Training Course ‘Impact of invasive alien species on biodiversity and ecosystem services in extreme environments’
03 – 04 April 2017, Sofia, Bulgaria

Benthic diatom monitoring and assessment of high-mountain lakes, Rila National Park, Bulgaria – standard methods

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**EVK1-CT-1999-00032 EMERGE project** (European Lake Ecosystem: Regionalisation Diagnostics and Socio-Economic Evaluation)
In the EMERGE project 235 lakes in several European ranges were investigated with the following specific aims:

1) to analyse the patterns of community assembly in several groups of phylogenetically related species;

2) to identify the main environmental descriptors explaining the species assemblages;

3) to investigate the commonalities among taxonomic groups from which some general ecological thresholds for alpine lake biota could be defined;
Nine lakes situated above the natural timberline in the highest massif on the Balkan Peninsula – Rila Mts. (Mousala peak – 2925 m. a.s.l.) are considered to be excellent indicators of environmental changes. The chosen lakes have to cover large gradients in altitude, bedrock type and limnological characteristics.
<table>
<thead>
<tr>
<th>Lake group</th>
<th>Seven lakes</th>
<th>Mousala lakes</th>
<th>Maritsa lakes</th>
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<tbody>
<tr>
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<td>Okoto</td>
<td>Bubreka</td>
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<tr>
<td>EMERGE lake code</td>
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**GEOGRAPHY & MORPHOMETRY**

<table>
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<th>Sulzata</th>
<th>Okoto</th>
<th>Bubreka</th>
<th>Bliznaka</th>
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<th>Alekovo</th>
<th>Karakashevo</th>
<th>Gorno Marichino</th>
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MATERIAL

- Sampling of both living diatom communities (epilithon) and sediment diatom assemblages (top and bottom) were carried out at all survey lakes. A total of 48 epilithic samples (3 samples per lake) were collected in July, September 2000 and July, October 2001.

- Four sediment cores (17.5-24 cm long) were taken from the deepest part of the lake Bubreka in July 2000. All these cores were extruded in the field at 2.5 mm intervals between 0 to 5 cm (to track the recent trends) and 5 mm intervals below 5 cm.
METHODS

• The samples were cleaned according to the standard methods described by Battarbee (1986);

• The cleaned diatoms were identified and counted under oil immersion at magnification of c. × 800 or × 2000, with a light microscope;

• A minimum of 500 valves was counted in every sample (Renberg 1990);

• In general, the nomenclature followed Krammer and Lange-Bertalot (1986-1991), Round et al. (1990), Lange-Bertalot and Metzeltin (1996), and Hofmann et al. (2011). For those taxa not listed by them, other taxonomic reference works were used, e.g. Reichardt (1999, 2001) for some *Gomphonema* taxa, as well as Lange-Bertalot *et al.* (2011) for the *Eunotia* taxa.

• Preparation for scanning electron microscopy follows Hasle & Fryxell (1970) and samples were examined on Jeol Superprobe 733;

• The spectra of physicochemical tolerance of the identified diatom taxa were based mainly on Lowe (1974), Krammer & Lange-Bertalot (1986-1991), Van Dam & al. (1994), and Lange-Bertalot *et al.* (2011);

• Water chemistry;

• Cluster and Multivariate analyses;
RESULTS:

CASE STUDY – 1: Diatoms & environmental variables

1. The aim of this case study is to provide comparable information about the current state of four selected lakes in Sedemte ezera cirque.
The total number of diatom taxa observed in the epilithon and the sediment samples from the surveyed lakes is 223.
The results show slight variability of the environmental conditions in the lakes during the two years of studying. The littoral diatom communities of the lakes were dominated by cosmopolitan, oligotraphenitic and pH-indifferent and alkaliphilous taxa, mainly belong to genera *Fragilaria* sensu lato, *Gomphonema*, *Cymbella* sensu lato, *Pinnularia*, and *Achnanthes* sensu lato. The “Sulzata” lake group is distinct from all others. This lake is the shallowest in the surveyed lakes, at the highest elevation, with the smallest catchment and surface area and it has relatively lower pH in one autumn sample – October’2001, compare with other.
The Shannon – Wiener Diversity Index of the studied epilithic diatom samples.
CASE STUDY – 2: Benthic diatom flora in relation to chemical and physical factors

The composition of the benthic diatom communities in the studied lakes showed relation between species diversity and the main chemical gradient - pH, alkalinity and conductivity. Ordination diagram based on detrended correspondence analyses (DCA) of epilithic diatom assemblages was used in order to distinguish major patterns in diatom distribution within each lake and the results plotted in the figure.

