Knowledge &
Management of
Aquatic
Ecosystems

Journal fully supported by Agence
française pour la biodiversité

RESEARCH PAPER

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Faunistic patterns and diversity components of leech assemblages in karst springs of Montenegro

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Abstract – The aim of this study was to reveal faunistic and diversity patterns and to assess the effects of environmental factors on the differentiation of leech communities. This study covers investigations of 82 karst springs in Montenegro from 2009–2017. The communities were analyzed in respect to five wellspring types – caves, sublacustrine, limnocrene, rheo-limnocrene and rheocrene. The percentage of substrate types and aquatic vegetation cover was recorded alongside water parameters. In total, 18 leech species were identified, of which two were recently described as new species for science (*Dina minuoculata* Grosser, Moritz and Pešić, 2007 and *Glossiphonia balcanica* Grosser and Pešić, 2016). *K*-means clustering was used to classify leech assemblages into three homogenous groups. The patterns of leech communities and the components of both *alpha* and *beta* diversity were examined in identified groups of assemblages. The significance of environmental factors and the impact of selected factors were assessed through forward selection analysis, CCA and RDA. Our results indicate that the type of spring and the environmental variables, as well as the combination of biotic and abiotic factors in a microhabitat dictate the distribution of leeches.

Keywords: hirudinea / crenobiology / alpha and beta diversity / cca/rda

Résumé – Structures faunistiques et composantes de la diversité des assemblages de sangsues dans les sources karstiques du Monténégro. L'objectif de cette étude était de révéler les schémas faunistiques et de diversité et d'évaluer les effets des facteurs environnementaux sur la différenciation des communautés de sangsues. Cette étude porte sur 82 sources karstiques au Monténégro de 2009–2017. Les communautés ont été analysées en fonction de cinq types de sources d'eau – grottes, sous-lacustres, limnocrènes, rhéo-limnocrènes et rhéocroènes. Le pourcentage de types de substrat et de couverture végétale aquatique a été enregistré en même temps que les paramètres de l'eau. Au total, 18 espèces de sangsues ont été identifiées, dont deux ont été récemment décrites comme de nouvelles espèces pour la science (*Dina minuoculata* Grosser, Moritz et Pešić, 2007 et *Glossiphonia balcanica* Grosser et Pešić, 2016). Le regroupement *K*-means a été utilisé pour classer les assemblages de sangsue en trois groupes homogènes. Les modèles des communautés de sangsues et les composantes de la diversité alpha et bêta ont été examinés dans des groupes d'assemblages identifiés. L'importance des facteurs environnementaux et l'impact de certains facteurs ont été évalués au moyen d'une analyse de sélection prospective, de CCA et de RDA. Nos résultats indiquent que le type de source et les variables environnementales, ainsi que la combinaison de facteurs biotiques et abiotiques dans un microhabitat dictent la distribution des sangsues.

Mots-clés : hirudinée / crénobiologie / diversité alpha et bêta / cca/rda

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1 Introduction

Due to complex trophic relationships, leeches are important components of wetland and aquatic ecosystems (Elliott and Mann, 1979; Borda and Siddall, 2004; Lunghi *et al.*, 2018). They are top predators and ectoparasites that control prey and host populations in spring ecosystems. At the same time, leeches are prey for many carnivorous species (Koperski, 2006; Kubová *et al.*, 2013; Cichocka *et al.*, 2015; Živić *et al.*, 2015).

Leeches are generally considered as useful indicators in the biological assessment and monitoring of water quality (Friese *et al.*, 2004; Koperski, 2006, 2017). However, their populations are endangered by industrialization and excessive exploitation of natural resources (Stendera *et al.*, 2012).

Physical and chemical variables of water, substrate type and food availability are the main factors determining the distribution patterns of free-living leeches (Koperski, 2006, 2010; Beracko and Košel, 2011; Kubová *et al.*, 2013; Cichocka *et al.*, 2015). Despite long term studying of leeches in Montenegro and adjacent areas (Blanchard, 1905; Augener, 1937; Rémy, 1937; Sket, 1968; Šapkarev, 1975; Utevsky *et al.*, 2013; Grosser and Pešić, 2005; Grosser *et al.*, 2007, 2014a; Živić *et al.*, 2017), the available information on species distribution and the ecology of leech assemblages is incomplete.

In general, species diversity involves *alpha* (within-community) diversity, *beta* (between-communities) diversity and *gamma* diversity or overall biodiversity within a region of concern (Whittaker, 1972). *Alpha* diversity depends on the number of species present in a community (species richness) and the evenness of the individuals that are distributed among these species (species equitability) or species evenness (Lloyd and Ghelardi, 1964; Pielou, 1969, 1974).

Beta diversity (between-community dissimilarity) can be partitioned into two additive components: the dissimilarity due to the difference in species richness (nestedness) and the dissimilarity associated with the replacement of some species by others between assemblages (Baselga, 2010; Podani *et al.*, 2013; Legendre, 2014).

We surveyed karst springs in Montenegro in order to (1) detect faunistic patterns of leech assemblages, (2) detect the patterns of diversity components in analyzed leech assemblages, and (3) to examine the effects of environmental factors (water parameters, substrate composition, aquatic vegetation and modification of springs) on the differentiation of leech communities.

2 Material and methods

2.1 Study area and sampling

Extensive limnological investigations were conducted from 2009 to 2017 at a total of 82 wellspring sites (Appendix 1) in Montenegro. Samples were collected two times per site, once in the summer and once in the winter. Leeches were collected from five types of karst springs – cave, sublacustrine, limnocrene, rheo-limnocrene and rheocrene. Rheocrene springs emerge on the ground surface as one or more flowing streams while limnocrene have discharge that emerge from aquifers into one or more lentic pools (Springer and Stevens

2009). In addition to these two commonly used ecomorphological spring types (Di Sabatino *et al.*, 2003), three additional ecomorphological types of springs were found in the studied area. Rheo-limnocrene with the characteristiscs of the above types, cave springs, and sublacustrine springs which are underwater depressions whose bottom extends deep below the lake floor (Barović *et al.*, 2018). Some springs were modified for human use (diverted into pipes) or turned into livestock troughs. The study area included springs from a wide territory, from the southern part of Montenegro (the drainage basin of Lake Skadar), to the northern part of the country (the Tara River) (Fig. 1). The Lake Skadar is the largest lake on the Balkan Peninsula and is located in the area of Dinaric karst, with a surface area that seasonally fluctuates between 370 and 600 km^2 (Pešić *et al.*, 2018) The lake is well-known for the occurrence of numerous karstic springs (Radulović *et al.*, 2015). Most of them are rheocrene and limnocrene springs. There are also numerous sublacustrine springs that occur along the coast of the Lake Skadar. Other karstic springs are located outside the drainage basin of the lake at higher altitudes.

