

The diatom communities from Apuseni Mountains: a first approach on crenic diatom flora

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Abstract. The unique geomorphological and hydrological characteristics along with the stable abiotic parameters have shaped springs into suitable aquatic habitats for a high number of microorganisms. Increasing anthropogenic impact, such as groundwater pollution, alteration or destruction of the eucrenal spring area and high demand for pristine water, are affecting the crenic biodiversity. The present study is the first to focus on taxa belonging to the Bacillariophyta phylum and includes diatom samples taken from 30 karstic springs located in the Apuseni Mountains, Romania. Because diatoms often display distinct preferences for specific substrates, 15 samples were collected from each spring: five from each of the three substrate types (bryophytes, stones, and fine sediments) within the eucrenal area. For qualitative assessment, three mixed samples were subsequently prepared for each substrate type, resulting in a total of 99 analysed samples. A total of 216 taxa were identified in the analysed samples, with 16% found only on bryophytes and 15% occurring only on sand. Two groups of frequent taxa, distinguished by their preferred substrate type and present in at least 25 springs have been observed: *Caloneis fontinalis* (Grunow) A. Cleve, *Cocconeis lineata* Ehrenberg and *Meridion circulare* (Greville) C. Agardh

in epibryon, followed by *Gomphonema parvulum* (Kützing) Kützing and *Planothidium dubium* (Grunow) Round & Bukhtiyarova in epipsammon; three taxa *Achnanthidium minutissimum* (Kützing) Czarnecki, *Amphora pediculus* (Kützing) Grunow and *Coccconeis placentula* Ehrenberg stand out as a dominant group across all three types of substrates. Moreover, in the epilithon these were the only taxa present in all samples. Microhabitat-specific richness was shaped primarily by light conditions and canopy cover, with oxygen availability contributing as a secondary factor. *Gomphonema elegantissimum* E. Reichardt & Lange-Bertalot and *Caloneis fontinalis*, along with other 13 taxa have been identified for the first time in Romania.

Keywords: Bacillariophyta, Carpathian Mountains, epibryon, epilithon, epipsammon.

Introduction

The unique geomorphological and hydrological characteristics have shaped springs into being a suitable aquatic habitat for a high number of microorganisms. Compared to other aquatic habitats, springs are smaller in size, have high biodiversity and often serve as refuges for sensitive, rare or endangered species (Cantonati *et al.*, 2006).

Springs provide several ecosystem services and play a key role in the conservation of aquatic biodiversity. The growing human demand for reliable drinking-water sources has threatened these habitats, degraded or even completely altered the eucrinal area through catchment development. These actions are carried out to the detriment of biodiversity, further intensifying the freshwater crisis. Moreover, in the European Water Directive (2000), springs are mentioned exclusively in the context of drinking water, without acknowledging the need to assess and monitor the ecological quality of spring ecosystems (vegetation near the springs, macroinvertebrates and phytobenthos).

The phytobenthos includes epiphytic (found on aquatic macrophytes), epilithic (growing on the surface of stones), epipsammic (living on fine sediments) diatoms. Being at the beginning of the food chain, diatoms are one of the key bioindicators of water quality and often display distinct preferences for a specific type of available substrate (Cantonati *et al.*, 2007). As geographically isolated environments that support crenic organisms, springs represent more stable ecosystems compared to other freshwater habitats. Consequently, diatom communities act as sentinel organisms, responding rapidly to both long or short term, abiotic or biotic influences.

Studies conducted in the Alps and other regions have shown interdependence between some algal groups and a specific bryophyte or vascular plant species (Mogna *et al.* 2015, Cantonati *et al.*, 2022), along with spring-type-specific benthic algal assemblages (Cantonati *et al.*, 2012a).

The Romanian Carpathians are rich in crenic microhabitats, nonetheless a low number of studies have been published about the algal flora, especially diatoms inhabiting them. One of these studies included qualitative samples taken only from stones in the Cerna Karst Spring (Izbucul Cernei) in the Southern Carpathians (Sinitean *et al.*, 2012), without addressing other substrate types. In contrast to karstic systems, another study investigated diatom assemblages in thermo-mineral springs at Băile Herculane (South-Western Romania), where preliminary qualitative and quantitative data were reported by Péterfi and Sinitean (2002). A more recent study investigates invertebrates alongside diatom communities in 5 limnocrene and rheocrene springs from the Transylvania region, central-western Romania (Micle *et al.*, 2018). Although diatoms were sampled from bryophytes, above sand and from the surface of stones, the paper reports diatom data aggregated per spring, not separately per microhabitat. The focus was on frequent taxa identified in the quantitative approach of the study.

A different geographic setting was considered by Motaş *et al.* (1962) on springs and groundwater from the catchment area of The Neajlov River, approximately 50 km northwest of Bucharest. The study included several types of communities: algae, mosses, vascular plants and invertebrates. Two other studies investigate the diatom communities from mineral springs, being among the earliest detailed diatom studies on Romanian springs, widely cited in later Romanian diatom literature: Péterfi *et al.* (1983 and 1985). Comparative analyses of benthic diatom assemblages across several mineral springs in Romania, focusing on species composition, diversity and the role of ionic composition on community stability.

The aim of the present research is to provide a first insight into the crenic diatoms communities found in karstic spring habitats located in the Apuseni Mountains (South-Eastern Carpathians, Romania). Our objectives are: (i) to identify the diatom species composition in the studied springs and (ii) to characterize the karstic springs based on their biotic and physico-chemical attributes. Furthermore, we aim to contribute to the knowledge of crenic habitats by identifying diatom communities that may display preferences for specific microhabitats within the eucrenal area. Together with the existing studies on Carpathian springs (Fránková *et al.*, 2009; Wojtal and Solak 2009; Wojtal and Sobczyk, 2012), our work provides new insights into crenic diatom distribution and substrate preference, contributing not only to the algal flora of Romania but also to the broader knowledge of Carpathian freshwater algal diversity.

Materials and methods

Study area

Crenic diatom communities were sampled during the summer of 2018 (May to August) from 30 springs located in different karstic regions of the Apuseni Mountains, South-Eastern Carpathians (Fig. 1). The investigated areas include the Pădurea Craiului Mountains, the Bihor-Vlădeasa Mountains, the Vaşcău Plateau and the Trascău Mountains, with altitudes ranging from 271 to 1229 m a.s.l. The studied springs belong to the main drainage basins of the rivers: Someşul Cald, Arieş, Crişul Repede, Crişul Alb and Crişul Negru. All sampled springs were known to have permanent discharge at the time of the sampling campaign, although their flow varies seasonally (Orăşeanu, 2016) and they do not undergo complete drought.

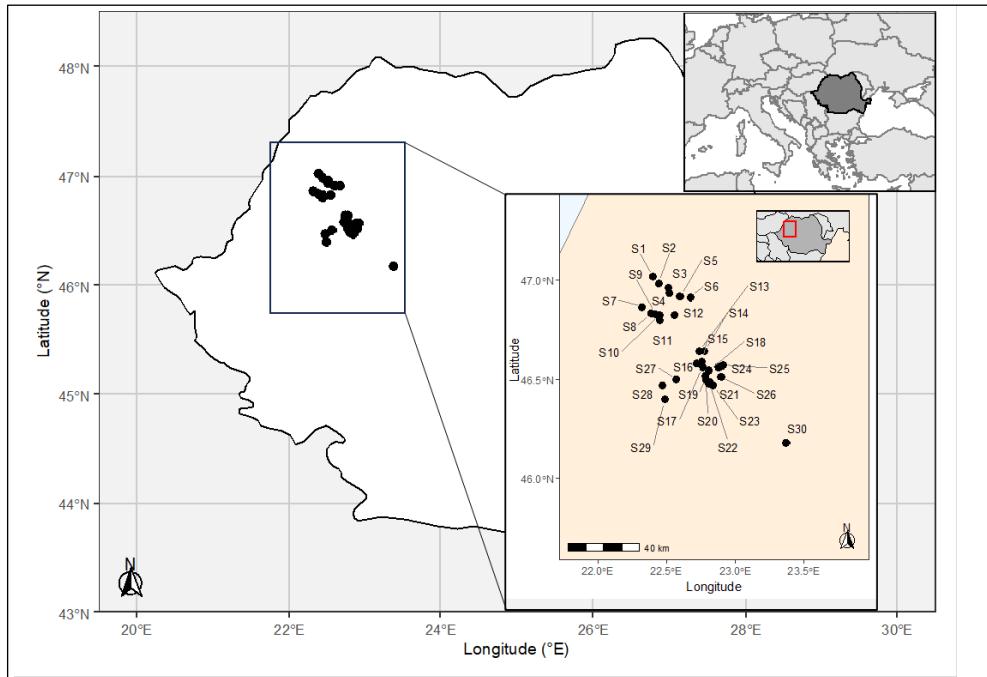


Figure 1. Map of the sampling sites (for spring codes see Table 1).

