

Water quality and biotic interaction of two cavefish species: *Typhleotris madagascariensis* Petit, 1933 and *Typhleotris mararybe* Sparks & Chakrabarty, 2012, in the Mahafaly Plateau groundwater system, Madagascar

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Abstract

The karstic subterranean aquatic system of the Mahafaly Plateau in south-western Madagascar is inhabited by two species of cavefish: *Typhleotris madagascariensis* and *Typhleotris mararybe*. Knowledge about both cavefish species is scant. In order to learn more about the distribution of the two species, 15 caves and sinkholes spread over the Mahafaly Plateau were inventoried for their presence. Abiotic water quality and interspecific relations of the two species were investigated in six of these caves and five of the sinkholes during the dry and the rainy seasons. *Typhleotris madagascariensis* was present in all sampled water bodies while *T. mararybe* was restricted to five sites in the region around the town of Itampolo. The inventories extend the known range of both species of *Typhleotris* on the Mahafaly Plateau. Abiotic water characteristics did not differ between seasons. The abundances of both species were negatively correlated with iron concentrations. Further correlations between the abundance of either fish species and abiotic water characteristics remained inconclusive as these water characteristics co-varied with geographical latitude that in turn was correlated with fish abundance. For both species neither the abundance nor a condition factor based on body mass showed any significant seasonal variation. Also the presence of *T. mararybe* had no influence on the abundance and the condition of *T. madagascariensis*. Thus, no evidence for competition was noticed between the two species.

Keywords

Mahafaly Plateau, limestone, subterranean water, cavefish, water quality

Introduction

Madagascar is known for its exceptional biodiversity and human reliance on goods and services provided by the original ecosystem, making it one of the world's most prominent biodiversity hotspots (Myers et al. 2000). The island is characterized by high levels of endemism for many groups of organism and a depauperate freshwater ichthyofauna (Benstead et al. 2003, Proudlove 2010). Since 1933, four endemic troglobites fish species have been described from Madagascar: *Glossogobius ankaranensis* Banister, 1994 (Gobiidae) and *Typhleotris madagascariensis* Petit, 1933, *Typhleotris pauliani* Arnoult, 1959 and *Typhleotris mararybe* Sparks & Chakrabarty, 2012 (Eleotridae).

The genus *Typhleotris* is restricted to a vast network of subterranean limestone (karst) habitats in arid regions of coastal south-western Madagascar (Sparks and Chakrabarty 2012). *Typhleotris pauliani* occur in the subterranean waters of Andalambezo-Morombe (Arnoult 1959) north of Tuléar while *T. madagascariensis* and *T. mararybe* are known from the Mahafaly Plateau groundwater network south of Tuléar. This plateau extends over 150 km between the Onilahy river in the north and the Menarandra river in the south (André et al. 2005) and ends in the west with a cliff of up to hundred meters, followed towards the sea by an alluvial plain of sand and sandstones (Dobrilla 2014). Many caves and sinkholes are present in the coastal plain and the western part of the plateau. Some of them end with subterranean lakes populated by blind cavefish. A total of six caves are known to be inhabited by *T. madagascariensis*. This species' geographic range extends from wells near Ambilailalike, located about midway between Soalara and Efoetse in the north, to the sinkhole of Nikotsy near the town of Itampolo in the south (Kiener 1964, Sparks and Chakrabarty 2012). For *T. mararybe* the type locality and only known occurrence is the «grotte de Vitany», a sinkhole located near Itampolo on the coastal plain (Sparks and Chakrabarty 2012).

Knowledge of the cavefish fauna from the Mahafaly Plateau is scant. Published studies focussed on species descriptions with very few data on habitats. Most research was restricted to the type localities. Thus, much basic information of Malagasy blind sleeper gobies of the genus *Typhleotris* is yet to be learned, especially about the subterranean habitat, their distribution, biology and ecology. In order to fill this gap, we inventoried several caves and sinkholes within the Mahafaly Plateau from October to November 2012 (dry season) and from February to April 2013 (rainy season) for the presence of *Typhleotris* spp. and measured physical aspects of water quality. Specifically, we asked the questions:

What are the distributions of *T. madagascariensis* and *T. mararybe*?

Is their occurrence related to abiotic characteristics of water quality?

Is there any evidence for competition between the two species as indicated by changes in morphology in the case of sympatry versus allopatry?

Materials and methods

Study area

Field surveys were conducted in the Mahafaly Plateau, in south-western Madagascar. This karst plateau is characterized a sub-arid climate with low precipitation (Battistini 1964). Annual precipitation varies from 150 to 300 mm in the coastal plain and between 500 and 700 mm on the plateau with extreme inter-annual variation (Juberthie and Decu 2001, Ratovonamana et al. 2013).

In addition to the occurrences of the genus *Typhleotris* reported by Petit (1933), Kiener (1964) and Sparks and Chakrabarty (2012), some subterranean waters known by local people as cavefish habitat were visited during this study. In total, 15 subterranean water bodies were checked for cavefish presence, 11 of them (5 sinkholes and 6 caves) were chosen for measurements of water quality and more detailed fish inventories because of accessibility (Fig. 1).

Water characteristics

Water sampling was carried out on two to four successive days during the dry season and during the rainy seasons at around 9 am. Eleven water physico-chemical parameters that can affect fish population were assessed for each study site. Measurements were performed directly in the field using portable meters. Temperature, dissolved oxygen and oxygen saturation were measured with a Voltcraft DO-100 dissolved oxygen meter. The pH, the electrical conductivity (EC) and total dissolved solids (TDS) were assessed with an HI 98129 electronic pH and EC meter of Hanna. Ammonia, nitrate, nitrite, phosphate and iron analyses were performed using specific reagents and the HI83205 photometer of Hanna. More details on the analytical methods are provided by Rasoloariniaina et al. (2015).

Fish sampling

Fish sampling was carried out seasonally during four successive days. Cavefish were caught for three hours a day using a triangular *Zebco* landing net and an aquarium net. The landing net was a 60×50×60 cm bow with adjustable handles (up to 2 m) and 6 mm mesh size. The aquarium net had a plastic coated metal handle and 200 micron mesh size.

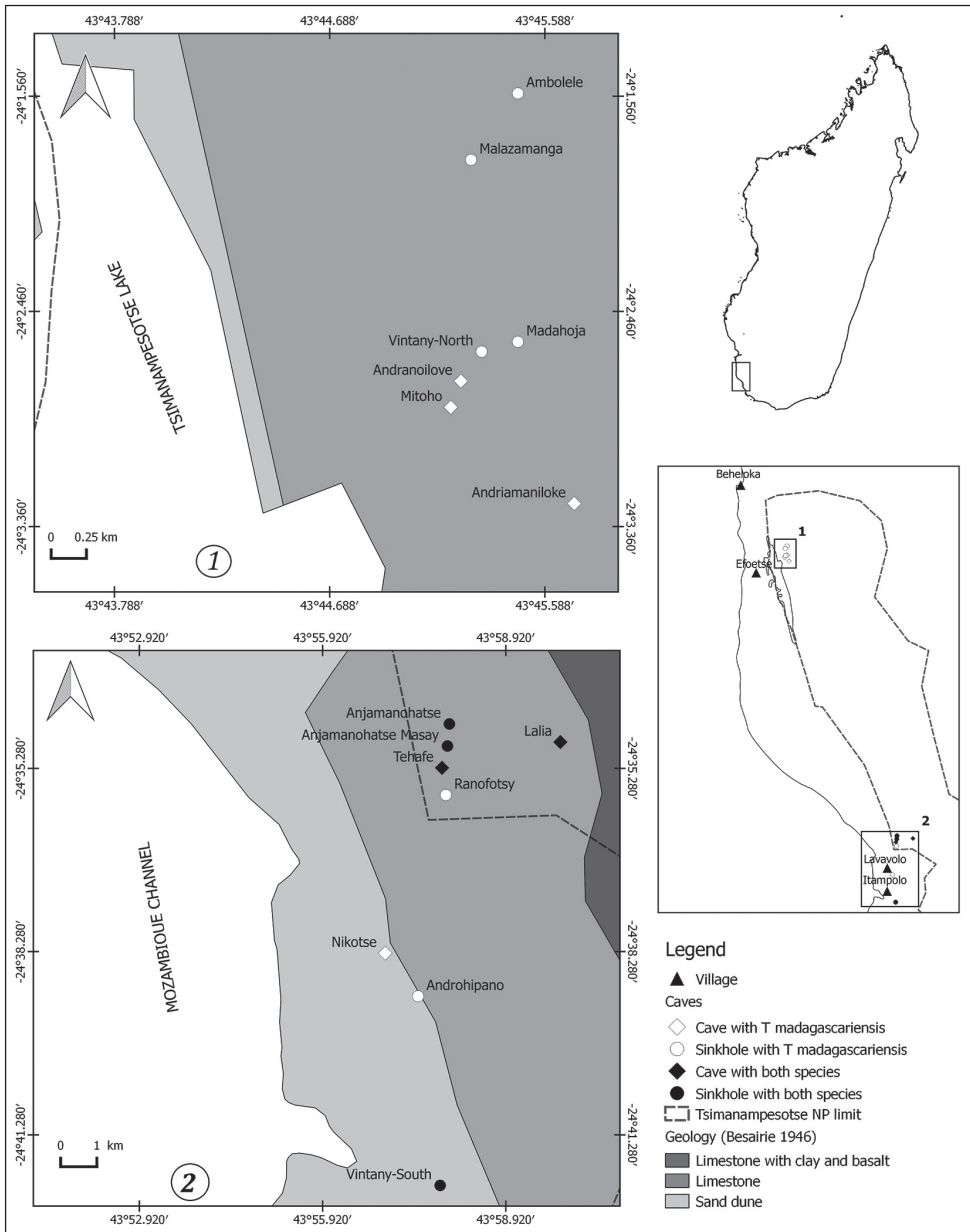


Figure 1. Study area and survey sites for *Typhleotris* spp.

Due to the large sample sizes, only subsamples of *T. madagascariensis* were measured for total length (TL) and standard length (SL) to the nearest 0.1 millimetre with a Vernier calliper, weighed to the nearest 0.1 gram using a 500 g Maul pocket scale (Baecck et al. 2013). After the measurements all animals were released in their natural habitat after having been marked.

Data analyses

The Fulton's condition index was calculated as a measure of body condition for each individual with the formula $K = 100 * W / TL^3$, where W is the body mass of the fish in grams and TL is the length of the fish in centimetre (Nielsen and Johnson 1983).

Statistical analyses were performed in R 3.0 and SPSS 22.0 for WINDOWS. Wilcoxon matched-pairs signed rank test was used to assess differences between seasons (dry and wet seasons). Mann-Whitney-U test was used to compare the abundance and condition of *T. madagascariensis* and the abiotic factors of the waters where *T. mararybe* was present against the waters where *T. mararybe* was absent. Correlations between water characteristics and fish abundances and body condition were calculated by Spearman rank correlations.

Data resources

The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility at <http://www.gbif.org/dataset/937bb0a3-e9e0-400a-8e07-0b33176953aa>.

Dataset citation provided by publisher: Rasoloariniaina, J.R., 2015. SuLaMa blind cave fish occurrence data. SuLaMa - Participatory research to support sustainable land management on the Mahafaly Plateau in southwestern Madagascar. doi: 10.15468/dluigi.

Results

Water quality

The mean values of the physicochemical parameters of subterranean waters are shown in Table 1. Values are means of two to four measurements per water body. The water temperature ranged from 26.35° to 29.08°C (median = 27.75°C) during the dry season and from 26.87° to 30.03°C (median = 27.90°C) during the rainy season. The pH values ranged from 7.19 to 7.68 (median = 7.32) during the dry season and from 7.04 to 7.67 (median = 7.35) during the rainy season. The dissolved oxygen ranged from 4.85 to 7.85 mg/l (median = 5.35 mg/l) during the dry season and from 2.10 to 6.58 mg/l (median = 4.00 mg/l) during the rainy season. The electrical conductivity (EC) varied from 1448.50 to 3043.00 µS/cm during the dry season (median = 1741.25 µS/cm) and from 979.00 to 3121.25 (median = 1665.00 µS/cm) during the rainy season.

The ammonium content ranged from 0.23 to 1.94 mg/l (median = 0.29 mg/l) during the dry season and from 0.17 to 24.62 mg/l (median = 0.56 mg/l) during the rainy season. The nitrate concentration ranged from 0.00 to 4.55 mg/l (median = 0.00 mg/l) during the dry season and from 0.00 to 5.40 mg/l (median = 0.00 mg/l) during the rainy season. The nitrite concentration ranged from 0.00 to 9.00 mg/l

Table 1. Number of *Typhleotris* spp. caught during 4 days * 3 hours (= 12 hours) of inventories, their body condition and abiotic characteristics of different water bodies along the Mahafaly Plateau. Values for abiotic conditions are means based on 1–4 measurements per season. For *T. madagascariensis* and *T. mararybe* body condition indices *K* were calculated for *N* = 828 and 70 individuals for the dry season and *N* = 1011 and 31 individuals for the rainy season, respectively.

Site	Season	<i>T. madagascariensis</i>		<i>T. mararybe</i>		Temp (°C)	pH	O ₂ (mg/l)	O ₂ (%)	EC (µs/cm)	TDS (ppm)	NH ₄ ⁺ (mg/l)	NO ₃ ⁻ (mg/l)	NO ₂ ⁻ (mg/l)	PO ₄ ³⁻ (mg/l)	Fe (mg/l)
		Abund	K	Abund	K											
Andranolove (Cave)	Dry	571	0.98			28.68	7.28	5.25	14.03	3020.50	1508.25	0.24	0.00	1.00	19.35	0.06
	Rainy	645	1.08			30.03	7.04	2.10	5.55	3121.25	1560.25	24.62	2.00	2.50	41.80	0.08
Andriamaniolo (Cave)	Dry	264	0.84			28.73	7.26	5.45	14.63	3043.00	1521.50	0.23	0.00	0.50	14.05	0.10
	Rainy	153	0.97			29.10	7.12	3.15	8.48	3025.75	1512.50	0.17	0.00	3.00	28.00	0.21
Anjamaohatse (Sinkhole)	Dry	5	0.87	2	0.95	26.60	7.38	5.20	13.17	1565.33	782.67	0.37	0.00	7.00	1.90	0.06
	Rainy	3	0.74	1	0.91	27.05	7.29	4.10	10.35	1505.00	752.00	0.41	0.00	2.50	38.95	1.32
Anjamaohatse Masay (Sinkhole)	Dry	2	0.85	13	1.01	26.35	7.32	4.85	12.33	1741.25	869.50	0.29	0.00	0.00	12.10	0.01
	Rainy	0	NA	12	1.01	26.87	7.35	3.90	10.03	1665.00	665.67	0.78	0.00	0.50	47.40	1.27
Lalia (Cave)	Dry	190	0.70	53	0.89	28.05	7.52	7.23	19.18	1488.25	743.75	0.26	0.00	0.00	5.50	0.00
	Rainy	154	0.79	24	1.00	28.33	7.61	6.58	20.45	1452.50	726.50	0.84	0.10	3.00	42.15	0.04
Mitoho (Cave)	Dry	192	0.88			29.08	7.26	5.33	14.50	3015.50	1509.00	0.26	0.00	1.50	23.45	0.10
	Rainy	290	0.99			29.05	7.15	3.48	9.03	2998.25	1499.00	0.32	0.00	2.50	41.50	0.15
Nikotse (Cave)	Dry	1	1.15			27.28	7.29	6.45	16.90	1582.80	785.60	0.41	4.40	2.67	6.37	0.09
	Rainy	0	NA			27.50	7.53	4.00	10.50	1157.00	579.00	0.40	0.00	0.00	21.25	0.73
Ranofotsy (Sinkhole)	Dry	8	0.78			27.55	7.33	5.35	14.00	1900.00	949.50	0.95	0.10	0.00	4.00	0.01
	Rainy	11	0.64			27.90	7.37	5.15	12.85	1791.50	895.00	0.57	0.00	10.00	26.70	0.20
Tehafe (Cave)	Dry	41	0.95	9	1.02	27.75	7.19	5.00	13.25	1740.75	870.50	0.32	4.55	9.00	11.70	0.03
	Rainy	44	0.90	23	0.97	27.48	7.45	4.57	11.57	1050.50	532.75	3.94	3.40	2.00	36.80	0.64
Vintany North (Sinkhole)	Dry	94	0.75			28.83	7.35	5.37	13.97	3015.33	1508.00	0.24	1.00	4.50	46.60	0.10
	Rainy	84	0.76			29.20	7.24	4.58	11.98	3007.75	1503.25	0.26	5.40	5.00	53.20	0.13
Vintany South (Sinkhole)	Dry	1	NA	8	0.84	27.40	7.68	7.85	20.35	1448.50	724.00	1.94	0.65	1.50	35.35	0.05
	Rainy	0	NA	0	NA	27.20	7.67	2.40	5.50	979.00	490.00	0.56	0.00	1.50	26.75	0.95

Abund: Abundance; K: Condition factor; Temp: Temperature, NA: not assessed; O₂ (mg/l): dissolved oxygen; O₂ (%): oxygen saturation; EC = Electric conductivity; TDS: Total dissolved solids

(median = 1.50 mg/l) during the dry season and from 0.00 to 10.00 mg/l (median = 2.50 mg/l) during the rainy season. The phosphorus concentration ranged from 1.90 to 46.60 mg/l (median = 12.10) during the dry season and from 21.25 to 53.20 mg/l (median = 38.95 mg/l) during the rainy season. The iron concentration ranged from 0.00 to 0.10 mg/l (median = 0.06 mg/l) during the dry season and from 0.04 to 1.32 mg/l (median = 0.21 mg/l) during the rainy season.