Leeches were collected using a benthological hand net (mesh size 500 μm); additionally, individuals were collected by tweezers from hard substratum and vegetation. Each animal was relaxed in 10% ethanol and then transferred to 70% ethanol for further analysis. In the laboratory, the stereo-microscopes Nikon SMZ8000N (magnification 10–80x) and Zeiss Stemi 2000-C (magnification 6.5–50x) were used for sorting and identification.

Leeches were identified up to the species level or to the lowest possible taxonomic level in the case of damaged or small-sized juvenile individuals according to Nesemann and Neubert (1999), Grosser *et al.* (2007, 2016). The qualitative composition of the leeches was determined for each site (Appendix 2), along with species occurrence frequencies (F = 0–1) per spring type. The ASTERICS software package Version 3.1.1. (AQEM, 2002) was used for assessment of data and calculation of metrics.

2.2 Environmental variables

The GPS position and altitude of each site was recorded using a Garmin eTrex 20x Handheld GPS Receiver.

Water temperature and pH were measured in the field with a pH meter (HI 98127, accuracy 0.1).

In each spring we categorized the substrate types and aquatic vegetation coverage according to von Fumetti *et al.* (2006). The substrate types included rocks (ROC), stones (STO), gravel (GRA), sand (SAN), clay (CLA) and anoxic mud (ANM), going from largest to finest fractions. Aquatic vegetation included algae (ALG), mosses (MOS) and macrophytes (MCP). Both substrate types and aquatic vegetation coverage were categorized into four classes of frequency based on the percentage of cover: 0 (0%); 1 (1–25%); 2 (26–50%); 3 (51–75%) and 4 (76–100%). Human impact (N/M variable) was assessed by assigning 1 to natural and 2 to modified springs (Appendix 3).

2.3 Data processing

K-means clustering (MacQueen, 1967; Hastie *et al.*, 2009) was used to obtain maximally homogeneous groups of
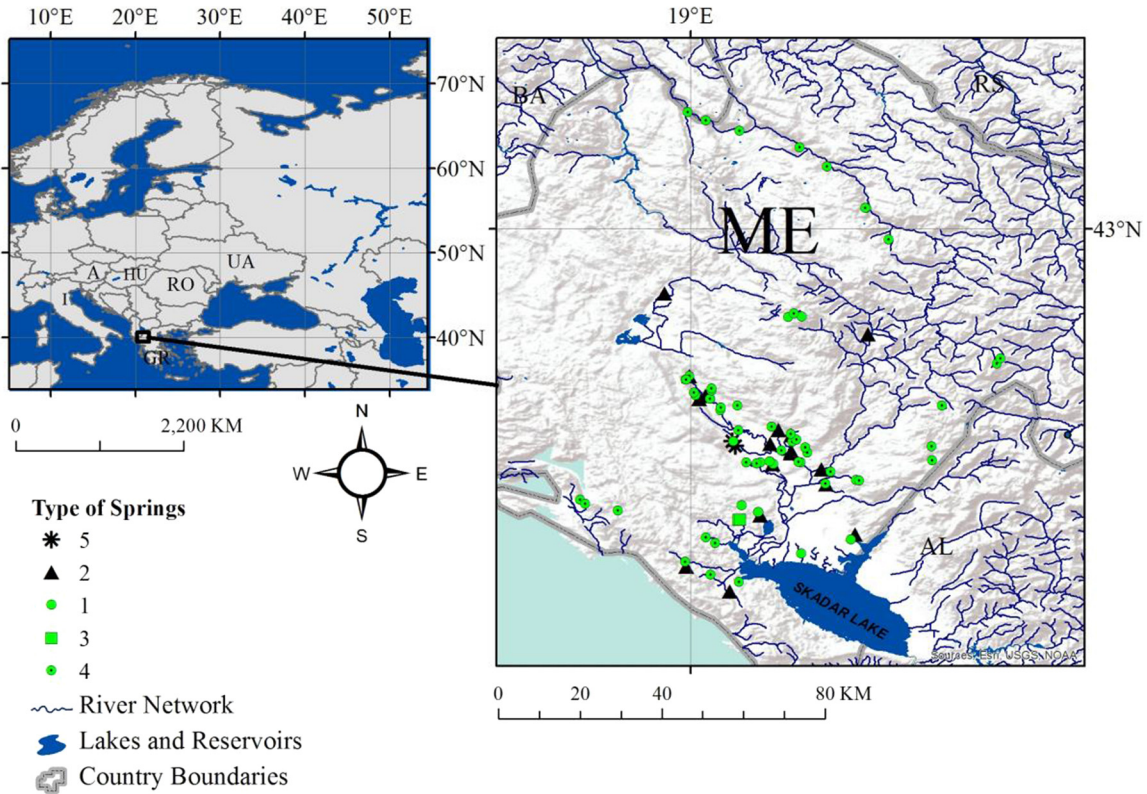
Fig. 1. Map of sampling sites for leeches in karst springs of Montenegro during 2009–2017 (1 – limnocrene; 2 – rheo-limnocrene; 3 – sublacustrine; 4 – rheocrene; 5 – cave springs).

communities. The method classifies a data set into a pre-specified number of clusters. The main drawback of the method is subjectivity in the initial guess of the number of clusters. To avoid this problem, we selected the number of clusters by maximizing the variance ratio:

$$VR = \frac{\sigma_b^2}{\sigma_w^2},$$

where σ_b^2 denotes the between-group variance (*i.e.* variance of cluster centroids), and σ_w^2 is the within-group variance (the sum of variances within each of k clusters). Maximization of the variance ratio assures that overlap of homogeneous clusters is minimized.

We used linear discriminant analysis – LDA (Fisher, 1936; Greenacre, 2010) to find a combination of species that maximally discriminates extracted clusters of communities.

Within each type of leech assemblage, we investigated components of *alpha* and *beta* diversity.

Alpha diversity was assessed using Shannon's entropy:

$$H = -\sum_{i=1}^{s} p_i \log p_i,$$

where p_i is the proportion of species i within a site, and s denotes the number of species within the site. Shannon's entropy varies from $H_{\min} = 0$ (in the case of one-species community) to $H_{\max} = \log(s)$.

