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Additional information on the distribution and ecology of the recently described diatom species *Geissleria gereckei*

by

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Abstract

The main objective of this paper is to report new information about the distribution and ecology of a recently described diatom species, *Geissleria gereckei*. The opportunity for updating the information on the distribution and ecology of the species was provided by the finding of well-developed *G. gereckei* populations on the lithic material and bryophytes in the Raška and Mlava rivers (Serbia). For several years after the first description, *G. gereckei* has been known only from the type locality and from another spring in the Dolomiti Bellunesi National Park (the south-eastern Alps). After accurate LM and SEM observations, we provide evidence for the occurrence of the species also in the two above-mentioned rivers in Serbia, as well as in the south-western and south-eastern Alps. After an extensive literature search, it appears that the species is known with certainty only from these sites. Our observations and details from the literature suggest that the species is able to occupy a much broader ecological niche than the very-specific one observed at the time of discovery. The two main determinants for the species' occurrence appear to be the carbonate nature of the catchments or aquifers, and the ability of the species to be competitive in habitats or microhabitats exposed to seasonal desiccation.

Key words: diatom, distribution, ecology, *Geissleria gereckei*, rivers, Serbia, south-eastern Alps

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Introduction

The genus *Geissleria* was originally described in 1996 by Lange-Bertalot & Metzeltin and is characterized by the presence of an annulus, groups of subterminal openings. *Geissleria* comprises species formerly included in the *Annulatae* section of *Navicula* s.l. According to Lange-Bertalot (2001), this genus belongs to naviculoid diatoms. However, recent studies have shown that the characteristics of the genus *Geissleria* are more related to cymbelloid diatoms (Nakov et al. 2014; Kulikovskiy et al. 2014). Species of the genus *Geissleria* are widespread in oligo- to eutrophic waters but generally occur with low abundance (Spaulding & Edlund 2009).

According to Lange-Bertalot, in 2001 about 30 species were known from the genus *Geissleria*, many still undescribed or included within other genera (in particular *Navicula* s.l.). Currently, the genus comprises 71 taxa, widely distributed in Europe, Asia, South and North America, and the Maritime Antarctic and sub-Antarctic regions. *Geissleria* species were recorded in different freshwater habitats, such as rivers, lakes, springs, waterfalls, and in soils (Novais et al. 2013).

Geissleria gereckei was described by Cantonati & Lange-Bertalot (2009) from a typical limestone/dolostone area. The type locality was Val-Lovatel Spring (Dolomiti Bellunesi National Park, the south-eastern Alps). At the time of its first description, this diatom was also found in the second spring (Casera-Maiolera Spring) in the same geographic area. In these two special freshwater habitats, the species was observed to be associated with the specific microhabitat of leaf-litter covered stones in shaded, forest springs emerging on the carbonate substrate. These low-elevation, small-discharge springs are likely to be affected by seasonal desiccation.

There is relatively little literature available on diatoms of the Raška River (Vidaković 2013; Vidaković et al. 2014), and there are no published data on the diatom microflora of the Mlava River. So far only five *Geissleria* species have been reported from Serbia: *G. decussis*, *G. ignota*, *G. schoenfeldii*, *G. similis*, and *G. thingvallae* (Krizmanić 2009; Andrejić 2012; Vidaković 2013).

The aim of this contribution was to collect and review information on the distribution and ecology of *Geissleria gereckei* in the period between the first description of the taxon and the present day in order to obtain a more comprehensive database and consequently a better description of its ecological preferences in nature.

Materials and methods

G. gereckei: The new riverine sites and their characterization. Diatom sampling and analysis

The Raška River and the Mlava River belong to the Black Sea drainage basin. The Raška River is a tributary of the Ibar River in the southwestern part of Serbia. The Mlava River is a tributary of the Danube in eastern Serbia (Marković 1980). The rivers have a carbonate bedrock and run through trout ponds. Samples were collected at the sites located downstream and upstream of the trout ponds. Some of the sites are exposed to seasonal desiccation when the discharge drops.

Diatoms were sampled from 5 localities in the Raška and Mlava rivers in April, June, August, and November 2011, and March and May 2012. Epilithon and epibryon were considered in the Raška River, while only epilithon was sampled in the Mlava River. Samples were immediately fixed with formaldehyde to a final concentration of 4%. Conductivity, oxygen, pH, and water temperature were measured in the field. Hydrochemical analyses were carried out at the Institute of General and Physical Chemistry, University of Belgrade, following standard methods (APHA 1998). Diatom samples were treated according to standard methods to obtain permanent slides (Krammer & Lange-Bertalot 1986). Permanent slides, prepared material, and aliquots of the samples were deposited in the diatom collection of the University of Belgrade, Faculty of Biology.

