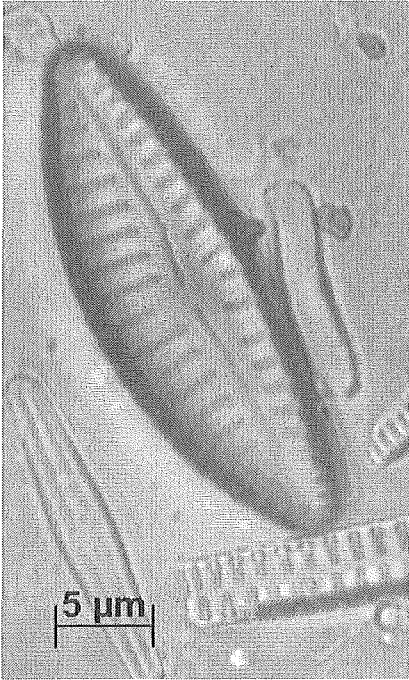
Denticula kuetzingii



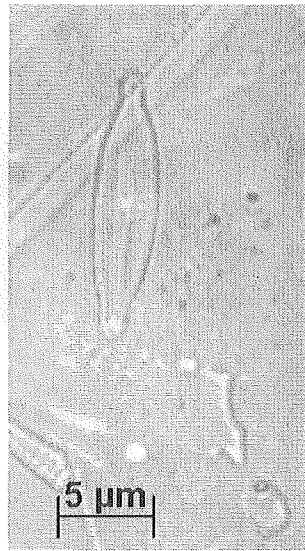
girdle view



Denticula tenuis



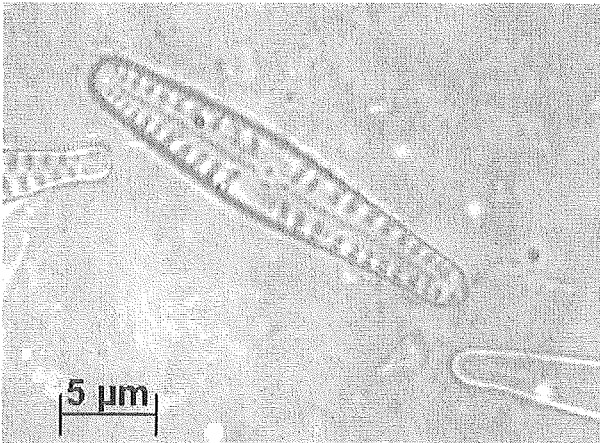
Encyonema alpinum



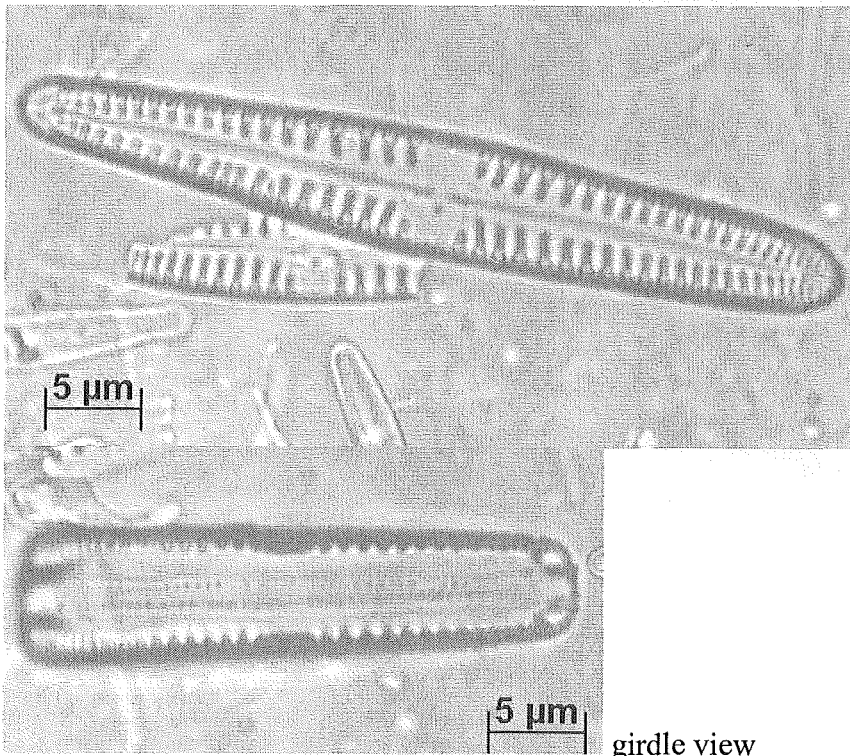
Encyonopsis minuta



Fragilaria distans



Gomphonema angustum