The equitability component of *alpha* diversity may be calculated using the equation:

$$E = H/H_{\max} = -\sum_{i=1}^{s} p_i \log p_i / \log s,$$

where $H_{\max}$ is the greatest possible entropy. Equitability is an indeterminate ratio 0/0 if a sample has only one species. To avoid such indeterminate cases, we calculated equitability using the modified equation:

$$E = -\sum_{i=1}^{s} p_i \log p_i / \log(s + 0.01).$$

After this modification, the equitability varied from 0, to a number that approximated 1.

In this study, we detected components of *beta* diversity using the procedures described by Baselga (2010) and Podani *et al.* (2013).

Forward selection (FS) was used to detect predictors (environmental variables) with statistically significant effects on the variability of the analyzed leech communities. The variable selection methods can operate using either parametric or non-parametric statistical tests (Miller, 1984). Compared to parametric tests, the non-parametric alternatives have an essential advantage (Anderson, 2001, 2005). Therefore, we tested the hypothesis on species-environment independence

Table 1. Selection of an optimal number of clusters based on the ratio of between-group to within-group variances.

Nc	BgV	WgV	VR
2	0.2507	0.7834	0.3199
3	0.3491	1.0625	0.3286
4	0.3811	1.2854	0.2965
5	0.3947	1.5954	0.2474
6	0.4403	1.6718	0.2634
7	0.4824	1.7994	0.2681
8	0.5306	2.1432	0.2476
9	0.4983	2.1653	0.2301
10	0.5959	2.1378	0.2788

Abbreviations: N – number of clusters; BgV – between-group variance; WgV – withingroup variance; VR – variance ratio (between-group to within-group variance).

using a non-parametric Monte Carlo permutation test (3000 permutations, $p < 0.05$).

Using statistically significant environmental variables, we performed both canonical correspondence analysis – CCA (Ter Braak, 1986) and distance-based redundancy analysis – RDA (Legendre and Anderson, 1999) to detect the simultaneous effect of selected variables on faunistic differentiation of leech communities.

Statistical analyses were performed using FLORA software (Karadžić, 2013), updated version.

3 Results

Over the seven-year study, in 164 samples taken from 82 karst springs of Montenegro, the subclass Hirudinea was represented by 844 individuals with 18 species. Representatives of Rhynchobdellida and Arhynchobdellida were recorded. Species belonged to 4 families and 9 genera.

Ordo Rhynchobdellida was represented with nine species belonging to the family Glossiphoniidae, mostly in sublacustrine and limnocrene springs. The species *Placobdella costata* (Fr. Müller, 1846) was found in all types of springs and was the most frequent. *Hemiclepsis marginata* (O.F. Müller, 1774) predominantly inhabited limnocrene springs, while *Glossiphonia balcanica* Grosser and Pešić, 2016, *Glossiphonia paludosa* (Carena, 1824), *Alboglossiphonia heteroclita* (L., 1761) and *Alboglossiphonia striata* (Apáthy, 1888) were found only in sublacustrine springs. *Glossiphonia concolor* (Apáthy, 1888) was only recorded in one cave and in one limnocrene spring.

Ordo Arhynchobdellida included species classified in the subordo Hirudiniformes and subordo Erpobdelliformes.

The subordo Hirudiniformes was represented by two species, *Haemopis sanguisuga* (L., 1758) and *Hirudo verbana* Carena, 1820.

H. sanguisuga was the most frequent leech species in limnocrene springs (F = 0.69), followed by *P. costata* (F = 0.54). The species *H. verbana* preferred caves, limnocrene and rheo-limnocrene springs.

Seven taxa belonging to the subordo Erpobdelliformes were recorded during this study. During our research, the erpobdellid leech, *Dina dinarica* Sket, 1968, was observed to be the most common species, occurring in more than 60% of springs in the southern part of Montenegro. It inhabited all five spring types and was the most common species in rheo-limnocrene (F = 0.7) and in rheocrene (F = 0.67) springs. *Erpobdella octoculata* (L., 1758) was found in all sublacustrine springs and in one limnocrene and one rheo-limnocrene spring. Other species of Erpobdelliformes are rare species and were only found at a few sites.

The glossiphoniid leech *P. costata* was the most common species in limnocrene springs, while in sublacustrine springs several species of the Glossiphoniidae family (*G. paludosa*, *G. balcanica*, *A. heteroclita* and *A. striata*) were recorded.

Five species that were recorded are considered endemic for the Balkan Peninsula, including two species recently described as new for science, *G. balcanica* and *D. minuoculata*. Other recorded species have large distribution ranges.

The majority of recorded taxa are predators (83.33%), while 16.67% are parasites (*H. verbana. H. marginata* and *P. costata*) (based on the autecological characterization of taxa provided by Moog, 2002). The recorded leeches prefer a hard substratum such as gravel, stone, and aquatic macrophytes, whereas the majority of taxa (42%) use walking/sprawling as a means of locomotion, and less than 10% are swimmers or (semi)sessile (for 48% of taxa the data on locomotion is lacking (Moog, 2002).

3.1 Classification of leech assemblages

We classified leech communities using two approaches. The first approach was based on the faunistic similarity of the analyzed communities. Alternatively, the communities were classified *a priori*, according to the types of springs in which they occurred.

Ratio of between-group to within-group variances for different number of clusters obtained through *K*-means clustering is shown in Table 1. The maximal variance ratio indicated three clusters form the most homogeneous groups of communities.

LDA provided a combination of species that maximally discriminated between three clusters of communities (Fig. 2a). The species *H. sanguisuga* dominated in the first group of communities, and was frequently associated with *H. verbana* and *Erpobdella vilnensis* (Liskiewicz, 1925). This group included species that occur rarely, such as *Trocheta dalmatina* Sket, 1968; *Dina montana* Sket, 1968; *D. minuoculata* and *Erpobdella nigricollis* (Brandes, 1900).

The dominant species in the second group of communities was *P. costata*. It was associated with *Glossiphonia complanata* (L., 1758), *Glossiphonia nebulosa* Kalbe, 1964, *G. paludosa*, *G. balcanica*, *H. marginata*, *A. heteroclita, A. striata* and *E. octoculata*.

The species *D. dinarica* was almost exclusive to the third type of community. *H. marginata, G. complanata* and *E. octoculata* occurred sporadically. *P. costata*, *D. dinarica*, *H. sanguisuga* and *H. verbana* were found in all types of assemblages (Tab. 2).