For both seasons, the abiotic factors showed no significant difference except for the electrical conductivity (as a proxy of salinity) which is significantly higher during the dry season than during the rainy season (Wilcoxon test: $W = 28.0$, $P = 0.017$, $n = 11$).

The pH increased and EC decreased significantly from north to south ($r_s = 0.80$ for pH and -0.84 for EC, $P < 0.01$, $n = 11$; Table 2).

Distribution, abundance and condition of *Typhleotris* spp.

Typhleotris madagascariensis is more widespread and more abundant than *T. mararybe*. *Typhleotris madagascariensis* was found in all eleven water bodies. In contrast, *T. mararybe* was encountered only at five sites, all located in the southern part of the study area (Fig. 1).

The relative abundance of *T. madagascariensis* (as measured by 4 days of 3 hours = 12 hours of capture efforts) varied from 1 to 571 individuals during the dry season and from 0 to 645 individuals during the wet season. The highest numbers were found in the Andranoilove cave in both seasons. The abundance of *T. mararybe* varied from 1 to 53 individuals during the dry season and from 1 to 23 individuals during the wet season. The abundances of neither species differed significantly between seasons (Wilcoxon test: $P > 0.05$; Table 1).

The condition factor K of *T. madagascariensis* varied from 0.70 to 1.15 during the dry season and from 0.64 to 1.08 during the rainy season. The condition factor of *T. mararybe* varied from 0.84 to 1.02 during the dry season and from 0.91 to 1.01 during the rainy season. There was no seasonal difference in K for either species (Wilcoxon test: $P > 0.05$; Table 1).

In order to assess correlations between water quality and the abundance and body condition of fish, we pooled the data for the wet and the dry season and calculated annual means for all variables which were then used in Spearman rank correlations. The abundance of *T. madagascariensis* was negatively correlated with the pH of the water body ($r_s = -0.76$, $P = 0.006$, $n = 11$) and positively and significantly correlated with various water characteristics that indicate the concentration of organic matter in the water, such as the electrical conductivity and total dissolved solid material ($r_s = 0.78$ for both variables, $P = 0.005$; $n = 11$; Tables 1, 2). These correlations might represent spurious relationships as the abundance of *T. madagascariensis* decreases significantly from north to south ($r_s = -0.78$, $P < 0.01$, $n = 11$; Table 2), thus following the correlations between geographic latitude, pH and EC (Table 2). Small sample size prohibited multivariate analyses. Iron concentrations were negatively correlated with the abundance

of *T. madagascariensis* ($r_s = -0.71$, $P = 0.017$; $n = 11$). The relationships are not linear but indicate thresholds below which the concentration of the component in question has a limiting effect and upper values beyond which any increase does not result in an increase of fish abundances (Fig. 2). Abundances of *T. mararybe* were also correlated negatively with the concentration of iron ($r_s = -0.90$, $P = 0.037$; $n = 5$).

Body conditions of *T. madagascariensis* and *T. mararybe* were uncorrelated with the abiotic measurements of water quality.

Interspecific relationships

The abundances of the two species of *Typhleotris* spp. were positively correlated ($r_s = 0.96$, $P = 0.011$, $n = 5$; based on annual means of abundances per site). Abundances of *T. madagascariensis* did not differ between sites where *T. mararybe* was present or not (Mann-Whitney-U test: $P > 0.05$ for both seasons and the annual mean abundance).

During both seasons, the body condition (K) of *T. madagascariensis* was uncorrelated with the body condition of *T. mararybe* and did not differ between sites where *T. mararybe* was present or not (Mann-Whitney-U test: $P > 0.05$ for both seasons and the annual mean abundance).

Discussion

The distribution, ecological requirements and interspecific interactions of the blind cavefish of Madagascar are poorly known. Recently, a new species of *Typhleotris*, *T. mararybe*, was described from the Mahafaly Plateau which occurs in sympatry with *T. madagascariensis* previously assumed to be the only species present in the area (Sparks and Chakrabarty 2012). Given the morphological similarity of the two species, the questions arises how the two species can coexist in a seemingly poorly structured environment that seems to offer few options for ecological niche separation. In order to come to a better understanding of the interactions of the species and their ecological requirements, we investigated the ranges of *T. madagascariensis* and *T. mararybe*, related their occurrence to physical and chemical water characteristics, and investigated whether or not there was any evidence for competition between the two species as indicated by changes in morphology in the case of sympatry versus allopatry.

Typhleotris mararybe is a sister taxon to *T. madagascariensis* (Chakrabarty et al. 2012). The former species inhabits the southern part of the Mahafaly Plateau (about 10 km around Itampolo) while *T. madagascariensis* is known from Ambilailalike (north-east of Beheloka) to Vintane (2 km south of Itampolo). *Typhleotris madagascariensis* reach higher abundances than *T. mararybe* and are very abundant in dark caves such as Andranoilove, Andriamaniloke and Lalia. Abundances of neither species, varied significantly between seasons, indicating sedentary populations.

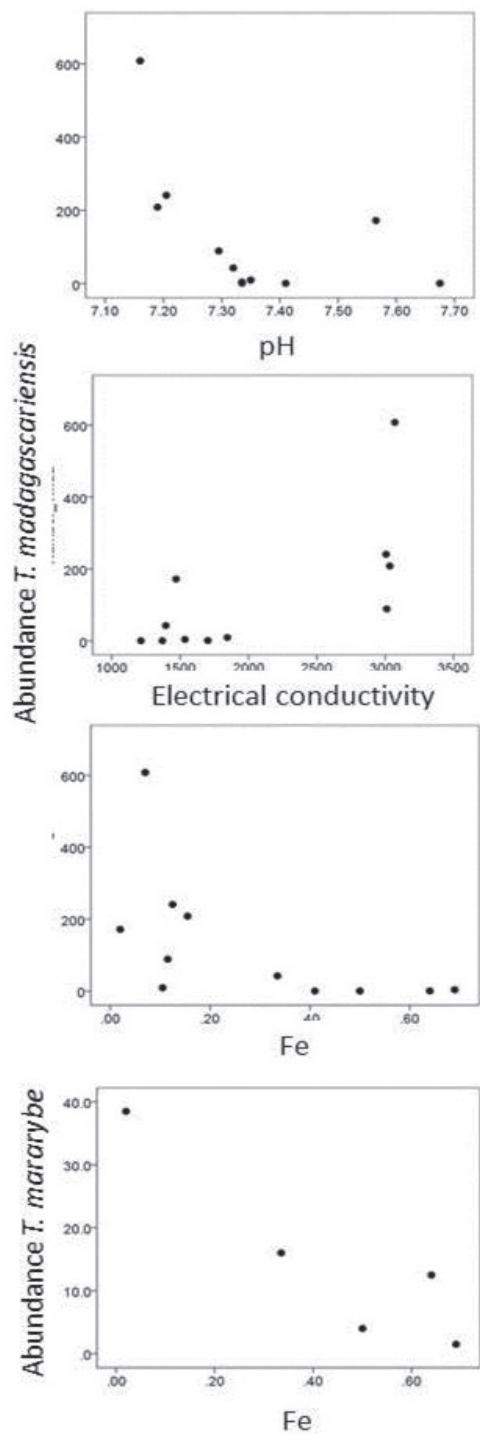


Figure 2. Significant relationships between the abundance of *Typhleotris madagascariensis* and *T. mararybe* and water characteristics.

Water quality

In the groundwater system of the Mahafaly Plateau water quality did not differ between the wet and the dry season. The low annual precipitation in this area of about 400 mm (Ratovonamana et al. 2013) and the feeding of the underground water bodies through subterranean influx over long distances (Guyot 2002, Dworak 2014) does not lead to any relevant change in water quality between seasons.

Analyses of the associations of the two cave fish species are hampered by several boundary conditions. First, the water characteristics (pH, electrical conductivity, total dissolved solids) that are correlated best with the abundance of *T. madagascariensis* are also the variables that show a linear relationship with latitude, increasing or decreasing from north to south. Since the abundances of *T. madagascariensis* co-vary with latitude and these water characteristics, it remains unclear whether the correlations between abundances and water characteristics are simply a consequence of latitudinal variation or whether they represent confining conditions. Furthermore, the relationships between water characteristics and abundances of *T. mararybe* are also difficult to evaluate due to small sample size and the low abundances. Yet, abundances of both species are negatively correlated with the concentrations of iron components on the regional scale (in case of *T. madagascariensis*) as well as on a local scale (in case of *T. mararybe*). Iron is moderately toxic for fish and some of the water bodies come close to inhibiting iron concentrations when compared to other fish species (Shuhaimi-Othman et al. 2015).

Similarly to many other troglobitic species (Poulson 2010), *T. madagascariensis* and *T. mararybe* are not exigent in terms of water quality. Our results indicate that most of the various water physico-chemical parameters recorded for the caves and sinkholes are within the tolerances for fish wellbeing. In caves and sinkholes, the water temperature is relatively high. According to Delince (1992) 30–35 °C is tolerable to fish. The pH was also within the optimal range for fish which is between 6.5 and 8.5 (Nisbet and Verneaux 1970, Boyd 1979). Fish can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0 and death is almost certain at a pH below 4.0 or above 11.0 (Ekubo and Abowei 2011). Electrical conductivity is an index of the total ionic content of water, and therefore indicates salinity (Ogbeibu and Victor 1995). EC of freshwater varies between 50 to 1500 µS/cm (Boyd 1979) and seawater has a conductivity around 52,000 µS/cm (Guyot 2002, Rasoloariniana et al. 2015). Stone and Thomforde (2004) recommended the suitable range for freshwater species of 100–2000 µS/cm. The measured water electrical conductivity during this study was within this range.

Compared with surface lakes, water quality at our sites does not seem ideal for fish development especially with respect of the dissolved oxygen (DO), the nitrogen content and the phosphorus concentrations. The adequate DO value for tropical fish is 5 mg/l (Bhatnagar and Devi 2013). The measured DO of the underground lakes is slightly below this limit during the rainy season (4 mg/l). The amount of oxygen that can be dissolved in water decreases with increasing temperature. The high nitrogen content is an indicator of organic pollution (Abdel-Rahman 2002, Jameel and Siraju-

deen 2006). Ammonia is toxic for fish but the toxicity depends on the water temperature and pH (Arrignon 1976). For aquatic organisms, the maximum limit of ammonia concentration is 0.1 mg/l (Meade 1985, Santhosh and Singh 2007). The ammonia content of the sampled waters was above this value during both seasons (0.29 mg/l during the dry season and 0.56 mg/l during the rainy season). Nitrate is scant in the study area (median = 0 mg/l during both seasons). However, some water bodies had high concentration (up to 5.40 mg/l). Yet, this does not represent a major problem for fish because nitrate is relatively nontoxic to fish and causes health hazard only at high levels above 90 mg/l (Stone and Thomforde 2004). Nitrite is also toxic for fish but the toxicity varies from one species to another. Recommended value should not exceed 0.2 mg/l in freshwater (OATA 2008, Bhatnagar and Devi 2013). The nitrite concentration of the groundwater is largely above these values (1.50 mg/l during the dry season and 2.50 mg/l during the rainy season). Because of accumulation of bat guano in some caves, the nitrogen content of the water was largely above the limit. From a management perspective, the use of some sinkholes for cattle watering worsens the nitrogen sewage. Phosphate is not directly harmful for fish. However, high phosphorus concentration permits excessive growth and reproduction of the algae and constitute a possible source of problem for fish due to the production of toxic substances or oxygen shortage caused by decomposition of organic matter (Arrignon 1976). Phosphate levels of about 0.06 mg/l are desirable for fish culture (Stone and Thomforde 2004). Probably because of the high concentration of phosphorus (up to 53.20 mg/l), different species of alga covered large surface areas of some of the sinkholes. The erosion of phosphate from rocks in the study area seem to be the main origin of this phosphate contamination (Rasoloariniaina et al. 2015).

Interspecific relations between *T. mararybe* and *T. madagascariensis*

In the southern part of the Mahafaly Plateau, the two cavefish species coexist in the groundwater system. In the field, no direct competition was observed between the two species. Furthermore, the abundance and the condition factor of *T. madagascariensis* did not differ significantly between sites where *T. mararybe* was present or not. Though subterranean habitats are nutrient-poor (David et al. 2009) and food availability is likely to be lower than in surface habitats (Poulson 2010), interspecific resource competition seems very low or even absent. This matches the findings by Poulson (2010) who has noticed that competition does not seem to be intense for cavefish. This is surprising as caves represent a stable ecosystem that should allow species to increase their population size up to the carrying capacity. Under these conditions, competition ought to be intense and species occupying the same niches should not be able to coexist, based on Gause's competitive exclusion principle (Gause 1934). From a human perspective, the underground water system seems to offer few options to adapt to different niches. Possible options are excretions from bats, an occasional cockroach dropping into the water, some crustaceans and material blown into the cave from the

cave entrance. Yet, this certainly leaves the question open which resources actually limit cavefish populations (Berti and Messina 2010).

Cave fishes are larger than most other stygobionts and tend to be at the top of subterranean food webs (David et al. 2009). The suggested diet of *T. madagascariensis* consist of either an aquatic micro fauna of epigean origin or plankton or more likely cave crustacean and insects (Berti and Messina 2010). A wide variety of organisms are found in subterranean habitats for example, the associated aquatic fauna of the Mitoho cave crustaceans (decapod *Typhlopatsa pauliani*, isopod *Anopsilana poissoni* and copepods: *Tropocyclops confinis* and *Diaptomus* sp.) and insects (Tanaidaceae *Microvelia mitohoi*) (Decary and Kiener 1970, Remillet 1973, Berti and Messina 2010). Because of the sun exposure, sinkholes are colonized by surface aquatic flora and fauna. This makes this ecosystem richer in terms of food availability with many invertebrate taxa found in open freshwater (dragonflies, water beetles, snails). Crustacean and insect larvae and/or adults from the surface habitats may provide additional resources for cavefish. Yet, despite the seemingly marked ecological differences between water bodies, such as sinkholes and caves, none of these variables seem to have a pronounced impact on the two cavefish species. This might either be a consequence of small sample size or could indicate high ecological tolerances that permitted the species to survive in the region for many millions of years.

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Linking spatial scale dependence of land-use descriptors and invertebrate cave community composition

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Abstract

Patterns of biodiversity respond to habitat disturbances and different land-uses. Those patterns possibly vary according to the spatial scale under analysis. Although other studies have shown such responses for different systems, no study has ever demonstrated spatial-scale influences in subterranean terrestrial communities. Therefore, the objective of this paper was to analyze how land use and cave physical structure could influence the terrestrial cave invertebrate species composition. We also determined the influence of different spatial scale on the structure of invertebrate cave composition. We collected environmental data at local scale (e.g. cave size, substrate and environmental stability). For spatial scale we determined land uses at three different landscape scales; we gathered these data into circular areas of different sizes (50, 100 and 250 meters) with centroids in the cave entrances. We finally performed three Distance Based Linear Modeling analyses to test for differences among the predictability of environmental variables when comparing different spatial scales. The best explanatory variable for cave invertebrate similarities was the percentage of covering of the external environment by limestone outcrops. We confirm the scale-dependence hypothesis through the different patterns showed among distinct buffer areas. Models become more precise when larger scales were analyzed to explain cave invertebrate composition. This suggests that larger scales capture important environmental features that explain the cave fauna similarities more precisely. Additionally, we found a strong influence of limestone outcrops at all landscape scale structuring cave communities.

Keywords

Subterranean, habitat heterogeneity, land-use, limestone outcrop, native vegetation, cattle pasture

Introduction

Environmental heterogeneity in natural landscapes has been historically replaced by anthropogenic mosaics around the world. As a consequence, several hypothesis describing how landscape characteristics affect biodiversity patterns have been proposed (Tscharrntke et al. 2012). Ecological functions and processes are dependent on larger spatial scales than a single habitat patch (Gustafson 1998, Steffan-Dewenter et al. 2002). Biodiversity is often positively correlated with the amount of available habitat (Fahrig 2003), but the effect of land-use changes on biodiversity depends on the landscape context (MacDonald et al. 2000) and on the spatial scale that has been analyzed (McGlenn and Hurlbert 2012, Dumbrell et al. 2008, Zimmermann et al. 2010, Morueta-Holme et al. 2013). Furthermore, ecological communities are structured under processes that act on the landscape, in which both regional and local scales are important factors (Harrison and Cornell 2008).

In the context of landscape influences on biodiversity distribution patterns, caves are good models since they represent simplified and fragile ecosystems (Culver 1982, Culver and Pipan 2009). Since the cave communities are dependent on the allochthonous input of nutrients, alterations in the availability, properties and abundance of these nutrients in the landscape surrounding the caves may affect cave biodiversity. Despite their fragility, caves are under several anthropogenic pressures, and only few studies evaluated how such human activities can affect the invertebrate cave communities, such as inadequate tourism (e.g. Poulson et al. 1995, Moldovan et al. 2003, Pellegrini and Ferreira 2012). Such studies are even scarcer when considering human impact at landscape scale, such as agriculture, urban development, deforestation and mineral resources extraction (Eme et al. 2014, Zagmajster et al. 2014). All these activities lead to aquifer pollution, cave destruction, and biodiversity loss (Beynen et al. 2012). Considering this, the current Brazilian legislation (Brazil. Decree n° 6.640/2008) imposes that caves should bear a protection area of 250 meters in radius surrounding the cave linear projection on the surface (Portaria IBAMA n° 887/1990). Although other studies have shown responses for different spatial scales under analysis (e.g. Steffan-Dewenter et al. 2002), few studies have demonstrated such influences in subterranean communities patterns (Eme et al. 2014, Zagmajster et al. 2014).