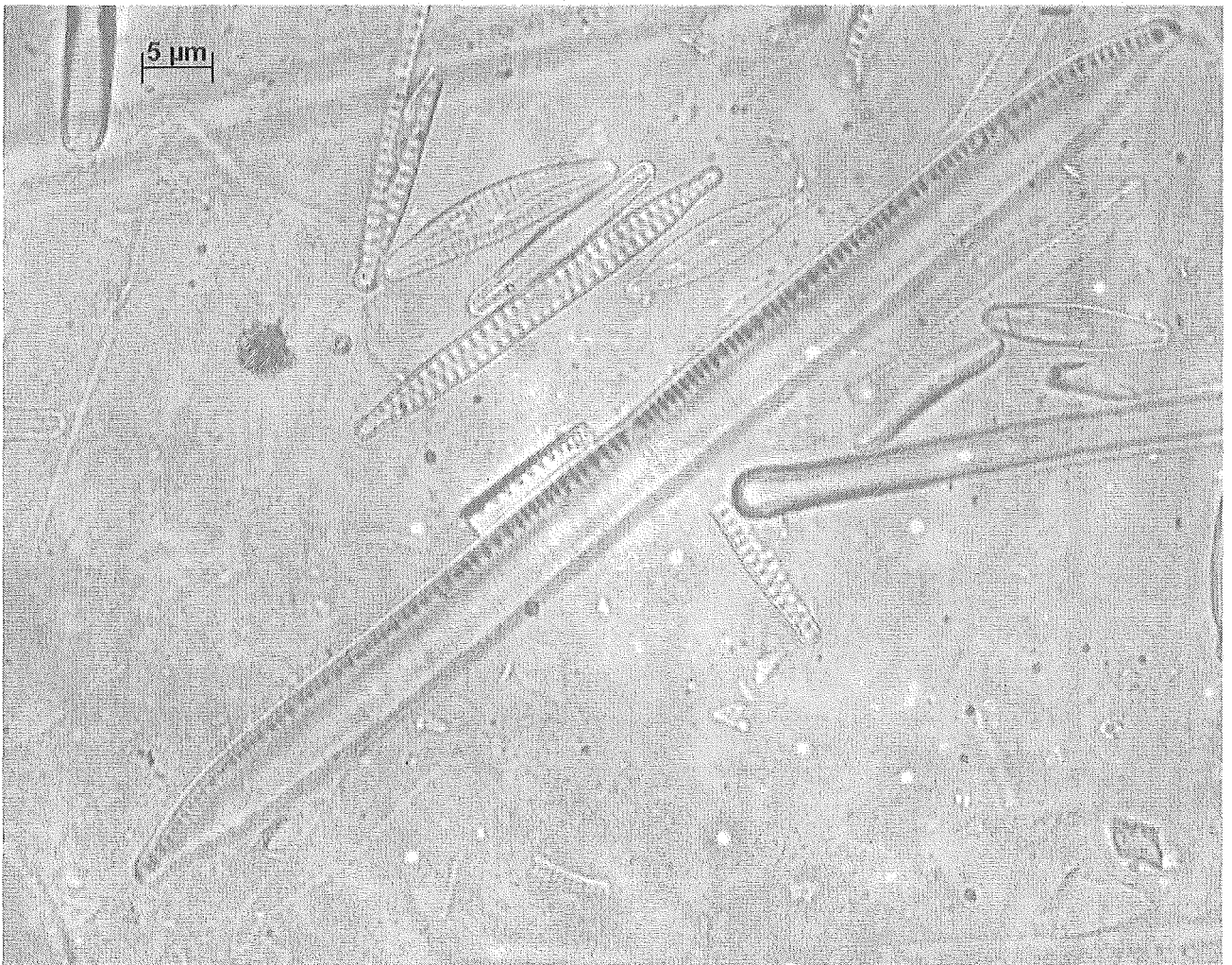


girdle view

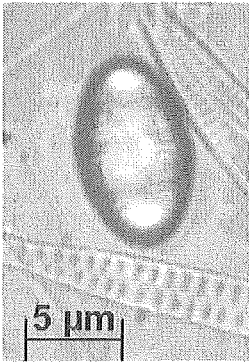
Gomphonema lateripunctatum



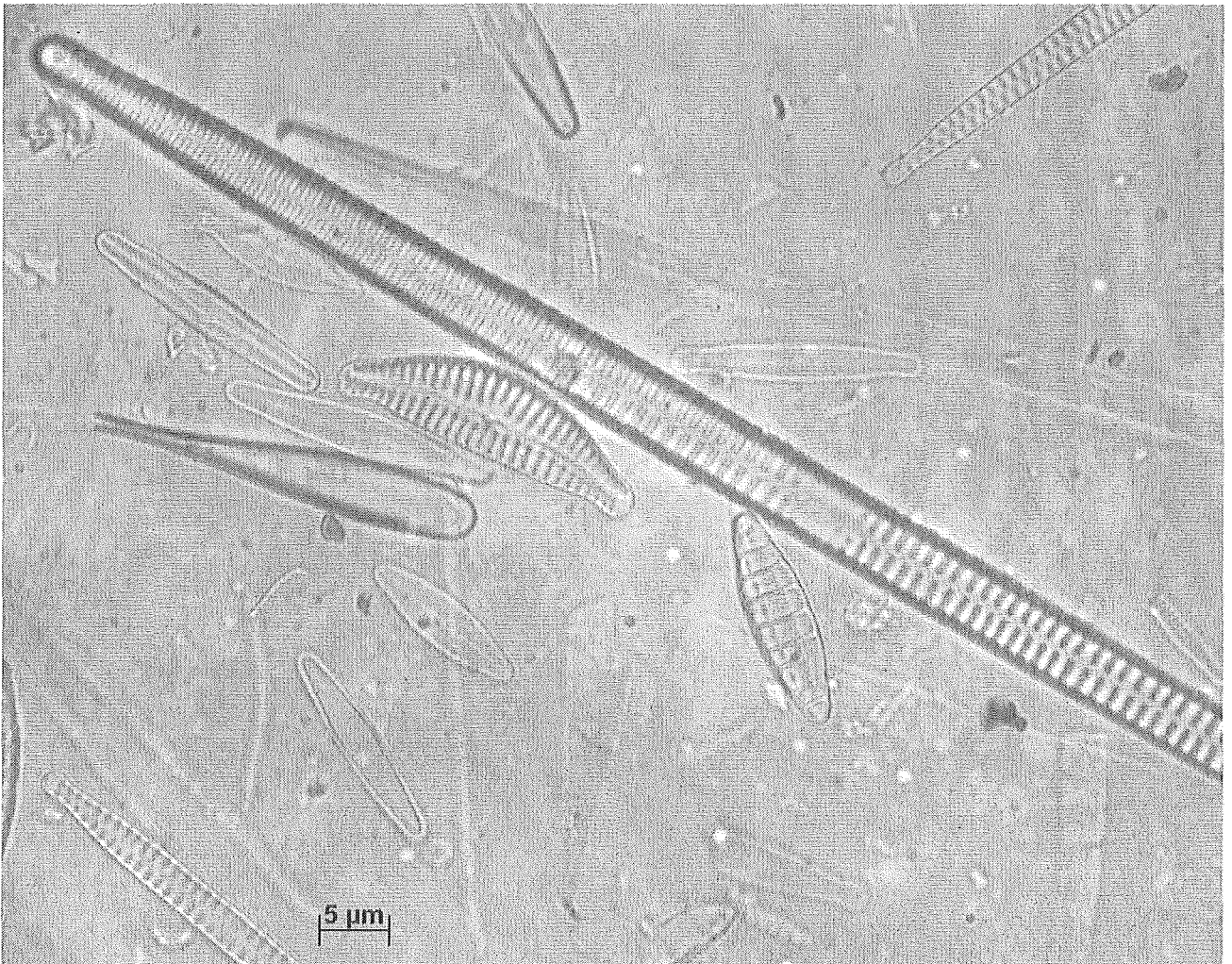
Navicula cryptotenella



Nizschia linearis

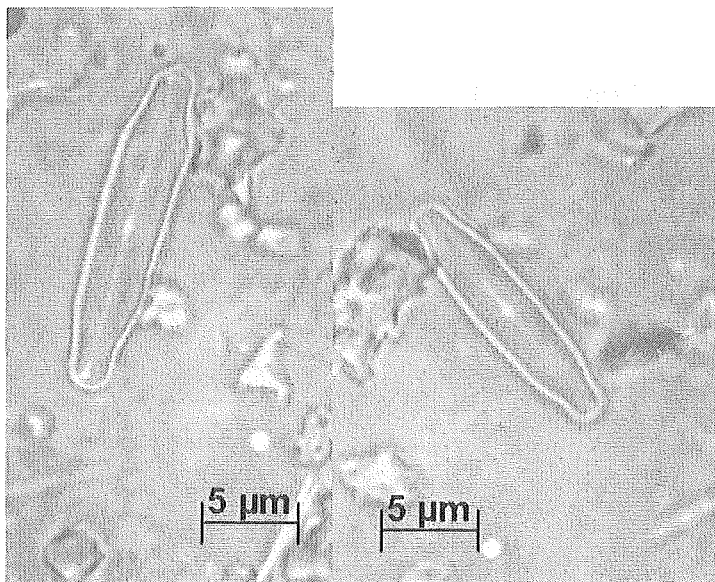


Tetracyclus rupestris

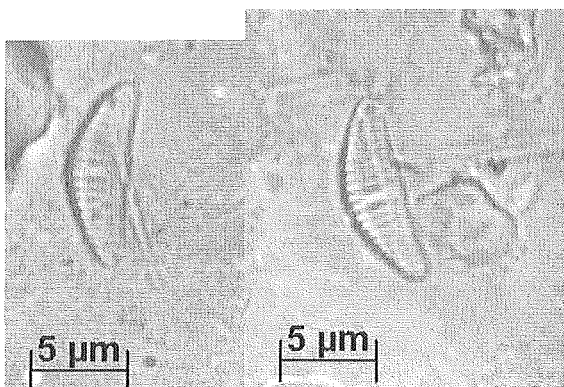


Ulnaria ulna