Beside faunistic classification, we categorized communities according to the type of springs where they occurred. The extracted groups exhibited a low variance ratio (VR = 0.0192). LDA provided similar results, centroid of all groups overlap, with exception of two sublacustrine springs (Fig. 2b).
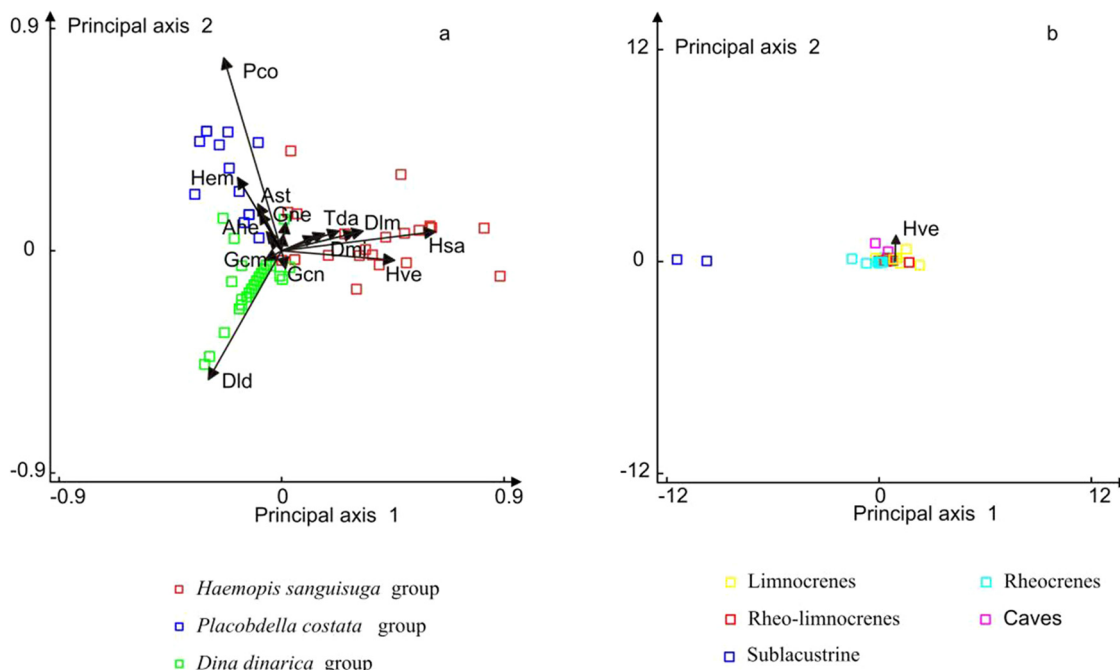
Fig. 2. a: LDA based on the groups of faunistically similar leech assemblages, dominated by the *Haemopis sanguisuga* (1), *Placobdella costata* (2) and *Dina dinarica* (3) groups. (for codes of species presented on the plots refer to Appendix 2); b: LDA based on groups of communities that occur in different types of springs (L – limnocrene; R-L – rheo-limnocrene; SL – sublacustrine; R – rheocrene; C – caves, for codes of species presented on the plots refer to Appendix 2).

Table 2. Frequency of species occurring in three groups of assemblages.

Species	*Haemopis sanguisuga* group	*Placobdella costata* group	*Dina dinarica* group
	F	F	F
Haemopis sanguisuga (Hsa)	0.78	0.21	0.11
Hirudo verbana (Hve)	0.35	0.07	0.02
Placobdella costata (Pco)	0.22	0.93	0.11
Dina dinarica (Dld)	0.09	0.29	1.00
Erpobdella vilnensis (Evi)	0.13	0.00	0.00
Erpobdella octoculata (Eoc)	0.00	0.21	0.02
Erpobdella nigricollis (Eni)	0.09	0.00	0.00
Dina minuoculata (Dmi)	0.04	0.07	0.00
Dina montana (Dlm)	0.09	0.00	0.00
Trocheta dalmatina (Tda)	0.09	0.00	0.00
Glossiphonia complanata (Gcm)	0.04	0.07	0.05
Alboglossiphonia heteroclita (Ahe)	0.00	0.14	0.00
Glossiphonia nebulosa (Gne)	0.00	0.14	0.00
Glossiphonia paludosa (Gpa)	0.00	0.14	0.00
Alboglossiphonia striata (Ast)	0.00	0.14	0.00
Glossiphonia concolor (Gcn)	0.09	0.00	0.00
Glossiphonia balcanica (Gba)	0.00	0.14	0.00
Hemiclepsis marginata (Hem)	0.04	0.14	0.02

Abbreviations: F – frequency of occurring in group of assemblages.

Between-group variance was low (0.0506) while within-group variance was high (2.634).

Correspondence between faunistic groups and spring types is presented in a histogram (Fig. 3). Leech assemblages with *P. costata* inhabit four types of springs, mostly limnocrene, rheo-limnocrene and sublacustrine. Communities dominated by *D. dinarica* usually occur in rheocrene and rheo-limnocrene springs. Leech communities with *H. sanguisuga* usually occur in limnocrene and rheocrene springs, but not in sublacustrine springs.

3.2 Distributional patterns of leech assemblages

Springs inhabited by different groups of leech assemblages significantly differ with respect to altitude (Fig. 4). The assemblages that belong to the *P. costata* group occur at the lowermost altitudes (mainly in springs around Lake Skadar). Most of the assemblages that belong to the *H. sanguisuga* group predominate in mountainous springs. However, these communities also occur in lowland and altimontane regions. Due to the wide altitudinal range of springs inhabited by the *H. sanguisuga* group of leech assemblages, their species composition was the most heterogeneous.

3.3 Diversity components

We analyzed the components of *alpha* (within-community) diversity within each of faunistically homogeneous groups of communities. Three types of leech assemblages differ significantly with respect to the components of *alpha* diversity
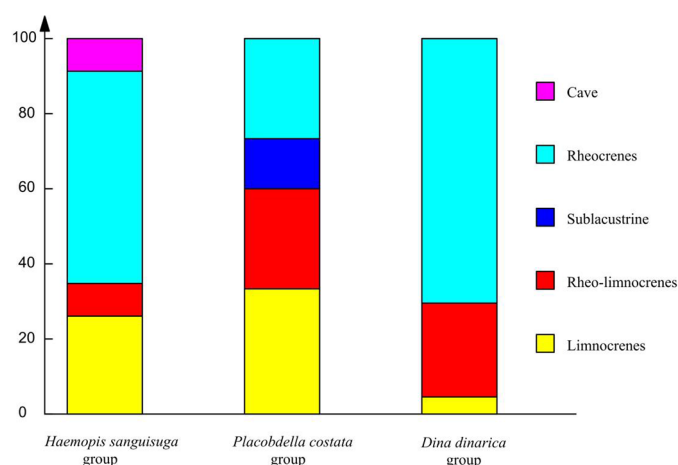
Fig. 3. Correspondence between faunistic groups of leech assemblages and types of springs.