The main goal of this paper is to explain cave invertebrate composition through environmental variables from within the cave and also from the landscape surrounding the caves at different spatial scales. To that end, we tested the hypothesis that the spatial scale affects the predictability of environmental variables. We also checked for grouping patterns among cave fauna according to the most explanatory variables.

Methods

Study area

The present study was carried out at the conservation unit “*Parque Estadual do Sumidouro*” – PESU (Sinkhole State Park), in the suburban mesoregion of Belo Horizonte, Minas Gerais state, Brazil (Fig. 1). The PESU is in a karstic area, presenting a Savanna wet tropical weather. The caves are located in an anthropic landscape, in which there are areas with native vegetation but most areas are covered with cattle pasture (Iniesta et al. 2012). We chose 10 caves in this park based on the main surrounding landscape matrix, vegetation cover and land-use types, thus encompassing a heterogeneous landscape around the caves. The caves present sizes ranging from 16.85 to 137.68 meters. We performed one sampling event at each cave.

Terrestrial invertebrate collection

We only used terrestrial invertebrates for our analysis because they account for most of cave richness and abundance in Brazilian caves (Pinto-da-Rocha 1995), especially considering the caves in study, poor in water bodies. In each cave, we collected invertebrates manually, using tweezers and brushes, with special attention to microhabitats such as under wood trunks and rocks, as well as other organic matter accumulations. In the laboratory, the collected specimens were identified to the lowest taxonomic level possible and separated into morphotypes in order to obtain species presence/absence for each cave (Oliver and Beattie 1996). Such morphospecies separation is sufficient for ecological biodiversity studies and conservation purposes. Oliver and Beattie (1996) demonstrated that morphospecies identified by non-specialists could led to estimates of richness comparable with those elaborated using species identified by specialists. Furthermore, Oliver and Beattie (1996a) have shown that the use of morphospecies provides results usually concordant with conventional species inventories. Biological material is deposited in the Zoology Collection (Coleção de Invertebrados Subterrâneos de Lavras), Seção de Invertebrados Subterrâneos (from ISLA 3478 to ISLA 3618).

Local environmental data collection

We considered environmental variables at different spatial scales, thus encompassing traits inside the cave (local scale), and those belonging to the landscape scale. At the local scale, we measured the linear extension of the cave and number and size of the entrances. We used those variables to estimate the cave Environmental Stability Index (*ESI*), proposed by Ferreira (2004) and also used by Bento et al. (in press). This

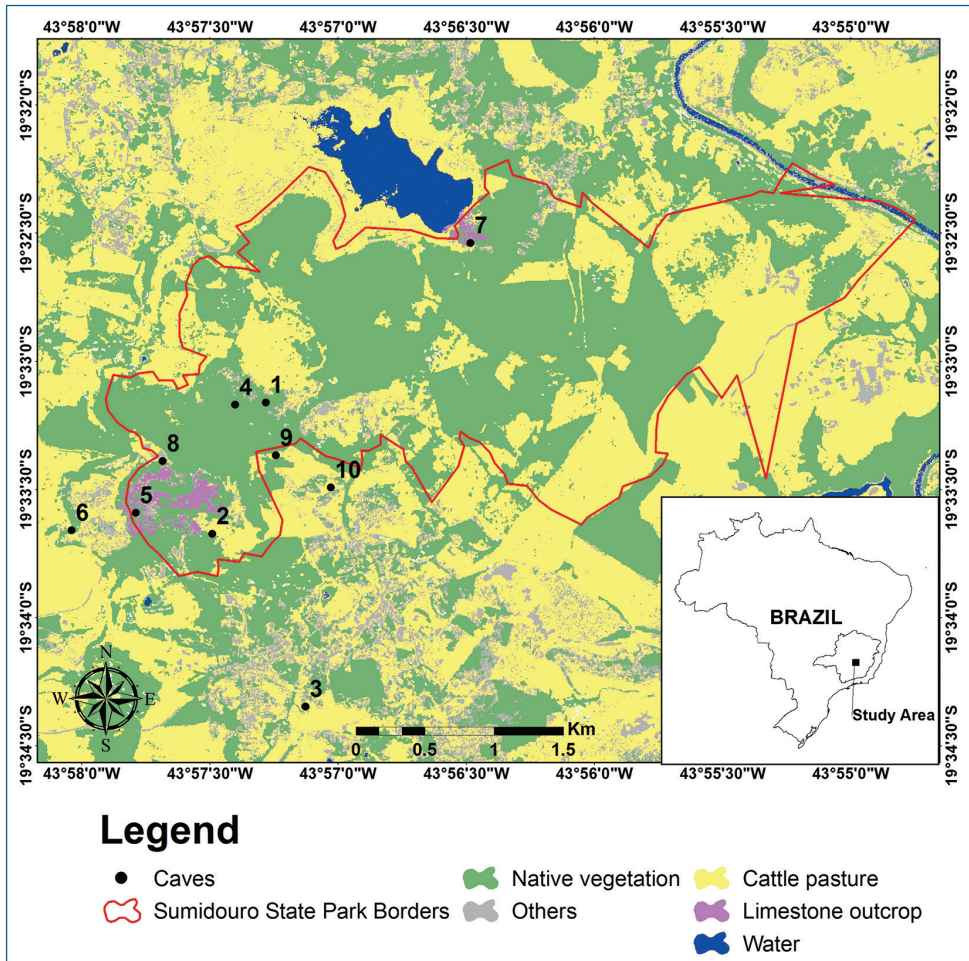


Figure 1. Spatial characterization of landscape at “Parque Estadual do Sumidouro”. Different colors represent distinct vegetation cover or land-use types. The numbers indicate the sampled caves, indicated by name. Legend: **1** Gruta Ninho de Pérolas **2** Gruta Macaco das Cavernas **3** Lapa da Várzea **4** Gruta do Grilão **5** Gruta Helictites **6** Lapa das Pacas **7** Gruta do Sumidouro **8** Gruta Lagoa Seca **9** Gruta do Feneme **10** Gruta do Lixo.

index accounts for outside interference on the cave environment, as a ratio between the number and size of entrances (as their spatial distribution) and the cave size (Eq. 1). In Eq.1, LE is the linear extension (total length) of the cave, $\sum EE$ is the sum of all entrances extension, NE is the number of entrances, and DEE is the average distance from all entrances to a reference cave entrance (remaining that all distance between entrances must be considered). Big caves with small entrances would be more stable than small caves with big entrances, which would possess an internal environment more disturbed by the external environment.

$$ESI = \frac{\frac{LE}{\sum EE}}{\frac{(NE)(DEE)}{LE}} \quad [1]$$

In order to determine the habitat heterogeneity inside each cave, we classified and quantified the different types of habitat. We divided each cave main conduit into at least 11 transects, equally distanced. Bigger caves were divided with more transects with a maximum distance between them corresponding to 15 meters. Each transect was then subdivided at five points, and at these points we visually examined substrate type (guano, water, trash, organic matter and the size of inorganic grains), along the five equidistant points, encompassing a minimal number of 55 measurements for each cave. The size of inorganic grains was classified into eight classes (bedrock, large boulders, boulders, cobbles, coarse gravel, fine gravel, sand, silt and hardpan). This methodology was modified from Peck et al. (2006) and from Hughes and Peck (2008). We used these values to assess habitat heterogeneity parameter as explained hereafter. Considering the local scale, the cavities under study showed different patterns in their physical variables. Based on the 55 habitat measurements, we calculated the proportion of each habitat class within each cave and estimated the habitat heterogeneity using Shannon's diversity index, using such values of habitat classes encountered in each cave. We calculate Shannon's index using PAST 3.11 software (Hammer et al. 2001).

Landscape environmental data collection

In order to obtain environmental variables at a landscape scale we quantified the main land-use types at different buffers through image classification and matrix characterization. These buffers were circular areas centered at each cave entrance, with a radius of 50 m, 100 m, and 250 m. For those caves with multiple entrances, the biggest entrance was used as reference in this analysis. Therefore, we delimited three circular areas, named respectively Buffer 50 m, Buffer 100 m, and Buffer 250 m (Figure 1). The percentages of each land-use type at each buffer were then included as environmental variables on further tests.

Spatial characterization of PESU required a RapidEye remote sensing image from 2010; images were obtained in LEMAF (*Laboratório de Estudos e Projetos em Manejo Florestal*), in the Federal University of Lavras, Lavras city, Brazil. We created an image subset delimiting only the park area. Then we segmented and classified that subset into five classes: native vegetation, water, cattle pasture, limestone outcrop, and others (which included roads, cities, constructions, bare land and general urbanized areas) (Figure 2), using algorithm K *Nearest Neighbor* with the software ENVI EX v.4.8 (ITT 2010).

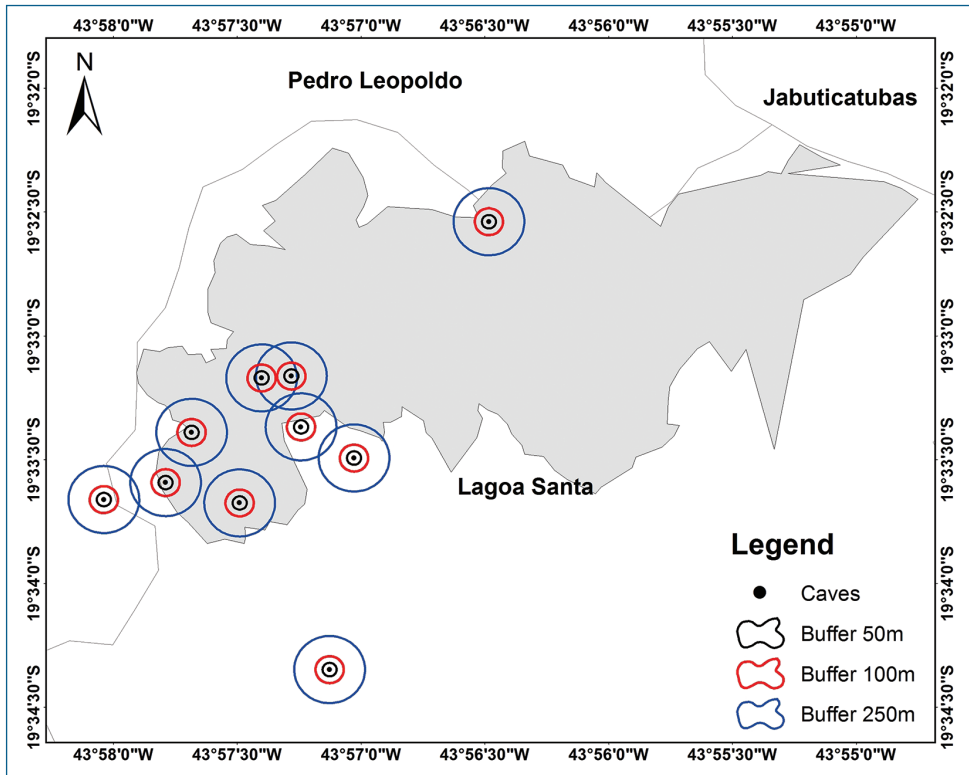


Figure 2. Study area location, sampling design used in sampled caves at “Parque Estadual do Sumidouro”, and the Buffers of 50m, 100m, and 250m for analyzing the effect of spatial scale on the explanatory power of environmental variables in the cave invertebrate communities.

Data analysis

In order to detect if the geographic distance is responsible for the highest similarities between the studied caves, we performed a Mantel test with PAST 3.11 software (Hammer et al. 2001). Environmental variables at different scales were used to analyze cave invertebrate communities. The influence of spatial scale (Buffer 50 m, Buffer 100 m, and Buffer 250 m) on invertebrate fauna composition was assessed with three independent Distance Based Linear Modeling (DistLM) analyses (software PRIMER 6.0). We performed the analyses based on species composition data, by a resemble matrix using *Jaccard* index (qualitative data) for calculating the species similarities between the caves. As predictor variables, we used environmental data: percentage of land-use types at each buffer scale (thus representing the landscape spatial scale), added to local scale information (linear extension, environmental stability index and Shannon's diversity index for substrate). We chose *adjusted R*² as a selection criterion using the *Best* procedure, which examines the value of the selection criterion for all possible combinations of predictor variables (Anderson et al. 2008).

Results

We found 186 invertebrate species, distributed along at least 78 families and 23 orders (Table 1). Among them, the order Diptera was the most representative, presenting 40 species, which belong to at least 17 families. The order Araneae, with 14 families and 33 species was the second richest. We found three troglomorphic species, two belonging to the order Collembola (Entomobryomorpha and Hypogastruroidea) and one Isopoda (Platyarthridae, *Trichorhina* sp.). Entomobryomorpha was found in two caves, while Hypogastruroidea was restricted to a single cave. *Trichorhina* sp. was more broadly distributed, being found in four caves.

We found no correlation between geographical distance and caves invertebrate similarity (Correlation $R_{\text{MANTEL TEST}} = -0.3366$; $p = 0.9111$).

Lapa das Pacas Cave had the highest Environmental Stability Index ($ESI=3.53$). Ninho de Pérolas Cave presented the smallest value, $ESI = -0.56$ (Table 2). The habitat varied highly among caves. Only two caves showed bat guano, Gruta do Sumidouro and Lapa da Várzea. Water bodies were also found in only two caves, Gruta do Sumidouro and Lapa das Pacas. Gruta do Sumidouro Cave presented 12 different types of habitats and the Shannon diversity index for this parameter was 2.25, the highest value among the caves analyzed. The lowest Shannon diversity value was found at the Lapa da Várzea Cave (1.30) with only 6 different types of habitats (Table 3).

At landscape scale we found four main types of land cover: limestone outcrop, cattle pasture, native vegetation and others. At all buffers the two principal land covers were pasture and native vegetation. The only buffer with water was the 250 m buffer of the Gruta do Sumidouro Cave (Table 4).

Limestone outcrop was the most important predictor variable of community composition (Jaccard index - considering species identity in the community) in all buffer scales, although other variables varied with landscape scale. The best model for the 50 m Buffer presented an *adjusted* R^2 value of 0.40, and used only two variables (limestone outcrop and others). The best model solution for Buffer 100 m presented an *adjusted* R^2 value of 0.45, and revealed three variables, limestone outcrop, cattle pasture and native vegetation. Finally, the best model for Buffer 250 m showed an *adjusted* R^2 value of 0.73 and used four variables (limestone outcrop, water, environmental stability index and substrate Shannon's diversity index) (Table 5). Therefore, large-scale models have higher R^2 values.

Discussion

There are few studies on spatial patterns of cave communities although some studies evaluate differences of spatial scale sampling on species patterns (e.g. Zagmajster et al. 2008, Eme et al. 2014). Few studies evaluated spatial-scale dependence on community-land uses relationships for cave invertebrates (e.g. Bento 2011). These studies are important to reliably identify essential features and patterns for conservation and management

Table 1. Taxon and families collected at Parque Estadual do Sumidouro Caves. NI: Not Identified. In each column is represented the number of morphospecies found at that cave.