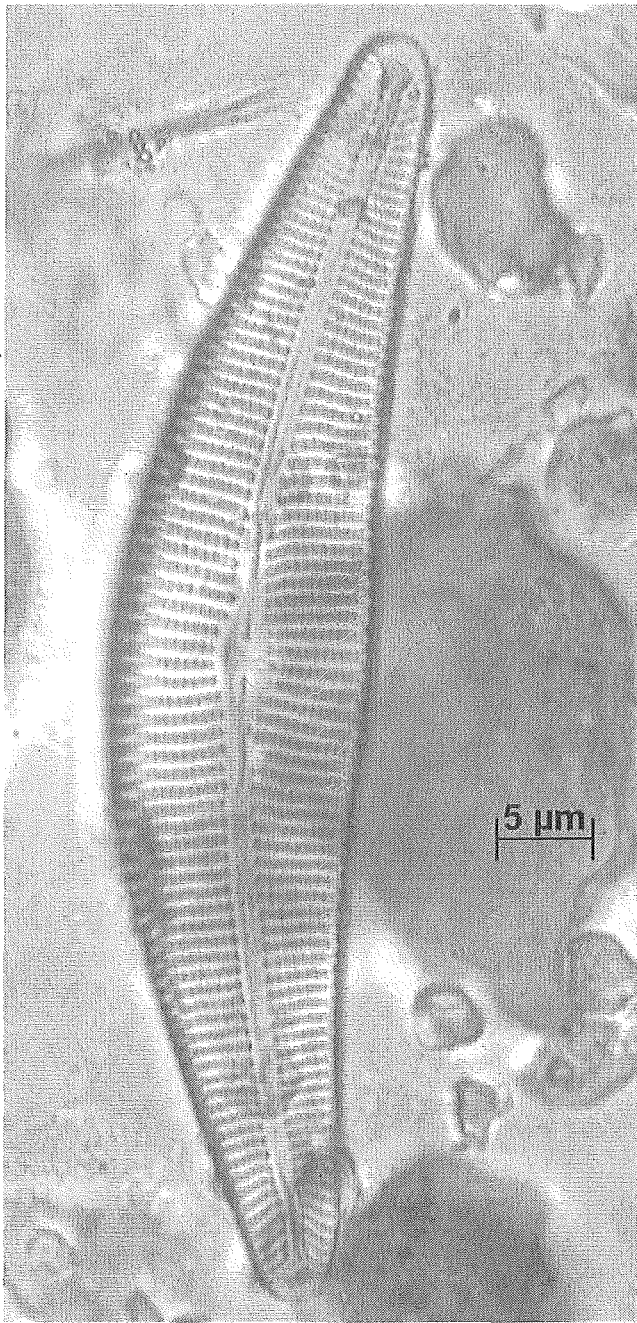
**Appendix 2 - Iconography of the littoral diatom microflora of the
Lake Sulzkarsee (material sampled from stones, -0.2 m).**



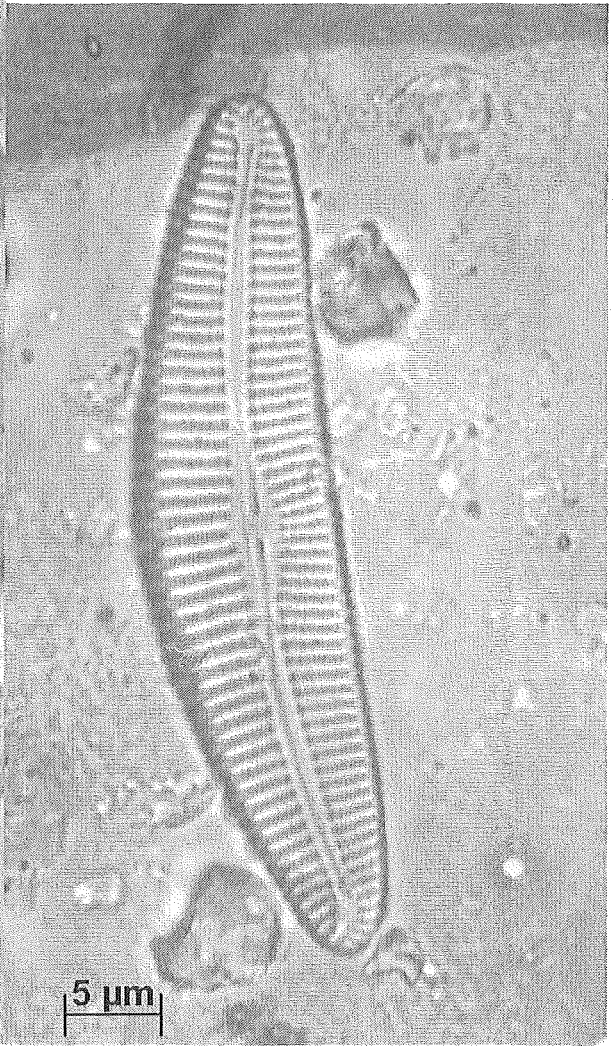
Adlafia bryophila



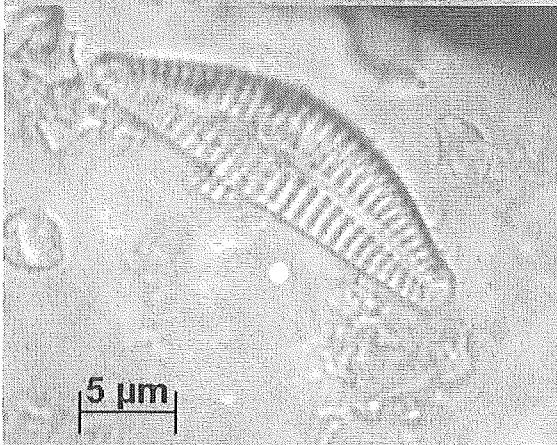
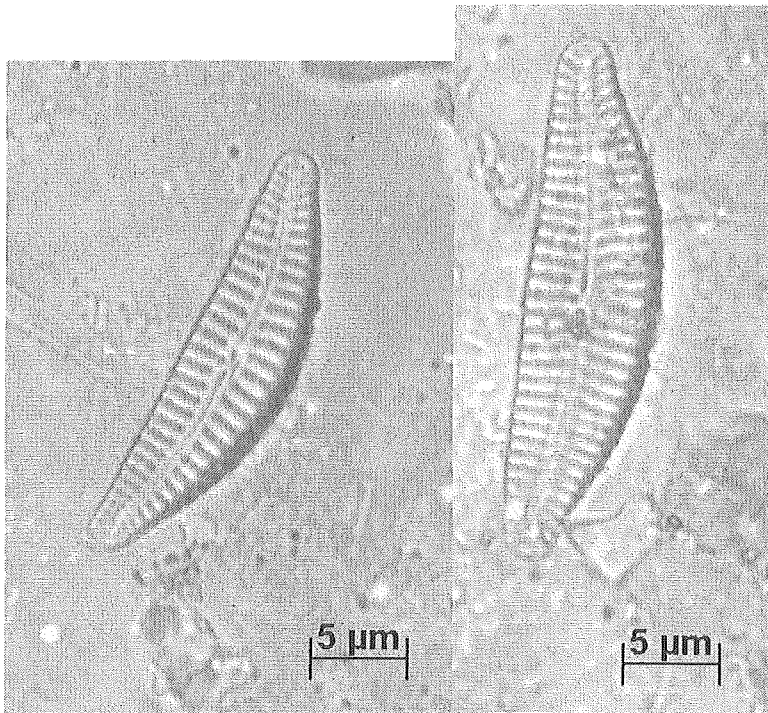
Amphora pediculus