Spring classification

The different aquatic substrates, on which diatoms prefer to grow, occupy varying proportions of the eucrenal area and the specific hydrological characteristics of each spring justify a spring classification.

In this study, we adopted the spring classification proposed by Springer and Stevens (2009), Cantonati *et al.* (2007): limnocrene springs (L) characterized by the emergence of confined aquifers in a pool (2 springs); helocrene springs (H), which emerge from low-gradient wetlands, with multiple sources seeping from shallow, unconfined aquifers (2 springs); rheocrene springs (R) where water emerges into one or more stream channels (4 springs); and rheohelocrene (RH), emerging from low-gradient wetlands, typically with indistinct or multiple sources seeping from shallow, unconfined aquifers (6 springs). Fifteen springs were categorized as rheocrene cave (RC), where the water emerges from or within a cave through large conduits (Fig. 2). Finally, one rheocrene (RI) spring shows intermittent discharge throughout the year.



Figure 2. The three main types of springs: limnocrene (L), rheocrene cave (RC) and helocrene (H).

Sampling and processing methods

In order to be sampled, a spring had to fulfil the following criteria: karstic geological substrate, permanent discharge and three available microhabitats for diatoms – bryophytes, stones and sand. Only the eucrenal area was sampled from each spring included in this study.

For each sampling site, the following procedures were carried out. Firstly, a map was drawn for the eucrenal area and the percentage cover of each preferred substrate for diatoms was estimated. Only submerged habitats were taken into consideration when estimating the relative surface of every substrate type at the time of sampling. A set of physical and chemical parameters was measured once in the field using portable multimeters: Hanna HI98130, Hanna HI98194 and YSI-52 for water temperature, pH, conductivity, TDS and dissolved oxygen. The measured values are listed in the supplementary material in Cîmpean *et al.*, 2022.

Table 1. Spring codes and other characteristic considered for the present study
(Br – epibryon; Sa – epipsammon; St – epilithon).

Spring codes	Name of the spring	Mountain groups	Altitude (m a.s.l)	Spring type	Canopy cover (%)	Number of diatom taxa			
						Total	Br	Sa	St
S1	Aştileu Cave	Pădurea Craiului	271	RC	65.08	69	44	44	20
S2	Moara Jurjii Cave	Pădurea Craiului	440	RC	64.78	34	15	28	9
S3	Vadul Crişului Cave	Pădurea Craiului	352	RC	81.13	39	26	23	17
S4	Izbucul Izbândiş	Pădurea Craiului	484	L	70.89	54	25	43	20
S5	Izbucul Bratcuţa Mare	Pădurea Craiului	384	RH	90.34	57	25	49	6
S6	Bulz Watery Cave	Pădurea Craiului	391	RC	71.39	56	26	48	16
S7	Topliţa de Vida Cave	Pădurea Craiului	303	RC	83.55	49	26	41	33
S8	Izbucul Toplita de Roşia	Pădurea Craiului	303	RC	84.81	36	26	19	4
S9	Izbucul Roşia Vally	Pădurea Craiului	363	RH	86.82	23	14	12	15
S10	Izbucul Toplicioara	Pădurea Craiului	436	R	62.05	35	21	25	6
S11	Izbucul Izbuneală	Pădurea Craiului	335	R	65.67	19	18	10	5
S12	Valea Leşului Watery Cave	Pădurea Craiului	674	R	77.63	31	15	15	24
S13	Pepii Cave	Bihor-Vlădeasa	1140	RC	61.41	39	24	25	19
S14	Izbucul Alunul Mic	Bihor-Vlădeasa	1178	RC	62.09	45	30	37	18
S15	The Meadow of Karst Springs	Bihor-Vlădeasa	1226	H	42.47	39	33	20	14
S16	Izbucul Ponor	Bihor-Vlădeasa	1094	RC	45.43	52	38	37	13
S17	Gura Apei Cave	Bihor-Vlădeasa	1225	RC	15.38	59	36	41	20
S18	Izbucul Vulturului	Bihor-Vlădeasa	1063	RC	53.04	47	41	27	5
S19	Izbucul Tăuzului	Bihor-Vlădeasa	923	L	65.24	41	33	22	8
S20	Corobană Cave	Bihor-Vlădeasa	834	RC	68.88	28	15	24	13
S21	Izbucul Poliţei	Bihor-Vlădeasa	874	RH	81.71	33	21	20	14

Spring codes	Name of the spring	Mountain groups	Altitude (m a.s.l)	Spring type	Canopy cover (%)	Number of diatom taxa			
						Total	Br	Sa	St
S22	Cotețul Dobreștilor Cave	Bihor-Vladeasa	840	RC	83.36	52	32	24	31
S23	Poarta lui Ionele Cave	Bihor-Vladeasa	850	RC	72.58	31	22	24	7
S24	Izbucul Lina Mare	Bihor-Vladeasa	1229	H	1	52	35	36	12
S25	Izbucul Apa Caldă	Bihor-Vladeasa	1106	R	77.6	37	33	13	13
S26	Izbucul Mătișești	Bihor-Vladeasa	966	RH	85.14	37	23	23	20
S27	Izbucul Bulzului	Bihor-Vladeasa	529	RC	66.98	54	35	33	13
S28	Izbucul Boiu	Platoul Vascau	321	RH	86.4	47	16	34	28
S29	Izbucul Călugări intermittent	Platoul Vascau	464	RI	21.9	41	34	21	9
S30	Izbucul Iezerului	Trascău	884	RH	62.12	36	22	24	10

Secondly, to avoid sample contamination, diatoms were surveyed in the following order: sand, stones and finally bryophytes. Five points were randomly selected for each preferred substrates, resulting in fifteen subsamples per spring. Within the ecrenial zone of each surveyed spring, one or two dominant bryophyte species were identified and sampled as substrates for the collection of epiphytic diatom communities. A bryophyte was considered dominant when it occupied at least five separate patches within the defined ecrenial sampling area, thereby qualifying it for sampling. Consequently, in several springs, multiple sets of epiphytic diatom samples were collected from each bryophyte taxa. In eight springs, two bryophyte species occurred in the ecrenial zone; consequently, five subsamples were collected for each bryophyte taxon. This resulted in a total number of 495 subsamples (150 for sand, 150 for stones and 195 for bryophytes).

For the qualitative assessment of crenic diatoms, mixed samples were prepared separately for each substrate type. From every sampling site, equal quantities of material (5 ml or a bryophyte tuft) were combined from the five subsamples to produce one mixed sample per substrate. Consequently, three mixed samples were prepared per spring (one for epipsammon, one for epilithon and one for each sampled bryophyte species), leading to a total of 99 Falcon tubes. All samples were preserved in ethanol.



Figure 3. Images with sampling methods applied in this study.