TAXON		FAMILIES	Gruta do Grilão	Gruta do Lixo	Gruta do Sumidouro	Gruta Feneme	Gruta Pacas	Helictites	Lagoa Seca	Lapa da Varzea	Macacos da Caverna	Ninho de pérolas
Annelida	Oligochaeta	NI	0	0	0	0	1	0	0	0	0	0
			1	0	1	0	2	1	0	1	0	0
		Platyarthridae										
		NI	0	0	0	0	2	1	0	2	1	1
Crustacea	Isopoda	Pseudonannolenidae	1	0	1	1	0	1	0	0	1	1
			0	0	0	0	0	0	0	1	0	0
		Polysxenidae	0	0	0	0	0	0	0	0	0	0
		NI	0	1	0	0	0	0	0	0	0	0
Gastropoda	Diplopoda	Scutigerridae	0	1	0	0	1	0	0	1	0	0
			0	0	0	0	0	0	0	3	0	0
		NI	0	0	0	0	0	0	0	0	0	0
		Anytidae	0	1	0	1	0	0	1	0	0	0
		Argasidae	1	1	0	0	0	1	0	1	1	1
		Ixodidae	0	0	0	0	1	0	1	1	0	0
		Macronyssidae	0	0	1	0	0	0	0	0	0	0
		Rhagidiidae	0	1	0	0	0	0	0	1	0	0
		Tydeidae	0	0	0	0	0	0	0	1	0	0
		NI	0	0	0	0	0	0	0	1	0	0
		Araneidae	0	0	0	1	0	0	1	0	1	0
		Ctenidae	1	1	2	2	0	1	1	1	2	1
		Deinopidae	0	0	0	0	0	0	0	0	1	0
		Linyphiidae	0	0	0	1	0	0	0	0	2	0
		Nemesidae	0	0	0	0	0	0	0	0	0	1
		Oonopidae	0	0	0	0	1	0	0	1	0	0
		Pholcidae	1	1	2	1	1	2	2	1	1	1
		Salticidae	0	1	0	0	0	3	2	0	2	1
		Sicariidae	1	1	1	0	0	1	1	1	1	1
		Symphytognathidae	1	1	0	0	0	0	0	0	0	1
Arachnida		Theraphosidae	0	0	0	0	1	0	0	0	0	0

TAXON	FAMILIES	Gruta do Grilão	Gruta do Lixo	Gruta do Sumidouro	Gruta Feneme	Gruta Pacas	Helictites	Lagoa Seca	Lapa da Varzea	Macacos da Caverna	Ninho de pérolas
Hexapoda	Theridiidae	0	0	2	0	2	0	0	3	2	0
	Theridiosomatidae	0	1	0	1	0	0	0	1	1	0
	Uloboridae	0	0	0	0	1	1	0	0	0	0
	NI	1	0	1	0	1	1	0	0	1	0
	Pseudoscorpiones	1	0	0	0	0	0	0	0	0	0
	Chetridiidae	0	0	0	1	0	1	1	0	0	0
	Chthoniidae	0	1	0	0	0	0	0	0	0	0
	Gonyleptidae	0	0	1	0	0	0	0	1	0	0
	Eukoeneiidae	0	1	1	0	1	0	0	0	1	0
	NI	0	0	0	1	0	0	0	2	0	0
	NI	0	0	0	0	0	0	0	1	0	0
	Carabidae	0	0	0	0	0	0	0	0	0	0
	Cholevidae	0	0	0	0	0	0	0	1	0	0
	Elateridae	0	0	1	0	0	0	0	0	0	0
	Histeridae	0	0	1	0	0	0	0	0	1	0
	Pselaphidae	0	0	0	0	1	1	0	0	0	1
	Ptilidae	0	0	0	0	0	0	0	1	0	0
	Ptilodactylidae	1	2	1	0	0	0	1	2	0	1
	Rhizophagidae	0	1	0	0	0	0	0	0	0	0
	Scarabaeidae	0	0	0	0	0	0	0	0	1	0
	Staphylinidae	0	0	0	0	0	0	0	0	1	0
	Tenebrionidae	1	0	0	0	0	1	1	1	0	1
	NI	3	0	2	1	2	1	0	4	3	1
	NI	0	1	2	0	1	0	0	2	0	0
	Collembola	0	0	0	0	0	0	0	0	0	1
	Anthomyiidae	0	0	0	0	0	0	0	0	0	0
	Bibionidae	0	0	0	0	0	0	0	1	0	0
	Calliphoridae	0	1	0	0	0	0	0	0	0	0
	Ceratopogonidae	1	0	0	0	0	0	0	0	1	0
	Chloropidae	0	1	0	1	0	0	0	0	1	0
	Culicidae	0	1	0	0	1	1	1	0	1	0
	Diptera	0	1	0	0	0	1	1	0	1	0

TAXON	FAMILIES	Gruta do Grilão	Gruta do Lixo	Gruta do Sumidouro	Gruta Feneme	Gruta Pacas	Helictites	Lagoa Seca	Lapa da Varzea	Macacos da Caverna	Ninho de pérolas
	Dolichopodidae	1	1	0	0	0	0	1	0	1	0
	Drosophilidae	0	1	0	1	0	0	0	3	2	1
	Lauxanidae	0	0	0	0	0	1	1	0	0	0
	Milichiidae	0	0	0	0	0	1	0	0	0	0
	Mycetophilidae	0	0	0	0	0	0	0	1	1	0
	Phoridae	0	0	1	1	0	0	0	2	1	0
	Psychodidae	0	0	0	0	1	0	0	1	1	1
	Sciaridae	0	1	0	0	0	0	0	1	1	0
	Stratiomyiidae	0	0	0	0	0	0	0	1	0	0
	Tabanidae	0	0	0	0	0	0	0	0	1	0
	Trichoceridae	0	0	0	0	0	1	0	0	0	0
	Gryllidae	0	0	0	0	1	0	0	0	0	0
Ensifera	Pahlangopsidae	1	0	1	2	1	1	0	1	2	1
	NI	0	0	0	0	0	0	0	2	0	0
Hemiptera	Cixiidae	1	1	0	1	0	1	0	1	1	0
	Miridae	0	0	1	0	0	0	0	0	0	0
	Ploiaridae	0	0	1	0	0	0	0	0	1	0
	Reduviidae	1	1	2	0	1	1	1	0	2	1
Hymenoptera	NI	0	0	0	0	0	0	0	0	0	1
	Formicidae	1	1	1	3	3	3	1	0	2	2
	Eulopidae	0	0	1	0	0	0	0	0	0	0
	Rhinotermitidae	0	0	0	0	1	0	0	0	0	0
Isoptera	Termitidae	0	0	0	0	1	0	1	0	0	0
	NI	0	1	0	1	0	0	0	1	0	0
Lepidoptera	Geometridae	0	1	0	0	0	0	0	0	0	0
	Hesperiidae	1	0	0	0	0	0	0	0	0	0
	Noctuidae	1	4	1	3	0	2	0	2	1	2
	Tineidae	1	2	2	2	1	2	0	2	2	1
Neuroptera	Myrmeleontidae	0	0	1	0	0	1	0	0	0	0

TAXON		FAMILIES	Gruta do Grilão	Gruta do Lixo	Gruta do Sumidouro	Gruta Feneme	Gruta Pacas	Helictites	Lagoa Seca	Lapa da Varzea	Macacos da Caverna	Ninho de pérolas
	Pauropoda	NI	2	0	0	1	1	0	0	2	2	1
	Psocoptera	NI	2	0	0	1	1	0	0	1	2	1
		Epipsocidae	0	1	0	0	0	0	0	0	0	0
		Lepidopsocidae	0	0	1	1	1	0	0	0	1	0
		Liposcelidae	0	0	1	0	0	0	0	0	0	0
		Psocidae	0	0	0	0	0	1	1	0	0	0
		Psyllipsocidae	0	0	0	0	0	1	1	0	0	0
		Ptiloneuridae	0	1	2	0	1	0	0	0	1	0
	Thysanura	Nicoletidae	0	1	0	0	0	0	0	0	0	0
TOTAL RICHNESS			27	37	35	29	34	34	20	58	51	26

Table 2. Values of physical variables found at each cave at PESU. ESI: Environmental Stability Index; LE: Linear Extension; Σ EE: Sum of Entrances Area; NE: Number of Entrances.

CAVE	ESI	LE	Σ EE	NE
Gruta do Grilão	0.5983	42.82	2.74	3
Gruta do Lixo	0.0829	16.85	8.28	1
Gruta do Sumidouro	3.3859	137.68	6.47	1
Gruta do Feneme	3.2335	26.13	0.8	1
Gruta Helictites	1.8935	69.48	3.45	1
Gruta Lagoa Seca	1.7884	28.39	7.59	3
Lapa da Várzea	2.806	134.35	2.53	1
Lapa das Pacas	3.5262	319.56	1.41	1
Gruta Macaco das Cavernas	1.5085	42.7	8.19	2
Gruta Ninho de Pérolas	-0.5618	27.22	11.42	1

actions. Our findings confirm the scale-dependence hypothesis on explaining similarities among cave communities. Models get more precise at larger scales, it is possible to incorporate new explanatory variables that may be absent at smaller scales. The combination of different scales variables explain better cave community composition.

Karst areas have different historical land uses and human impacts vary according to landscape characteristics (Frumkin 1999). While karst depressions are more easily cultivated, rocky karst slopes and carbonate outcrops are less suitable for such uses (Frumkin 1999). At PESU we could clearly observe this pattern, since impacted areas, with cattle pasture, are mainly those located in depressed areas. Additionally, the best-preserved areas were those located on limestone outcrops or in their surroundings and they were the best predictor of cave community structure for all landscape scales. Such areas also host denser vegetation ensuring better conditions for cave invertebrate communities, most identified species in this study were troglophiles, also present in the surface habitats.

The second factor that explained the cave similarity in the 50 m buffer was “*others*”, represented by cities, human constructions, roads and bare land, as results of urbanization. According to McIntyre and Hobbs (1999), urbanization is one of the most destructive human activities generating habitat change and loss of ecological function. As urbanization may cause a strong reduction in invertebrate diversity (Buczkowski and Richmond 2012), this effect could influence cave communities in two ways: *i*) we suggest that caves could be a refuge for invertebrate species, especially for those caves near such areas or *ii*) urbanization could reduce both, epigeal and hypogean invertebrate communities. Here, smaller scales indicated an intensified urbanization impact near cave entrances (Figure 3), thus suggesting that caves may offer shelter to invertebrate species, offering optimal conditions for many invertebrate species, especially for edaphic species that establish a continuum of life from the surface soil to the deep subterranean environment (Gers 1998, Ortuño et al. 2013).

The 100 m buffer indicated, in addition to limestone outcrops, cattle pasture and native vegetation as important variables. The landscape cover determines food availability

Table 3. Environmental variables at local scale. Kinds of substrates found at each cave at PESU study.

CAVE	Gruta do Grilão	Gruta do Lixo	Gruta do Sumidouro	Gruta Feneme	Gruta Helicites	Lagoa Seca	Lapa da Varzea	Lapa das Pacas	Macacos das Cavernas	Ninho de pérolas
Bat guano	0	0	2	0	0	0	3.5	0	0	0
Waterbody	0	0	1	0	0	0	0	28	0	0
Trash	0	3	0	0	0	0	0	0	0	0
Organic matter	3.5	3	10.5	11.5	0	5.5	12.5	0	2	1.5
Bedrock	17	5	3	6	17	37	0	13	7	0
Large boulders	1	5	2	0	2	2	0	0	8	3
Boulders	0	9	9	12	2	2	0	2	16	6
Cobbles	8	3	2	5.5	2	0	0	3	12	9
Coarse gravel	3	2	2	8	3	2	0	2	12	12
Fine gravel	15	12	7.5	5	18	4.5	2	1	15	1
Sand	17	4	5	2	4	6	3	0	8.5	0.5
Silt	0.5	0	5.5	0	6	0	44	68	3	58
Hardpan	0	9	10.5	15	1	11	25	8	11.5	4
Substrate Richness	8	10	12	8	9	8	6	8	10	9
Dominance	0.2105	0.1332	0.1219	0.1563	0.2271	0.3242	0.3386	0.3622	0.1218	0.4048
Shannon_H	1.709	2.148	2.295	1.963	1.754	1.527	1.307	1.338	2.189	1.347

Table 4. Environmental variables at landscape scale. Percentage of each land-use type at all Buffer sizes.

	Limestone Outcrop			Others			Cattle Pasture			Native Vegetation			Water	
	50	100	250	50	100	250	50	100	250	50	100	250	50	250
BUFFER AREA SIZE														
Gruta do Grilão	0.0	0.0	0.0	0.0	2.2	5.8	9.1	7.1	7.0	90.9	90.6	87.2	0.0	0.0
Gruta do Lixo	0.0	0.0	0.0	17.8	22.0	11.9	76.0	56.2	57.7	6.2	21.8	30.4	0.0	0.0
Gruta do Sumidouro	37.6	29.1	2.6	10.1	8.8	1.5	16.9	16.2	3.5	35.4	45.9	15.8	76.7	0.0
Gruta do Feneme	0.0	0.0	0.0	3.5	3.2	2.4	47.7	28.6	30.8	48.8	68.2	66.8	0.0	0.0
Gruta Helictites	33.0	35.0	20.7	14.9	20.1	18.3	1.9	5.3	20.4	50.2	39.6	40.6	0.0	0.0
Gruta Lagoa Seca	2.8	12.9	9.4	35.9	19.2	9.9	19.2	20.2	18.3	42.2	47.7	62.4	0.0	0.0
Lapa da Várzea	0.0	0.0	0.0	22.1	16.5	13.4	59.5	69.7	63.4	18.4	13.7	23.2	0.0	0.0
Lapa das Pacas	0.0	0.0	0.0	4.9	14.7	12.1	43.8	47.1	70.7	51.3	38.2	17.2	0.0	0.0
Gruta Macaco das Cavernas	0.0	1.9	9.1	3.0	2.9	6.9	52.0	53.7	35.0	45.0	41.5	49.1	0.0	0.0
Gruta Ninho de Pérolas	0.0	0.0	0.0	0.1	9.0	8.5	1.3	6.1	26.8	98.6	84.9	64.8	0.0	0.0

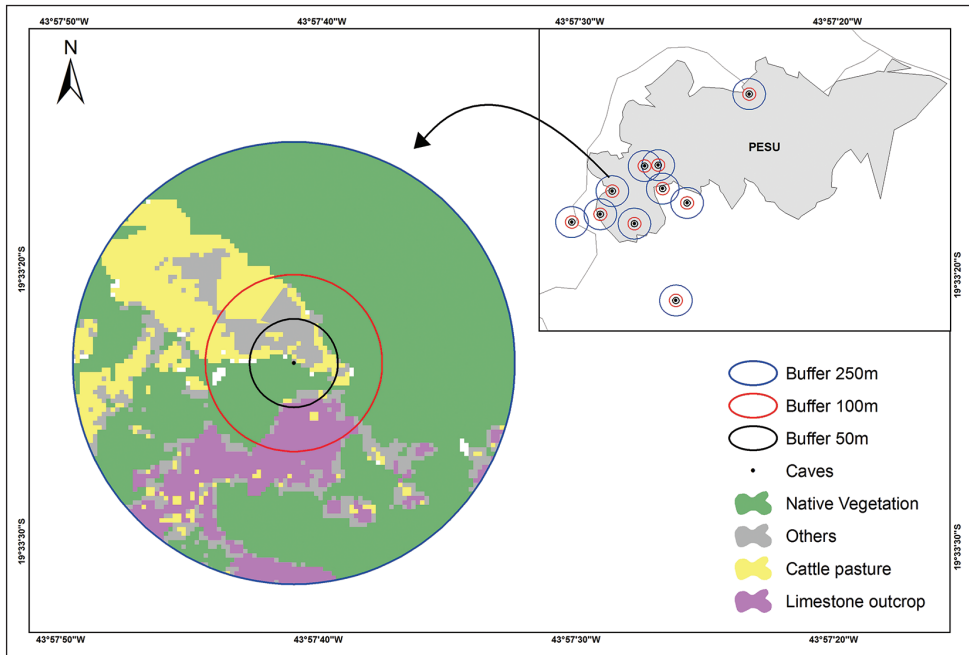


Figure 3. Detailed figure of Gruta Helictites showing different land uses within the 50, 100 and 250 m buffers.

inside caves, which possibly affects cave communities. Although one would expect to have richer communities in caves surrounded by forests (as a rich source of organic matter that can be brought inside caves), guano also constitutes an important resource for many cave invertebrates. Some species are highly dependent on guano, and cave communities associated to this resource can be relatively complex (Ferreira and Martins 1999, Ferreira et al. 2007). The conversion of forests to pastures may favor some species (Gillieson and Thurgate 1999), aside from also reducing foraging habitats for several bat species. Habitat loss and fragmentation seems to favor *Desmodus rotundus*, a hematophagous bat species that remains in areas transformed into rural landscapes (Aguar et al. 2010). The change in the organic resource quality can result in a remarkable invertebrate substitution (Souza-Silva et al. 2011), which, in turn, could result in several changes in patterns and processes inherent to the cave fauna. Such changes could eventually enhance the cave community instability, which can become more vulnerable to environmental disturbances.

Considering the 250 m buffers, the model incorporated three different explanatory variables aside from the limestone outcrops: water bodies, environmental stability index and habitat heterogeneity. The importance of the allochthonous nutrient input through water transport is well known (e.g. Hawes 1939, Culver 1982, Romero 2009). Water acts as a molding agent and a vehicle in which nutrients, gases, minerals and even microorganisms are transported underground (Culver and Pipan 2009). The only cave that presented epigeal water in the 250 m buffer was the Gruta do Sumidouro. That water is a lake connected to the cave by a sink and its invertebrate fauna

is unique because flood pulses continually disturb and remove the accumulated food resource (Souza-Silva et al. 2011). In addition, floods may help maintain a regular food supply to caves, thus operating as distribution agents (Hawes 1939), contributing to the faunal singularity.

The higher community similarity among caves with similar values of *ESI* was expected. Stable associations by community in some cave sectors, which exhibit optimal climatic conditions, were reported for some invertebrate species (Di Russo et al. 1997). In this context, Bento et al. (in press) conducted a work in 24 cavities in the Brazilian Caatinga (semi-arid landscape) and found that more stable caves showed less variation in the invertebrate community composition when comparing their communities in both seasons (rainy and dry) than less stable caves. In this paper cave stability was calculated by the same index used in this study. Considering these, the more stable the cave environment the more similar the faunal elements at PESU, favoring some species over others (Tobin et al. 2013); especially species with high specialization for cave life, or edaphic spaces.

The habitat diversity hypothesis proposes that species diversity in a landscape will increase as the greater structural complexity increases, because of the higher resource abundance and the potential addition to the number of partitionable niche dimensions (MacArthur and MacArthur 1961). Environmental heterogeneity is correlated to patterns of groundwater crustacean richness (Eme et al. 2014), although it is not correlated to distribution patterns of this group (Zagmajster et al. 2014). Furthermore, cracks and stones, which increase environmental heterogeneity, may provide shelter for small invertebrates in caves (Carchini et al. 1982). We found that habitat heterogeneity is an important factor explaining community composition.