(Fig. 5). Species-poor leech assemblages, dominated by *D. dinarica*, were usually represented by one, and in some cases only three species. Compared to leech assemblages dominated by *D. dinarica*, assemblages with *P. costata* and *H. sanguisuga* had significantly higher numbers of species (Shannon's entropy and equitability).

The greatest number of species was recorded in leech communities dominated by *P. costata*. These communities occurred in springs with the most diverse microhabitats (sublacustrine, limnocrene and rheo-limnocrene springs) that offered many ecological niches supporting high species richness. However, this group of communities also occurred in rheocrene springs with only one or a few species. Shannon's entropy and species richness had similar trends. Equitability of species abundances decreased from leech communities dominated by *H. sanguisuga* to communities with *D. dinarica*. The relatively low equitability in communities with *P. costata* can be explained by the effects of species-poor rheocrene springs.

We assessed total *beta* diversity, and its components using two alternative approaches (Baselga, 2010; Podani *et al.*, 2013); however, both approaches provided essentially the same result.

Beta diversity increased from leech assemblages with *D. dinarica* to assemblages dominated by *H. sanguisuga*.

To detect percentage contributions of nestedness and species turnover to the *beta* diversity, we used a ternary plot (Fig. 6).

The lowest values of both nestedness and species turnover were detected in leech assemblages with a domination of *D. dinarica*. Nestedness (differences in species richness) was more important than species replacement in assemblages dominated by *P. costata*. This indicated that the relative contribution of species loss to the *beta* diversity was more important than species replacement. Quite an opposite trend was observed for leech assemblages dominated by *H. sanguisuga*.

3.4 Effects of environmental variables on the variability of leech assemblages

After performing FS, we observed that all geographic variables (latitude, longitude and altitude) had a significant

effect on the differentiation of analyzed communities (Tab. 3). Water parameters, acidity (pH) and temperature recorded in winter (T°C_w) and in summer (T°C_s), had statistically insignificant effects on the leech assemblage due to low fluctuations during the sampling season. The lowest temperature recorded in winter was 6.9°C and the highest was 13.4°C, while the average value was 10.4°C. The acidity ranged from pH 6.92 to 7.59, with an average pH of 7.3. Only two out of six substrate characteristics (percentage of sand and rock particles) were significant. Considering the vegetation characteristics, the most influential predictor was the percentage of moss cover. Other variables were statistically insignificant. The habitat variables (type of springs and anthropogenic modification of habitats) had statistically significant effects on the leech assemblage patterns.

The synergic effect of all (statistically significant) variables on the faunistic variability of leech communities was analyzed using canonical correspondence analysis (CCA).

The first two CCA axes explained 52.4% of the variation of the fitted data, obtained by multiple regressions. Environmental predictors explained a relatively small portion of the total variability of leech distributions ($R^2 = 0.307$). CCA indicated that the spatial variables (altitude and longitude – eastward) had the most prominent effects on data variability (Fig. 7a). In two subalpine springs (altitude 1800 m), only one species (*D. montana*) was recorded. Most springs with *E. vilnensis* and *H. sanguisuga* were also located at high altitudes. CCA emphasized these species and communities where they occurred.

The importance of other environmental variables was much lower. As CCA results indicated, the type of spring had a small effect on the faunistic differentiation of the analyzed leech communities.

The application of distance-based RDA to our data set confirmed that environmental predictors explained a relatively small part of the total variability of leech distributions ($R^2 = 0.279$). The first two d-b RDA axes explained 86% of the variation of the fitted data.

As with CCA, the d-b RDA also emphasized the importance of *H. sanguisuga*, *E. vilnensis*, *D. montana* and subalpine springs where they occurred. The vector of the altitude variable is orientated along the second principal axis, contrary to CCA. The d-b RDA produced more interpretable results with respect to the effects of other factors (types of habitats, variables specifying bottom structure, vegetation, modification of springs) on faunistic variability. Communities dominated by *D. dinarica* were separated from other communities (Fig. 7b).

4 Discussion

The leech fauna of Montenegro includes 29 taxa, 26 species and *D. lineata* represented by three subspecies (Grosser *et al.*, 2014a). The taxa list also includes two species recently described as new for science (Grosser *et al.*, 2007, 2016). Our study of karstic springs revealed the presence of 18 species (62% of the total number of recorded taxa in Montenegro). Leech fauna of the Balkans, particularly the family Erpobdellidae, still remains relatively unknown. According to Sket (1968), in the Dinaric karst areas of the western Balkan Peninsula, as well as in the southeastern pre-Alpine region, four distinct erpobdellid leech taxa are known.
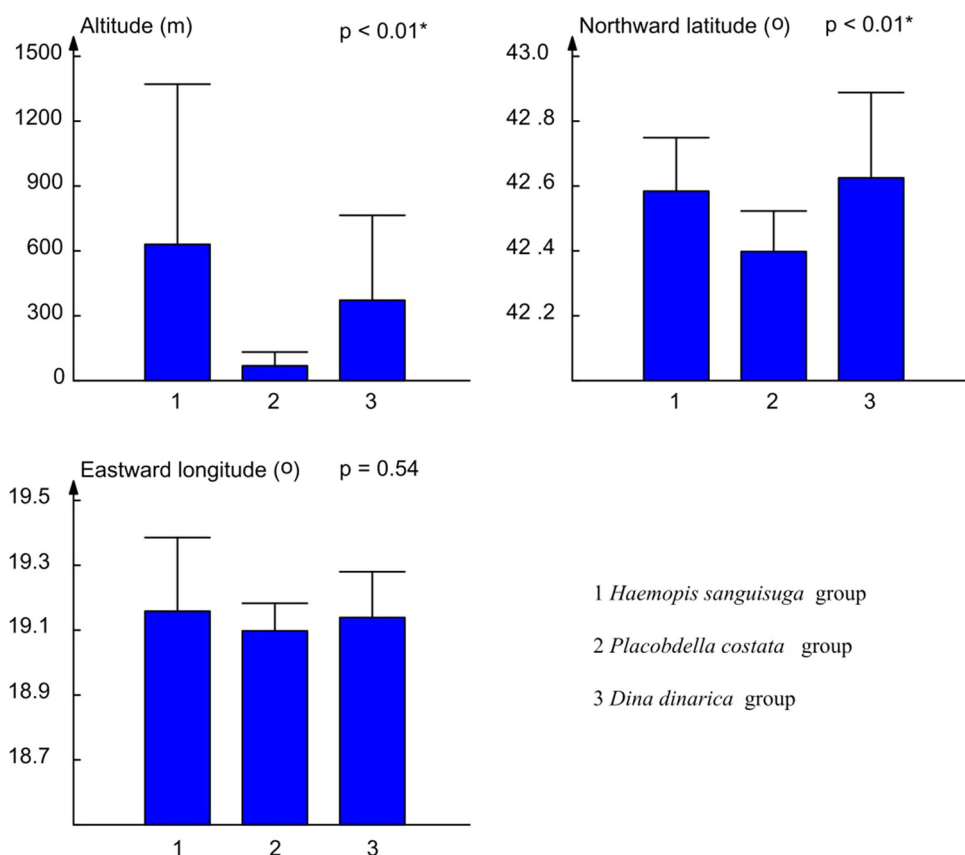
Fig. 4. Altitude and geographic positions of springs inhabited by three groups of leech assemblages. For each group of assemblages, the mean values and variances of altitude, Northward latitude and Eastward longitude of springs are represented by bars and lines, respectively.