Using a 37 mm diameter tube fitted with a non-absorbent sponge at its end, a surface of 10.75cm^2 was scraped from the selected stones. The tube was pressed firmly against the stone surface, 25ml of spring water were added inside the tube and the biofilm was detached using a brush. The resulting suspension was extracted with a syringe and transferred into a Falcon tube (Fig. 3). Epipsammic diatoms were sampled by pipetting the surface layer of sediments 0,063 – 6,3mm in size (sand) from an area of 25-100 cm^2 or until 25ml were obtained. Epibryon samples consisted of tufts of the area-dominant bryophyte species present in the eucrenal area, placed in Falcon tubes with spring water. All samples were preserved in a known volume of ethanol.

From the middle of the eucrenal area of each spring, a photo was taken with a wide-angle camera, directed toward canopy above. Each photo was processed using an imaging software named Gap Light Analyzer (GLA), Version 2.0: to extract canopy structure and gap light transmission indices from photographs (Fig. 4) (Frazer *et al.*, 1999). Afterwards, the images were analysed to calculate the percentage of shaded areas across each the sampled spring.

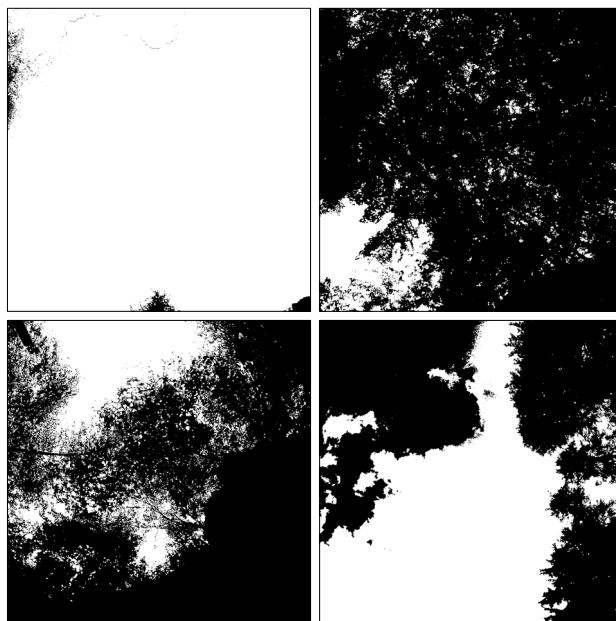


Figure 4. Examples of canopy cover above four of the sampled springs (S24, S05; S27, S15).

To obtain clean frustules, 10ml from each mixed sand and stone sample were added to approximately 20ml of hydrogen peroxide and left to react for at least five days or until the organic matter was removed. Because washing and squeezing the bryophytes in water does not fully remove the epiphytic diatoms, bryophyte tufts were treated with a mixture of H_2SO_4 and $KMnO_4$. After seven days of daily mixing, all samples were rinsed repeatedly with distilled water until the remaining diatom frustule sediment was clean.

Following standardized diatom slide preparation methods (Kelly *et al.*, 1998), a total of 198 slides (two for each mixed sample) were created. The entire coverslip area was examined during identification, with particular emphasis on detecting rare taxa. Identifications were made to species level using a Nikon Eclipse E400 optical microscope, with a few exceptions, where the ventral side of the valve was not visible. The following identification keys were used: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Lange-Bertalot *et al.*, 2017.

Data analysis

The frequency of common taxa in the three types of samples was calculated as the percentage from the total number of samples discriminated between: rare species (those with less than 20% frequency, i.e., present in seven springs),

commonly occurring species (a frequency between 21% and 79%) and frequent species (a frequency above 80%, i.e., found in more than 25 springs).

Taxonomic occurrence, referred as number of taxa present in a sample, was arranged as a matrix with the value one for the species being present in a sample and the value zero representing its absence. Afterwards the matrix was used in software PAST version 4.14c (Hammer *et al.*, 2001) to perform a multivariate analysis of clustering based on the Jaccard indices, a specific distance coefficient for presence/absence data. The similarity was indicated between the epiphytic, epilithic and epipsammic samples corresponding to each karstic spring.

To evaluate how each type of sampled microhabitat availability shapes diatom richness within karstic spring microhabitats, we analysed: the proportion (%) of each type of substrate in the ecrenal zone (epibryon – Br; epipsammon – Sa and epilithon – St) as explanatory variables and the number of taxa recorded on each corresponding substrate as biological response variables. Given that both variables comprise continuous values (expressed as percentages and richness counts, respectively) we employed two complementary correlation approaches: Pearson's correlation, which assumes linearity and approximate normality, and Spearman's rank correlation, which is non-parametric and detects monotonic trends irrespective of distribution form.

Prior to analysis, the frequency distribution of taxa richness values was inspected. Richness associated with bryophytes and sand substrates exhibited near-unimodal distributions with only slight skewness, whereas stone-associated richness showed moderate right-skew but no extreme outliers. On this basis, data transformation was not applied; richness values occurred within a comparable numeric range across springs, percentage predictors were already standardised on a 0–100 scale, thus transformation would risk obscuring ecological interpretability without improving model robustness. Furthermore, the application of Spearman correlation ensured methodological robustness in the presence of non-normal data distributions.

The Pearson's correlation and Spearman's rank correlation were performed using the PAST software. Correlation coefficients and p-values were analysed for both statistical measures. Patterns were interpreted as ecologically reliable only when correlation direction and significance were consistent between the two methods.

Multivariate analyses were used to visualize and interpret the data. Redundancy analysis (RDA) was used to project the data on a two-dimensional map and identify the relationship between environmental drivers and the biological communities. Two separate RDA analyses were performed and are presented as distinct figures. RDA-I examines how each type of sampled

microhabitat availability shapes diatom richness, whereas RDA-II illustrates the relationships between environmental variables and substrate-specific richness.

Given the linear and monotonic relationships between substrate cover and diatom richness confirmed by both Pearson and Spearman correlations, we used redundancy analysis (RDA I) to visualise how taxa richness on each microhabitat responds to different substrate availability. Prior to performing the RDA analysis, environmental predictors were automatically centered by Canoco 5 version 5.15 (ter Braak *et al.*, 2012), which is standard practice in linear ordination. No further standardisation or transformation was required because variables were already comparable in scale and ecologically interpretable. Moreover, the substrate cover data is compositional and has a gradient 1.1 SD units long, so a linear method is recommended.

Environmental and taxa richness relationships were analysed using two datasets: the diatom communities found on each sampled substrate (number of taxa recorded on bryophytes, sand and stones in each spring), and environmental variables measured *in situ* (altitude, pH, dissolved oxygen, conductivity, temperature, total dissolved solids, and canopy cover).

Prior to multivariate analysis, all environmental predictors were z-standardised (mean-centred and scaled by standard deviation) using the R function *scale()*. Response variables were not standardized for the reasons mentioned above, at the first RDA analysis data preparation.

Spearman rank correlations were calculated between each environmental variable and each taxa richness, because Spearman's ρ does not require normally distributed data and remains reliable for monotonic yet non-linear ecological relationships. Statistical significance was evaluated using *cor.test()* with two-tailed p-values, and the correlation matrices with corresponding probabilities were interpreted.

Multivariate relationships were subsequently tested by performing a Redundancy Analysis (RDA II) using the vegan package (function *rda()* with the software Rstudio version 2023.03.0). A response matrix was constructed from the three richness variables (NbBr, NbSa, NbSt), and the matrix of standardised environmental variables served as the explanatory dataset. Significance of the global RDA model, individual canonical axes, and individual environmental predictors was assessed using permutation tests (999 permutations; functions *anova(rda_rich)*, *anova(rda_rich, by = "axis")*, and *anova(rda_rich, by = "term")*). Ordination was visualised with scaling = 2, and species-response vectors were extracted using scores (*rda_rich, display="species", scaling=2*) to interpret substrate-specific richness patterns along environmental gradients.