Slides from the Raška (14 samples) and Mlava (8 samples) rivers were used for microscope observations. Light microscope observations and micrographs were made using a Zeiss AxioImagerM.1 microscope with DIC optics and AxioVision 4.8 software. SEM observations were made at the Institute of Physics, University of Belgrade, using a TESCAN MIRA 3 scanning electron microscope with maximum accelerating voltage of 30 kV. Sample surfaces were sputtered with gold using QUORUM TECHNOLOGIES MINI SPUTTERCOATER SC7620 for enhanced conductivity.

G. gereckei: Further sites of occurrence found after the species description

Trying to gather as much data on the distribution of *Geissleria gereckei* as possible, the following databases were carefully checked: Pesio and Tanaro Valleys Nature Park (Ligurian Alps, the south-western extreme of the Alps; Mogna et al. 2015), central to eastern Switzerland and the Jura (the north-western/

central Alps; Taxböck, L., Karger, D.N., Kessler, M. & Cantonati, M. 2016 submitted paper), CRENODAT (Trentino, the south-eastern Alps; Cantonati et al. 2012), CESSPA (Province of Verona, Veneto Region, Italy; Cantonati et al. 2016), Berchtesgaden National Park (Bavaria, Germany; Cantonati & Lange-Bertalot 2010), Gesäuse National Park (Styria, Austria; MC unpublished data), Julian Pre-Alps Nature Park (Friuli Venezia Giulia Autonomous Region, Italy) (the south-eastern Alps; Cantonati 2003). In the case of the database established before the description of *G. gereckei*, also direct inspection of the permanent mounts was carried out to check for possible occurrence of the species. Finally, repeated literature searches were carried out ("*Geissleria*gereckei*") in the Web of Science (WOS) and Google Scholar.

Results

Geissleria gereckei populations found in the two rivers in Serbia

Main morphological characteristics. Valve outline linear-elliptic to elliptic. Ends subcapitate and protracted. Length 9.9-18.5 µm, breadth 5.0-7.1 µm; length/width ratio 2.0-2.8. Raphe filiform; axial area narrow, linear; central area characterized by longer and shorter striae. Single stigma placed in the center of the valve. Striae 13-17/10 µm, radiate throughout (Fig. 1a-g).

SEM: external view (Fig. 2b): Proximal ends of the raphe expanded and moderately deflected whilst distal ends are hooked. Striae uniseriate throughout. Areolae apertures as short slits (55-60 in 10 µm). Annulus evident as three rows of areolae larger than in stria areolae, apically elongated. In each row, from 3 to 6 apically elongated areolae are present.

SEM: internal view (Fig. 2 a and c): Distal raphe ends slightly deflected in a small, aureole-shaped helictoglossa, central raphe endings straight. Annulus structure with warty outgrowths.

Distribution. The Raška and Mlava River. *G. gereckei* was found both in the epilithon and in the epibryon. Relative abundance in epilithic diatom communities of the Raška River ranged from 0.5 to 3%. Only one specimen of *G. gereckei* was recorded in epiphytic diatom assemblages of the Raška River and in epilithic diatom assemblages of the Mlava River.

Associated diatoms. The most frequent diatoms which were found together with *G. gereckei* (Raška River epilithon) were: *Achnanthidium affine* (1.2-18%), *A. minutissimum* (9.2-48%), *Amphora pediculus* (11-32%), *Cocconeis lineata* (0.5-32%), *Diatoma vulgaris* (0.2-20%), *Gomphonema parvulum* (0.2-2.8%), *G. tergestinum* (0.3-39%), *Navicula cryptotenella* (0.2-4.2%), and *Nitzschia dissipata* (0.5-5%).

Ecology. The Raška and Mlava rivers are small mountain rivers, running at altitudes between 296 and 557 m a.s.l. Samples were collected at the sites located downstream and upstream of the trout ponds, which explains the high nutrient values. Conductivity values are consistent with the carbonate bedrock (Table 1). The highest abundance of *G. gereckei* was observed at microhabitats (river wetted surface and bryophytes) exposed to seasonal desiccation.