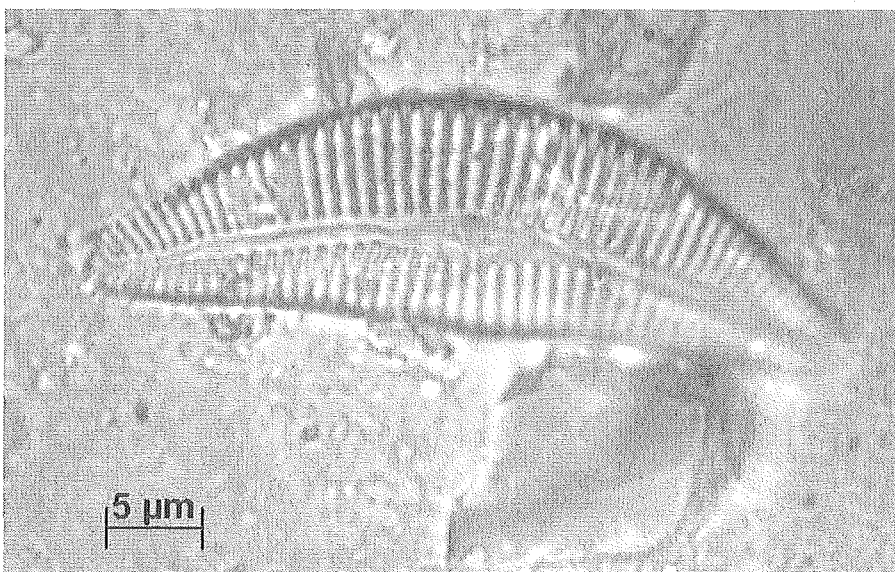
Cymbella cymbiformis



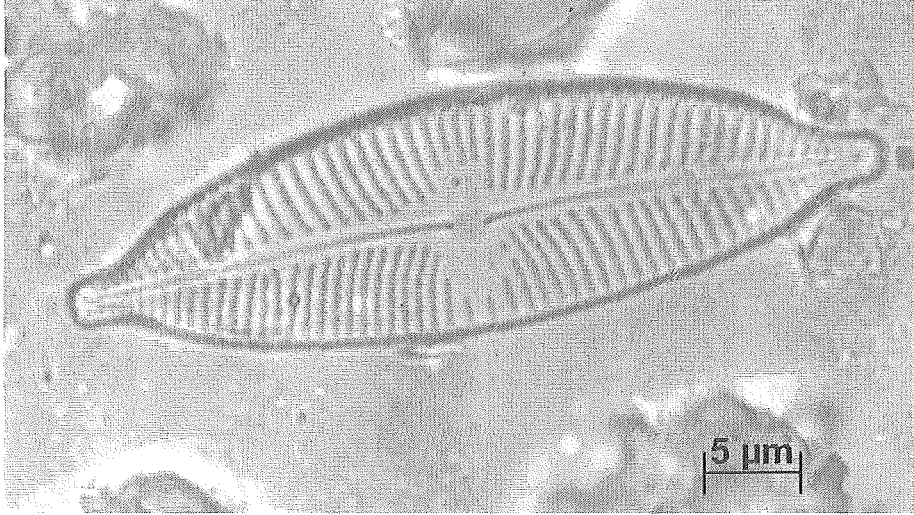
Cymbella compacta



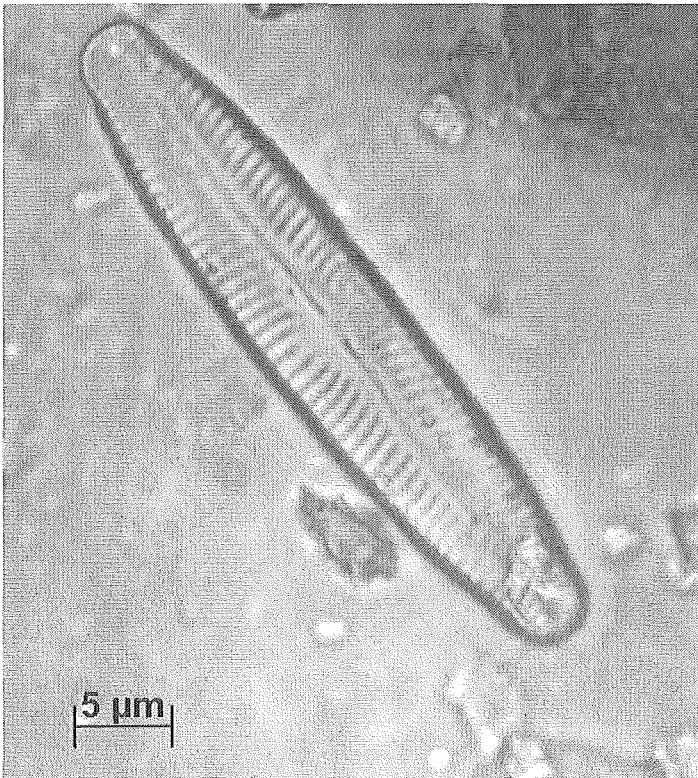
Cymbella excisa var. *angusta*



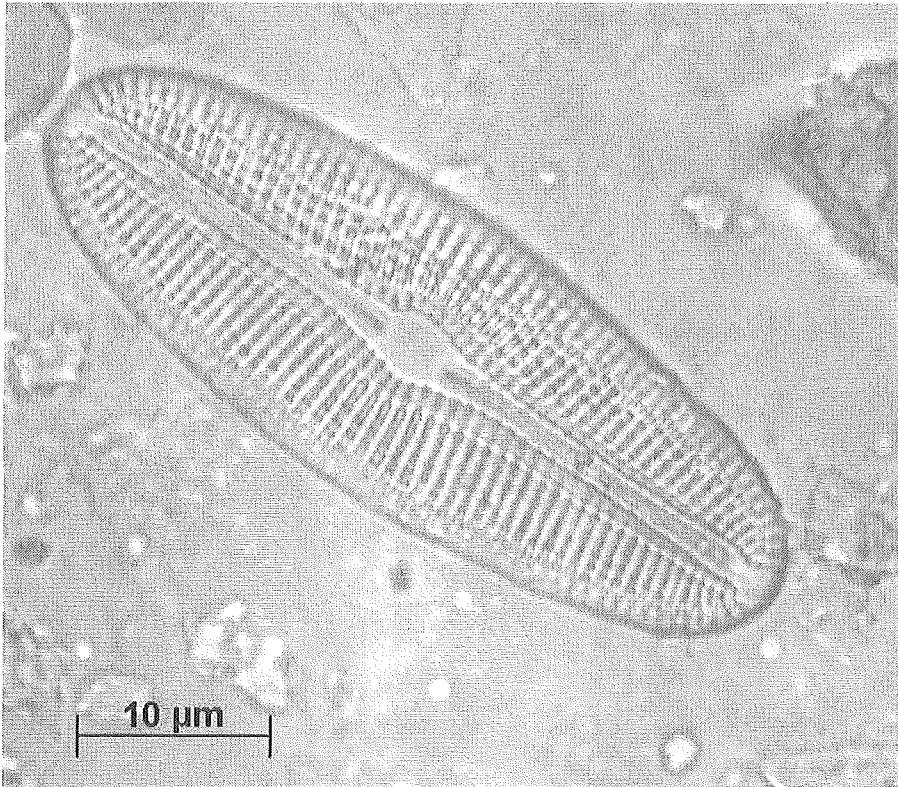
Cymbella subcystula