To explore relationships among sampling sites based on multiple environmental parameters, a Hierarchical Cluster Analysis (HCA) was performed

using the *pvclust* function from the *pvclust* package (with Ward's linkage method and Euclidean distances) in RStudio. Prior to performing the HCA, conductivity and dissolved oxygen values were log10-transformed to reduce skewness and improve comparability with linear variables. All variables (including altitude and pH, which were not log-transformed) were then standardized using Z-scores normalization to ensure equal weighting.

A heatmap was generated to visualize the relative frequency of diatom taxa across the three sample types (Br, St and Sa). The dataset was imported into R and converted into a numeric matrix, and no data scaling was applied. We used the *viridis* colour gradient, which allows intuitive interpretation of abundance intensity, with lower values represented by darker shades and higher values by yellow.

Results

Species composition

Across the 198 slides of epiphytic, epilithic and epipsammic samples a total number of 216 taxa were identified. A large number of species (n=83) was found in only one of the studied springs, either as a few frustules (*Cyclotella bodanica* var. *lemanica* (O. Müller ex Schröter) Bachmann) or as a more abundant population (*Achnanthidium pyrenaicum* (Hustedt) H. Kobayasi, *Fragilaria pinnata* Ehrenberg cf). *Achnanthidium minutissimum*, *Amphora pediculus*, *Cocconeis lineata*, *C. placentula*, *Meridion circulare* and *Planothidium lanceolatum* are the six species found in all the 30 karstic springs. Another nine taxa were present in more than 75% of the springs, such as: *Cocconeis placentula* var. *euglypta* (Ehrenberg) Cleve, *Gomphonema parvulum*, *Navicula cryptotenella* Lange-Bertalot, *Caloneis fontinalis*, *Planothidium dubium*, *Odontidium mesodon* (Ehrenberg) Kützing, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot and *Navicula tripunctata* (O.F.Müller) Bory (supplementary data).

The lowest number of taxa (n=19) was recorded in spring S11, with more than three quarters of them belonging to the most commonly occurring species found in the studied karstic springs (listed above). In contrast, spring S1 showed highest richness, with 69 taxa identified, of which only four were not present in the other springs (Fig. 5). The average number of taxa per spring was 42.

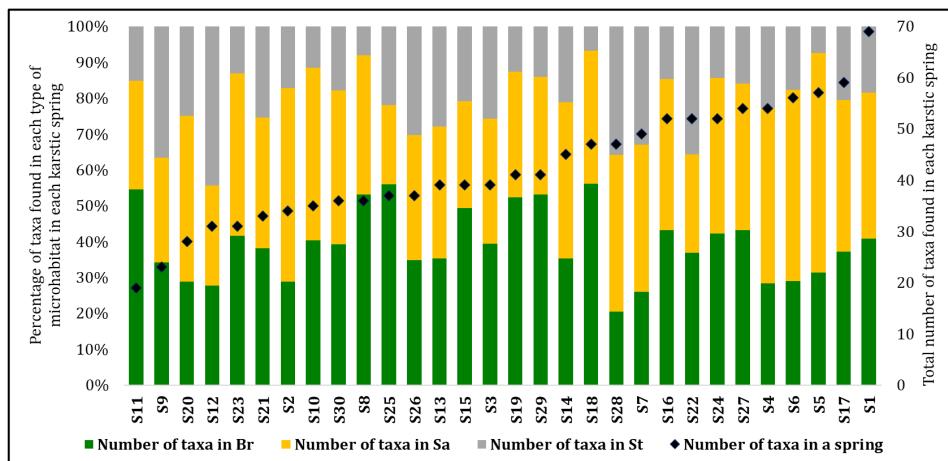


Figure 5. The number of taxa identified on bryophytes (Br), sand (Sa), and stones (St) shown as percentage bars, versus total number of taxa in a spring, represented by black rhombic symbols.

Epibryon diatom communities accounted for 66.6% of the species identified in the studied karstic springs. The number of taxa ranged from a minimum of 13 (in S9) to a maximum of 44 (in S1). In most springs, the number of taxa on bryophytes was relatively low; in twenty springs, richness ranged between 13–22 taxa, and in another group of springs it ranged between 23–32 taxa. Frequent species in the epiphytic samples were *Cocconeis placentula* and *Planothidium lanceolatum* (Fig. 6). Seven taxa were found in more than 75% of the bryophyte samples: *Meridion circulare*, *Cocconeis lineata*, *Achnanthidium minutissimum*, *Navicula cryptotenella*, *Amphora pediculus*, *Caloneis fontinalis* and *Cocconeis placentula* var. *euglypta*. In total, no more than 60 taxa occurred only once within the epibryon samples, of which 35 were found exclusively on bryophytes (i.e. *Amphora copulata* (Kützing) Schoeman & R.E.M. Archibald, *Cocconeis neothumensis* Krammer, *Gomphonema pumilum* (Grunow) E. Reichardt & Lange-Bertalot). The most species-rich genera were *Navicula* (20 taxa), *Gomphonema* (16 taxa), and *Nitzschia* (12 taxa), out of a total of 43 identified genera.

Of the total number of taxa identified in the karstic samples, fewer than half were present in the epilithic samples ($n=102$). Among these, 45 taxa occurred in only a single slide (i.e. *Cocconeis pediculus* Ehrenberg, *Cymbopleura subaequalis* (Grunow) Krammer, *Gomphonema acuminatum* Ehrenberg, *Hannaea arcus* (Ehrenberg) R.M.Patrick, *Navicula cari* Ehrenberg, *Placoneis elginensis* (W.Gregory) E.J.Cox). Seven taxa from this microhabitat had a frequency above 50%: *Achnanthidium minutissimum*, *Amphora pediculus*, *Cocconeis placentula*,

Navicula cryptotenella, *Meridion circulare*, *Planothidium lanceolatum* and *Gomphonema parvulum*. The number of taxa found on the surface of stones in a spring ranged from a minimum of 4 (in S8) to a maximum of 32 (S7). In half of the springs, taxa richness ranged between 12 and 21 taxa, and the average number of taxa in epilithic samples was 14.

The presence/absence rate was low in the epilithic samples, only one taxon, *Achnanthidium minutissimum*, was identified in almost all analysed slides (n=29). *Amphora pediculus* and *Coccneis placentula* were the only two taxa found in more than 75% of the samples (Fig. 6). The genera *Gomphonema* and *Navicula* comprised the highest number of taxa in the samples collected from stones, out of a total of 40 identified genera.

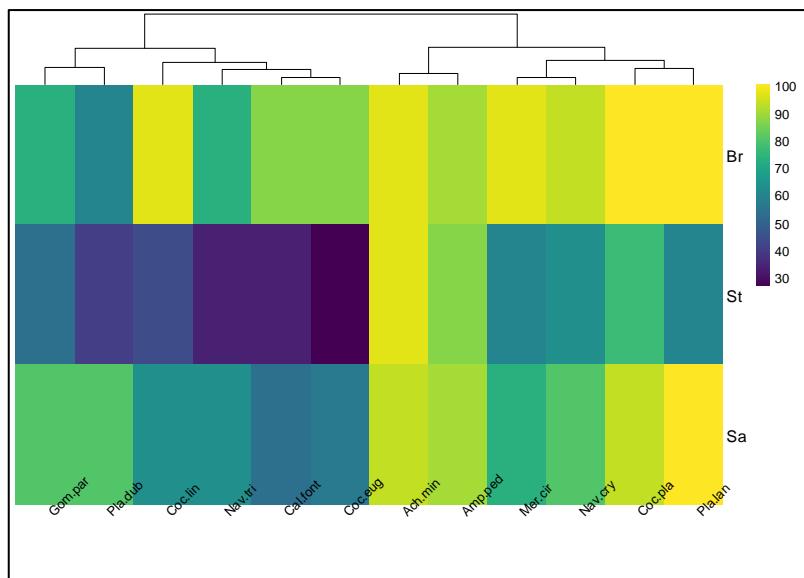


Figure 6. Heatmap showing taxa with frequencies above 50% across the three sampled substrates (Gom.par – *Gomphonema parvulum*; Pla.dub – *Planothidium dubium*; Coc.lin – *Coccneis lineata*; Nav.tri – *Navicula trivalis*; Cal.font – *Caloneis fontinalis*; Coc.eug – *Coccneis placentula* var. *euglypta*; Ach.min – *Achnanthidium minutissimum*; Amp.ped – *Amphora pediculus*; Mer.cir – *Meridion circulare*; Nav.cry – *Navicula cryptotenella*; Coc.pla – *Coccneis placentula*; Pla.lan – *Planothidium lanceolatum*; Sa – sand; Br – bryophytes; St – stones).

