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Recent ostracods (Crustacea: Ostracoda) of Alpine springs and adjacent springbrooks of the Southern Limestone Alps, Slovenia

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Abstract. The ecology and distribution of ostracods in Alpine springs and springbrooks from Slovenia is presented. Benthos sampling was performed and major environmental characteristics (discharge, substrate composition, temperature, dissolved oxygen, conductivity, pH, alkalinity, sulphate, nitrate, calcium, magnesium) were measured in 12 springs and adjacent springbrooks. Sampling campaign was carried out on six sampling occasions (spring, summer, autumn in 2009 and 2010). Fourteen ostracod species were found among other fauna. The commonest and most abundant species were *Psychrodromus fontinalis* (Wolf, 1920) and *Cavernocypris subterranea* (Wolf, 1920), while the other species occurred at one or two sites at the most. Substrate composition and water temperature were statistically significant variables in explaining ostracod assemblages composition in this study.

Key words: microcrustacea, species-environment relationship, biodiversity, distribution

Izvleček. Recentni dvoklopniki (Crustacea: Ostracoda) v alpskih izvirih in izvirskih potokih Južnih Apneniških Alp v Sloveniji – Prispevek predstavi ekologijo in razširjenost dvoklopnikov (Ostracoda) v alpskih izvirih in izvirskih potokih Slovenije. Vzorčenje bentosa in meritve okoljskih dejavnikov smo opravili spomladi, poleti in jeseni v letih 2009 in 2010 v 12 izvirih in izvirskih potokih. Najdenih je bilo 14 vrst dvoklopnikov. Najbolj pogosti in številčni sta bili vrsti *Psychrodromus fontinalis* (Wolf, 1920) in *Cavernocypris subterranea* (Wolf, 1920). Druge vrste so se pojavljale posamično, na eni ali dveh lokacijah. Sestava substrata in temperatura vode sta bila edina statistično značilna okoljska dejavnika, ki sta določala sestavo združbe dvoklopnikov.

Ključne besede: nižji raki, odnos med vrstami in okoljem, biodiverziteta, distribucija

Introduction

Research in ecology and distribution of recent ostracods in spring environments has progressed rapidly in Europe over the past 20 years (Roca & Baltanas 1993, Särkkä et al. 1997, Stoch 1998, Gerecke et al. 1998, Stoch 2003, Gerecke et al. 2005, Rossetti et al. 2005, Pieri et al. 2007, Bottazzi et al. 2008, Stoch et. al. 2011, Zhai et al. 2014). However, in the Alps, only the springs in Trentino Province, Italy (Stoch 1998, Stoch et al. 2011), in the Regional Nature Park of the Julian Pre-Alps (Friuli-Venezia Giulia Province), Italy (Stoch 2003), and in the Berchtesgaden National Park, Bavaria, Germany (Gerecke et al. 1998), have been systematically investigated for ostracods (among other taxa). In Slovenia, such studies are lacking.

Within this study, 12 springs and springbrooks in the Alpine Region of Slovenia, which is part of the Southern Limestone Alps, were investigated over three seasons and two years in order to provide a better understanding of the spatio-temporal variability in environmental factors and macro- and meio-benthos. In this paper, the data on ostracod species distribution and their relationship to the major environmental factors are presented.

Materials and methods

The investigated Alpine springs are located within the catchment of the Sava River and are distributed across three mountain ranges (Julian Alps, Karavanke, and Kamniško-Savinjske Alps) (Fig. 1). The majority of springs are situated along deep, narrow Alpine valleys at altitudes from 600 to 943 m a.s.l., while three of them are located at higher altitudes (1,108–1,232 m a.s.l.) (Tab. 1). All selected springs are located within forested areas and are rheocrene where the groundwater discharges through the spring directly into the confined stream channel (width 0.2–1.2 m).