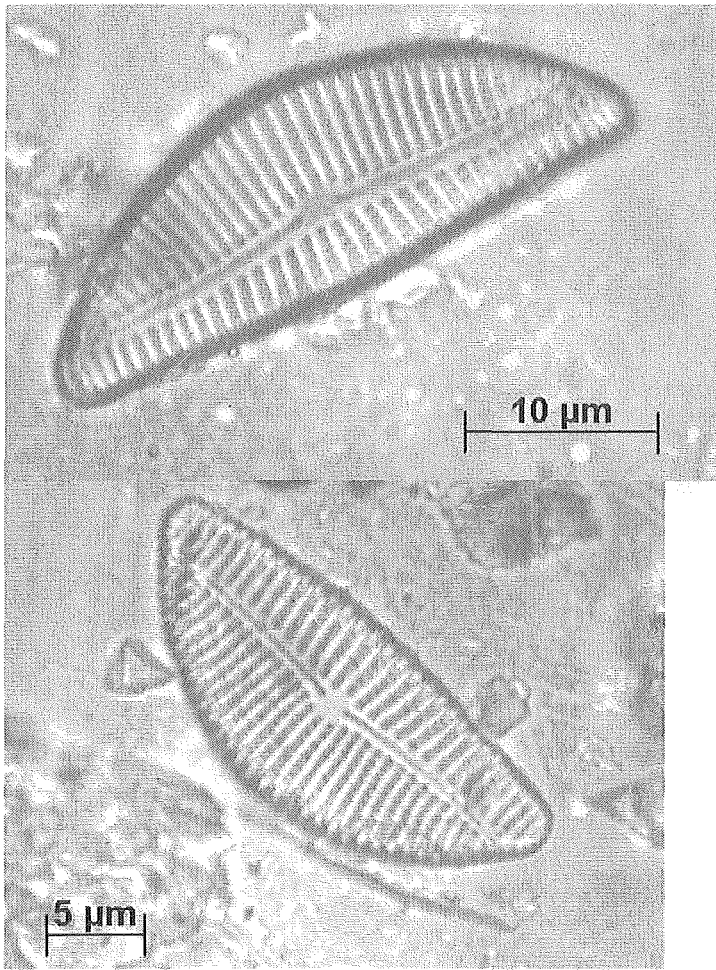
Cymbopleura cf. *perangelica*



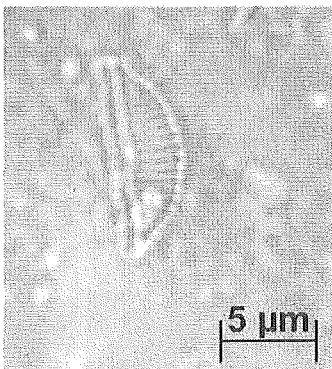
Cymbopleura *subaequalis*



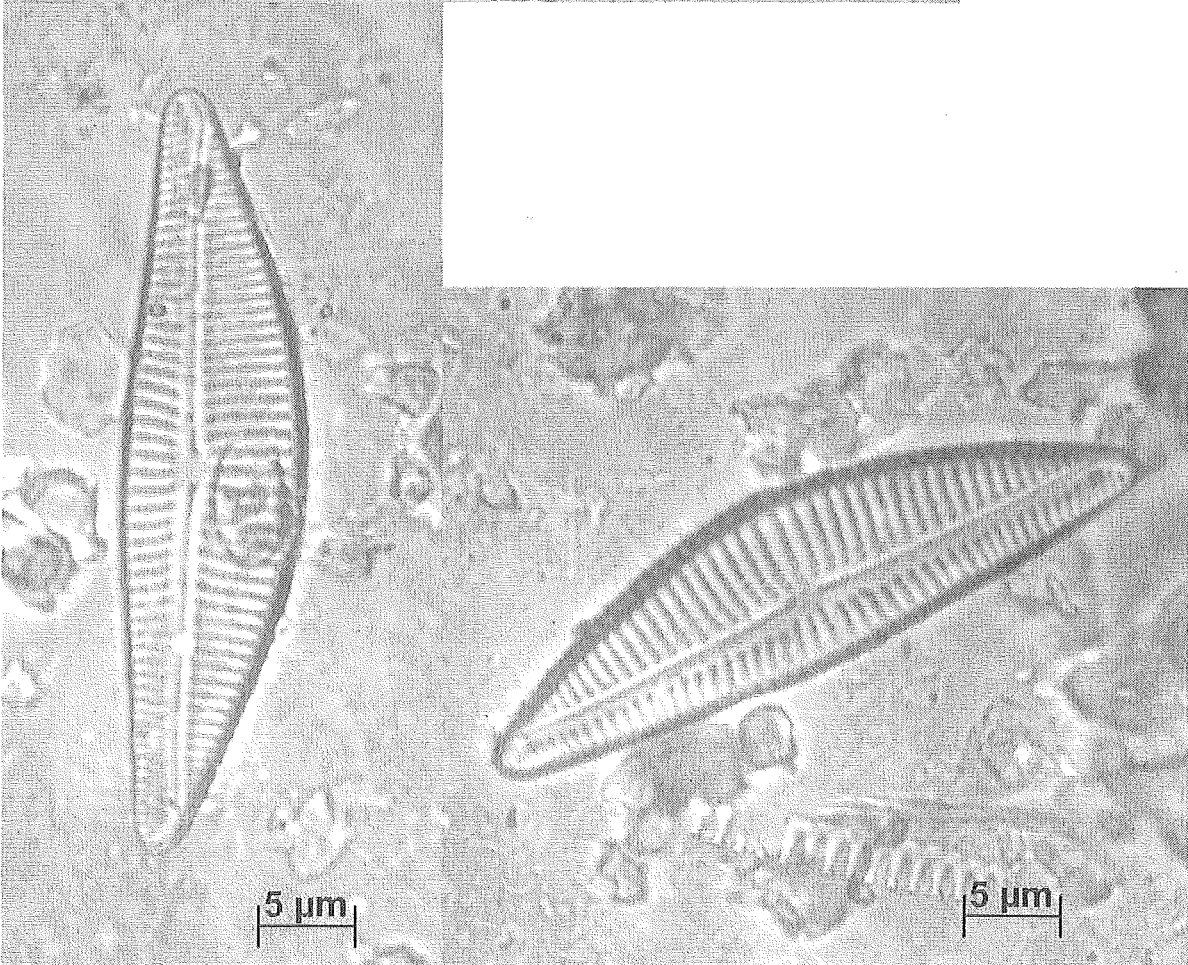
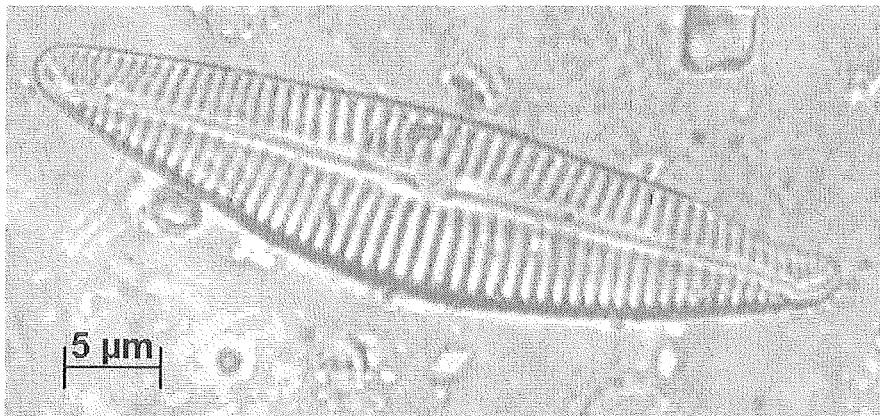
Diploneis krammeri