The aim is to determine the relation of the benthic diatom flora to physical and chemical features in these lakes.

CASE STUDY – 3: Diatoms and lake sediments

Chemical and biological sedimentary records of the high alpine lake “Bubreka”, were used to reconstruct palaeoecological conditions and to determine the phases in the ontogeny of the lake.

- Four sediment cores (17.5-20.0 cm long) were taken from the deepest part of the lake in July 2000. All these cores were extruded in the field at 2.5 mm intervals between 0 to 5 cm (to track the recent trends) and 5 mm intervals below 5 cm.

- The sediment cores were dated by $^{210}$Pb and $^{137}$Cs and analysed for percentage of dry weight (%DW), loss-on-ignition (LOI), pigments, diatoms, chrysophyte stomatocysts, cladocerans, chironomids and speroidal carbonaceous particles (SCPs).

Radiometric chronology of Bubreka core RI0002A, showing CRS model $^{210}\text{Pb}$ dates together with the 1963 depth determined from the $^{137}\text{Cs}/^{241}\text{Am}$ stratigraphy. Also shown are the corrected $^{210}\text{Pb}$ dates and sedimentation rates.

Prof. Peter Appleby
Dept of Mathematical Sciences
University of Liverpool
Liverpool, UK
Spheroidal carbonaceous particles (SCP) analysis

A first presence of SCPs is identified in the 1950s and the profile is erratic throughout. Two main peaks in concentration (> 5000 gDM-1) occur in 1984 and 1991 while a third lower peak occurs in the 1960s.

Dr. Neil Rose
University College, London
**Lake Bubreka** - core C: Organic carbon (Corg), nitrogen (Ntot), sulphur (Stot) and the organic carbon to nitrogen ratio (C:N) sediment profiles. Units are in percentages of dry weight.

**Pigments**

**Lake Bubreka** - core C. Profile of chlorophyll derivatives (CD), total carotenoids (TC), ratio of the spectrophotometer wavelength at 430 and 410nm (430:410).

**Andrea Lami & Simona Musazzi**
CNR-Istituto per lo studio degli Ecosistemi, Verbania-Pallanza, Italy
### Diatom remains

**DAZ 1 (17.5 - 13.5 cm)** - The diatom flora is dominated by epiphytic species such as *Achnanthes curtissima* (=*Psammothidium curtissimum*), *Eunotia bilunaris* and *Gomphonema parvulum*.

**DAZ 2 (13.5 - 8.0 cm)** - The diatom flora is rich and diverse. An increase in the abundance of *Aulacoseira alpigena* is observed.

**DAZ 3 (8.0 - 1.25 cm)** - There is a peak in the planktonic diatoms (30.9%), mainly *Aulacoseira valida*. *Fragilaria* sensu lato species as well as *Gomphonema parvulum* increase concomitantly.

**DAZ 4 (1.25- 0.00 cm)** – The centric planktonic species decrease markedly. The diatom flora shows an increase in periphytic species, such as *Fragilaria species* and *Diatoma mesodon*.

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<table>
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<tr>
<th>Age AD 2000</th>
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<th>DAZ 2 (13.5 - 8.0 cm)</th>
<th>DAZ 3 (8.0 - 1.25 cm)</th>
<th>DAZ 4 (1.25 - 0.00 cm)</th>
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<tr>
<td>Depth in core (cm)</td>
<td>Achnanthes curtissima</td>
<td>Aulacoseira alpigena</td>
<td>Aulacoseira valida</td>
<td>Aulacoseira valida</td>
</tr>
</tbody>
</table>
**Habitat**: Periphytic species predominate throughout the core, with epiphytic species ranging between 63 and 72%.

