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# SURVIVAL OF THE EPIGEAN DENDRODRILUS RUBIDUS TENUIS (OLIGOCHAETA: LUMBRICIDAE) IN A SUBTERRANEAN ENVIRONMENT

# PREŽIVETJE POVRŠINSKEGA DEŽEVNIKA DENDRODRILUS RUBIDUS TENUIS (OLIGOCHAETA: LUMBRICIDAE) V PODZEMELJSKEM OKOLJU

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**Abstract** UDC 595.142:551.442(497.4Huda luknja)
***Tone Novak, Csaba Csuzdi, Franc Janžekovič, Tanja Pipan, Dušan Devetak & Saša Lipovšek: Survival of the epigean Dendrodrilus rubidus tenuis (Oligochaeta: Lumbricidae) in a subterranean environment***
The peregrine earthworm *Dendrodrilus rubidus tenuis* was regularly sampled in a percolating water drip originating in a habitat inaccessible to humans in the Huda luknja pri Doliču cave, Slovenia. The reconstruction of this habitat includes both a larger passage with bat colonies supplying wet bat guano sediments, on which the earthworms feed, and narrow channels, which drain water from this passage into the sampling drip. Fresh guano is deposited in autumn shortly before the earthworms become inactive. Then, it is exploited by moulds and additionally depleted by water washing out the nutrients before the earthworms reactivate in spring. Thus, this is a rather poor food resource for the earthworms. Despite this, and apart from their short size and delayed maturation, no other disturbance or damage was found caused by malnutrition, which was confirmed in individuals submitted to starvation in captivity. We suggest that *Dd. r. tenuis,* which shows neither disturbance from nor adaptation to living in a subterranean environment, can subsist there because of its euryoecious character. Nevertheless, in temperate climates, this is rather a harsh habitat for this earthworm.
**Keywords:** dripping water, earthworms, karstic cave.

**Izvleček** UDK 595.142:551.442(497.4Huda luknja)
***Tone Novak, Csaba Csuzdi, Franc Janžekovič, Tanja Pipan, Dušan Devetak & Saša Lipovšek: Preživetje površinskega deževnika Dendrodrilus rubidus tenuis (Oligochaeta: Lumbricidae) v podzemeljskem okolju***
V vzorcih curka prenikajoče vode v Hudi luknji pri Doliču je bila peregrina vrsta deževnika *Dendrodrilus rubidus tenuis* redno prisotna. Curek doteka iz neznanega, človeku nedostopnega habitata. Na osnovi zbranih podatkov sklepamo, da sestoji ta habitat iz dveh habitatnih tipov. Prvi je večji rov s kolonijami netopirjev, ki proizvajajo gvano, s katerim se deževniki hranijo. Drugi je sistem ozkih kanalov, po katerih odteka voda iz tega rova v vzorčevani curek. Netopirji proizvedejo sveže gvano jeseni, malo preden deževniki prenehajo z aktivnostjo. Nato gvano izkoristijo plesni, dodatno se osiromaši zaradi kapljajoče vode, ki iz njega izpere nutriente, preden se spomladi deževniki reaktivirajo. To je torej zanje dokaj reven vir hrane. Kljub temu razen njihove kratke dolžine in poznega spolnega dozorevanja ne kažejo nobene motnje ali poškodbe, ki bi bile posledica slabe prehrane; enako smo ugotovili pri osebkih v ujetništvu. Predlagamo razlago, da lahko *Dd. r. tenuis,* ki ne kaže niti motenj niti prilagoditev na življenje v podzemeljskem okolju, tam preživi zaradi svojega evriekega značaja. Kljub temu so razmere v podzemeljskem okolju v območjih z zmernim podnebjem za to vrsto dokaj ostre.
**Ključne besede:** deževniki, kraška jama, prenikajoča voda.