A higher proportion of taxa (75% of the total number of identified taxa in this study) was present in the epipsammic samples. Moreover, approximately a quarter were found only once in the analysed slides. *Caloneis silicula* (Ehrenberg) Cleve, *Cyclotella bodanica* var. *lemanica* and *Tabellaria flocculosa* (Roth) Kützing

were among the 34 taxa identified exclusively on sand. The average number of taxa per spring was 27, ranging from a minimum of 10 (in S11) to a maximum of 48 (S5 and S6). *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot was observed in all analysed slides, with high number of individuals. *Achnanthidium minutissimum*, *Cocconeis placentula*, *Amphora pediculus*, *Gomphonema parvulum*, *Navicula cryptotenella*, and *Planothidium dubium* were present in 80% of the samples. The genus *Navicula* was the most species-rich (20 taxa), followed by *Nitzschia* (12 taxa) and *Gomphonema* (9 taxa). Overall, the sand samples displayed the highest genus-level diversity (55 genera).

The frequent species, occurring in the three substrate types and in all analysed slides were: *Achnanthidium minutissimum*, *Amphora pediculus* and *Cocconeis placentula*. *Planothidium lanceolatum*, *Navicula cryptotenella*, *Meridion circulare* and *Gomphonema parvulum* were commonly occurring taxa found in epipsammon and epibryon, usually with high number of individuals.

The highest similarity in the presence – absence patterns was observed among samples belonging to the same substrate type rather than between springs (using Jaccard index). Almost 70% of the taxa were shared between the sand samples from S18 and S20. In contrast, samples S8_St and S19_St showed the lowest similarity to all other samples, with fewer than 10% shared species. The bryophytes microhabitat showed the most homogeneous communities, with more than half of the samples clustering into two groups with similarity values of 40-55%. Epipsammic samples were more similar to the epibryon than to those collected from stones. Smaller clusters of epipsammic samples were also observed, and a general tendency was noted for samples from springs in the same area to cluster together. Seven epilithic samples formed a distinct cluster, sharing only 20% of their taxa (S5, S10, S11, S18, S23, S29, S30).

Karstic springs characteristics

The eucrenal area was assessed in order to estimate the proportion of each sampled substrate. Overall, the microhabitat offered by bryophytes was dominant in most of the karstic springs (Fig. 7), reaching up to 60% coverage of the eucrenal area.

Although rocks and boulders were a common feature in the rheocrene cave springs, the stone substrate formed a stable microhabitat in all karstic springs, covering 30–40% of the eucrenal area. Despite the wide percentage range of sand coverage (5–60%), in most springs it did not exceed 30% of the eucrenal area.

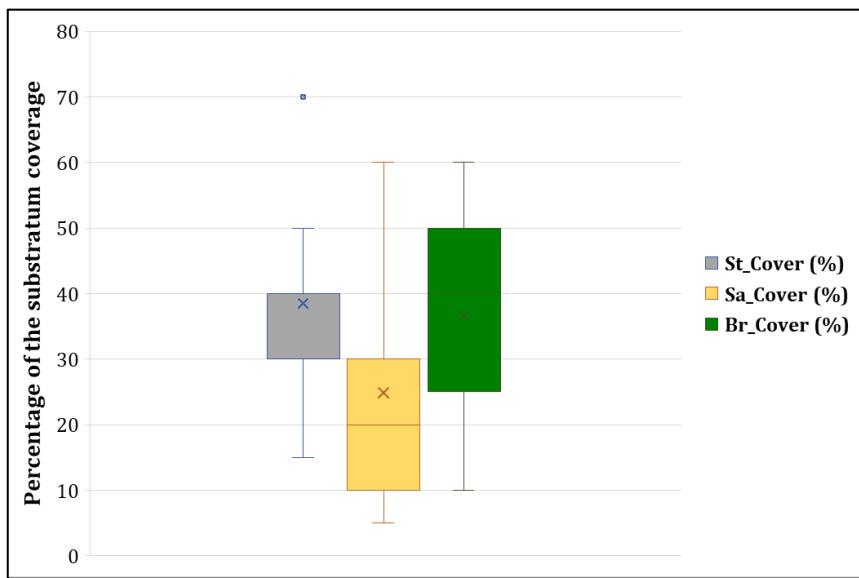


Figure 7. Boxplot with the percentage cover area of each microhabitat found in the eucrenal area of the sampled karstic springs.

The Spearman rank correlations and Pearson correlation matrix (Fig. 8) and associated p-values were calculated to assess the statistical significance of the relationships between substrate cover and taxonomic richness in the qualitative samples. In the eucrenal area, sites with higher coverage of stones (St%) supported fewer sand-associated taxa, likely reflecting reduced habitat availability for epipsammic colonisation. This observation is supported by a statistically significant negative correlation (Pearson $r = -0.47$, $p = 0.0082$; Spearman $\rho = -0.48$, $p = 0.0071$) between number of taxa in epipsammic samples and the percentage cover of stones (St%).

Similarly, sand cover was negatively correlated with stone-associated taxa ($r = -0.36$, $p = 0.0476$; $\rho = -0.43$, $p = 0.0185$). It demonstrates a pattern of mutual exclusion between these two types of substrates.

In contrast, bryophyte cover displayed the strongest significant positive relationship, correlating with increased richness of epilithic taxa ($r = +0.48$, $p = 0.0067$; $\rho = +0.47$, $p = 0.0081$). The observed pattern implies that bryophyte patches with greater structural complexity could enhance surface heterogeneity, consequently facilitating the increase in epilithic species.

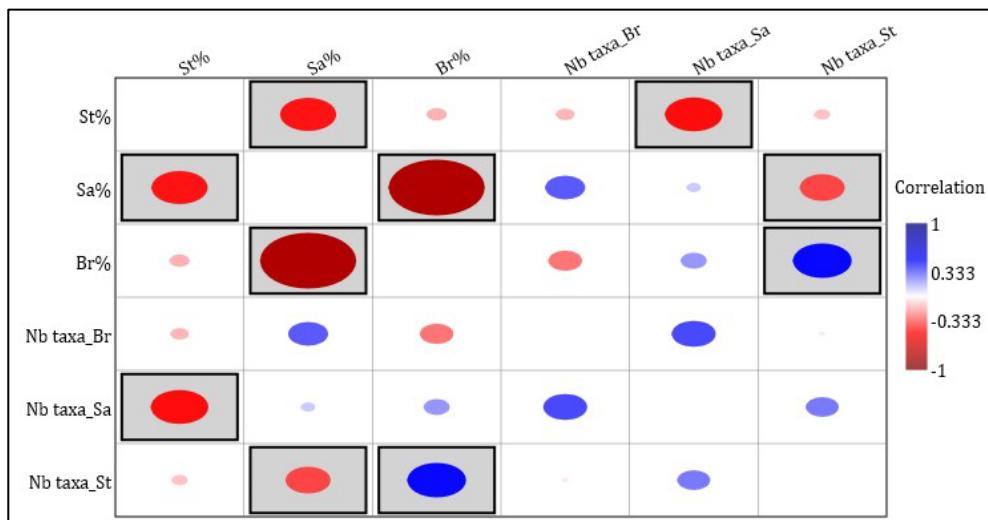


Figure 8. Pearson correlation matrix of the variables: percentages of each substrate type and the number of taxa found in each one (Nb taxa – number of taxa identified in the samples, St – epilithon, Sa – epipsammon, Br – epibryon).