**Salinity**: Indifferent oligohalobic taxa predominate, ranging for 66 to 81%.

**pH**: It is difficult to establish which pH groups are dominant.

**Phytogeography**: The cosmopolitan group is the most abundant (60 to 73%), followed by the north-alpine (20 to 37%) and boreal (3 to 11%) groups.

**Diagram of the different diatom ecological groups**

Chrysophyte cysts are most abundant at 5.5 - 6.0 cm (50%) coinciding with low taxonomical diversity of the diatom assemblage.
Four zones can be distinguished through the core.

**Dr. A. Brancelj**
National Institute of Biology,
Ljubljana, Slovenia
PCA of the chironomid taxon assemblages from the 26 sites (core levels) examined in the present study with the vectors of the most common taxa marked. The sites are categorised into clusters I-IV.

*Site numbers will be transformed to ages.*

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**Dr. Gaute Velle**
Museum of Zoology, University of Bergen, Norway
Four distinct successional phases can be described in the lake history during the last ~ 250 years, revailing complex interactions between anthropogenic impact and climate.

- **Phase 1** (ca. AD 1762 -1834) is characterised by stable oligotrophic conditions.
- **Phase 2** (ca. AD 1834 -1928) there is a change from oligotrophic to eutrophic conditions.
- **Phase 3** (ca. AD 1928 - 1994 ± 2) shows an increase in LOI due to the high proportion of organic material. The shorter ice cover period in the lake may have created conditions more suitable for the growth of phyto- and zooplankton and changed the overall primary production from benthos to plankton.
- **Phase 4** (c. AD 1994 ± 2 - 2000) there is increased LOI, increasing percentages of periphytic diatoms, less favorable conditions for cladocerans, and rheophilic chironomid assemblages which indicate rapid input of nutrients causing mesotrophication. The SCP record and comparisons with other European mountain lakes suggest there is a moderate impact from atmospheric deposition compared with similar lakes elsewhere in Europe.
A method of top-bottom comparison approach was used in assessment of regional diatom assemblage change across Bulgarian high-altitude mountain lakes.

DCA plot of the top and bottom sediment diatom samples from nine Rila Mts lakes. The suffixes B and S are used for bottom and top sediment samples from each site.

Alkalinity, pH and conductivity summarize the main chemical characteristics of the studied lakes. pH changes were detected in the group of lakes located in Musalenski cirque (Ledeno, Alekovo and Karakashevo) over the last 150 years.

The increase in planktonic diatoms in the lakes could be affected by changes in the ice-cover regimes. Owing to the limited number of sites (9) and the limited gradients from which our lakes have been chosen (i.e. oligotrophic, bedrock type lakes), our conclusions for the changes in ice-cover regimes could be considered preliminary. Further monitoring is needed to evaluate the direction and size of past and future changes, and to investigate the effects of the increasing load of atmospheric pollutants.
Lake Bliznaka
close-up of stop 2, stop 3, and stop 1
October’2001
single frustules - *Didymosphenia geminata*
East and South European Network for Invasive Alien Species – a tool to support the management of alien species in Bulgaria (ESENIAS-TOOLS)

Funding: Financial Mechanism of the European Economic Area in the period 2009-2014 in the frame of Programme BG03 Biodiversity and Ecosystem Services

Project Coordinator: Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences

Project Partners:
3 from Bulgaria
7 from other European countries – Greece, Croatia, Serbia, Romania, Turkey, Iceland, R. Macedonia

-2 State Environmental Agencies, 4 Universities, 5 Research Institutes
Marine | Freshwater | Plants | Invertebrates | Vertebrates
---|---|---|---|---
WG2 | WG3 | WG4 | WG5 | WG6

CS1
CS2
CS3
CS4

WG1
ESENIABS database and IAS tools

WG8
WG9
WG10

Research, management, legislation

Capacity building | Awareness raising
EASIN, NOBANIS, DIAS, IUCN SSC/ISSG, IAS Cost Actions
**Case Studies**

Case study 1: Biological and ecological traits of invasive alien freshwater mussels in Bulgaria (Bulgaria – Italy)