Encyonema caespitosum



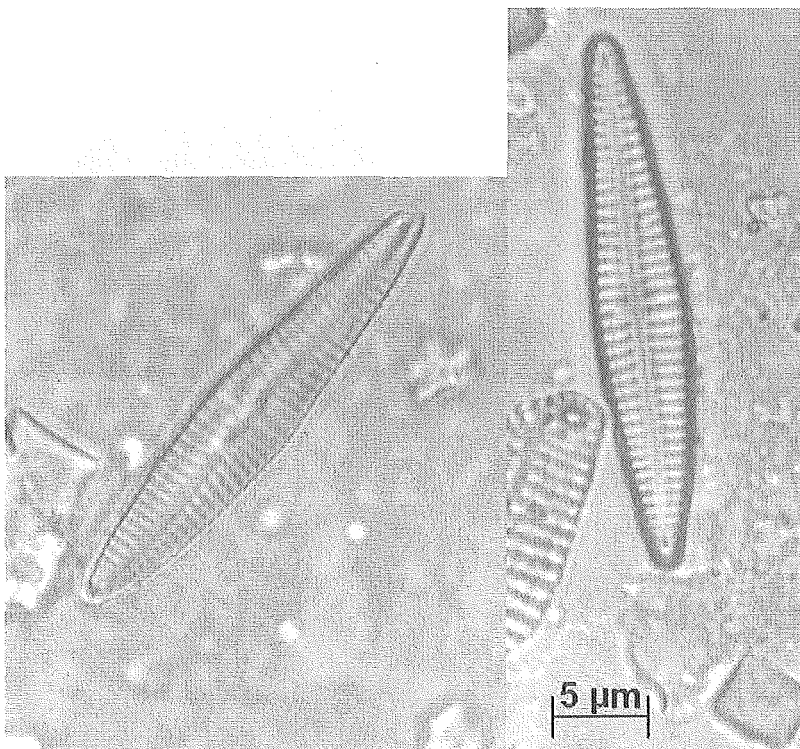
Encyonema minutum



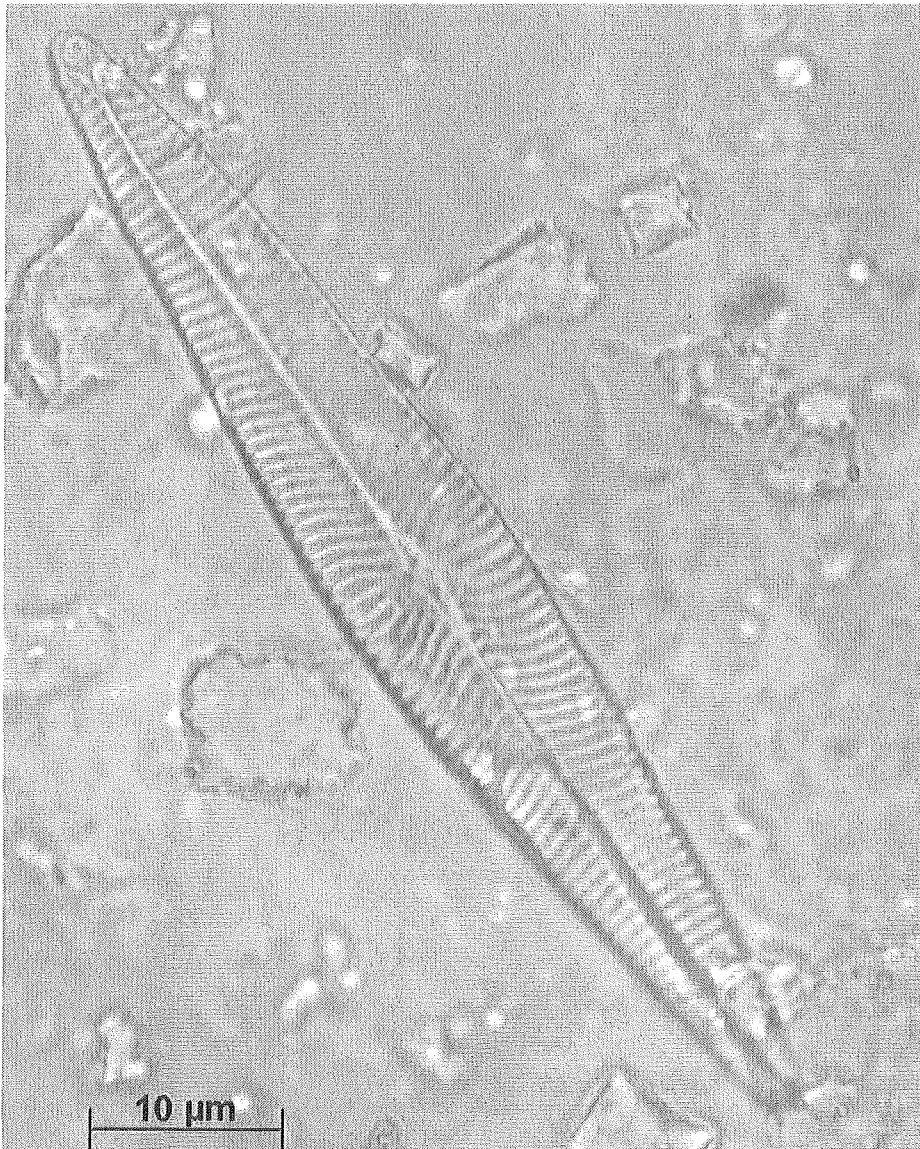
Encyonema silesiacum var. *distinctepunctatum*