The climate of the study area is alpine, with a mean annual temperature of 4 to 9 °C and annual precipitation from 1,721 to 2,335 mm (Ogrin 1998). The catchment is composed of carbonate rock, predominantly Triassic age limestone with some dolomite and dolomitized limestone. The dominant hydrogeological units that are recharging the springs are highly permeable karst aquifers with fissured porosity. More detailed hydrogeological descriptions of springs are presented in Kanduč et al. (2012).

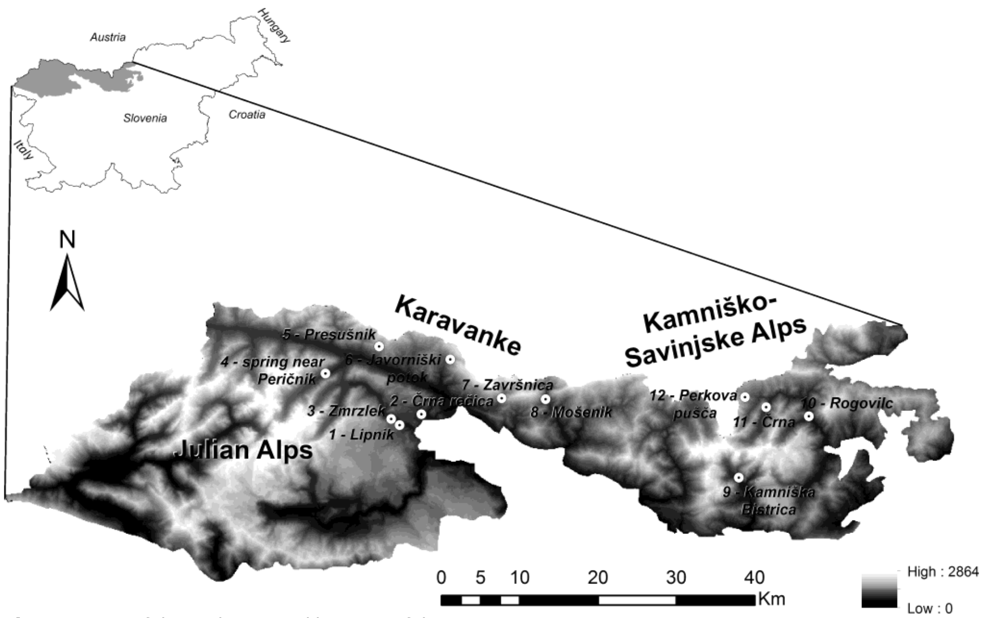


Figure 1. Map of the study area and locations of the springs.
Slika 1. Karta območja in lokacije izvirov.