A weaker positive association was observed between sand cover and bryophyte taxa in the Spearman analysis ($\rho = +0.36$, $p = 0.0487$), although not supported by Pearson correlation. All other relationships between substrate cover and taxa richness were weak and non-significant ($|r| < 0.32$, $p > 0.05$). Indicating that substrate abundance may not consistently predict diatom richness in the respective microhabitat.

Redundancy analysis (RDA I) showed that substrate cover remains an important driver of richness when considered across the entire eucrenal zone, not just at a microhabitat scale (Fig. 9). The analysis explained 27.12% of total variation in the dataset. Cumulatively, the first two axes captured 100% of the fitted variation, confirming that substrate gradients adequately describe the major richness shifts observed among springs. Permutation tests for the full model were significant (pseudo- $F = 3.2$, $p = 0.016$), demonstrating that substrate composition has a non-random, directional influence on diatom assemblage richness.

Structurally, the RDA analysis reflected the correlation patterns described previously: stone-dwelling taxa aligned positively with bryophyte cover and opposite the sand vector, indicating that bryophyte-rich microhabitats facilitate bigger lithic communities, while sand-dominated substrates suppress them.

Overall, the RDA highlights cross-microhabitat effects as key drivers of diatom assemblage structure in karstic eucrenal springs, with the strongest variance associated with gradients between bryophyte-enhanced and sand-suppressed lithic diversity.

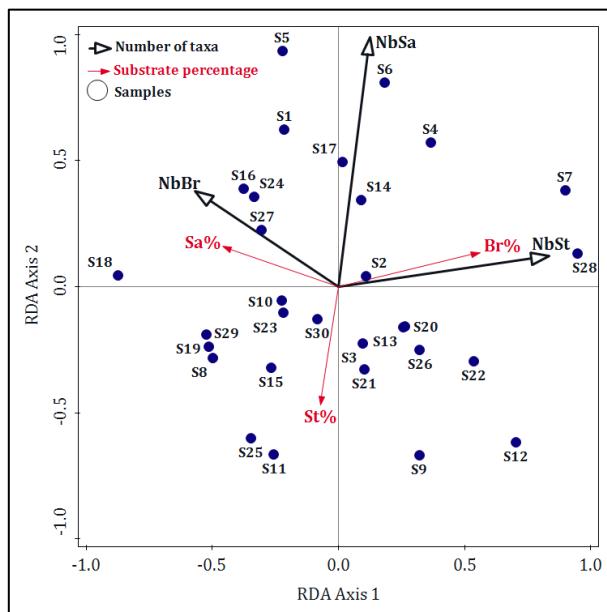


Figure 9. Redundancy analysis (RDA I) biplot (explained variation: 21.65% on Axis 1; 5.47% on Axis 2, $p=0.016$) showing substrate percentage cover in relation to taxa richness (NbSt – number of taxa in epilithon; NbSa - number of taxa in epipsammon; NbBr - number of taxa in epibryon).

Diatoms, as ecological bioindicators, reflect the physical and chemical characteristics of the aquatic habitat they inhabit. The 1000 m difference in elevation between the S1 and S24 springs provided a wide altitudinal range (Tab. 1), which may influence the structure of crenic diatom communities. Water temperature showed little variation among the studied springs, ranging from a minimum of 6°C and a maximum of 11.79°C in S30. Field measurements indicated circumneutral to slightly alkaline pH values, with a maximum of 9.68 recorded in S5, and 58% of the total identified diatoms indicate the same trend, reflecting their preference for alkaline environments.

Dissolved oxygen concentrations were consistently high (>8 mg/L) in most karst springs, which is supported by the fact that 40% of the crenic diatom community consisted of taxa preferring elevated oxygen levels; however, lower values were measured in the eucrenal zone of a few springs, likely reflecting drier sampling periods when reduced flow and stagnation may have decreased oxygen levels. Half of the diatom communities identified in this study thrive on wet and moist habitats or mainly occur in water bodies, however 10% of the taxa could survive when spring flow is significantly reduced. Information on ecological indicator values of diatoms and bioindicating characteristics was sourced from the framework presented by Van Dam *et al.* (1994).

Most springs exhibited conductivity values typical of waters flowing on calcareous substrate, between 300-500 $\mu\text{S}/\text{cm}$ (Pricope, 1999). The only exception was S1, which recorded a maximum conductivity of 894 $\mu\text{S}/\text{cm}$.

To explore the relationships among sampling sites based on multiple environmental parameters, a Hierarchical Cluster Analysis (HCA) was performed. This method grouped the sites according to their overall similarity, and the resulting dendrogram (Fig. 10) identifies clusters of environmentally similar springs. Based on the environmental data, four different clusters were formed. The red rectangular clusters shown below have approximately unbiased values (AU) $\geq 95\%$ and p-values < 0.05 .

The first hierarchical cluster comprises a large group of 14 high elevated karstic springs located in the Bihor-Vlădeasa Mountains and characterized by cold and high-oxygenated waters, moderate pH and conductivity (from S13 to S26).

The second largest group of sampling sites which form a cluster are mid-altitude springs: S2, S3, S6, S8, S9, S10, S11 and S12 (located in the Pădurea Craiului Mountains), S27 (from the Bihor-Vlădeasa Mountains), S28 and S29 (from Platoul Vașcău) and S30 (in the Trascău Mountains). Moderate mineral content and good oxygenation are dominant in this group, with more stable environmental parameters.

A smaller cluster (S1, S4 and S7) forms a group of low altitudes sampling sites from the Pădurea Craiului Mountains, with low oxygenated waters, high values of conductivity. A single outlier can be observed in the dendrogram, the rheohelocrene karstic spring Izbuclul Brătcuța Mare (S5), due to its highly alkaline water (pH=9.68). The value measured on the field was correct, as it has been confirmed by the Administration of the Valea Groșilor Nature Reserve.

From the ecrenal area of each karst spring, an upward-facing photograph of the canopy was taken to estimate the proportion of light passing through the cormophyte foliage. Of all studied springs, 48% were half-shaded; in some rheocrene cave springs, shading was caused by the cave walls. A total of 36% of the springs were almost completely shaded during the spring–summer season (Fig. 11). Springs S15 and S16 were partially sunny, S17 and S29 were fully sun-exposed, and S24 was the only spring in complete sunlight, situated in a meadow at 1229 m.

The RDA term-by-term permutation test showed that shade was the only significant predictor of diatom richness across the sampled substrates ($F = 2.94$, $p = 0.044$) (Fig. 11), while dissolved oxygen exhibited a marginal trend ($F = 2.44$, $p = 0.077$). Conductivity, pH, and altitude showed no significant effect after accounting for the influence of shade ($p > 0.25$). These results suggest that light availability and canopy cover play the primary role in structuring microhabitat-specific richness, with oxygen availability exerting a secondary influence.