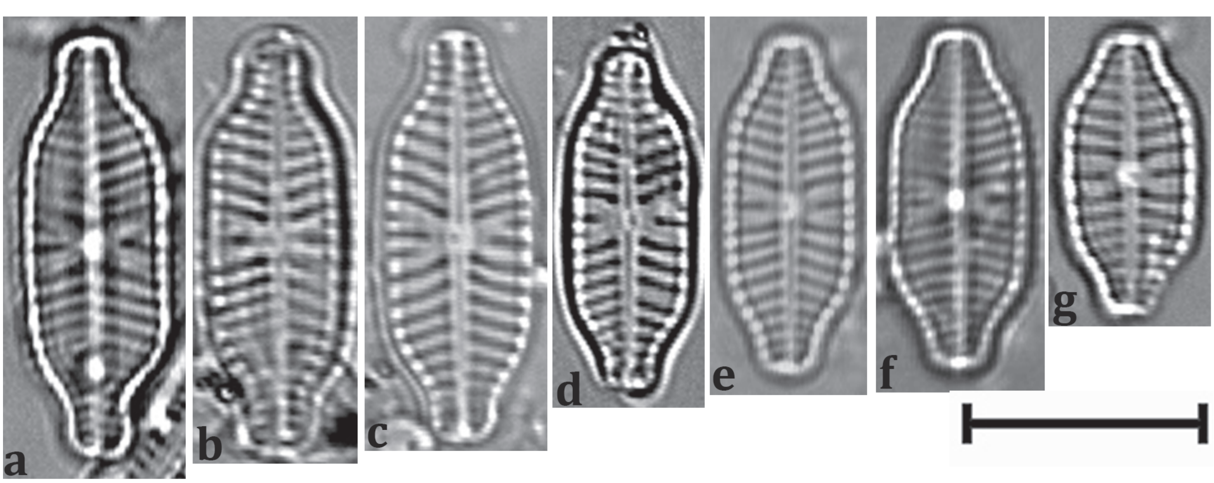

Figure 1

(a-g) Light microscopy (LM) micrographs of *Geissleria gereckei* Cantonati et Lange-Bertalot. **Scale bar = 10 µm**

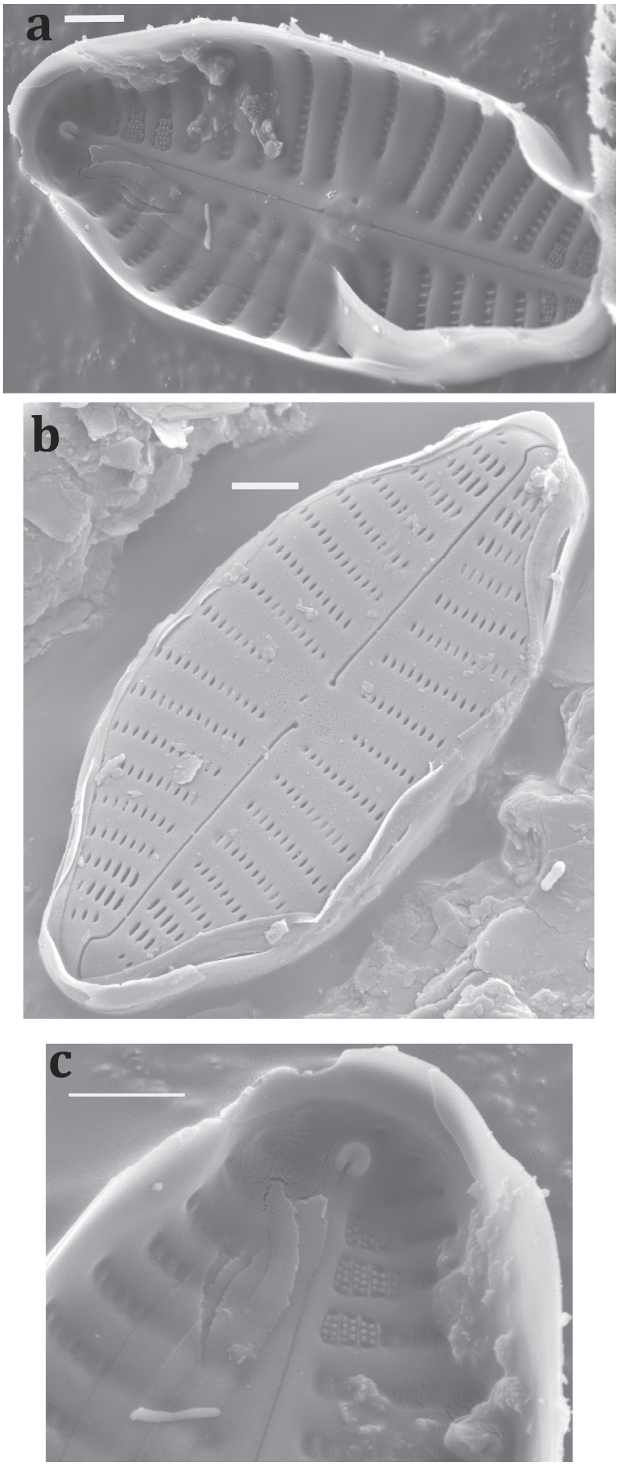

Figure 2

Scanning electron microscopy (SEM) micrographs of *Geissleria gereckei*. (a) Inside view of the valve. (b) Valve outside view of the frustule; apically elongated areolae (annulus). (c) Distal ends (internally) and helictoglossa; annulus structure with warty outgrowths. **Scale bar = 1 μm**