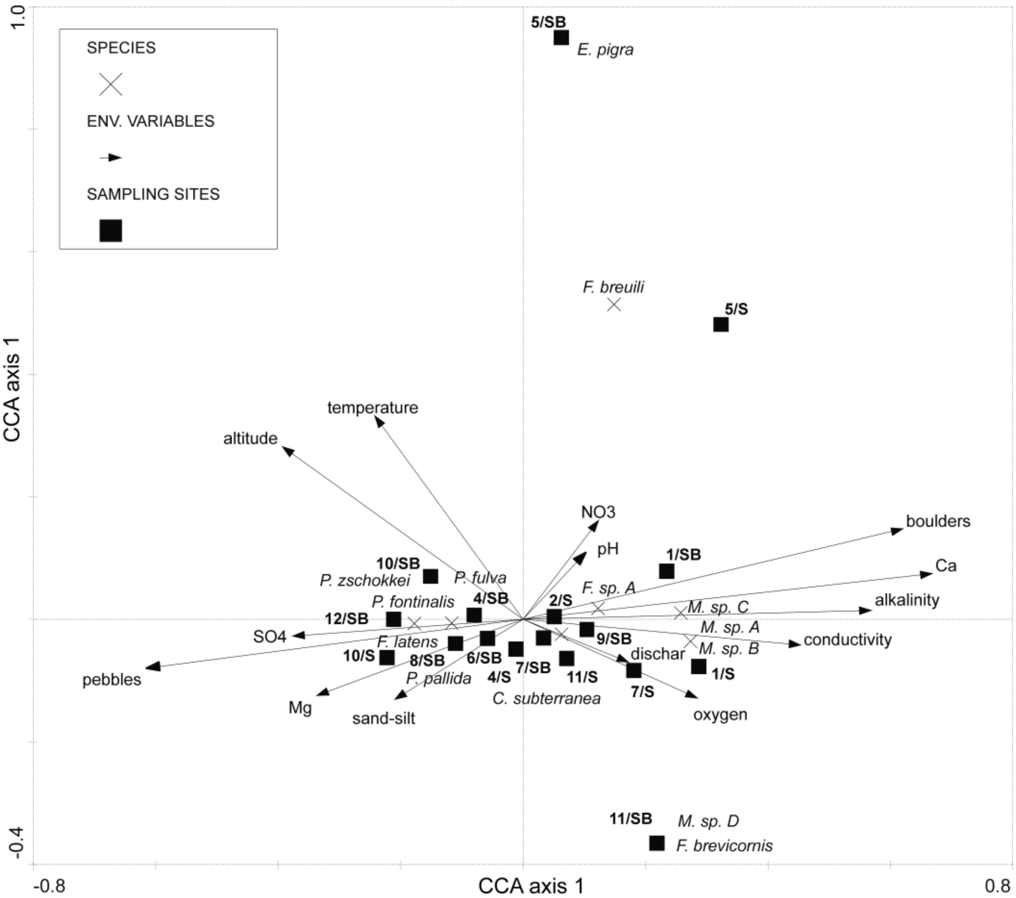
Table 1. The geographical coordinates and altitudes of the studied springs.
Tabela 1. Koordinate in nadmorska višina raziskovanih izvirov.

Spring code*	Spring name	Gauss-Krueger X	Gauss-Krueger Y	Altitude (m)
	Julian Alps			
1	Lipnik, Radovna, Bled	5138308	5425554	650
2	Črna rečica, Spodnje Gorje	5139683	5428322	645
3	Zmrzlek, Radovna, Bled	5139090	5424476	710
4	sping at Peričnik waterfall, Mojstrana	5144888	5416086	720
	Karavanke			
5	Presušnik, Mojstrana	5148330	5422931	1220
6	Javorniški potok, Jesenice	5146689	5432032	1108
7	Završnica, Jesenice	5141732	5438568	943
8	Mošenik, Tržič	5141621	5444173	807
	Kamniško-Savinjske Alps			
9	Kamniška Bistrica, Kamnik	5131603	5468851	600
10	Rogovilc, Mozirje	5139436	5477797	669
11	Črna, Luče	5140596	5472340	740
12	Perkova pušča, Pavličevo sedlo	5141860	5469638	1232

*Spring numbers correspond with the codes used in the CCA ordination diagram (Fig. 2).

Figure 2. CCA ordination diagram with sampling sites, species and environmental variables. Codes for the sampling sites are composed of the number that correspond with the spring number in Tab. 1 and Fig. 1 and indicate the geographical location and abbreviation S/SB that gives information whether the sample was taken from the spring (S) or springbrook (SB).

Slika 2. CCA ordinacijski diagram z vzorčnimi mesti, vrstami in okoljskimi spremenljivkami. Oznake za vzorčna mesta so sestavljene iz številke lokacije, ki je navedena v Tab. 1 in na Sl. 1 kot številka izvira in označuje geografsko lokacijo. Dodatna oznaka S pomeni izvir, SB pa izvirski potok.