Although the landscape scale explains better species composition, the local scale model suggests an influence of the habitat heterogeneity and stability on cave community. The variables *ESI* and habitat heterogeneity were important only in Buffer 250 m, the largest evaluated scale. In smaller buffers, such local variables were not important probably because other landscape variables had already explained the community variation. In that case, including *ESI* and habitat heterogeneity in smaller buffers would not increase the model explanation. It has long been known that cave ecosystems are highly vulnerable to external events, even those occurring at some distance from the cave (Gillieson and Thurgate 1999). This indicates the importance of studies on larger areas, which could avoid erroneous conclusions on the real influence of each epigean environmental variable on the cave communities. It is worthy to mention that in situations of geographically closer caves, as Ninho de Pérolas Cave and Gruta do Grilão Cave, the results found could be at least in part due to the 250 meters buffer superposition. However, the distance between caves was not related to caves invertebrate similarity.

Landscape use can even make terrestrial troglophile populations more isolated, severely reducing their dispersal possibilities, by conversion from forest to pasture (Eberhard et al. 1991). Hence, results of community influences by landscape structure (predominance of natural vegetation or disturbed spaces) clearly indicate the need to conserve the adjacent landscapes of caves in karstic zones. Furthermore, attention should be given not only for intact landscapes, but also to continuous forest corridors

between caves, that promote fauna dispersal between habitat patches, that should be also protected. In this study, the caves surrounding landscapes show impacts of the human use, and the small remaining forest patches were important for cave invertebrate species maintenance.

Considering the current Brazilian legislation (Brazil. Decree nº 6.640/2008), there is an obligation to protect the area corresponding to the cave linear projection on the surface and also a radius of 250 meters around this projection (Portaria IBAMA nº 887/1990). Unfortunately, there are no studies showing an eventual efficiency of such radius to preserve cave communities. Our study could be the first step to improve Brazilian legislation, since it provides a new methodology to evaluate three aspects: cave invertebrate communities, cave physical traits and surface land use.

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New distribution records of cave-dwelling gekkonid lizards (Sauria, Gekkonidae and Phyllodactylidae) in the Zagros Mountains of Iran

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Abstract

The distribution of cave-dwelling lizards of the families Gekkonidae and Phyllodactylidae (Sauria) was investigated in the Zagros Mountains of Iran. Obtained information includes new distribution records of species from 15 caves. The caves are Bendireh, Taigeh, Ban, Zarrinabad, Ghadah, Kulkani, and Darham-reh in Ilam Province, Pelazh, Gavbar and Asmari in Khuzestan Province, Tadovan, Manian and Sangeshkan in Fars Province and Dalaki and Khesht in Bushehr Province. In this study, five species belonging to the Gekkonidae and Phyllodactylidae families were recorded including: *Asaccus elisae*, *Asaccus nasrullahi*, *Hemidactylus persicus*, *Cyrtopodion scabrum*, and *Cyrtopodion gastrophole*.

Keywords

Cave-dwelling lizards, Gekkonidae and Phyllodactylidae, Zagros Mountains, Western Iran

Introduction

In recent years, there has been a rapid increase in information available on the fauna of caves (Chapman 1983, Culver and Sket 2000, Bauer et al. 2002, Ngo et al. 2008, Afrasiab 2011). There is only one record for cave dwelling lizards, *Asaccus kermanshahensis* Rastegar-Pouyani, 1996, in Iran which, so far, has not been reported from other habitats, although the diversity of caves fauna in Iran is not well known (Esmaili-Rineh and Sari 2013). The northern and western borders of the country are bounded by two large mountain ranges, the Elburz and Zagros, respectively. These two mountain ranges have played an important role in shaping the past and present distribution patterns of various Agamid, Scincid and Gekkonid taxa (Macey et al. 2000, Torki et al. 2010, Afrasiab et al. 2013). The Zagros Mountains include a series of parallel ridges interspersed with plains that bisect the country from northwest to southeast. The Zagros Mountains represent 55% of all karstic-carbonate formations in Iran, and more than 250 caves, most dry, are known from this region (Raeisi 2004). A number of species of animals live permanently or temporarily in these caves, including bats, blind crustacean, blind fishes and arthropods in deeper part or lizards, snakes, and birds near the entrance (Akmali et al. 2011, Esmaili-Rineh and Sari 2013, Sharifi et al. 2014).

Although there are no obligate cave-dwelling (i.e., troglobionts) reptiles, caves are used on a temporary to long-term basis by several snakes and lizards around the globe (Afrasiab 2011). Some colubrid snakes hunt bats at cave entrances or enter caves to hunt roosting bats (Akmali et al. 2011, Sharifi et al. 2014). Among lizards, geckos are considered the most common cave-dwelling lizards (Culver 2000, Bauer et al. 2002). There are nearly 50 described species of lizards belonging to 10 families that occur in the Zagros Mountains (Anderson 1999). Gekkonidae is the largest family of geckos consisting of over 950 described species belonging to 51 genera (Uetz and Hošek 2015). Gekkonids along with Lacertid lizards are among the most diverse family of lizards in the Zagros Mountains (Anderson 1999).

In this study, we describe the lizard fauna of caves in the Zagros Mountains. The purpose of this study is to identify cave dwelling lizard's species as important elements of life in caves of central and southern parts of the Zagros Mountains.

Methods

We surveyed 15 caves for lizards in the southern, southwestern and western parts of Iran in Bushehr, Fars, Ilam and Khuzestan provinces, which is the primary region with caves and karst development in the Zagros Mountains (Figures 1 and 2; Table 1). Sampling of lizards was done by using long pans and special bags, and the latitude and longitude of caves were recorded using a Garmin GPS unit (GPSMAP 60CSx; Garmin International, New York, USA). Captured lizards were photographed and identified in the field.

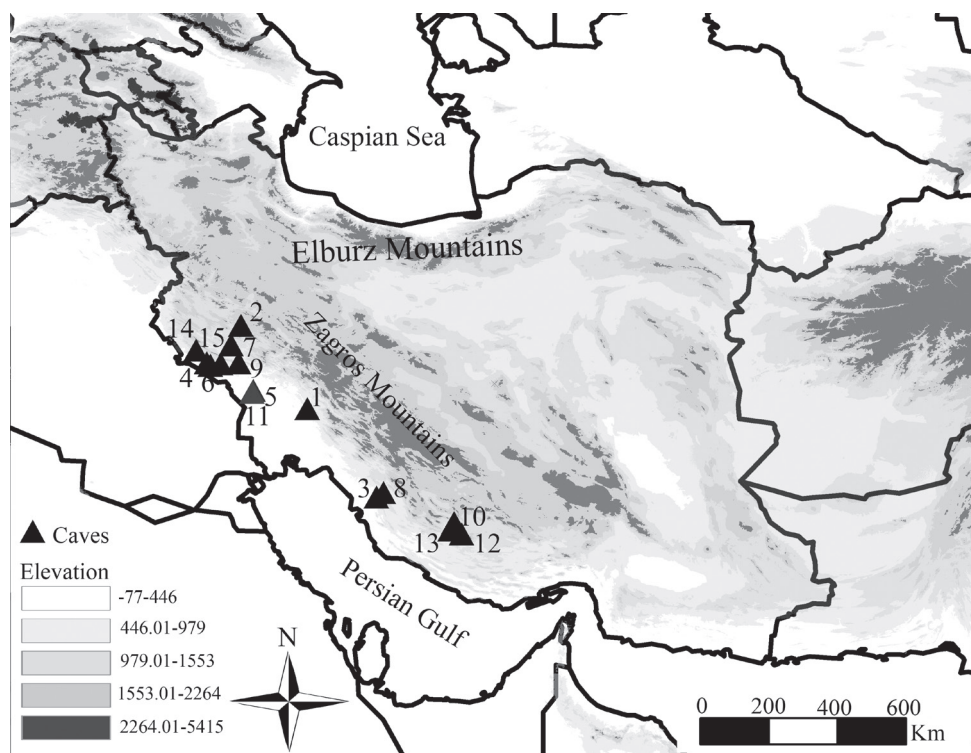


Figure 1. Location of the visited caves in the Zagros Mountains. (Numbers show caves: **1** Asmari **2** Bendirh **3** Dalaki **4** Darhamreh **5** Gaybar **6** Ghadah **7** Ban **8** Khesht **9** Kulkani **10** Manian **11** Pelazh **12** Sangeshkan **13** Tadovan **14** Taigh **15** Zarinabad).

Results

Five species of Gekkonid lizards belonging to *Asaccus*, *Hemidactylus* and *Cyrtopodion* genera were collected from the 15 caves throughout the Zagros Mountains in western and southwestern Iran (Table 1 and Figure 3A–F) as follow.

Cyrtopodion scabrum (Heyden, 1827)

Figure 3A

Keeled rock gecko, rough-tailed gecko and rough thin-toed gecko

Remarks. Mean snout-vent length 38.9 mm and tail 49.2 mm.

This species has a wide distribution range from Egypt through southwest Asia to northwestern India (Anderson 1999). It is mostly distributed in western Iran, rarely found out of residential places, and it is the most common house gecko in western



Figure 2. Some of the caves visited in the studied area. For more details and coordinations refer to Table 1. Dalaki (A), Taigh (B), Tadovan (C), Manian (D), Zarrinabad (E) and Gavbar (F).

Iran (Anderson 1999). Caves are not the common habitat for this gecko, but we could observe this species at two caves in Ilam and Fars provinces (Table 1). Darhamreh cave is limestone cave with a small and bright entrance. Internal environment of this cave is completely dry and there is no water. The other cave (Sangeshtkan) is a system of artificial underground spaces (disused sandstone mines) in a hill at the southern margin of Jahrom town (Fars province). The cave consists of some chambers with broken and fallen ceilings, creating a number of smaller spaces, fissures and crevices, well useful as roosts.

Table 1. Coordinates, altitudes, length (m) and entrance size of caves and list of the Gekkonid lizards observed and collected in this study.

Cave	Province	Coordinate	Altitude (m)	Length (m)	Entrance size	Species	Number of samples	Family
Asmari	Khuzestan	31°46'N; 49°30'E	434	-	Small	<i>Asaccus elisae</i>	4	Phyllodactylidae
Bendireh	Ilam	32°58'N; 47°46'E	413	40	Large	<i>Asaccus elisae</i>	4	Phyllodactylidae
Darhamreh	Ilam	32°55'N; 46°58'E	615	20	Small	<i>Asaccus elisae</i>	7	Phyllodactylidae
Gavbar	Khuzestan	32°15'N; 48°05'E	90	47	Small	<i>Asaccus elisae</i>	3	Phyllodactylidae
Ghadah	Ilam	32°57'N; 47°14'E	1029	220	Large	<i>Asaccus elisae</i>	5	Phyllodactylidae
Khesht	Bushehr	29°34'N; 51°30'E	416	15	Large	<i>Asaccus elisae</i>	8	Phyllodactylidae
Zarrinabad	Ilam	32°59'N; 46°52'E	796	100	Large	<i>Asaccus elisae</i>	3	Phyllodactylidae
Kulkani	Ilam	32°59'N; 47°42'E	512	70	Large	<i>Asaccus nasrullahi</i>	2	Phyllodactylidae
Darhamreh	Ilam	32°55'N; 46°58'E	615	20	Small	<i>Cyrtopodion scabrum</i>	5	Gekkonidae
Sangeshkan	Fars	28°29'N; 53°33'E	1091	300	Large	<i>Cyrtopodion scabrum</i>	4	Gekkonidae
Dalaki	Bushehr	29°27'N; 51°19'E	259	20	Small	<i>Cyrtopodion gastrophole</i>	3	Gekkonidae
Ban	Ilam	33°27'N; 47°30'E	1391	90	Large	<i>Hemidactylus persicus</i>	1	Gekkonidae
Manian	Fars	28°35'N; 53°15'E	1077	40	Large	<i>Hemidactylus persicus</i>	8	Gekkonidae
Pelazh	Khuzestan	32°12'N; 48°03'E	110	36	Large	<i>Hemidactylus persicus</i>	5	Gekkonidae
Sangeshkan	Fars	28°29'N; 53°33'E	1091	300	Large	<i>Hemidactylus persicus</i>	4	Gekkonidae
Tadovan	Fars	28°47'N; 53°21'E	1200	300	Large	<i>Hemidactylus persicus</i>	6	Gekkonidae
Taigh	Ilam	33°19'N; 46°35'E	1204	25	Small	<i>Hemidactylus persicus</i>	2	Gekkonidae

***Cyrtopodion gastrophole* (Werner, 1917)**

Figure 3B

Werner's bent-toed gecko, Persian Spider-gecko

Remarks. Mean snout-vent length 44.8 mm, tail 54.5 mm.

This species is an endemic species for Iran, known only from its type locality, the Fars province. But we collected this species in a cave in Bushehr province. We collected this species inside Dalaki cave, in humid and cool places used as a shelter for physiological thermoregulation during mid-day hours. Usually active at night but in the middle of the day it was found in shaded areas and feed on Lepidoptera insects.

***Asaccus elisae* (Werner, 1895)**

Figure 3C, D

Werner's leaf-toed gecko

Remarks. Mean snout-vent length 42.5 mm, tail 29.8 mm.

This species was mostly observed and collected in caves in Iran (Anderson 1999). Other researchers (Weber 1960, Wettstein and Löffler 1951) reported it as a house gecko in Iraq on the Mesopotamian plain. This species was collected in seven caves

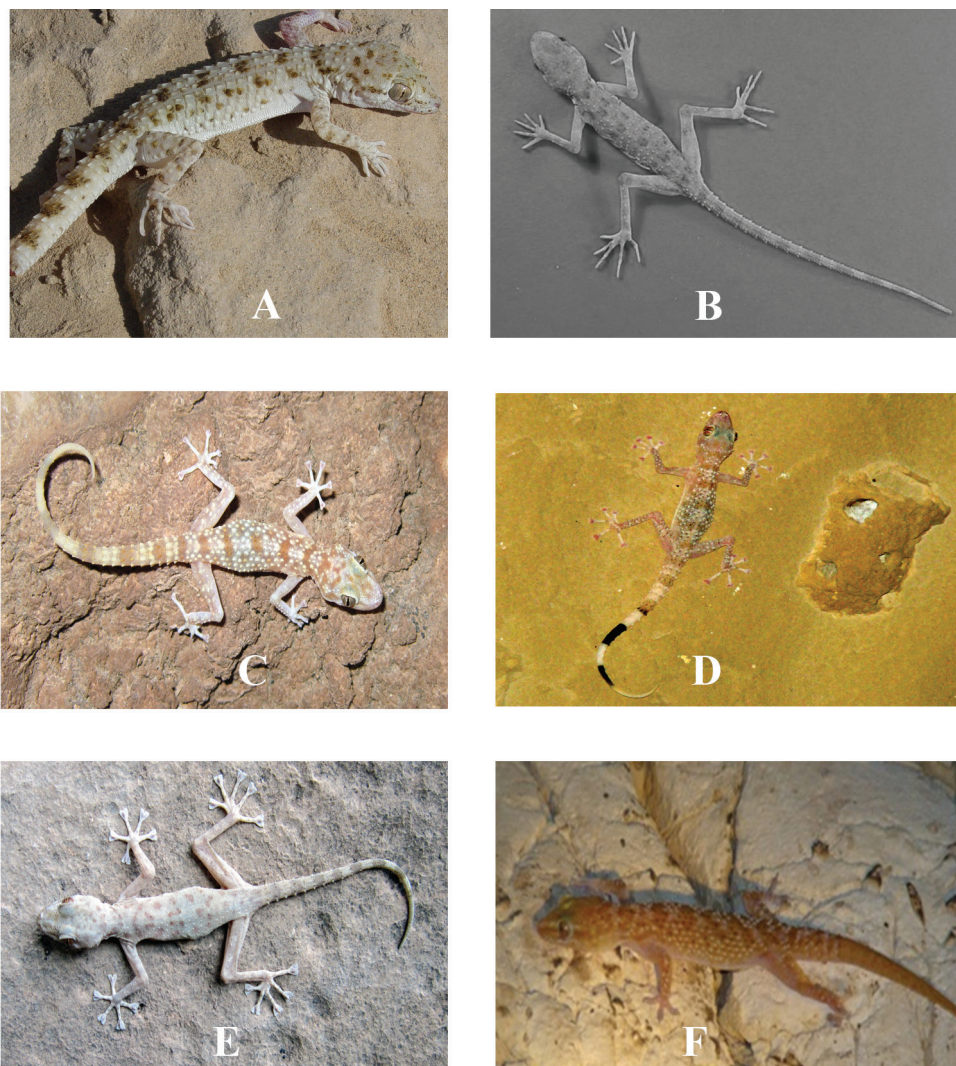


Figure 3. The collected species in this study in the caves located in Table 1. *Cyrtopodion scabrum* (A), *Cyrtopodion gastrophole* (B), *Asaccus elisae* (C, D), *Asaccus nasrullahi* (E), *Hemidactylus persicus* (F).

(Table 1). Six of them are limestone caves with dry and bright entrances. One of these cave (Zarinabad) have gypsum structure and at the time of the year there is running water inside it. Due to its tunnel-like shape air flows through it.

***Asaccus nasrullahi* Werner, 2006**

Figure 3E

Nasrullah's leaf-toed gecko

Remarks. Mean snout-vent length 70 mm, tail 66.8 mm.

The species is distributed in parts of the West and Southwest of the Zagros Mountains, mainly in Loristan province. In this study we report a new record of this taxon from Kulkani cave in Ilam province. Kulkani is limestone cave with large and bright entrance. The cave can be divided into two parts. The first part is dry and bright where specimens were found in it. The second part is smaller and dark with little humidity.

***Hemidactylus persicus* Anderson, 1872**

Figure 3F

Persian gecko

Remarks. Mean snout-vent length 53.3 mm, tail 62.9 mm.