The northwestern parts of this range are occupied by *Trocheta cylindrica* Örley, 1886, in the southern part they are replaced by *Dina lineata dinarica* Sket, 1968 (Dalmatia (Croatia), Hercegovina and Montenegro), the southernmost area of this range is occupied by *D. lineata montana* Sket, 1968 (the alpine region of Montenegro), and the fourth taxon, *Dina krasensis* (Sket, 1968), occurs in a relatively narrow area between the first two taxa, which inhabit southern Slovenia and northwestern Croatia (Grosser *et al.*, 2014b).

The taxonomical status of two former subspecies of *D. lineata* (*D. l. dinarica* and *D. l. montana*) recorded in this study and their relation with the subspecies *D. l. lineata* (O.F. Muller, 1774) is unclear. They should be treated as separate species or as subspecies of *Dina dinarica* Sket, 1968 (Grosser *et al.*, 2016).

T. dalmatina is an endemic erpobdellid species that inhabits small streams in the narrow zone of the Adriatic coast from Croatia, Dubrovnik to Montenegro, Lake Skadar (Nesemann and Neubert, 1999; Grosser *et al.*, 2014a).

The two approaches used to classify leech communities in karst springs gave different results. The first approach, based on the faunistic similarity of the analyzed communities, resulted in three distinct groups that had low within-group variability, while between-group variability was high. Classification of communities based on the type of spring gave opposite results.

The low variance ratio of extracted groups (VR = 0.0192) clearly indicated that the classification based on the type of

springs is suboptimal when compared to classification based on faunistic similarity. Linear discriminant analysis confirmed this conclusion, since the centroids of all groups, with the exception of sublacustrine springs, overlap. Due to the close proximity of centroids, between-group variance is low (0.0506). On the other hand, within-group variance is high (2.634).

The diversity of groups based on faunistic similarity differs greatly. In groups of assemblages dominated by *D. dinarica* all diversity components are very low. This type of leech assemblages occurred mainly in rheocrene springs with sandy and rock substrates. Distance based RDA highlighted that the type of spring and substrate are important factors in determining this group. Extremely low species turnover within these types of communities can be explained either by the unfavorable environmental conditions in rheocrene springs for other leech species, or by the competitive superiority of *D. dinarica*. Further investigations of competitive interactions of *D. dinarica* and other leech species are required.

The frequent occurrence of *D. dinarica* in rheocrene springs is expected because mountain and subalpine streams are typical habitats of this species (Sket, 1968). These findings are in agreement with the investigation of Kubová *et al.* (2013), where *D. lineata* was only found in lotic ecosystems.