At each spring, benthos from the spring (S) and from the adjacent springbrook (SB) approximately 5–10 m downstream from the spring was collected by a semi-quantitative kick sampling method. The substrate was kicked by foot for 3 min and a hand net (mesh size 100 µm) was used to collect benthos. Additionally, larger stones were picked and washed out into the hand net. Biological samples were preserved in 70% ethanol and taken to the laboratory for further processing. Substrate composition, percentage of boulders and cobbles (>6.4 cm), pebbles and gravel (6.4–0.2 cm), sand and silt (<0.2 cm) were determined by visual estimation (Armitage et al. 1995). Oxygen (WTW Multiline P4, CellOx 325), temperature and conductivity (WTW Multiline P4, TetraCon 325), and water levels and flow velocities (OTT

ADC flow meter) were measured on sites. For the laboratory analyses, 250 ml of water was collected in polyethylene bottles. The sampling campaign was carried out over three seasons (spring, summer, autumn) and two years (2009, 2010) (N=6).

In the laboratory, pH was measured using a WTW pH 540 GLP, with a TetraCon 325 probe. Alkalinity was determined using titration after Gran, and was expressed as $CaCO_3$ equivalent per litre. Nitrate, sulphate, calcium and magnesium were analysed by ion chromatography (Metrohm, 761 Compact IC). Biological samples were sorted, the specimens counted and ostracods identified to the species level using Meisch (2000) identification key.

The Kruskal-Wallis non-parametric test was performed on environmental variables to determine if there are statistically significant differences between the sampling sites. Canonical correspondence analysis (CCA) was used to investigate the relationship among species data and environmental variables. The species abundance data were log(x+1) transformed to down-weight highly abundant species. Significance of environmental variables in CCA was tested by Monte Carlo permutation test (999 permutations) using forward selection procedure. CCA was run by CANOCO 4.5 program (ter Braak & Smilauer 2002).

Results

Environmental characteristics of Alpine springs and springbrooks

The discharge varied greatly between the springs (Tab. 2). The highest was in the Kamniška Bistrica spring (over 212 l s^{-1}), whereas in other springs discharges were substantially lower (<28 l s^{-1}). The lowest variation in discharge was observed in Perkova Pušča spring and in the spring at Peričnik waterfall. The predominant substrates were boulders, cobbles, pebbles and gravel, although in some springs sand and silt prevailed (e.g. Črna and Perkova Pušča). Mean water temperature per spring ranged between 4.9 to 7.8 °C. Seasonal and yearly variation in water temperature was low for all springs (SD<0.4 °C) except Presušnik (SD=1.4 °C). Mean pH per spring ranged from 6.8 to 8.1, mean alkalinity from 927 to 3,062 µeq l^{-1}, mean conductivity from 90 to 275 µS cm^{-1}, and oxygen concentrations from 8.3 to 12.5 mg l^{-1}. Mean concentrations of ions in the water varied from 1.4 to 2.7 mg l^{-1} for nitrate, from 1.1 to 12.5 mg l^{-1} for sulphate, from 13.4 to 64.2 mg l^{-1} for calcium and from 3.5 to 10.6 mg l^{-1} for magnesium (Tab. 2). The Kruskal-Wallis non-parametric test was significant ($p<0.001$) for all measured physico-chemical variables, indicating that at least one sampling site is significantly different from other sampling sites when considering individual variables.

Ostracod assemblages

The number of ostracod specimens varied greatly between the spring and springbrook (Tab. 3). Similarly, variation in abundance was high across the studied springs. The highest total abundances were found at the Peričnik waterfall (158 specimens in the spring and 119 specimens in the springbrook) and in the springbrook of Perkova pušča (128 specimens). No ostracod specimens were collected in the Zmrzlek spring and adjacent springbrook, in the springs of Javorniški potok, Mošenik, Kamniška Bistrica and Perkova pušča and in the Črna rečica springbrook. The highest species richness was observed in the Lipnik springbrook (5 species). Only one species was collected in the Črna rečica, Presušnik and Završnica springs.

Altogether, 14 ostracod species and 588 specimens were collected during 6 sampling campaigns. The commonest and most abundant species were *Psychrodromus fontinalis* (Wolf, 1920) and *Cavernocypris subterranea* (Wolf, 1920) with 321 and 215 specimens collected altogether. *P. fontinalis* was collected from 2 springs and 5 springbrooks and *C. subterranea* from 6 springs and 7 springbrooks. *Fabaeformiscandona brevicornis* (Klie, 1925), *Eucypris pigra* (Fischer, 1851) and two *Mixtacandona* species (sp. C, sp. D) were collected only from one site.