**Case study 2:** Comparative study on the effect of hydrological regime on the distribution of the invasive diatom *Didymosphenia geminata* in extreme environments (Icelandic rivers and lakes, and Bulgarian high-mountain lakes) (Iceland – Bulgaria)

Case study 3: Assessment of the impact of alien species on the biodiversity and endemism of ancient Balkan lakes (Lake Ohrid case study) (R. Macedonia – Bulgaria)

Case study 4: Estimating dispersal routes for IAS (Romania – Bulgaria)
Case study 2: Comparative study on the effect of hydrological regime on the distribution of the invasive diatom *Didymosphenia geminata* in extreme environments (Icelandic rivers and lakes, and Bulgarian high-mountain lakes)

**Leader:** David Finger, Reykjavik University, Iceland

**Participants from:**
- Reykjavik University, Reykjavik, Iceland
- Marine and Freshwater Research Institute, Reykjavik, Iceland
- Institute of Biodiversity and Ecosystem Research, BAS, Sofia, Bulgaria
**Didymosphenia geminata** (Lyngbye) M. Schmidt

**BASIONYM:** Echinella geminata Lyngbye 1819

**SYNONYM(S):** Gomphonema geminatum (Lyngbe) Agardh

**REPORTED AS:** didymo (common name) | rock snot (common name)

- *Didymosphenia geminata* (Lyngbye) M. Schmidt is an invasive diatom commonly referred to as “didymo” or “rock snot”;
- Historically, *D. geminata* was described as a rare, cosmopolite lotic diatom usually recorded in oligotrophic mountain rivers with cold water and moderate flow;
- The type material of *D. geminata* has been recently documented with light and electron microscopy photographs (Metzeltin and Lange-Bertalot 2014);

1. Valves large
2. Headpole capitate
3. Stigmata 2-5
4. Apical porefield present
5. Distal raphe deflected

The valves are large and robust. The headpole is distinctly capitate, while the footpole may be capitate or not. The number of stigmata is variable, but depends on valve size. A pore field is present at the footpole. The distal raphe is deflected at the footpole, rather than bisecting it.

*D. geminata* cells produce extracellular stalks in order to attach to rocks or other available submerged substrates. These stalks constitute the main component in biomass in natural colonies (Spaulding et al. 2005; Elwell 2006; Lagerstedt 2007). They are predominantly composed of polysaccharides.
Introduction and study site

• In Iceland and high mountain areas the diatom *Didymosphenia geminata* (didymo) is an invasive species
• Anthropogenic activities and climate change are the suspected main cause for the enhanced spreading
• Two case studies were monitored to investigate the spreading:
  • River Elliðaár in the capital area of Iceland
  • The Seven Rila lakes in the high mountains of Bulgaria
Study area is of great importance for the locals. Firstly, Heiðmörk is nature reserve from where drinking water (groundwater) is extracted and provided to Reykjavik and surrounding municipalities. Secondly, study area is also used for recreational activities, such as angling in river Elliðaár or hiking in Heiðmörk. Furthermore, river Elliðaár was also utilised for energy production.

River Ellida´ar - Stops 1 and 2
The tributaries Suðurá and Hólmsá - Stops 5 and 6
Lake Elliðavatn - Stop 7
Preliminary results

• In River Ellíðaár:
  • Didymo has spread into the tributaries during the last 20 years

The total number of diatom taxa recorded in the epilithon was 130, among which one was determined to genus only. They were referred to 49 genera, belonging to three classes: Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae. In terms of taxonomic diversity, the classes Fragilariophyceae and Bacillariophyceae prevailed (93%).
Data analyses showed that ratio of *Didimosphenia geminata* with total taxa number in the sample has significantly increased since the first measurements held by Björnsson (1998). In 1994 in downstream of River Elliðaár *D. geminata* ratio was just 1.9% (Björnsson, 1998), whereas in the current data set *D. geminata* ratio increased up to 3.4% in June and 5.2% in November.