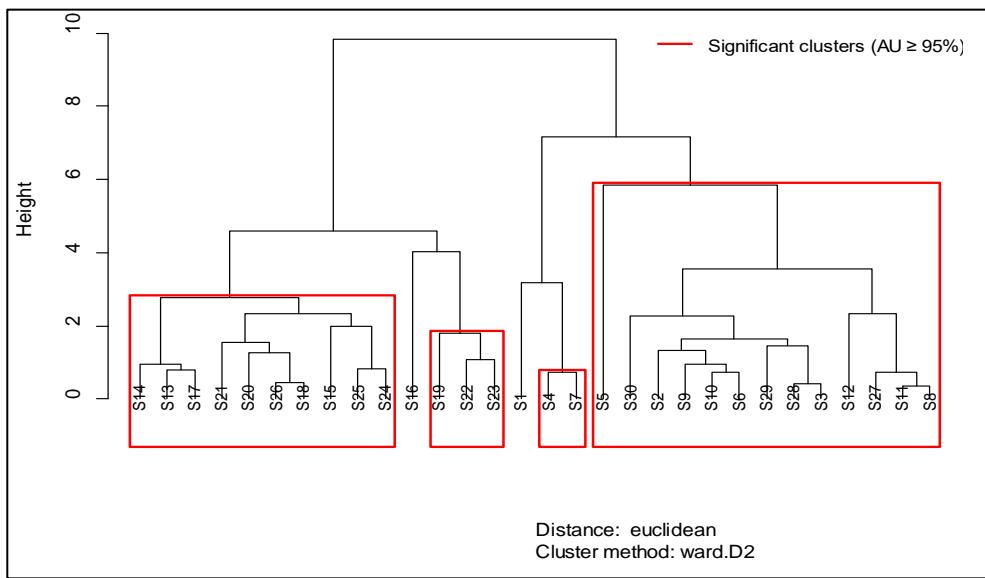


Figure 10. Hierarchical Cluster Analysis (HCA) dendrogram showing the clustering of the 30 springs based on their measured physico-chemical characteristics.

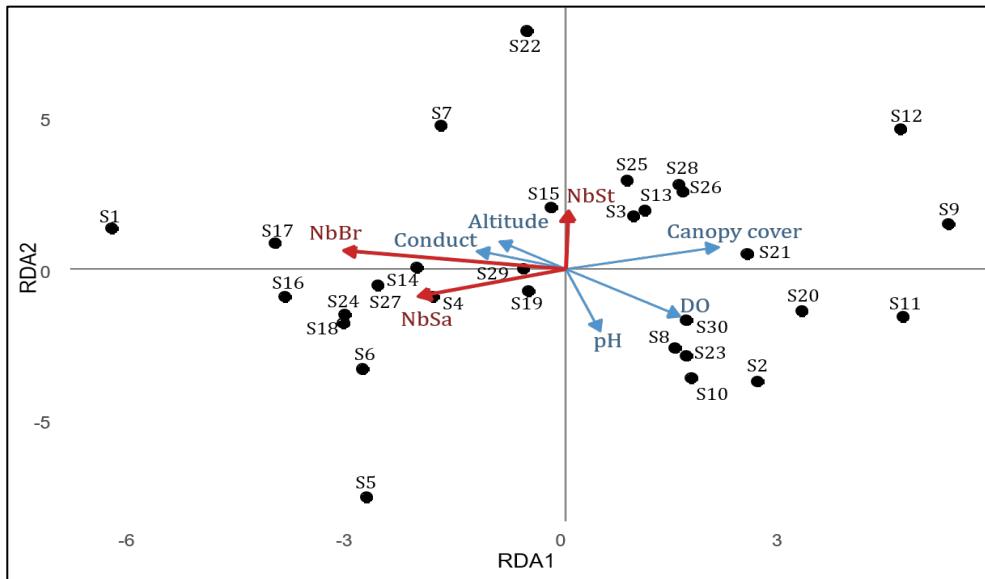


Figure 11. Redundancy analysis (RDA II) biplot (explained variation: 16.25% on Axis 1; 5.78% on Axis 2, $p=0.002$) illustrating the relationship between environmental variables (Altitude (m a.s.l.); Conduct – conductivity ($\mu\text{S}/\text{cm}$); DO – dissolved oxygen (mg/l); Canopy cover (%)) and diatom communities (NbSt – number of taxa in epilithon; NbSa - number of taxa in epipsammon; NbBr - number of taxa in epibryon).

New cited taxa and endangered species

In the present study 13 taxa were cited for the first time in Romania: *Amphora minutissima* W. Smith, *Caloneis fontinalis*, *Diploneis fontanella* Lange-Bertalot cf, *Diploneis krammeri* Lange-Bertalot & E. Reichardt cf, *Gomphonema elegantissimum*, *Gomphonema lateripunctatum* E. Reichardt & Lange-Bertalot, *Gomphonema procerum* E. Reichardt & Lange-Bertalot, *Gomphonema holmquistii* Foged, *Gomphonema minusculum* A. Cleve, *Gyrosigma obtusatum* (Sullivant & Wormley) C. S. Boyer, *Kolbesia gessneri* (Hustedt) Aboal, *Neidium cuneatiforme* Levkov, *Stauroneis reichardtii* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito, *Surirella bohemica* (G. W. Maly) Schönfeldt and *Surirella birostrata* Hustedt. According to the Algae of Romania: a distributional checklist of actual algae (Cărăuș, 2017), these 13 taxa were not cited in Romania.

A group of 62 taxa from our study were identified in the Red List of diatoms (Hofmann *et al.*, 2018) which included: species from the category early warning list (*Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *Diploneis elliptica* (Kützing) Cleve, *Diploneis krammeri*, *Eunotia arcus* Ehrenberg, *Eunotia minor* (Kützing) Grunow, *Eunotia paludosa* Grunow, *Gomphonella calcarea* (Cleve) R. Jahn & N. Abarca, *Gomphonema lateripunctatum*, *Gomphonema sarcophagus* W. Gregory, *Halimphora normanii* (Rabenhorst) Levkov, *Nitzschia dissipata* var. *media* (Hantzsch) Grunow, *Nitzschia acidoclinata* Lange-Bertalot, *Pinnularia microstauron* C. A. Agardh, P. T. Cleve, and C. G. Ehrenberg, *Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round, *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg and *Surirella spiralis* Kützing); extremely rare (*Diploneis pseudovalis* Hustedt, *Gomphonema subtile* Ehrenberg, *Pinnularia subrostrate* Lohman & G.W. Andrews, *Surirella bohemica* (G.W. Maly) Schönfeldt, *Surirella birostrata* Hustedt); 15 taxa with a status “hazard of unknown magnitude”(); endangered taxa like *Achnanthidium petersenii* (Hustedt) C.E. Wetzel, Ector, D.M. Williams & Jüttner, *Amphora aequalis* Krammer, *Caloneis tenuis* (W. Gregory) Krammer, *Caloneis schumanniana* (Grunow) Cleve, *Cymbella helvetica* Kützing, *Cymbella laevis* Nägeli, *Cymbopleura subaequalis* (Grunow) Krammer, *Delicatophycus delicatulus* (Kützing) M.J.Wynne, *Encyonema vulgare* Krammer, *Eunotia trinacria* Krasske, *Frustulia erifuga* Lange-Bertalot & Krammer, *Gomphonema procerum* E. Reichardt & Lange-Bertalot; highly endangered species: *Cymbella affinis* Kützing, *Eucocconeis flexella* (Kützing) F.Meister, *Eunotia praerupta* Ehrenberg, *Navicula angusta* Grunow, *Navicula densilineolata* (Lange-Bertalot) Lange-Bertalot, *Navicula gottlandica* Grunow, *Navicula wildii* Lange-Bertalot and *Neidium iridis* (Ehrenberg) Cleve. In springs S4 and S8 two threatened with extinction species were identified: *Cyclotella bodanica* var. *lemanica* and *Cyclotella planctonica* Brunthaler, but only as a few frustules.

Discussion

Our study describes the diatom communities found on different microhabitats, such as bryophytes, stones and small sediments. It provides a first insight into the high taxa richness found in the ecrenal area of karstic springs in the Apuseni Mountains. The identifications and resulting data offer new information on the diatom communities inhabiting the karst crenic habitats located in the South-Eastern Carpathians, Romania.

We propose a straight-forward and easy to reproduce diatom sampling protocol which could be applied to many types of aquatic habitats and help with the monitoring of the diatom communities in all protected areas of the Carpathian Mountains.

In the present study, the three frequently occurring taxa were: *Achnanthidium minutissimum*, *Amphora pediculus* and *Cocconeis placentula*. Similar findings were cited from south-eastern Alps (Angeli *et al.*, 2010), listed as the most frequent and abundant taxa, with a frequency of 92.6% in epibryon and epilithon samples. The first two species mentioned above were found also as frequent taxa in epilithic samples in another study (Delgado *et al.*, 2013).