The canonical correspondence analysis (CCA) performed on species abundance and measured environmental characteristics (altitude, discharge, substrate, temperature, dissolved oxygen, conductivity, pH, alkalinity, sulphate, nitrate, calcium, magnesium), based on the first two axes explained 41.6% of total variation in the species data. The first axis explained 25.8% of species-environment relationship and the second axis an additional 22.9%. The first axis was primarily a gradient in substrate granulometric composition, and conductivity and alkalinity linked to calcium content, and the second axis a gradient in altitude and water temperature. A high correlation between water temperature and altitude is due to rather high temperatures in the Presušnik that is located at the highest altitude. Based on Monte Carlo permutation test, percentage of the boulders and cobbles in the substrate and water temperature showed to be statistically significant ($p<0.05$) for shaping ostracod assemblages. Other important, but not significant variables were percentage of pebbles and gravel, pH, conductivity and calcium content. CCA ordination diagram indicates that species of the genus *Mixtacandona* prefer oxygenated waters with higher conductivity, Ca contents and alkalinity. The sample sites were distributed mainly along the first axis, where the most distant were the groups of Lipnik spring, Lipnik springbrook and Završnica spring, and Perkova Pušča springbrook and Rogovilc spring and springbrook. Along the second axis, Presušnik spring and springbrook and Črna springbrook differed mostly from the other sites.

Table 2. Main environmental characteristics of the springs studied. Mean values and standard deviation for two years (2009, 2010) and three sampling campaigns (spring, summer, autumn) are presented. Since the differences between the springs and springbrooks were low, only the values for the springs are presented. Temp – water temperature; Cond – conductivity.

Tabela 2. Glavne značilnosti raziskovanih izvirov. Prikazane so srednje vrednosti in standardne deviacije za obe leti (2009, 2010) in tri sezonska vzorčenja (pomlad, poletje, jesen). Zaradi majhnih razlik med izviri in izvirskimi potoki so prikazane samo vrednosti za izvire. Temp – temperature vode; Cond – prevodnost.