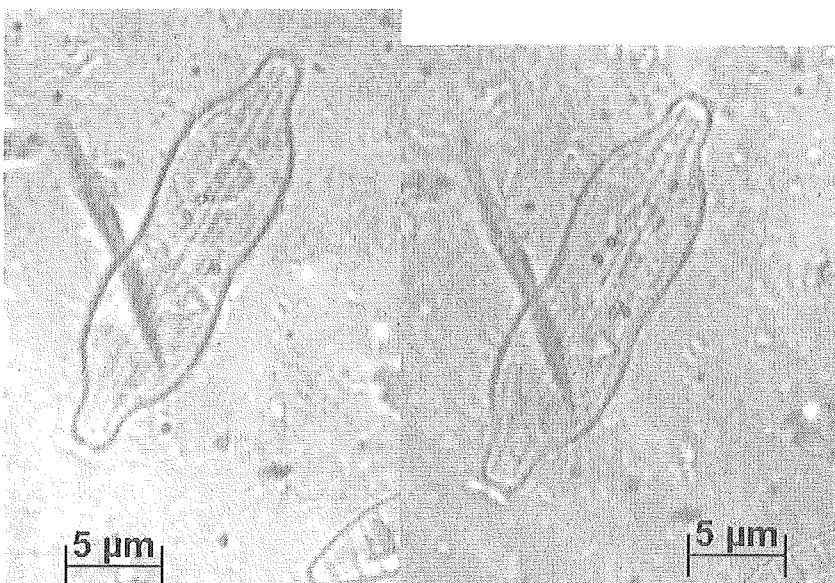
Encyonopsis cesatii



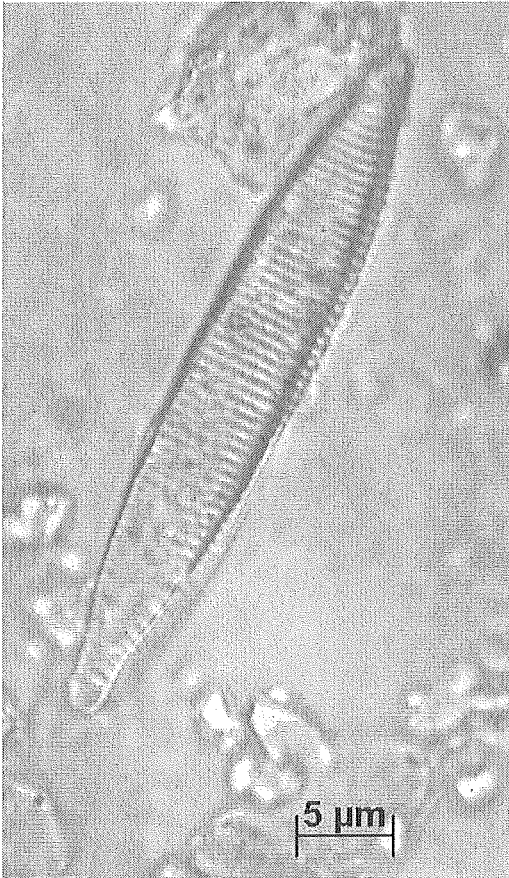
Gomphonema auritum



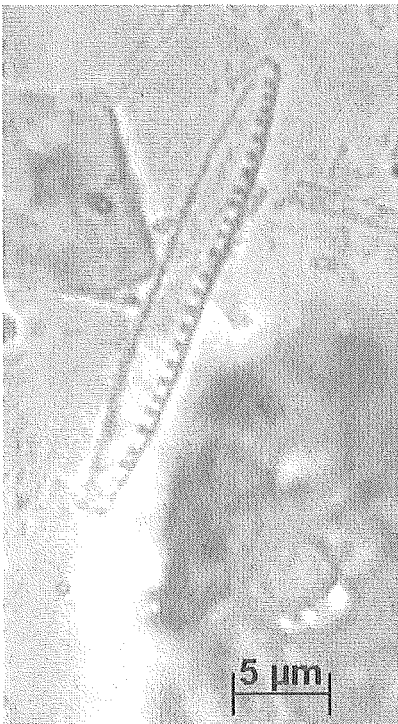
Navicula radiosa



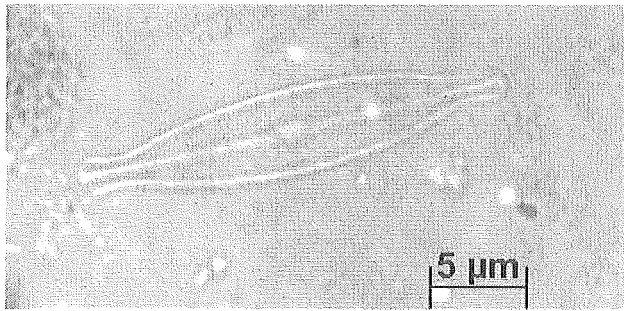
Neidiomorpha binodiformis



Nizschia angustata



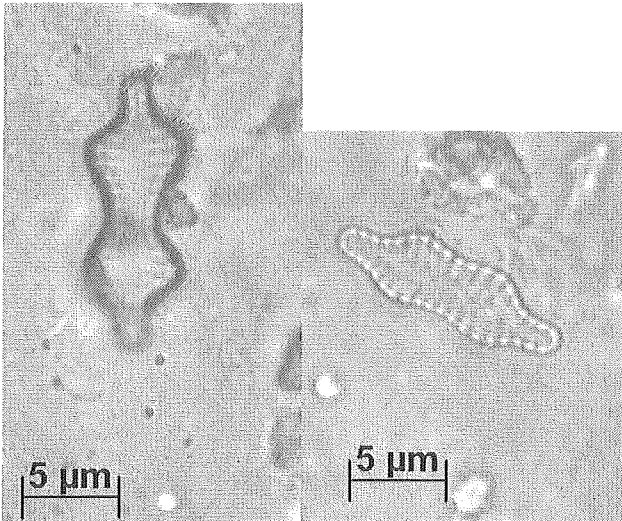
Nitzschia perminuta



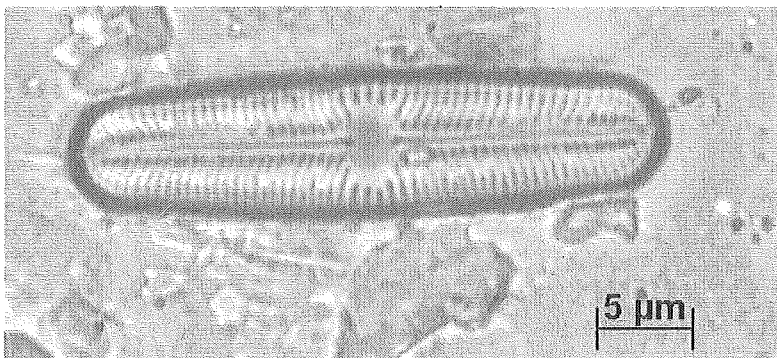
Nupela sp.