The species is widely distributed from coastal Arabia north to southern Iran and Iraq, east to Sind Waziristan, Pakistan (Anderson 1999). In Iran it is known from Khuzestan, Fars, Kerman, and Baluchistan Provinces. We collected this gecko in six caves (Table 1). All of these caves are located in different parts of the Zagros Mountains and are formed in limestone. The caves are almost similar in terms of habitat conditions such as moisture content, hydrology, entrance size and brightness. One of these caves is Tadovan located at a height of 1200 meter above the sea level. This cave is large and complex with a large entrance within a rocky overhang and is situated in a steep cliff on the left, eastern side of the Ghare-Aghaj river valley. The cave is a series of well passable corridors and domes of different width (ca. 1–5 m) and height (ca. 2–7 m). Immediately inside the entrance is a large pit of about 6 m in depth. After the pit, in the twilight zone, is a large gallery with 36% humidity and 30°C temperature. *Hemidactylus persicus* was found in this gallery which has the lowest humidity (36%) compared to other galleries in which their humidity ranges from 45% to 97%. This lizard was not found in other parts of the cave with different humidity.

Discussion

We visited 15 caves and recorded five species of Gekkonid lizards, *Asaccus elisae*, *Asaccus nasrullahi*, *Hemidactylus persicus*, *Cyrtopodion scabrum*, and *Cyrtopodion gastrophole*. Of the above-mentioned recorded species, only *Asaccus elisae* was previously reported by Anderson (1999) from caves and non-caves habitats. Anderson (1999) found it in a cave in Fars Province and in Lorestan Province under a large flake of exfoliated sandstone on a cliff face above a stream. This species is sympatric with *Cyrtopodion scabrum*, *Hemidactylus persicus*, and *H. flaviviridis* in western and southwestern regions of Iran (Parsa et al. 2009).

Geckos are chiefly surface active animals but there are great varieties of Gekkonid lizards adapted to live in darkness situations (such as caves) with some pre-adaptations for occupying cave environments and deep rock grooves such as long snout, reduced keeled tubercles, absence of caudal tubercles, all for crawling in rock cracks inside the caves, adhesive paddles, and slender limbs for climbing (Bauer et al. 2002). *Cyrtopo-*

dion scabrum is mostly known as a house gecko and there is no record of this species in other habitats as was sustained by Anderson (1999). We observed this species in two caves, hence extended the knowledge of habitat characteristics of this species. Although many of collected and identified lizard species used to caves as resting areas during the day and were observed around the caves in the rocky habitats, none of them is a true cave-dwelling species in Iran.

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Distribution and a comparative analysis of the aquatic invertebrate fauna in caves of the western Caucasus

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Abstract

The freshwater fauna of nine caves in central Abkhazia, western Caucasus, revealed 35 species of stygobionts, including 15 new species to be described elsewhere. The number of species per station increased from the depth towards the entrance in caves Golova Otapa and Abrskila, becoming the highest in the epigean part. In both caves, two abundance peaks of aquatic invertebrates were registered: one in the entrance area, associated with the development of amphibiotic insect larvae, the other in the depths due to the high numbers of stygobionts. In Cave New Athos, the highest species richness and abundance were observed in large lakes. In caves Golova Otapa and Abrskila, two faunistic complexes with complementary distributions were found, the first due to amphibiotic insects in the cave entrance area, the second one composed of stygobionts in the deep areas. The impact of anthropogenic factors on aquatic cave communities was also noted. The stygobiotic faunas of all caves studied were clearly divided into three groups, following the number of river valleys in which they were situated. The stygobiont faunas of the caves located within one river valley appeared to be 50% similar. In contrast, the fauna composition of the stygobionts from caves situated in different valleys shared not more than 12% species in common. Stygobiotic shrimps and gastropod mollusks show profound local endemism. Stygobiotic Amphipoda penetrating the ground waters revealed wide distributions between cave systems within a single karst massif.

Keywords

Abkhazia, abundance, distribution, stygobiont species complex

Introduction

Cave biotopes are often inhabited by unique complexes of aquatic species. At least some of these species are completely absent in epigeal ecosystems. Such a refugial character of stygobiotic communities is developed in consequence to the constant environmental parameters in subterranean waterbodies which remain stable not only for some years, but also on a geological time scale (Birstein and Borutzky 1950). Thus, the freshwater fauna of karst caves of the Caucasus is known to exist in more or less stable conditions since the Tertiary. Not surprisingly, the fauna includes a great number of endemic taxa both at the specific and generic levels (Birstein 1950).

The study of the stygobiotic fauna in the western Caucasus started with the works of Zhadin (1932, 1952), Starobogatov (1962), Birstein (Birstein 1950, Birstein and Ljovuschkin 1965, Birstein and Lopashov 1940) and Borutzky (Borutzky 1940, 1971, Borutzky and Mihajlova-Nejkova 1970). They all focused on mollusks and/or crustaceans. However, those early evaluations of the species diversity in the stygobiotic groups clearly indicated that the region was underexplored (Birstein 1950).

In the recent years, inventorying and cataloguing the subterranean fauna of the western Caucasus, in particular stygobiotic invertebrates, have been carried out (Kniss 2001, Barjadze et al. 2015). These studies predominantly concern the fauna of large karst caves of the Krasnodar Krai, Russia, Abkhazia and Imeretia, Georgia. The greatest number of new species has been described among stygobiotic Amphipoda, even though this is predicted to grow significantly in the future (Sidorov 2014, 2015a, b). Some species of the cavernicolous shrimp genus *Troglocaris* Dormitzer, 1853 have been redescribed (Anosov 2016, Marin and Sokolova 2014). A zoogeographic regionalization of the stygobiotic isopod fauna of the Caucasus has appeared recently (Turbanov 2015). A taxonomic revision and a species richness analysis of cavernicolous gastropods were published (Vinarski et al. 2014, Palatov and Vinarskiy 2015).

At the same time, a number of invertebrate groups inhabiting the caves of the western Caucasus remain almost untreated taxonomically. Descriptions of cavernicolous Turbellaria have just begun (Shumeyev 2008). The data existing on Oligochaeta and Hirudinea diversity are restricted to the identification of the stygobiotic genera most abundant in the region (Bizin et al. 2015). Thus, taxonomic studies on the aquatic cave fauna of the western Caucasus remains in its initial stage. Almost all biospeleological investigations are hampered by the inability to identify organisms to species, sometimes even to the generic level.

Not surprisingly, both the ecology and community structure of stygobiotic fauna in the western Caucasus are far from well-known. Investigations which would comprise all taxa of aquatic invertebrates inhabiting individual cave systems are extremely rare. Usually such studies concern both stygo- and troglofaunas, only partially com-

plete though (Koval 2003, 2004, Sendra and Reboleira 2012, Sidorov et al. 2014). Comparisons of macrobenthos compositions between different habitats of a single large cave or between different karst massifs are virtually absent (Ljovuschkin 1975). A similar situation is observed even in the far better prospected stygobiotic fauna of the Balkan Peninsula (Franjević et al. 2010).

Complex studies of cavernicolous species communities are of importance to biodiversity analyses and biogeographical reconstructions, as well as to the ecological monitoring of troglobitic ecosystems. There are some fundamental problems that remain unresolved: first of all, what is the real species richness of a karst massif? How far can different taxonomic groups of stygobionts disperse? Which changes or successions in aquatic communities can be observed with an increasing distance from the entrance? Which is the influence of anthropogenic impacts on those changes? The latter question is extremely topical, allowing for the use data on stygobiotic species as indicators of anthropogenic pressure in touristic caves (Marinskiy et al. 2015).

In the current study, an analysis of the freshwater fauna in nine caves of central Abkhazia, western Caucasus is presented. There are three different aims in this study: (1) to provide an inventory of the species richness of freshwater invertebrates; (2) to elucidate the patterns of species distribution within a single cave considering the anthropogenic factor; and (3) to perform a comparative analysis of the stygobiotic faunas inhabiting the different regions of Abkhazia.

Material and methods

Explored area

Our investigation focused on the Gumishinsko-Panavskiy speleoregion (Dublyansky et al. 1987) of central Abkhazia. The material was collected during two weeks of February 2012 and 2015 in six large and three small caves in the Gudautsky, Gulrypshtsky and Ochamchirsky regions. All cave waterbodies had low water levels during the sampling time, so the flood aspects were absent. The locations of the examined caves are shown on Figure 1.

- (1) *Gudautsky region*. Samples were taken in Cave New Athos (Novoafonskaya) and in Cave Simona Kananita. The former is a huge karst cave with several large halls and a total of approximately 1 mil. m³ volume. There are lakes in two of the halls, small puddles and streams being scattered almost all over the cave. The material was collected in nine halls, six of which are visited by organized tourist groups. Cave Simona Kananita supports a stream with the part available for sampling extending for no more than 20 m.
- (2) *Gulrypshtsky region*. The material was collected in Nizhnyaya Shakuranskaya and Srednyaya Shakuranskaya caves, as well as in Tsebel'dinskaya cave. All the three karst caves are horizontal and have a flooded central gallery with a stream or river

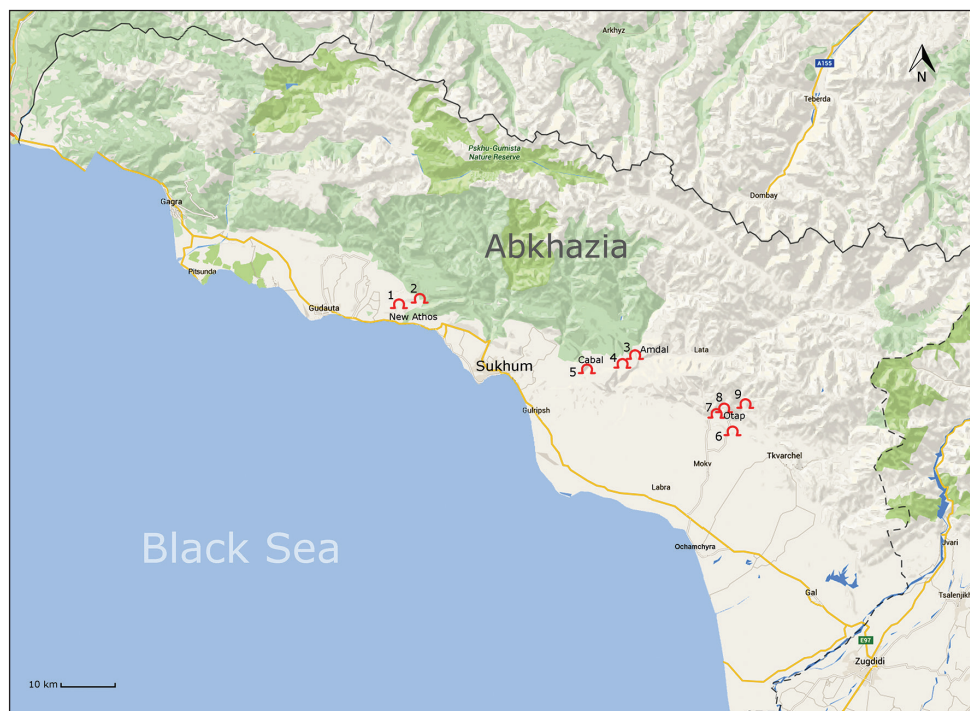


Figure 1. Map of the study regions of Abkhazia. Caves: **1** New Athos **2** Simona Kananita **3** Nizhnyaya Shakuranskaya **4** Srednyaya Shakuranskaya **5** Tsebel'dinskaya **6** Abrskila **7** Golova Otapa **8** Well Uapatyh **9** Well 85 m.

flowing all along. The length of the explored parts of these caves did not exceed 200 m.

- (3) *Ochamchirsky region*. Sampling was carried out in Abrskila and Golova Otapa caves, as well as in Uapatyh (Nad Golovoy Otapa) and the 85 m rock wells. The firsts two caves are represented by horizontal branching gallery systems, the largest of which supports a river. The total length of the explored part of Abrskila Cave was nearly 2.5 km, that of Golova Otapa Cave, 500 m. The parts of the streams available for sampling in both vertical wells were located near their bottoms.

Anthropogenic impact

Tourists mostly visit the New Athos Cave (Ekba and Dbar 2007), which has a special visiting tunnel. The cave is supplied with air-shafts, tourist trails, bridges, and illumination affecting at least 50% of the cave. Abrskila Cave is rarely visited by tourists. Artificial illumination in this cave extends to a distance of 400 meters from the entrance, i.e. less than a quarter of the total length of the cave. In Golova Otapa Cave, which is also frequented by tourists, the human influence can be restricted to the construction

of approximately 50 meters long wooden paths located to nearly 100 meters off the entrance. At the time of our research, the cave had no artificial illumination. Nizhn-yaya Shakuranskaya and Srednyaya Shakuranskaya caves, as well as Tsebel'dinskaya and Simona Kananita caves were only occasionally visited by local people. Both Uapatyh and 85 m wells were not accessible to man without special speleological equipment.

Sampling strategy

In all of the karst caves, quantitative macrobenthos sampling was undertaken. In the long horizontal Abrskila and Golova Otapa caves, the sampling stations were set in transects along the river from the deepest halls to the entrance area. One station was situated above ground not far away from a cave entrance. The transect in Abrskila Cave comprised 11 stations, versus nine in Golova Otapa Cave (Figures 2, 3). In New Athos Cave, which had only artificial outlet to the surface, besides of a sinkhole and cracks in the vaults of some halls, 12 sampling stations were established (Figure 4). The stations thus covered all of the main types of cave waterbodies. Three samples per station were taken in every cave.

In Nizhnaya Shakuranskaya and Srednyaya Shakuranskaya caves, as well as in Tsebel'dinskaya Cave, macrobenthos samples were collected at five stations each. All stations were located along the first 200 meters of the main flooded gallery. In both wells examined and in Simona Kananita Cave, sampling was done in only one station. However, this latter station included all available aquatic habitats.

At all of the stations, the basic hydrological and physical parameters of waterbodies (width, depth, flow rate, type of sediments, silting, and water temperature) were measured. The general characteristics of the collected material are presented in Table 1.

Quantitative samples of macrobenthos were taken with a hemispherical scraper with an area of 0.02 m² and a mesh size of 1 mm. Each sample included organisms pooled from five scrapers collected at a distance of five meters from each other. The sample area was approximately 0.1 m². Macrobenthos was collected from the samples and preserved in 90% ethanol.

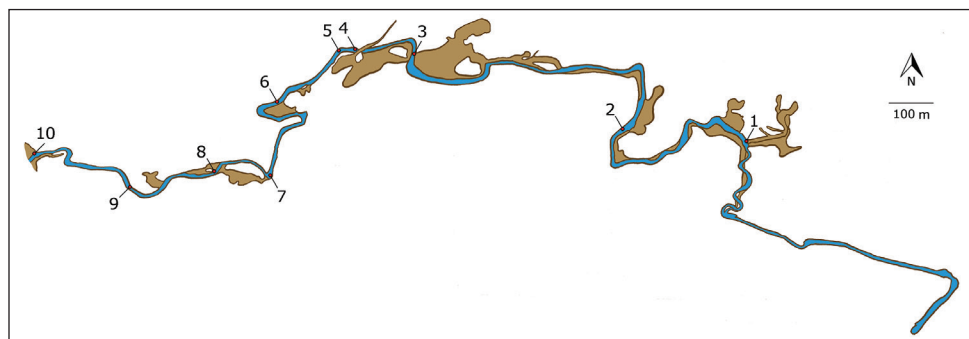


Figure 2. Map of Cave Abrskila. Sampling stations marked by red points (accordingly Benze et al. 1965).

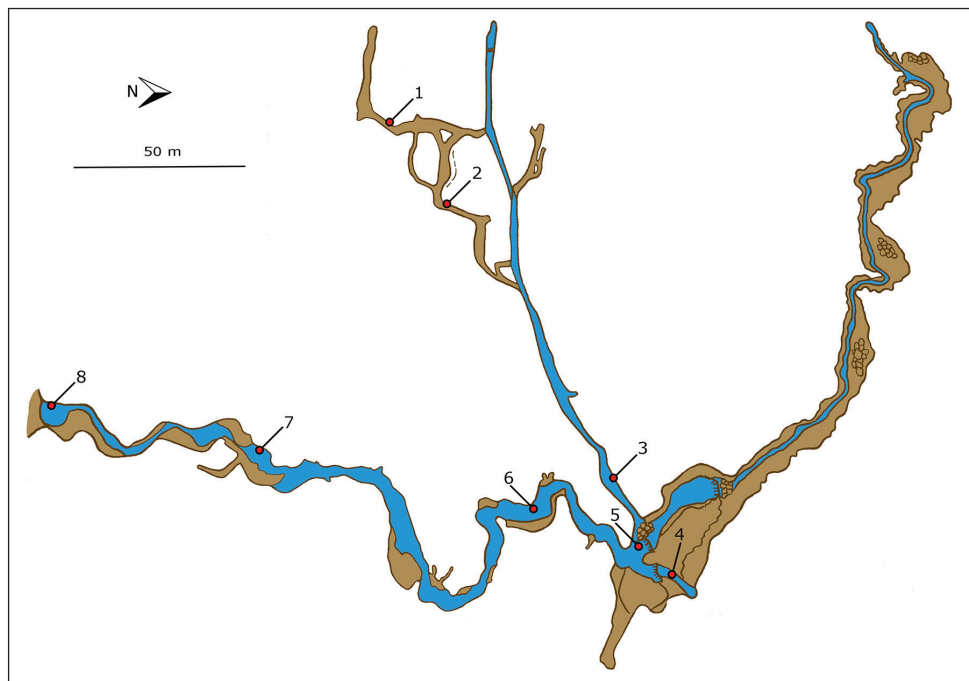


Figure 3. Map of Cave Golova Otapa. Sampling stations marked by red points (accordingly Grigorjan 1973).