In the epipsammon and epibryon samples *Planothidium lanceolatum*, *Navicula cryptotenella*, *Meridion circulare* and *Gomphonema parvulum* were typically present in high numbers.

The percentage of the species identified only once in the analysed samples was 38%, a high number of taxa with one occurrence being common in spring habitats from southeastern Alps (Cantonati *et al.*, 2012b). Moreover, both in epibryon and epipsammic samples the percentage of these taxa was high, even though in other studies the epilithon samples had the highest number of single occurrence species (Cantonati *et al.*, 2012b; Wojtal and Sobczyk, 2012). The low number of the epilithic taxa may be attributed to the smaller sampling surface or to the carbonate substrate as a similar phenomenon was observed in another study (Cantonati *et al.*, 2022).

A high number of diatom taxa associated with bryophytes has been reported in other studies (Mogna *et al.*, 2015). Our results indicate that bryophyte-associated diatoms do not increase proportionally with bryophyte cover; instead, richness was highest where bryophyte growth occurred in smaller, compact patches rather than large bryophyte mats.

Corroborating the physico-chemical data with the biotic one, several trends could be identified in case of studied karstic springs: although 48% of the sampling sites are half-shaded springs, taxon richness appears to be unaffected, similar findings were presented in a study from Italy (Cantonati, 1998); oxygen availability represents an important driver of the taxa richness.

Higher richness in shaded springs suggests that crenic diatom assemblages benefit from the moderated temperature regime, reduced UV exposure, and sustained moisture typical of well-canopied headwaters. Consistent findings could be observed in 16 springs in the Northern Apennines (Cantonati *et al.*, 2020). Conductivity and pH of the spring waters did not appear to affect taxa richness at the spring scale, but may still influence compositional turnover.

As the altitude increases, the occurrence of taxa listed in Red List of diatoms (Hofmann *et al.*, 2018) may also increase. High numbers of species included in Red List of diatoms were also found in the following studies: Sherwood and Sheath (1999), Cantonati *et al.* (2010).

Conclusions

The present study illustrates for the first time the composition of crenic diatom assemblages sampled from the eucrenal area of 30 karstic springs located in the South-Eastern Carpathians, Romania. We investigated three eucrenal microhabitats and qualitatively characterized their diatom assemblages.

Diatom communities in the karstic springs of the Apuseni Mountains are characterized by: (1) high taxon richness within a small sampling area; (2) a high percentage of the canopy cover and elevated dissolved oxygen levels are significant predictors of diatom richness across substrates; (3) the presence of taxa newly reported for the Romanian algal flora; (4) numerous threatened species presented in the Red List(s).

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Conflicts of Interest: The authors declare no competing interests.

Ethics declarations: not applicable.

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Name of diatom taxa and author	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30		
<i>Achnanthidium biasolettianum</i> (Grunow) Lange-Bertalot	-	1	1	1	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-		
<i>Achnanthidium minutissimum</i> var. <i>jackii</i> (Rabenhorst) Lange-Bertalot	1	-	-	-	-	-	-	1	-	-	-	1	1	1	1	1	-	1	-	-	-	-	1	1	1	-	1	1	1			
<i>Achnanthidium minutissimum</i> (Kützing) Czarnecki	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<i>Achnanthidium petersenii</i> (Hustedt) C.E.Wetzel, Ector, D.M.Williams & Jüttner	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-		
<i>Achnanthidium pyrenaicum</i> (Hustedt) H.Kobayasi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		
<i>Achnanthidium subatomus</i> (Hustedt) Lange-Bertalot	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Amphipleura pellucida</i> (Kützing) Kützing	1	1	-	-	-	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Amphora aequalis</i> Krammer	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Amphora copulata</i> (Kützing) Schoeman & R.E.M.Archibald	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Amphora inariensis</i> Krammer	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Amphora libyca</i> Ehrenberg	1	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	1	1	1	1	1	1	-	-	1	-	-	1	-	-	-	
<i>Amphora minutissima</i> W.Smith	1	-	-	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	
<i>Amphora ovalis</i> (Kützing) Kützing	1	1	-	1	-	1	1	1	-	-	-	1	-	-	1	-	1	-	-	-	-	-	-	1	-	-	1	-	-	-	-	
<i>Amphora pediculus</i> (Kützing) Grunow	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<i>Caloneis bacillum</i> (Grunow) Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	
<i>Caloneis schumanniana</i> (Grunow) Cleve	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Caloneis silicula</i> (Ehrenberg) Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Caloneis tenuis</i> (W.Gregory) Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Caloneis fontinalis</i> (Grunow) A.Cleve	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<i>Caloneis molaris</i> (Grunow) Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	
<i>Cocconeis placentula</i> Ehrenberg	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehrenberg) Cleve	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<i>Cocconeis lineata</i> Ehrenberg	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
<i>Cocconeis neothumensis</i> Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-		
<i>Cocconeis pediculus</i> Ehrenberg	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	-	1	-	1	-	1	-	1	-	-	-	
<i>Cocconeis pseudolineata</i> (Geitler) Lange-Bertalot	1	1	1	1	1	1	1	1	-	1	1	1	-	1	1	1	-	1	1	1	-	1	-	1	-	1	-	1	-	1		
<i>Cyclotella bodanica</i> var. <i>lemanica</i> (O.Müller ex Schröter) Bachmann	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Cyclotella meneghiniana</i> Kützing ¹	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
<i>Cyclotella iris</i> Brun & Héribaud	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
<i>Cyclotella planctonica</i> Brunnthaler	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cymatopleura apiculata</i> W. Smith ²	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-	

<i>Stauroneis phoenicenteron</i> (Nitzsch) Ehrenberg	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1			
<i>Stauroneis reichardtii</i> Lange-Bertalot, Cavacini, Tagliaventi & Alfinito	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-		
<i>Stauroneis smithii</i> Grunow	1	1	-	1	-	1	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	1	-	-	-			
<i>Staurosira venter</i> (Ehrenberg) Cleve & J.D.Möller	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Staurosirella pinnata</i> (Ehrenberg) D.M.Williams & Round	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	1		
<i>Surirella angusta</i> Kützing	1	-	1	1	1	1	1	-	-	-	-	-	-	-	-	1	1	1	1	1	-	-	1	1	1	1	-	1	1	-		
<i>Surirella bohemica</i> (G.W.Maly) Schönfeldt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Surirella brebissonii</i> Krammer & Lange-Bertalot	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		
<i>Surirella brebissonii</i> var. <i>kuetzingii</i> Krammer & Lange-Bertalot	1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1	-	-	
<i>Surirella librile</i> (Ehrenberg) Ehrenberg	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	
<i>Surirella spiralis</i> Kützing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	
<i>Surirella birostrata</i> Hustedt	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Surirella minuta</i> Brébisson ex Kützing	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	1	-	1	1	1	
<i>Tabellaria ventricosa</i> Kützing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tabellaria flocculosa</i> (Roth) Kützing	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ulnaria acus</i> (Kützing) Aboal	1	-	-	1	-	1	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ulnaria ulna</i> (Nitzsch) Compère	1	1	1	1	-	1	1	1	1	-	-	-	-	-	-	1	-	1	1	1	-	1	-	-	-	1	1	-	-	-	-	

Currently accepted names:

¹ *Stephanocyclus meneghinianus* (Kützing) Kulikovskiy, Genkal & Kociolek 2022

² *Surirella microlibrile* Van de Vijver, Pottiez & Jüttner 2024

³ *Cymbopleura naviculiformis* (Auerswald ex Heiberg) Krammer 2003

⁴ *Rhopalodia gibba* (Ehrenberg) O.Müller 1895

⁵ *Aerophilus roeseana* (Rabenhorst) Danz, Van de Vijver & Kociolek 2024

⁶ *Staurosirella pinnata* (Ehrenberg) D.M.Williams & Round 1988