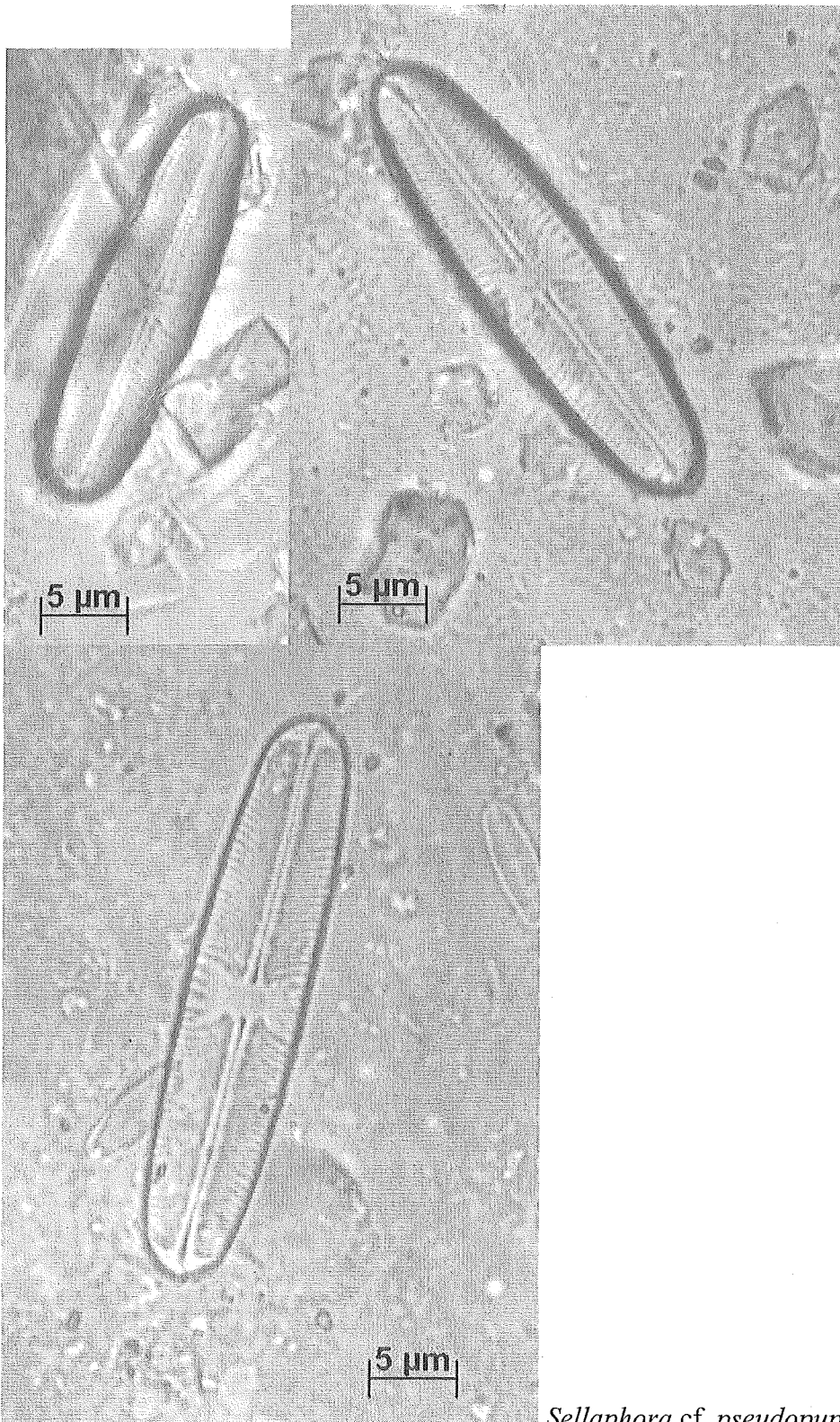
Pinnularia borealis



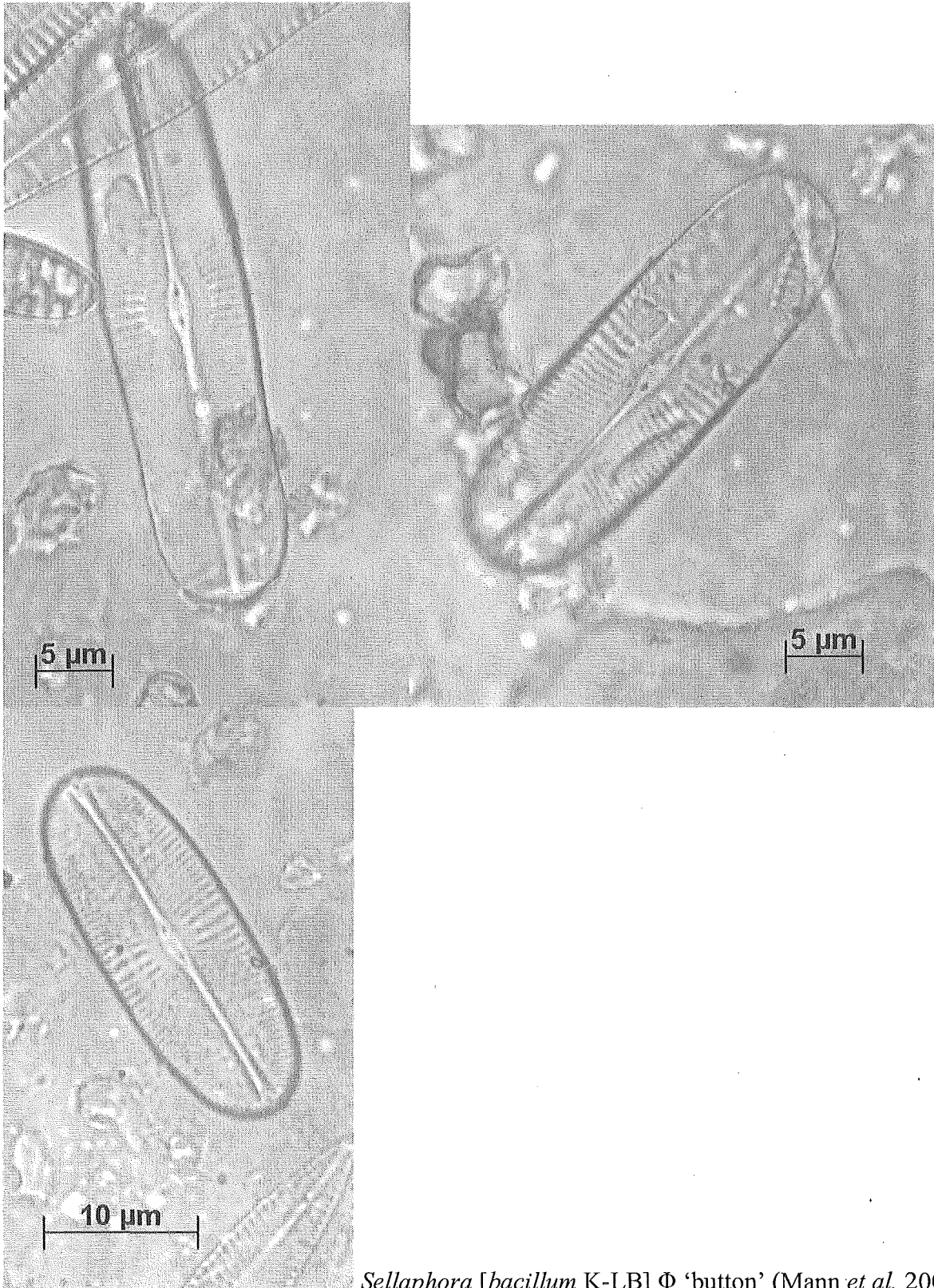
Pseudostaurosira robusta Teratological form



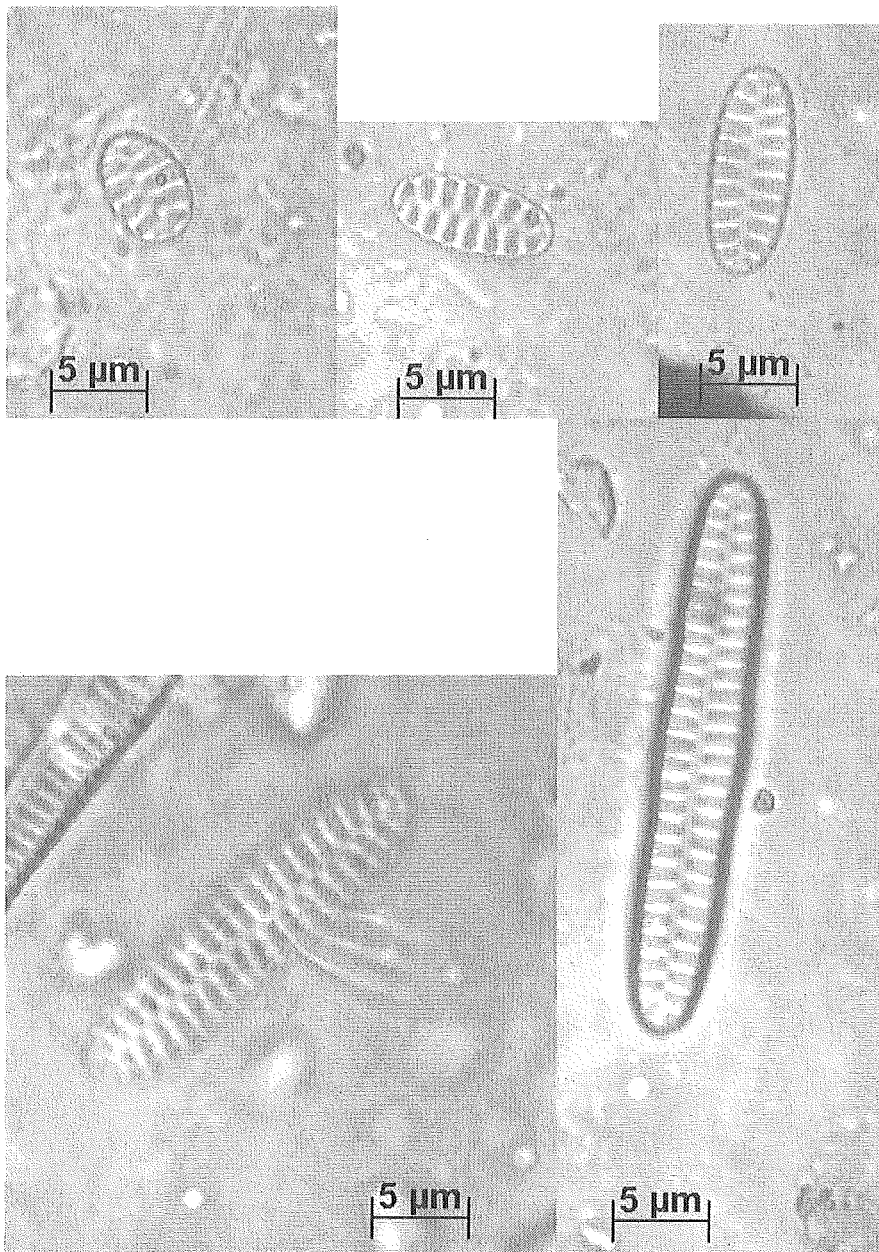
Sellaphora [*laevissima* K-LB] Φ 'normal' (Mann *et al.* 2008)



Sellaphora cf. pseudopupula



Sellaphora [bacillum K-LB] Φ 'button' (Mann *et al.* 2008)



Staurosirella pinnata