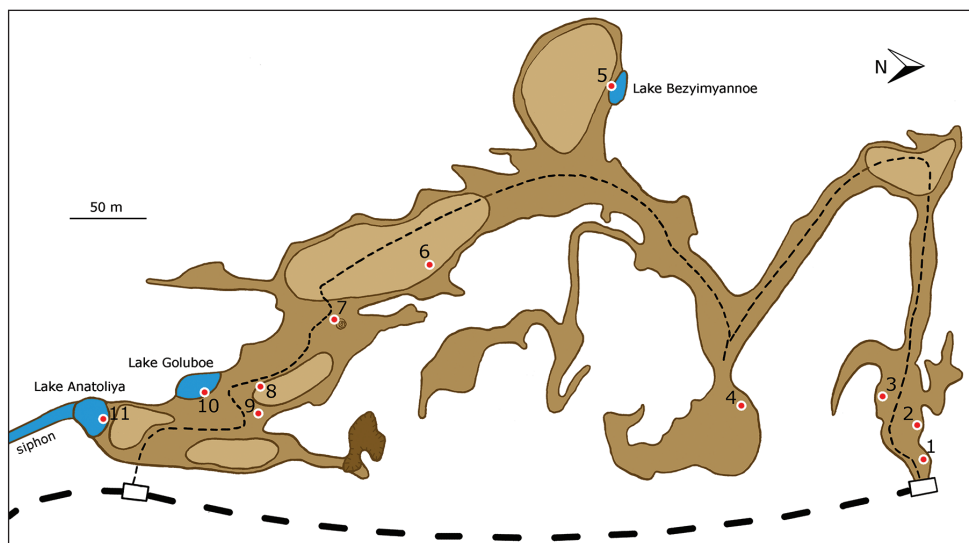


Figure 4. Map of Cave New Athos. Sampling stations marked by red points (accordingly Abhastur 2009).

Table 1. The main characteristics of material from the study caves of Abkhazia, western Caucasus.

Cave/Well	Number of stations	Number of samples (macrobenthos)	Number of taxa
New Athos	12	36	11
Simona Kananita	1	3	1
Nizhnyaya Shakuranskaya	5	15	17
Srednyaya Shakuranskaya	5	15	11
Tsebel'dinskaya	5	15	14
Abrskila	10	30	14
Golova Otapa	9	27	10
Uapatyh	1	3	5
85 m	1	3	2

Taxonomy

For species identifications, reference material representing most of the invertebrate groups and kept in the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) and the Zoological Museum of the Moscow State University was used. Identification keys are only known for the Caucasian members of the genus *Niphargus* (Birstein 1952), the family Typhlogammaridae (Starobogatov 1995), as well as stygobiotic shellfish (Starobogatov 1962). However, due to the high probability of new species encounters, in some cases no exact identification was possible based on these publications.

Statistical analysis

Species diversity. An analysis of species diversity in the study stations used the Shannon-Weaver index (Shannon and Weaver 1963). This index is applicable to assess community structure and takes into account both the number of species in a sample and the extent of their domination (% of total organisms abundance). The Shannon-Weaver index (H) is less dependent on the sample amount. In addition, this index is sensitive to changes in the abundance of rare species.

Comparing of caves faunas

Pairwise similarity of the species composition from different samples in one cave was evaluated using the Czekanowski index (D) for quantitative data (Magurran 2004):

$$D(x, y) = \sum_{i=1} \min(X_i, Y_i), \quad (2)$$

where X_i , Y_i are the proportion of individuals belonging to the each species of all individuals found in samples X and Y , respectively.

To compare the composition of troglobitic faunas, the taxonomic overlap (in %) was calculated between the species lists of different caves. The similarity in faunal composition between the caves was estimated using the Kulczynski index (K) for presence/absence data (Clarke and Gorley 2006):

$$K(x, y) = (a / (a + b) + a / (a + c)) / 2, \quad (3)$$

where a is the number of common species in fauna groups x and y ; and b and c are the numbers of species restricted to one of the groups. This index is independent of joint absence and moderately sensitive to the difference in the total length of the compared lists, making it useful for potentially insufficient or fragmentary data. It is often used for biogeographic analyses of recent faunas (Murray et al. 2002, Azeria 2004).

We applied the multi-dimensional scaling method in PRIMER (version 6) for a graphical presentation of species composition similarity between caves (Clarke and Gorley 2006). This method allows for comparing the objects in a bi-dimensional space so that all distances between points correspond to a certain value. Consequently, this approach helps to visualise the cluster structure of selected stations.

Results

Species richness

In the waterbodies of the study caves, 46 taxa of aquatic invertebrates were found to populate subterranean and epigean habitats. Among them, there are 35 stygobiotic species: four species of Turbellaria (the genera *Dendrocoelum* Ørsted, 1843 and *Dugesia* Girard, 1850), four Oligochaeta species (*Rhyacodrilus* Bretscher, 1901, *Emboloccephalus* Randolph, 1892, *Eisenia* Michaelsen, 1900), one leech (Hirudinea) (*Dina* Blanchard, 1892); 15 species of mollusks: Gastropoda (*Belgrandiella* Wagner, 1928, *Paladilhiopsis* Pavlovic, 1913, *Plagigeyeria* Tomlin, 1930, *Pontohoratia* Vinarski et al., 2014) and Bivalvia (the subgenus *Euglesa* Jenyns, 1832 of *Pisidium* Pfeiffer, 1821); 12 species of crustacean: Isopoda (*Proasellus* Dudich, 1925), Amphipoda (*Niphargus* Schiöde, 1849, *Synurella* Wrzesniowski, 1877, *Zenkevitchia* Birstein, 1940) and Decapoda (*Troglocaris* Dormitzer, 1853). Insect larvae and amphipods typical of epigean waterbodies included only nine species found mostly near the entrances of the caves. Approximately half of the stygobiotic invertebrates (14 species) are presumably new and will be described elsewhere (Table 2).

Taxonomic remarks

The taxonomic status of many stygobiotic groups from the western Caucasus is still questionable. This problem especially applies to Gastropoda. Many species of this class

are described by single shells and assigned to the European genera *Horatia* Bourguignat, 1887, *Plagigeyeria*, and *Paladilhiopsis* (Starobogatov, 1962). The inadequacy of such an approach was previously confirmed, *Horatia*-like mollusks taken as an example (Vinarski et al. 2014). Anatomical studies of other groups of Caucasian subterranean Hydrobiidae show similar results. Thus, all Caucasian species of “*Plagigeyeria*” and some species of “*Paladilhiopsis*” must be defined as new genera on the basis of anatomical features (Palatov and Vinarski 2015).

The situation concerning the Caucasian stygobiotic bivalve subgenus *Euglesa* of *Pisidium* is also complicated. All forms described from caves are close to the crenobiotic *Pisidium* (*Euglesa*) *personatum* Malm, 1855, which is common in epigean waterbodies of the region. The main differences between species lie in shell shape and the organization of the hinge teeth. These characters vary significantly in populations from different caves, so a correct identification cannot be provided at this stage. Furthermore, part of the material of Caucasian stygobiotic bivalves is still unpublished. Starobogatov (unpublished) revised stygobiotic *Euglesa* from the western Caucasus and noted that each cave had at least one endemic species. For example, the bivalve from Tsebel'dinskaya Cave which had previously been identified as *Pisidium cavaticum* Zhadin, 1952 (Starobogatov 1962), was later split into two new endemic species to be described.

Structure of cavernicolous communities

The aquatic faunas of the small caves (Simona Kananita, Uapatyh and the 85 m wells) are poor, and represent taxonomically depauperated derivatives from the faunas of the neighboring larger caves. In this regard, an analysis of the community structure was performed based on the three largest caves, Abrskila, Golova Otapa, and New Athos, that have been studied in detail.

Diversity and abundance. In Golova Otapa and Abrskila, variations in species complexes along the gradient of environment conditions were estimated. In both, the species richness and diversity increased from the most remote stations towards the cave entrance area and had the highest values in the epigean part of the streams (Figure 5). The average species richness varied from three species at the furthestmost station from the cave entrance to 7.5 species in the entrance area, and 10.5 species outside. The average value of the Shannon-Weaver diversity index varied from 0.8 in the distant parts to 1.7 near the entrance, becoming the highest beyond the cave on the surface, 2.7. Variations in the abundance of aquatic fauna along the environmental gradient in Golova Otapa and Abrskila were similar. In both caves, two peaks of abundance were observed (Figure 5). The stygobiotic density in Abrskila reached 220 individuals/m², in Golova Otapa 110 ind/m². The numbers at the epigean stations were close to or exceeded that in the cave entrance areas.

A different situation was observed in New Athos that had no natural horizontal gallery with an entrance to the surface. This cavity offered virtually no conditions for

Table 2. The list of stygobiontic fauna from the examined caves of Abkhazia. (Caves: 1 — New Athos, 2 — Simona Kananita, 3 — Nizhnyaya Shakurskaya, 4 — Srednyaya Shakurskaya, 5 — Tsebel'dinskaya, 6 — Abrskila, 7 — Golova Otapa, 8 — Well Upatykh, 9 — Well 85 m).

Species	Cave								
	Gudautsky region		Gulrypskysky region			Ochamchirsky region			
	1	2	3	4	5	6	7	8	9
Turbellaria									
<i>Dugesia</i> sp. 1							+	+	
<i>Dugesia</i> sp. 2			+	+					
* <i>Dendrocoelum</i> sp. 1	+								
* <i>Dendrocoelum</i> sp. 2					+				
Oligochaeta									
* <i>Rhyacodrilus</i> sp. 1						+			
* <i>Rhyacodrilus</i> sp. 2			+	+					
<i>Embolocephalus velutinus</i> (Grube, 1879)				+	+				
<i>Eisenia</i> sp. 1							+		
Hirudinea									
* <i>Dina</i> sp. 1						+			
Gastropoda									
* <i>Belgrandiella</i> sp. 1						+			
<i>Belgrandiella abchasica</i> Starobogatov, 1962			+		+				
<i>Paladilhiopsis schakuranica</i> Starobogatov, 1962			+		+				
<i>Paladilhiopsis shadini</i> Starobogatov, 1962	+								
* <i>Paladilhiopsis</i> sp. 1						+			
* <i>Paladilhiopsis</i> sp. 2							+		
* <i>Paladilhiopsis</i> sp. 3			+	+					
<i>Plagigeyeria horatieformis</i>			+						
* <i>Plagigeyeria</i> sp. 1			+						
* <i>Plagigeyeria</i> sp. 2							+		+
<i>Pontohoratia birsteinii</i> (Starobogatov, 1962)			+		+				
<i>Pontohoratia smyrni</i> Vinarski et al., 2014	+								
Bivalvia									
<i>Euglesa</i> cf. <i>cavatica</i>			+	+	+				
<i>Euglesa liovushkini</i> Starobogatov, 1962			+	+	+				
<i>Euglesa</i> sp. 1						+			
Isopoda									
* <i>Proasellus</i> sp. 1	+								
Amphipoda									
* <i>Niphargus</i> sp. 1	+	+							
<i>Niphargus iniochus</i> Birstein, 1941			+		+				
<i>Niphargus inermis</i> Birstein, 1940			+	+	+	+	+		
<i>Niphargus ablaskiri</i> Birstein, 1940	+					+	+		+
* <i>Synurella</i> sp. 1	+								
<i>Zenkevitchia yakovi</i> Sidorov, 2015			+	+	+	+	+	+	
* <i>Zenkevitchia</i> sp. 1	+								
Decapoda									
<i>Troglocaris ablaskiri</i> Birstein, 1939						+	+	+	+

Species	Cave								
	Gudauskys region			Gulryphshsky region			Ochamchirsky region		
	1	2	3	4	5	6	7	8	9
<i>Troglocaris fagei</i> Birstein, 1939	+								
<i>Troglocaris osterloffi</i> Juzbaschjan, 1940			+	+	+				
Number of species on the station	1.6	1	3.2	2.9	3.2	2.7	3.4	3	3
Total number of species	9	1	14	9	11	9	8	3	3

* — species are still to be described.

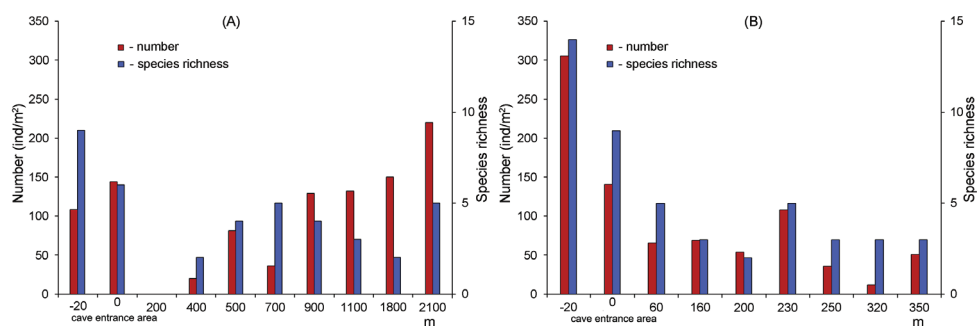


Figure 5. Spatial variations in abundance and species richness of the fauna along the gradient of subterranean environmental conditions. (X-axis: 0 — the cave entrance area, negative values — epigean zone, positive values — cave zone). **A** Cave Abrskila **B** Cave Golova Otopa.

studying a gradient of environmental conditions, nor did it allow for an evaluation of the distance variations in species complexes to be made. The samples collected in New Athos were located near the touristic trails and in halls further away making possible the study of the human impact. The highest number of hydrobionts was found in Goluboe, Anatolija and Bezymjannoe lakes, which all lie away from the tourist trails (abundance 100–150 ind/m²; the average number of species per station, 5.5). On the contrary, in waterbodies located near touristic trails, stygobionts were almost absent. Only some of them occurred in the halls with limited access by tourists, where the number of organisms reached 30 ind./m², the average number of species per station being 2.5.

Types of species complexes. Two main ecological-faunistic complexes were distinguished in the waterbodies of Abrskila, Golova Otopa, and New Athos caves.

A. Complex of amphibiotic insects from the cave entrance area. This community was characterized by the predominance of epigean organisms both in abundance and species richness. Larvae of amphibiotic insects formed the bulk of those communities (50–90% of the total fauna). Stygobionts found there must have been driven from the remote parts of the caves. The dominants were represented by epigean mayflies (Ephemeroptera), *Electrogena zimmemanni* Sowa, 1984, *Baetis (Rhodobaetis) cf. gemellus*, and the Amphipoda, *Gammarus komareki* Schäferna, 1922 (> 50% of the total abundance). The subdominants were caddisflies (Trichoptera), *Notidobia forsteri* Malicky, 1974 and *Lithax incanus* (Hagen, 1859). The number of species totaled 14. The

similarity estimated by the Czekanowski index (D) within this type of species complex was 0.62 ± 0.13 .

Individual specimens of stonefly larvae *Capnia* sp. (Plecoptera), were found in Abrskila Cave on a piece of wood approximately 400 meters from the entrance, in the zone affected by artificial light. This suggests a principal capability of epigean fauna for penetrating deep into caves with some extraneous substrates.

B. *Complex of stygobionts* in remote (> 10 m) parts of caves.

In Abrskila and Golova Otapa, the density of stygobiotic organisms gradually increased with distance from the entrance. However, the distribution of species was highly heterogeneous along the subterranean part: spots of high abundance are interspersed with areas with few specimens. Local aggregations of stygobionts seem to be in places where water seeps into the cave. The main dominant species is *Troglocaris ablaskiri* Birstein, 1939, up to 100% of the total abundance. The subdominants are *Paladilhiosis* (Gastropoda) and *Niphargus ablaskiri* Birstein, 1940 (Amphipoda). The number of species totals eight. The average similarity of the samples within the species complex is high ($D = 0.75 \pm 0.23$).

In New Athos, the complex of stygobiotic fauna is only present in the large lakes, dominated by the shrimp *Troglocaris fagei* Birstein, 1939, and the amphipods *Niphargus ablaskiri* and *Zenkevitchia* sp. 1 (67–100% of the total abundance). In other water bodies, hydrobionts are scarce and mainly represented by *Niphargus ablaskiri*. The number of species totals nine. The similarity within the species complex is 0.61 ± 0.12 .

Successions in the species complexes in the streams of Abrskila and Golova Otapa are shown in Figure 6. The distributions of the dominant *Troglocaris ablaskiri* and amphibiotic insects are clearly complementary.

Comparative analysis of the faunas

A comparative analysis of the stygobiotic invertebrate fauna of nine caves from central Abkhazia was conducted. These caves are located in three river valleys. Caves of the Ochamchirsky region (Abrskila, Golova Otapa, Upatyh and the 85 m wells) are located within the water catchment area of Mokvi River, caves of the Gulrypshsky region (Nizhnyaya Shakuranskaya, Srednyaya Shakuranskaya and Tsebel'dinskaya) in the valley of Kodori River, while caves of the Gudaitsky region (New Athos and Simona Kananita) in the valley of Psyrtskha River. The distance between the most separated caves, those from the Gudaitsky and Ochamchirsky regions, is approximately 60 km (Figure 1).