Furthermore, it was observed that dominant species in the river Elliðaár also has changed, i.e. *Staurosira construens* Ehr., *Fragilaria vaucheriae* (Küt.) Petersen and *Aulacoseira italica* (Ehr.) Sim. (Björnsson, 1998) were changed by *Pseudostaurosira pseudoconstruens* (Marciniak) Williams et Round and *Achnanthidium minutissimum* (Kütz.) Czarnecki.
Overall, the statistical analysis of the hydro-meteorological reveal that both temperature and cumulative precipitation over last 60 years increased in the study area. Mean air temperature between period 1 (from 1956 to 1985) and period 2 (1986-2015) increased from 4.48 °C to 4.95 °C, revealing an increase of 0.47 °C, with standard deviation of 0.64 in both periods.

Similar trends were observed in the precipitation observations. Comparison of mean yearly precipitation between period 1 and period 2, indicate an increase from 798.7 mm/yr to 861.7 mm/yr, revealing an increase of 63mm in 30 years. Standard deviation in period 1 is also lower than in period 2, 126.57 and 133.18 respectively.
The study included:

- Standardization of methods regarding sampling/identification/monitoring of Didymo/diatoms;

- Collecting available data on existence of Didymo in the Bliznaka Lake and tributaries and data about other diatoms/algae in the study area;

- Collecting chemistry and hydrological data;

- Organising the data into database and linking the data;

- Field work to check the existence/abundance of Didymo in the previous sampling location in Bliznaka Lake and new sampling in the connected lakes, situated downstream in the cirque Sedemte Ezera;

- Identifying difference in these factors between sites where the diatom is found and it is not.

The following water bodies were studied: Bubreka Lake, Okoto Lake, Sulzata Lake, Bliznaka, Detelina, Ribno and Dolno Lake.
In the period 09-10 July and 30 October-1 November 2015, a field trips for collecting diatom samples were organised in the Rila Mountains, Bulgaria Seven high-mountain lakes were sampled. Epilithic diatom samples were collected from 14 sites according to standard methods.

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<td>2440</td>
<td>42°12'01.0&quot;</td>
<td>23°18'18.0&quot;</td>
</tr>
<tr>
<td>10.07.2015</td>
<td>Okoto Lake – Site 3</td>
<td>2440</td>
<td>42°11'57.0&quot;</td>
<td>23°18'30.0&quot;</td>
</tr>
<tr>
<td>10.07.2015</td>
<td>Sulzata Lake – Site 1</td>
<td>2535</td>
<td>42°11'52.0&quot;</td>
<td>23°18'39.0&quot;</td>
</tr>
<tr>
<td>10.07.2015</td>
<td>Sulzata Lake – Site 2</td>
<td>2535</td>
<td>42°11'48.0&quot;</td>
<td>23°18'39.0&quot;</td>
</tr>
</tbody>
</table>

Collected data were compared with the previous data set (2000-2013) for the same control stations located in the same lakes, presenting no evident infestations.

EVK1-CT-1999-00032 EMERGE project (European Lake Ecosystem: Regionalisation Diagnostics and Socio-Economic Evaluation)
RESULTS

In 2015 a total of 184 diatom taxa were found in the epilithon samples. Three forms could not be identified beyond the generic level, and they were listed as “sp.”. The diatoms were attributed to 56 genera, from three classes: Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae.

Only 7.8% of the diatom taxa belonged to class Centrophyceae. The genus *Aulacoseira Thw.* has diverse species composition (8 taxa), but most of these species are rare in the investigated samples. Exceptions to this are *Aulacoseira alpigena (Grun.) Kramm.*, which is dominant and subdominant in the samples from lake Sulzata, and *A. valida (Grun.) Kramm.* subdominants in Bliznaka, Dolnoto Lakes.

In terms of taxonomic diversity, the classes Fragilariophyceae and Bacillariophyceae prevailed (92.4%). The majority of frequent taxa belong to genus *Fragilaria Lyngbye sensu lato*
The order Naviculales showed the greatest generic diversity.