During our research, the first of the two recently described species, *D. minuoculata* was recorded in epirhithral sectors in mountain streams of Montenegro, where it was also found for the first time (Grosser *et al.*, 2007).
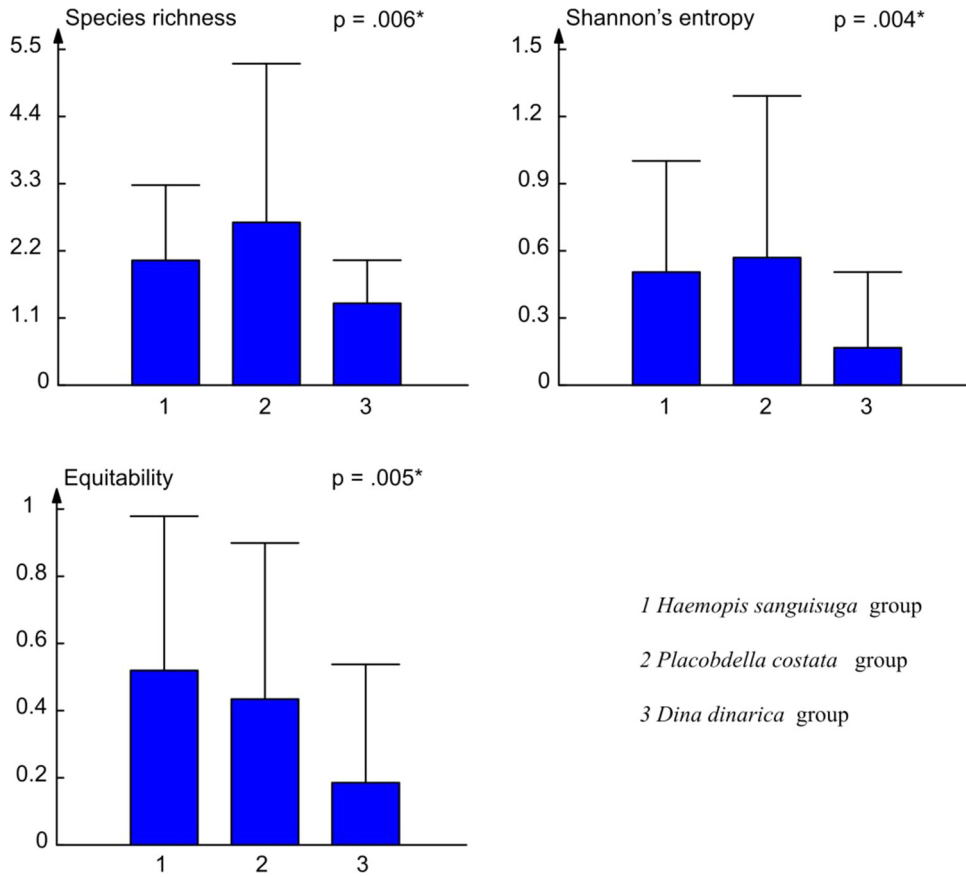
Fig. 5. Components of *alpha* diversity in leech assemblages dominated by *Haemopis sanguisuga* (1), *Placobdella costata* (2) and *Dina dinarica* (3).
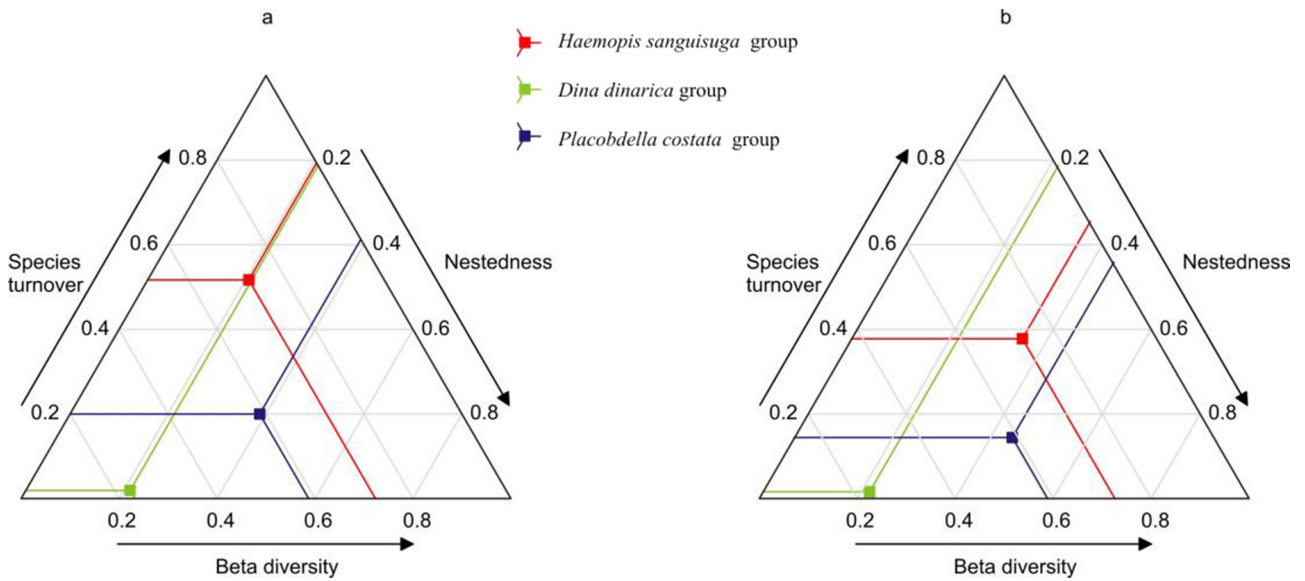
Fig. 6. Components of *beta* diversity in three groups of leech communities. Components obtained according to: a: Baselga, 2010; b: Podani *et al.*, 2013.

Other leech assemblage groups show greater species diversity. Both *H. sanguisuga* and *P. costata* groups have higher *alpha* and *beta* diversity components than the group represented by *D. dinarica*. The significant difference between leech assemblages dominated by *H. sanguisuga* and *P. costata* with respect to the species turnover component of *beta*

Table 3. Results of forward selection analysis.

Variables		F ratio	Probability
Geographic	E	8.072	0.001*
	Alt	3.584	0.001*
	N	2.483	0.016*
Water parameters	pH	1.894	0.335
	T°C_w	2.375	0.126
	T°C_s	1.734	0.254
Substrate	SAN	2.873	0.010*
	ROC	2.733	0.040*
	GRA	1.742	0.270
	CLA	2.234	0.172
	STO	1.302	0.166
	ANM	1.352	0.110
Vegetation	MOS	3.122	0.028*
	ALG	2.172	0.440
	MCP	1.857	0.265
Habitat	N/M	3.654	0.001*
	TOS	2.757	0.004*

Abbreviations: E – longitude (Eastward); Alt – altitude; N – latitude (Northward); pH – acidity; T°C_w – winter temperature; T°C_s – summer temperature; SAN – sand; ROC – rocks; GRA – gravel; CLA – clay; STO – stones; ANM – anoxic mud; MOS – mosses; ALG – algae; MCP – macrophytes; N/M – natural/modified springs; TOS – types of springs. Asterisk (*) denotes parameters with statistically significant effects on the variability of the analyzed leech communities.

diversity can be explained by the heterogeneity of the habitats where these assemblages occur (Pianka, 1974). While leech assemblages with *H. sanguisuga* were recorded in springs located on a wide range of altitudes, from lowland to subalpine regions, leech assemblages dominated by *P. costata* were mainly recorded in sublacustrine, limnocrene and rheo-limnocrene springs at lower altitudes of the Skadar Lake Basin.

The assemblages dominated by *H. sanguisuga* usually occur in limnocrene, rheo-limnocrene and rheocrene springs that form a diverse mosaic of microhabitats. Due to heterogenous microhabitats, the greatest *alpha* diversity was observed in leech assemblages dominated by *P. costata*. However, these communities occur in rheocrene springs also, where the number of species was low. Microhabitat complexity decreases from sublacustrine to rheocrene springs as follows: sublacustrine → limnocrene → rheo-limnocrene → rheocrene → cave springs. Significant reduction of microhabitat complexity is a selective pressure that reduces the number of species. Therefore, the high *beta* diversity in communities dominated by *P. costata* is attributable to the difference in species richness (nestedness) and not to species replacement. Quite the opposite trend was observed in communities dominated by *H. sanguisuga*. These communities occur in a wide altitudinal range. The high species turnover in this group of leech assemblages can be explained by the changes in environmental conditions along the altitudinal gradients. A

long environmental gradient can result in the diversification of ecological niches (MacArthur, 1984) and increased species replacement along the gradient.