Using the Kulczynski similarity index, the stygobiont lists of the investigated caves can be seen as being very different from one another ($K = 0.12 \pm 0.15$). However, when we consider the species lists from caves located within the same river valley and lying not more than 2–3 km apart, their faunas are quite similar ($K = 0.77 \pm 0.08$). This applies to Abrskila, Golova Otapa, and two adjacent wells located in the Mokvi River valley. Another group of similar cave faunas is in both Shakuranskaya and Tsebel'dinskaya. However, the subterranean faunas of neighboring river valleys lying

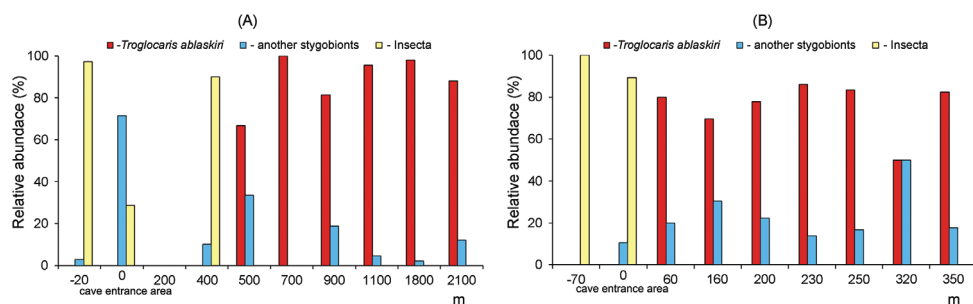


Figure 6. Spatial variations in relative abundance of main invertebrates groups along the gradient of subterranean environmental conditions. (X-axis: 0 — the cave entrance area, negative values — epigean zone, positive values — cave zone). **A** Cave Abrskila **B** Cave Golova Otapa.

at a distance of several dozen kilometers show no more than 12% of shared species from the total list, for example the species complexes of the Gulrypshsky and Ochamchirsky regions. A comparative analysis of the species lists based on the Kulczynski index demonstrates a clear separation of the faunas into three groups, each corresponding to its own river valley (Figure 7).

Estimating the distribution of the main taxonomic stygobiont groups shows that species of cavernicolous shrimps, gastropods, turbellarians, and oligochaetes are highly local in range (Table 2). In neighboring caves, the species lists of these taxonomic groups sometimes overlap completely, but in caves separated from each other by 30 km or more, common species are absent. In contrast, on average, the Amphipoda species appear to be distributed more widely. Three of them (*Zenkevitchia yakovi* Sidorov, 2015, *Niphargus iniochus* Birstein, 1941 and *N. ablaskiri*) are found in cave systems of two of the three river valleys concerned. The Amphipoda fauna in the caves from the Gulrypshsky and Ochamchirsky regions overlap by 50%. At the same time, all of these amphipod species are regional endemics of the northwestern Caucasian karst formations.

Discussion

Specificity of stygobiotic fauna of studied region

Taxonomic structure. A list of the currently known stygobiotic multicellular invertebrates from the western Caucasus totals nearly 110 species (Kniss 2001, Shumeyev 2008, Sidorov 2014, Sidorov et al. 2015a, b, Vinarskiy et al. 2014, Barjadze et al. 2015). This fauna is highly specific and contains more than 90% of presumed endemics not yet found outside the region. The composition of groundwater invertebrates of the western Caucasus includes almost all of the main subterranean groups typical of the Mediterranean: Turbellaria, Nematoda, Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Crustacea (Copepoda, Ostracoda, Syncarida, Amphipoda, Isopoda, and Decap-

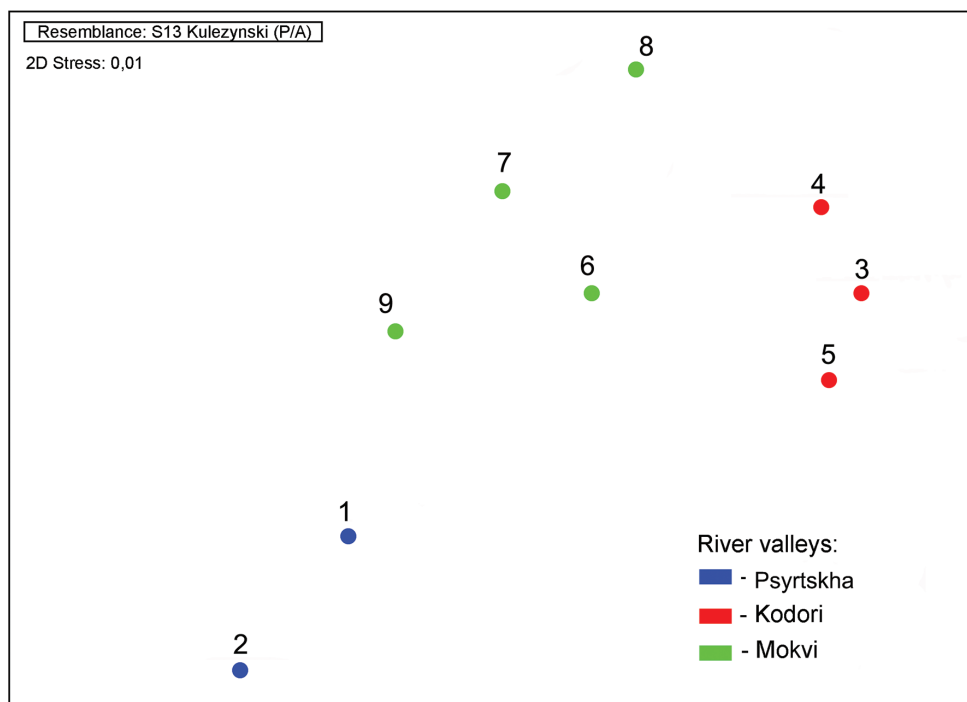


Figure 7. Two-dimensional ordination with superimposed clusters of the stygobiotic faunas from different caves, based on Kulczynski similarity index. River valleys are shown by different colors. Caves: **1** New Athos **2** Simona Kananita **3** Nizhnyaya Shakuranskaya **4** Srednyaya Shakuranskaya **5** Tsebel'dinskaya **6** Abrskila **7** Golova Otapa **8** Well Upatyh **9** Well 85 m.

oda) (Kniss 2001). From amongst the cavernicolous taxa of higher taxonomic rank, the documented fauna of the western Caucasus lacks sponges, cnidarians, polychaetes, several groups of crustaceans (Cladocera, Thermosbaenacea, Mysidacea), as well as aquatic insects with full development found in the hypogean waters of Balkan (Ivković et al. 2013, Jalžić and Pavlek 2013, Van Damme and Sinev 2011), Apennine (Morselli et al. 1995), and Iberian (Trieno De Figuerono and Lopez-Rodrigues 2010) peninsulas. In addition, the Syncarida fauna of the western Caucasus is very scarce, represented by only two genera, as opposed to the Balkan and Iberian peninsulas, with six and eight genera, respectively (Camacho and Valdecasas 2008).

In our material, all main groups of the Caucasian stygobionts were found, except Syncarida. The number of stygobiotic taxa totals 35. The most diverse are Amphipoda (seven species) and Gastropoda (the family Hydrobiidae, 12 species), which are dominant in the groundwater of the western Caucasus. Comparatively high rates of species richness of Amphipoda and Hydrobiidae are typical of some other Mediterranean regions, such as Croatia (Jalžić and Pavlek 2013).

At present, information on the taxonomic composition of the western Caucasian stygobiotic fauna is far from complete. New research in even readily accessible and

regularly visited caves alone is deemed to inevitably increase the list of cavernicolous taxa. In the western Caucasus, biological studies have hitherto been carried out in at least 200 caves (Kniss 2001), but actually the region harbors several thousand caves (Dublyansky et al. 1987).

Spatial distribution of abundance. In the Abrskila and Golova Otapa caves, two peaks of abundance were distinguished. The first, which corresponded to the parts of the caves that lay especially far from the entrances (> 200 m), was due to the high numbers of the shrimp, *Troglocaris ablaskiri*. This species showed very high numbers locally (200 ind./m^2). Such patchiness in some invertebrates (Insects) distribution in caves seemed to be confined to places where water, enriched with particulate organic matter, seeped inside the cave from the surface (Souza-Silva et al. 2011). The second peak of abundance was observed in the cave entrance area and was primarily associated with the development of epigean amphibiotic insects. In New Athos Cave, high stygobionts abundance was observed in the large lakes; no epigean aquatic organisms were found inside the cave. A clear spatial division of the abundance of epigean and stygobiotic species is long known to be typical of caves of the western Caucasus (Birstein 1950).

The stygobiotic fauna of the Abkhazian study caves showed very high abundance rates, as a rule. This is especially clearly visible in large flooded cave systems.. However, in many caves of the Balkan Peninsula (Jalžić and Pavlek 2013) and in caves of Brazil (Simões et al. 2015), the numbers of troglobionts average $10\text{--}20 \text{ ind./m}^2$. Due to the low fauna abundance, quantitative sampling is often ineffective. In such cases, only qualitative material is collected by trapping, scraping, and manually using forceps (Halsel and Pearson 2014, Simões et al. 2015). Therefore, the central Abkhazian caves, which are distinguished by a highly abundant stygobiotic fauna, are unique as testing grounds for analyzing the variability of quantitative characteristics of cave communities.

Factors determining the structure of troglobiont assemblages

The structure and the hydrological regime of caves. The species richness and abundance of stygobionts inhabiting the studied caves might to be dependent on the morphology: size, width of entrance holes and subterranean landscape. The highest diversity was in long caves with large cave entrance areas such as the Abrskila, Golova Otapa, Nizhnyaya Shakuranskaya, Srednyaya Shakuranskaya, and Tsebel'dinskaya. The fauna of these caves included 8–14 species. The richest stygobiotic fauna (> 10) was observed in Nizhnyaya Shakuranskaya and Tsebel'dinskaya caves. In contrast, no more than three species were found in small caves with vertical or narrow horizontal entrances (well Uapatyh and Cave Simona Kananita). This is in agreement with a previously reported positive correlation between species richness and cave volume (Culver et al. 2004, Matheus et al. 2015, Souza-Silva et al. 2011).

Besides this, the community structure significantly depended of the hydrological type of water bodies and heterogeneity in aquatic habitats. Species richness is also

higher in the caves with streams and rivers in comparison with those with stagnant ponds (Simões et al. 2015). Firstly, this can be accounted for by high air-humidity levels preferred by most of the troglobionts¹. Another reason is the availability of organic matter, which is deposited by water streams and consumed by troglobionts (Souza-Silva et al. 2011). However, we found no relationship between the number of stygobiotic species and the hydrological type of water basins.

Anthropogenic pressure. Preliminary assessments of the anthropogenic influences were made for Abrskila, Golova Otapa and New Athos caves. The faunas of the small caves were too poor for comparative purposes.

In the entrance areas of Abrskila and Golova Otapa caves, stygobionts were fully replaced by epigeal species. In Cave Abrskila Cave, apparently due to artificial light, larvae of epigeal Plecoptera, *Capnia* Pictet, 1841, were found approximately 400 m far from the entrance. These larvae inhabited a piece of rotten wood probably brought inside during the construction of tourist trails. *Troglocaris* were completely absent from the illuminated part of the cave, but remained rather abundant in the dark parts. Earlier, this shrimp occurred throughout the whole cave except the entrance area (cave owner Adleba V, pers. comm.). Thus, both artificial illumination and external substrates brought from the surface served as good examples of anthropogenic impact promoting amphibiotic insect larvae to moving upstream from the surface to deep in caves, in colonizing the subterranean habitats, and in replacing the stygobionts.

In New Athos Cave, the anthropogenic influence was remarkable near the walking trails, especially close to basins. Many of coins left behind by tourists could change the hydrochemical composition of water. On the other hand, cleaning up the speleothems from microalgae growing in the lighted places led to intrusions of cleansers into rimstone pools and puddles. This may be the reason of macrofauna was virtually absent from all basins near the touristic trails.

A number of methods for the assessment of anthropogenic pressure have been developed for marine or freshwater ecosystems, but these are not applicable to subterranean waters. The first criterion of a cave community disturbance is reduction of troglobiont abundance (Gutjahr et al. 2013). High-level human disturbances result in decreased abundance, observed in several microponds in New Athos, as well as in some parts of the river in Abrskila. Communities inhabiting caves are extremely sensitive to changes in air humidity, temperature, and illumination (Gutjahr et al. 2014). Even modest actions may cause significant changes in the communities. Therefore, the conservation of an endemic fauna requires special zones of restricted or no access and reduced nature management in separate caves or in whole karst massifs (Georgiev 2014).

The dispersal ability of taxa. A comparative analysis of the speleofauna of the study regions of Abkhazia revealed high degrees of similarity in composition between the caves from the same river valley, about 3 km apart from each other. In contrast, the species lists from the adjacent valleys varied greatly. Stygobiotic taxa differ notice-

¹ Here, the term “troglobionts” means subterranean animals *in sensu lato*, while “stygobionts” inhabit the subterranean waters.

ably by their dispersal capacities. The slow-moving Hydrobiidae gastropods inhabiting the surface of hard substrate, as well as relatively large benthoplanktonic shrimps (*Troglocaris*), were endemics to individual cave systems. All species of these taxa were found in some adjacent caves close by. Local endemism of *Troglocaris* and some mollusks (*Belgrandiella*, *Paladilhiopsis*, *Pontohoratia* etc.) has also been found in caves of the Balkan Peninsula (Franjević et al. 2010, Jalžić and Pavlek 2013). Therefore, local endemism is typical of this stygobiotic groups. The ranges of these taxa often do not exceed several kilometers. In contrast, the subterranean Amphipoda were relatively widely distributed. Among them, two species of the genus *Niphargus* and one of the genus *Zenkevitchia* were the only stygobionts recorded in the different cave systems of Abkhazia. This species inhabit entire karst massifs at least of a few dozen kilometers in span (Sidorov 2014, Sidorov et al. 2014, Skalski 1980). Amphipoda, as very common inhabitants of underground waters and hypotelminorheic habitats, show increased dispersal capacities (Culver and Pipan 2008, Culver et al. 2006, Marmonier et al. 1993).

Amphipods are often considered to be wider distributed across the karst regions than other stygobiotic taxa (Kniss 2001, Jalžić and Pavlek 2013). Recently, genetic analysis of the *Niphargus* genus revealed many cryptic species (Trontelj et al. 2009). Most of these species have narrow local ranges, but about 25% of them have been found in caves 100 km or more apart (Lefébure et al. 2006, Lefébure et al. 2007, Trontelj et al. 2009). The latter is peculiar to *N. arbiter* Karaman G., 1984, *N. fontanus* Bate, 1859, *N. illidzensis* cf. *dalmatinus* 1, *N.* cf. *longicaudatus* 2, *N. salonitanus* Karaman S., 1950, and to some cryptic lineages of *N. virei* Chevreux, 1896 and *N. rhenorhodanensis* (Schellenberg, 1937). Thus, a narrow local endemism is not obligate for stygobiotic amphipods.

Conclusion

Further biospeleological investigations are necessary to reveal more detailed patterns in the distribution and diversity of the stygobiotic fauna of the western Caucasus. More research will almost certainly extend the ranges of some species. On the other hand, molecular analysis may help in detecting additional taxa with restricted distributions. Estimating the real species richness and ecological preferences of stygobionts may lay the grounds for tracing consistent patterns of cave fauna formation in the region.

An analysis of the stygobiotic fauna from nine caves of central Abkhazia, western Caucasus, provides the following ecological observations:

- (1) In the studied caves were found 35 species of stygobionts: Turbellaria (4 species), Oligochaeta (4), Hirudinea (1); Gastropoda (12), Bivalvia (3); Crustacea (12). Of these, 15 species are still to be described as new to science.
- (2) In the subterranean rivers of Abrskila and Golova Otapa caves, the species richness and faunal diversity was revealed as increasing from the most remote stations inside the caves to the entrance areas, reaching the highest values in the epigean area. In

both caves, two abundance peaks of aquatic invertebrates were registered: one in the entrance area, associated with the development of amphibiotic insect larvae, the other one in the depth of the caves due to the high numbers of stygobionts. In Cave New Athos, the highest species richness and fauna abundance were observed in large lakes.

- (3) In caves Abrskila and Golova Otapa, two ecofaunistic complexes with complementary distributions were revealed. The first is a complex of amphibiotic insects in the cave entrance area, the second a complex of stygobionts in the remote parts of the caves. In Cave New Athos, which is devoid of an entrance area, only a stygobiotic faunistic complex was found.
- (4) The most significant anthropogenic impact on stygobionts was observed in Cave New Athos, where almost all waterbodies located near the excursion trails were devoid of a macrofauna. Hydrobionts mostly inhabited large lakes which were distant from the touristic routes. Both artificial illumination and extraneous material brought inside from the surface are the anthropogenic impacts that help epigean amphibiotic insect larvae colonize the subterranean habitats.
- (5) The stygobiotic faunas of the study karst caves are clearly divided into three groups, according to the number of river valleys in which they are located. The fauna compositions of the stygobionts from caves situated within one river valley are similar to no less than 50%. The compositions of the stygobionts from caves located in neighboring valleys (30 to 60 km apart) contain not more than 12% of shared species.
- (6) In the stygobiotic shrimps and Gastropoda (Hydrobiidae) that inhabit stone surfaces, local endemism is typical and their ranges often fail to span more than a few kilometers. In contrast, the stygobiotic Amphipoda that penetrate the ground waters show high dispersal capacities and are widely distributed between cave systems within individual karst massifs.

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