	Discharge	**Boulders /cobbles**	**Pebbles /gravel**	**Sand /silt**	**Temp**	**pH**	**Cond**	**Oxygen**	**NO_3^-**	**SO_4^{2-}**	**Ca^{2+}**	**Mg^{2+}**	**Alkalinity**
	$l\ s^{-1}$	**%**	**%**	**%**	**°C**		**$\mu S\ cm^{-1}$**	**$mg\ l^{-1}$**	**$mg\ l^{-1}$**	**$mg\ l^{-1}$**	**$mg\ l^{-1}$**	**$mg\ l^{-1}$**	**$Eq\ l^{-1}$**
Julian Alps													
Lipnik	14.5±7.3	80	10	10	6.7±0.1	7.7±0.2	247±64	10.7± 0.7	2.1±0.3	2.5±0.6	64.2±6.4	3.5±0.6	2754 ±226
Črna rečica	18.2±7.9	60	30	10	7.5±0.1	7.7±0.2	261±74	12.2±2.2	2.7±0.3	3.1±0.6	60.4±3.9	9.8±3.0	3062±270
Zmrzlek	10.1±4.8	80	20	0	6.0±0.1	7.9±0.2	204±56	11.3±0.5	1.7±0.1	1.7±0.3	53.2±5.4	4.6±0.7	2446±227
Peričnik	3.4±0.4	30	60	10	6.0±0.2	8.1±0.1	166±39	11.0±0.6	1.7±0.1	1.8±0.2	35.6±1.5	6.8±0.3	1937±81
Karavanke													
Presušnik	1.6±0.6	40	40	20	7.5±1.9	7.9±0.2	203±44	9.6±0.8	2.4±0.2	3.4±0.7	51.9±3.1	6.4±0.6	2529±115
Javorniški potok	28.0±5.1	80	20	0	4.9±0.1	8.0±0.1	170±39	12.5±0.9	2.5±0.1	2.6±0.1	37.9±2.3	6.2±0.3	1972±95
Završnica	21.8±5.7	50	50	0	5.8±0.3	7.9±0.1	179±38	11.3±0.7	2.5±0.1	2.7±0.3	42.9±2.2	4.5±0.3	2049±70
Mošenik	24.9±4.2	0	90	10	7.2±0.4	7.9±0.1	207±43	11.4±3.5	2.4±0.1	12.5±1.8	41.8±2.6	8.7±0.4	2079±43
Kamniško-Savinjske Alps													
Kamn. Bistrica	212.7±87.3	80	20	0	5.4±0.2	8.0±0.1	148±28	12.0± 0.6	1.5±0.2	1.1±0.4	32.5±1.6	3.5±0.5	1574±102
Rogovilc	1.5±0.7	40	50	10	7.8±0.2	8.0±0.1	275±43	10.9±0.5	2.6±0.2	10.6±3.8	55.1±3.8	9.4±2.5	2822±183
Črna	8.0±1.9	0	40	60	7.3±0.4	7.8±0.1	259±37	10.7±1.2	2.0±0.1	3.5±0.1	49.2±0.9	10.6±0.3	2811±88
Perkova pušča	1.1±0.2	0	40	60	7.4±0.1	6.8±0.4	90±13	8.3±0.3	1.4±0.2	2.6±0.4	13.4±1.1	3.9±0.3	927±64

Table 3. List of ostracod species and their total abundances in the springs (S) and springbrooks (SB).
Tabela 3. Seznam vrst in skupno število osebkov v izvirih (S) in izvirskih potokih (SB).

	Lipnik		**Črna rečica**		**Zmrz-lek**		**Perični k**		**Presuš-nik**		**Jav. potok**		**Završ-nica**		**Moše-nik**		**Kamn. Bistrica**		**Perkov a pušča**		**Rogo-vilc**		**Črna**		**Total**
	S	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	**S**	**SB**	
Psychrodromus fontinalis (Wolf, 1920)							11	104				12		2		19				121	4	48			**321**
Cavernocypris subterranea (Wolf, 1920)	4	18	1				147	7	1			6	1	11		9		4					4	2	**215**
Mixtacandona sp. B	4	7																							**11**
Potamocypris zschokkei (Kaufmann, 1900)																2				4	2	2			**10**
Potamocypris fulva (Brady, 1868)								8																	**8**
Mixtacandona sp. A	6	1																							**7**
Fabaeformiscandona sp. A		2												3											**5**
Fabaeformiscandona latens (Klie, 1940)																				3					**3**
Fabaeformiscandona breuili (Paris, 1920)		1								1															**2**
Potamocypris pallida Alm, 1914																2									**2**
Mixtacandona sp. C	1																								**1**
Fabeaformiscandona brevicornis (Klie, 1925)																								1	**1**
Mixtacandona sp. D																								1	**1**
Eucypris pigra (Fischer, 1851)										1															**1**
Total abundances per spring/spring brook	**15**	**29**	**1**	**0**	**0**	**0**	**158**	**119**	**1**	**2**	**0**	**18**	**1**	**16**	**0**	**32**	**0**	**4**	**0**	**128**	**6**	**50**	**4**	**4**	**588**