The *P. costata* group is mostly comprised of various Glossiphoniidae species. These species prefer stagnant water bodies (Nesemann and Neubert, 1999). They were usually found in limnocrene and sublacustrine springs in the Skadar Lake Basin in which lentic conditions prevail. *P. costata* is reported to build assemblages with numerous species of leeches usually in stagnant water bodies. Its abundance is associated with the presence of its food source; it feeds on the blood of pond turtles and amphibians (Spyra and Krodkiewska, 2013) so that the high frequency of this species could be the result of food availability in the investigated springs. High species nestedness in this group could be explained by a loss of favorable microhabitats in different types of springs. Sublacustrine and limnocrene springs could have more microhabitats with favorable conditions that Glossiphoniidae leeches prefer compared to rheo-limnocrene and rheocrene springs.

During our research, the second newly described species for science, *G. balcanica*, was found in two sublacustrine springs (Karuč and Volač) that occur along the coast of Lake Skadar. These two springs and the Mareza spring near Podgorica were the localities from which this species was described for the first time (Grosser *et al.*, 2016).

CCA and d-b RDA confirmed that environmental predictors can explain the relatively small part of total variability of leech distributions. CCA indicates that spatial variables (altitude and eastward latitude) have the most influence on data variability. This finding can be explained by the specific topography of Montenegro (a strong altitudinal gradient in a southwest to northeast direction). In two subalpine springs (altitude above 1800 m), only one species (*D. montana*) was recorded. Most springs with *E. vilnensis* and *H. sanguisuga* were also located at high altitudes. CCA emphasized these species and the communities where they occur. *E. vilnensis* is usually described as a low- to intermediate- altitude species (Nesemann and Neubert, 1999), while Agapow and Piekarska (2000) describe it as species characteristic for mountain streams in Poland. Our record at an altitude of 1786 m is considerably higher than the findings of Utevsky *et al.* (2012) from Ukraine (960 m) and Kazanci *et al.* (2015) from Turkey (900 m). The lower temperature of water could be the factor that separates this species from a similar species, *E. octoculata* (Kubová and Schenková, 2014).

Forward selection and distance-based redundancy analysis show that the presence/absence of modification influences leech communities. Concrete or wooden livestock troughs with hard flat bottoms are a good habitat for leeches. The accumulation of organic sediment and slowing of water could favor some prey items (oligochaeta and chironomidae larvae), thus providing good conditions for leeches (Adamiak-Brud *et al.*, 2018). Modified springs were usually located in or close to urban areas and are vulnerable to inputs of pollutants, which reduce both water quality and habitat quality (Koperski, 2010).

The importance of other environmental variables is much lower. As CCA results indicate, the type of spring has a small effect on the faunistic differentiation of the analyzed leech
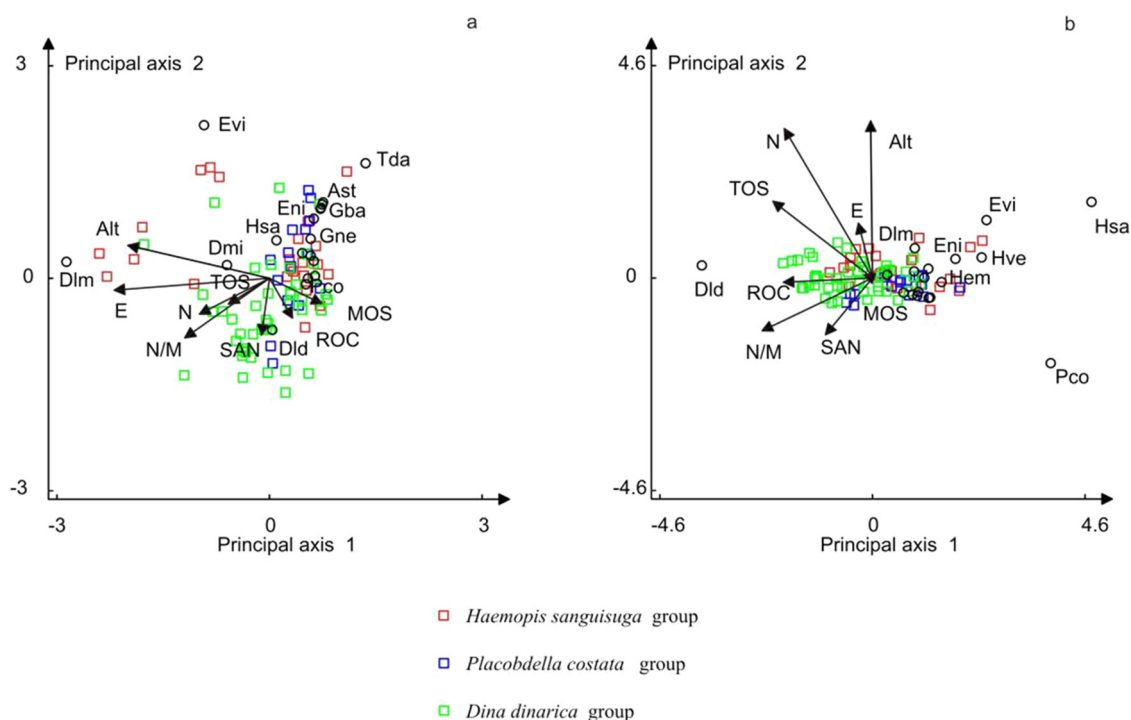
Fig. 7. a: results of canonical correspondence analysis of impact of selected factors on leech assemblages; b: results of distance-based RDA analysis of impact of selected factors on leech assemblages (Alt – altitude; N – latitude (Northward); E – longitude (Eastward); N/M – human impact: natural or artificially modified springs; TOS – type of spring; ROC – rock; SAN – sand; MOS – moss).

communities. This finding is in accordance with conclusions of Kubová *et al.* (2013) that environmental variables are a less significant predictor of leech assemblage composition than biotic variables (*i.e.* prey availability).

To conclude, the analyzed environmental factors have a slight influence on the distribution and diversity of leeches in spring ecosystems. It is likely that the combination of a specific set of biotic and abiotic factors in microhabitats determines the distribution of this interesting group of aquatic macro-invertebrates.

Supplementary Material

Appendix 1. Name of the karst springs with GPS-position and description of the types of springs.

Appendix 2. a: Leeches taxonomy list; b: Qualitative composition of leeches.

Appendix 3. Classes of frequency based on percentage of cover for habitat in five types of karst springs of Montenegro.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2019019/olm.

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