Further occurrence of *Geissleria gereckei*

G. gereckei was also found in one spring in the south-eastern Alps (Ziola Bassa, AT0756, Vigolana Upland), in springs Alpine foothills (Verona Province, Lessinia, ML0622) and in eight springs of the south-western Alps (Table 1). These springs are small, with low discharge (probably <0.5 l s^{-1} in most cases), and likely to be affected by periodic desiccation. All of these springs are fed by carbonate aquifers, and have medium conductivities (200-400 μS cm^{-1}).

Discussion

The morphological features of the *Geissleria gereckei* populations observed during this study correspond with those reported at the time of the species description (Cantonati & Lange-Bertalot 2009). However, our observations, based on the populations in the two rivers in Serbia, advance the knowledge about the size range of the species.

Our data increase the number of known sites of the species from 2 to 13, and the populations are no longer limited to the Alpine area.

The ecological characteristics of the new sites revealed that *G. gereckei* can also successfully colonize sites where light intensity is not a limiting factor. The niche described at the time of the species description (carbonate stones covered by leaf litter in small shaded springs in broadleaf forests) must therefore be considered an extreme situation that shows that the species has the capability to be competitive also in microhabitats with very low light availability.

Our data and observations provide insights into the niche of *G. gereckei* in terms of nutrient concentrations, suggesting that although the species thrives well and is frequent at oligotrophic sites, it can colonize places with high nutrient (in particular P) concentrations. The spring and river sites also reveal that above-average sulfate values are well tolerated by the species.

A preference for carbonate stony substrate is confirmed, although it is shown that the species can thrive on bryophytes substrate as well.

The two main determinants for the species occurrence appear to be the carbonate nature of the catchments or aquifers of the environments colonized, and the ability of the species to be competitive in habitats (small springs) or microhabitats (river wetted surface and bryophytes) exposed to seasonal desiccation because of fluctuating and low discharge values.

Table 1

Value ranges of physical and chemical parameters of the sites with *Geissleria gereckei*

Parameters/Sampling sites	Rivers in Serbia		Spring SE Alps (Trentino)	Springs Alpine foothills (Verona Province, Lessinia)	Springs in the south-western Alps							
	Raška River	Mlava River	AT0756	ML0622	DC1620	RF1477	MT1300	SB1050	MC1650	MC1652	MC1655	RP1800
Shading (1-5 scale)			2	1	1	1	4	3	1	1	1	2
Temperature (°C)	7.5-14	9.1-14.6	9.4	11	8.4	8.1	5.8	7	4	3.4	3.2	2
pH	7.02-8.23	6.95-7.95	7.6	7.3	8.4	7.39	8.33	8.31	8.48	8.56	8.43	8.9
Conductivity (µS cm^{-1})	305-420	389-490	356	411	200	105	125	175	198	200	201	170
Alkalinity (as mg $CaCO_3$ l^{-1})	7-120.8	12.6-120.6	195	240	25	15.2	17.7	26	23	23	24	18.4
N-NH_4^+ (µg l^{-1})	<18.9-473.8	<18.9-355.8	< 20	<100	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
N-NO_3^- (µg l^{-1})	6300-7900	6900-9500	224	3160	45	147	481	565	361	373	230	319
TP (µg P l^{-1})	13.5-104.2	8.7-112.8	4	-	-	-	-	-	-	-	-	-
SRP (µg P l^{-1})	5.2-51.7	3.7-46.8	2	-	-	-	-	-	-	-	-	-
Cl^- (mg l^{-1})	1.2-2.2	0.9-2	1.0	8.0	0.4	0.4	0.3	0.3	0.5	0.5	0.4	0.2
SO_4^{2-} (mg l^{-1})	1.4-3.4	5.5-22.4	21.0	10.0	-	1.6	1.8	4.3	-	-	-	-
% *Geissleria gereckei*	0.5-3	-	<0.2	4.5	1.0	2.3	0.2	0.5	1.0	0.8/0.5	0.5	0.8
Substrate	epil./epib.	epilithon	epilithon	epilithon	epilithon	epilithon	epilithon	epilithon	epilithon	epil./epib.	epilithon	epilithon

– No data

Data from the springs in the Alps were provided by Marco Cantonati.

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