Discussion

The ostracods were present at all studied locations with the exception of Zmrzlek, which is an intermittent karst spring and was dry during both summer samplings. The overall species richness of ostracods was relatively high, with a total of 14 species. The other studies on spring ostracods carried out throughout Europe resulted in species numbers from 3 to 34 (Tab. 4). Due to different numbers of springs that were sampled during those investigations, species richness cannot be directly compared. But the calculated mean number of species per spring ranged from 0.1 to 0.7 for listed studies and 1.2 for this study, indicating high ostracod biodiversity in the Slovenian part of the Southern Limestone Alps. For example, only 18 species (0.2 species per spring) were recorded for 110 Alpine springs of Trentino Province investigated during the CRENODAT project (Stoch et al. 2011). Similarly, only three species (0.2 species per spring) were collected in 20 springs of the Julian Pre-Alps, Italy, a low number probably related to the temporary regime of these environments (Stoch 2003). Bottazzi et al. (2008) summarized previous unpublished investigations carried out in the Alpine and Pre-Alpine regions in Italy and reported a total of 21 ostracod species. Unfortunately, in this summarization there are no data on the number of sampled springs. Furthermore, in the Northern Limestone Alps in Germany, 11 species (0.6 species per spring) were collected from 19 springs located in the Alps of Berchtesgaden (Upper Bavaria) (Gerecke at al. 1998). One of the reasons for much higher average number of species per spring in this study could be due to higher sampling intensity, where each sampling site was sampled on six occasions. It was previously shown that increased temporal sampling intensity increases the observed species richness (Mori & Brancelj 2013).

Table 4. Number of ostracod species collected during different studies of spring meiofauna across Europe.
Tabela 4. Pregled raziskav izvirov v Evropi z navedenim številom najdenih vrst dvoklopnikov ter številom vzorčevanih izvirov.

No of species	No of springs	Average No of species per spring	Type of spring	Region	Reference
3	20	0.2	Rheocrene	Regional Nature Park of the Julian Pre-Alps, Italy	Stoch 2003
9	23	0.4	Rheo/Limno/Helocrene	Adamello Brenta National Park, Italy	Stoch 1998
11	19	0.6	Rheo/Helocrene	Alps of Berchtesgaden, Germany	Gerecke at al. 1998
12	31	0.4	Limnocrene	The river Po, S sub-catchment, Italy	Rossetti et al. 2005
13	19	0.7	Rheo/Rheo-limnocrene	Northern Apennines, Italy	Bottazzi et al. 2008
16	28	0.6	Limnocrene	The river Po, N sub-catchment, Italy	Pieri et al. 2007
17	31	0.5	Not known	Finland	Särkkä et al. 1997
18	110	0.2	Rheocrene	Trentino Province, Italy	Stoch et al. 2011
21	149	0.1	Helocrene	Central Pyrenees, Spain	Roca & Baltanas 1993
28	41	0.7	Rheo/Limno/Helocrene	Luxemburg	Gerecke et al. 2005
34	74	0.5	Helocrene	Western Carpathians, Czech Republic, Slovakia	Zhai et al. 2014

The commonest and most abundant species, present in the half of the studied springs, were *P. fontinalis* and *C. subterranea*. In this study, the temperature and altitudinal range of those two species was from 4.9±0.1 to 7.8±0.2 ºC and above 600 m a.s.l. During investigation of ostracods in Friuli-Venezia Giulia Region (NE Italy), Pieri et al. (2009) observed that those two species, accompanied with *Cryptocandona vavrai* Kaufmann 1900*, Cyclocypris mediosetosa* Meisch 1987 and *E. pigra*, were present only in the northern part of the region, in springs and peat bogs at altitudes higher than 840 m a.s.l. Meisch (2000) classified those two species as typical cold stenothermal, stygophilic species frequently found in springs, springbrooks and also groundwaters (caves, interstitial habitats) (Meisch 2000). Their distribution is Palaearctic (Martens & Savatenalinton 2011).