The most species-rich genus was *Pinnularia* Ehr. (19), followed by *Gomphonema* and *Eunotia* (13), *Nitzschia* Hassal, *Fragilaria* Lyngbye and *Cymbella* Ag. (9 taxa). The greatest species diversity was identified within the genus *Pinnularia* Ehr. Most of these species were well represented in almost all the samples, but only a few of them were subdominants in the diatom association.
July’2015
The total number of species found in the different lakes (cirque Sedemte Ezera) ranged between 42 and 89. The lowest number was recorded in lake Ribnoto.

Detrended correspondence analysis (DCA) was performed on epilithic diatom data separately from July and October 2015 to determine the gradient length of the data set to select the most suitable ordination technique. The gradient length was shorter than 3 standard deviation units indicating that a linear response model should to be used. Principal Component Analysis (PCA) was undertaken on epilithic diatom assemblages to explore any underlaying structure inherent in the data.

Correlation biplot based on Principal Component Analysis of the sample taken in July 2015.
The first two principal components ($\lambda_1=0.637$, $\lambda_2=0.205$) cumulatively explained 84.2% of total variance and accounted for 63.7% and 20.5% of the variance, respectively.
Detelinata Lake
stop 1 – July’2015
brocken frustule - Didymosphenia geminata
October’2015

The total number of species found in the different lakes (cirque Sedemte Ezera) ranged between 55 and 90. The lowest number was recorded in Dolno lake.

Correlation biplot based on Principal Component Analysis of the sample taken in October 2015. The first two principal components ($\lambda_1=0.402$, $\lambda_2=0.225$) cumulatively explained 62.7% of total variance and accounted for 40.2% and 22.5% of the variance, respectively.
Bubreka Lake
stop 1 – October’2015
single frustule - *Stephanodiscus binderanus* (Kütz.) Krieger
The Red List data categories

Ex – *Extinct or probably extinct.* Species which are no longer known to exist in Rila lakes after repeated searches of the type localities and other known or likely places;

E – *Endangered.* Taxa in danger of extinction and whose survival unlikely if the caused factors continue to operate;

1. – Threatened with extinction;
2. – Strongly endangered;
3. – Endangered;

G – *Supposed to be endangered;*

V – *Vulnerable:* Taxa believed likely to move into endangered category in the near future if the caused factors continue to operate;

R – *Extremely rare taxa.* They are not endangered or vulnerable, but at risk of becoming so;
The Red List status (Lange-Bertalot and Steindorf, 1996) was available for 88.2% of the taxa. Some additions were supplemented according to recent proposals (Sieminska et al., 2006; Hofmann et al., 2011). Forty two percent of the taxa are classified as rare and endangered to various degrees.

Six taxa were assigned to category strongly endangered (E-2). *Aulacoseira valida* (Grun.) Krammer, *Eunotia arcus* Ehr., *E. monodon* Ehr., *E. bigibba* Kütz., *E. tetraodon* Ehr., and *Boreozonacola hustedtii* Lange-Bertalot, Kulikovskiy & Witkowski.
Navicula pseudosilicula Hust. is so far known only from Spitzbergen (Hustedt, 1942). According to the DIATCODE system of EMERGE project this was unique for the Rila region, but recently there are new data for its distribution – it is determined also in the Retezet Mts, Carpathian region (Buczkó, pers. comm.). Kulikovskiy et al. (2012) reported that this taxon could be belonged to newly described genus Boreozonacola Lange-Bertalot, Kulikovskiy & Witkowski, 2010, but there are missing sufficient morphological investigation on the type materials.
Type material – Hustedt Collection No E2171, Mayrhofen 157, Zillertal

Figs 1-9. SEM. External view;
Fifteen taxa were classified as ‘Endangered’ (E-3).

The most interesting within this group is Navicula/Naviculadicta digitulus Hust. It is most abundant in top/bottom sediments of lake Okoto and lake Sulzata, but it was collected also in the epilithic samples. A comparison of specimens found in lakes from the Carpathian and Balkan Mountains with the type material of Genkalia digitulus (Hustedt) Lange–Bert. et Kulikovskiy resulted in description of a new species – Genkalia boreoalpina.

Type material – Hustedt Collection № E1319, Davos, Schweiz 126, Schwarzsee, Grund

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Thank you for your attention!