With the exception of *P. zschokkei* (collected from 4 sites), all other species in this study were collected only from one or two sites (either spring or springbrook). This pattern is similar to that observed during the study of 110 springs in Trentino Province, Italy (Stoch et al. 2011), where the commonest species, i.e. present in more than 10 springs, were *P. fontinalis*, *P. pallida*, *P. zschokkei*, and *E. pigra*, and to the pattern observed for 19 springs in the Alps of Berchtesgaden, where the commonest and most abundant species were *E. pigra* and *P. fontinalis*, followed by *Candona neglecta* Sars (Gerecke et al. 1993), while all other species occurred scattered in the small number of springs. Bottazzi et al. (2008) reported a similar species composition for Italian alpine and pre-alpine springs as in our study, with the exception of the genus *Mixtacandona* that was found only during this study. *Mixtacandona* is an exclusively groundwater genus with an array of species inhabiting karst as well as alluvial aquifers that exhibit high degree of endemism at the species level (Rogulj & Danielopol 1993, Mori & Meisch 2012). Most probably, the historical events in the studied area are the reason for four different *Mixtacandona* species found during this investigation.

The CCA analysis of environmental data and ostracod assemblages revealed the distribution of sampling sites along the gradient of substrate composition, conductivity, calcium content and alkalinity. Significant variables for shaping ostracod assemblage composition were water temperature and substrate composition. Those results indicate the importance of hydrogeochemistry for shaping the studied assemblages. Stoch et al. (2011) demonstrated that altitude, water chemistry, and water flow were the main environmental descriptors of meiofaunal distribution patterns in Trentino springs, while substrate was not so important. The main reason for that was highly heterogeneous substrate in the studied springs and strong altitudinal and geological gradient that override the influence of the substrate type. Zhai et al. (2014) have shown that mineral content of spring water (conductivity) and total organic carbon in the substrate significantly affected the ostracod assemblages in the western Carpathian springs, while the substrate composition and altitude were not important. However, the altitudinal gradient in their study was quite low (640±146 m a.s.l.). Based on those findings, it is clear that environmental predictors for ostracod assemblage composition differ among the studies due to different altitudinal and environmental range of the springs studied.

The presented study revealed high species richness of ostracods in Slovenian Alpine springs and adjacent springbrooks and contributed new knowledge on their distribution and ecology. The commonest and most abundant species were the widespread species occurring in the springs and groundwaters across Europe, while several species from the genus *Mixtacandona* and *Fabaeformiscandona* are most probably endemic for this area, indicating the effect of historical events (glaciations). Among the measured variables, water temperature and substrate composition were significant for shaping ostracod assemblages, while conductivity linked to calcium content and pH were also important.

Povzetek

V zadnjih 20 letih so bile v Evropi opravljene številne raziskave ekologije in razširjenosti dvoklopnikov (Crustacea, Ostracoda) v izvirih. Kljub temu je poznavanje razširjenosti in dejavnikov, ki vplivajo na pojavljanje dvoklopnikov v alpskih izvirih, z izjemo Italije, slabo. V tem prispevku so predstavljeni rezultati dveletne raziskave, kjer smo v 12 izvirih na območju alpske biogeografske regije v Sloveniji sistematično vzorčili bentos v izvirih in izvirskih potokih ter hkrati merili okoljske dejavnike. Vzorčenje je potekalo spomladi, poleti in jeseni, v letih 2009 in 2010. Za raziskovane izvire, ki ležijo med 600 in 1.232 m n. m. v., so značilne nizke srednje vrednosti temperatur vode (4.9–7.8 °C) z majhnim sezonskim in medletnim spreminjanjem, srednjimi pretoki od 1,1 do 212,7 l s^{-1}, heterogen substrat ter visoke vrednosti kisika. Med raziskavo smo našli 14 vrst dvoklopnikov, ki se, z izjemo dveh vrst (*Psychrodromus fontinalis* in *Cavernocypris subterranea*), večinoma pojavljajo na eni ali dveh lokacijah. Zanimivost raziskave je pojavljanje rodu *Mixtacandona*, ki je tipična podzemna vrsta, pogosta v kraških podzemnih vodah. Na podlagi obravnavanih spremenljivk smo ugotovili, da sta sestava substrata in temperatura vode statistično značilna okoljska dejavnika, ki vplivata na sestavo združb v raziskovanih alpskih izvirih in izvirskih potokih.

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