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## INTRODUCTION

A dozen lumbricid species have been reported exclusively from caves, such as *Allolobophora cryptocystis* Černosvitov, 1935, *Helodrilus kratochvili* (Černosvitov, 1935), and *H. mozsaryorum* (Zicsi, 1974), mostly as single finds. Some have later been recognized as accidental individuals of epigean species (Černosvitov 1939, Pop 1968, Zicsi 1974, Dumnicka & Juberthie 1994). In central and northern Slovenia, 17 lumbricid species and one subspecies have been occasionally recorded in caves and artificial tunnels (Mršić 1990a, 1990b; Novak 2005). These are *Allolobophora leoni* (Michaelsen, 1891), *Aporrectodea rosea* (Savigny, 1826), *Ap. smaragdina* (Rosa, 1892), *Dendrobaena byblica* (Rosa, 1893), *D. octaedra* (Savigny, 1826), *Dendrodrilus r. rubidus* (Savigny, 1826), *Dd. r. tenuis* (Eisen, 1874), *Eisenia lucens* (Waga, 1857), *E. spelaea* (Rosa, 1901), *Eiseniella t. tetraedra* (Savigny, 1826), *Lumbricus castaneus* (Savigny, 1826), *L. r. rubellus* Hoffmeister, 1843, *Octodrilus transpadanus* (Rosa, 1884), *O. meroandricus* Mršić, 1985, *Octodriloides kamnensis* (Baldasseroni, 1919), *O. zupancici* Mršić, 1987, *Octolasion l. lacteum* (Örley, 1881) and *Proctodrilus antipae* (Michaelsen, 1891). Zicsi (1974) declared *H. mozsaryorum* from the famous Hungarian cave Baradla to be a troglobiotic aquatic species, although he did not exclude the possibility of its finding in surface habitat in the future. However, despite the intensive research carried out in the Aggtelek karstic region, *H. mozsaryorum* has not been found in surface habitats; therefore, the troglobiotic nature of this species was later corroborated (Zicsi *et al.* 1999). Beside this species, no other exclusively cavernicolous lumbricid earthworm is known so far (Dumnicka *in lit.*).

Hypogean communities of epigean earthworm species can always be found in caves among accumulations of various types of organic matter, like torrential deposits and the sediments of underground watercourses and guano (Dumnicka & Juberthie 1994; Sambugar & Sket 2004; Dumnicka 2005). After the discovery of the importance of epikarst for subterranean biota (Pipan 2005), intensive investigations of this habitat started in Slovenia and the USA (Pipan *et al.* 2006, 2008; Pipan & Culver 2005, 2007a, 2007b; Culver & Pipan 2008, 2014). During similar investigation of the fauna in percolating water drips in a cave in northern Slovenia, we found a viable population of *Dendrodrilus rubidus tenuis* (Lumbricids in Pipan *et al.* 2008). Cave-dwelling, troglophile populations of this species have also been reported from other caves (McAlpine & Reynolds 1977; Reeves *et al.* 1999). Our present scope is to analyze the main parameters allowing this epigean earthworm species to survive in a subterranean habitat, and to reconstruct the environmental features of this hypogean habitat inaccessible to humans. For this purpose, we considered both the dripping water data, and the physical condition of the *Dendrodrilus* specimens found in the drip. We hypothesized a rich food resource supply in the habitat, which is crucial for the survival of the earthworms.

## MATERIAL AND METHODS

Systematic monthly sampling of water from 12 trickles was carried out in the Medvedji rov passage in the Huda luknja pri Doliču cave (Fig. 1; entrance 46°24´53″ N, 15°10´44″ E, altitude 508 m) from November 2005 till October 2006 (details in Pipan *et al.* 2008). These trickles provided different types of percolating water: a tiny permanent current (min ca. 0.5 dL day$^{-1}$), constant dripping water (0.05–0.2 dL sec$^{-1}$) and drips immediately reacting to precipitation (up to 1.5 dL sec$^{-1}$). Water drips were collected using a funnel 30 cm in diameter into a 15 cm high container of 10 cm diameter, with side overflow holes covered with net (mesh size 60 µm) to retain the animals and solid particles. The water temperature was measured in situ; concentrations of $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^-$, $NO_3^-$ and $SO_4^{2-}$ ions as well as Ca- and total hardness, and conductivity were determined using Standard Methods for the Examination of Water and Wastewater (1989) in the laboratory. The samples of fauna were fixed *in situ* with formaldehyde to a final concentration of 2–3 %. After extraction, the organisms were stored in 70 % ethanol. To provide further information on earthworms, each bat guano accumulation in the cave and the "lumbricid-trickle" in control were additionally inspected for lumbricids 14 times between 2006 and 2009. The fauna of the small water pools formed in guano piles by trickling water was sampled using a spoon.

For light microscopy, three earthworms were treated immediately with gut contents intact, and two were kept alive on a water-gelatine gel, analogous to the water-agar gel method (Pokarzhevskii *et al.* 2000), for four days at 8 °C until the gut content was voided. The earthworms were fixed in 2.45 % glutaraldehyde and

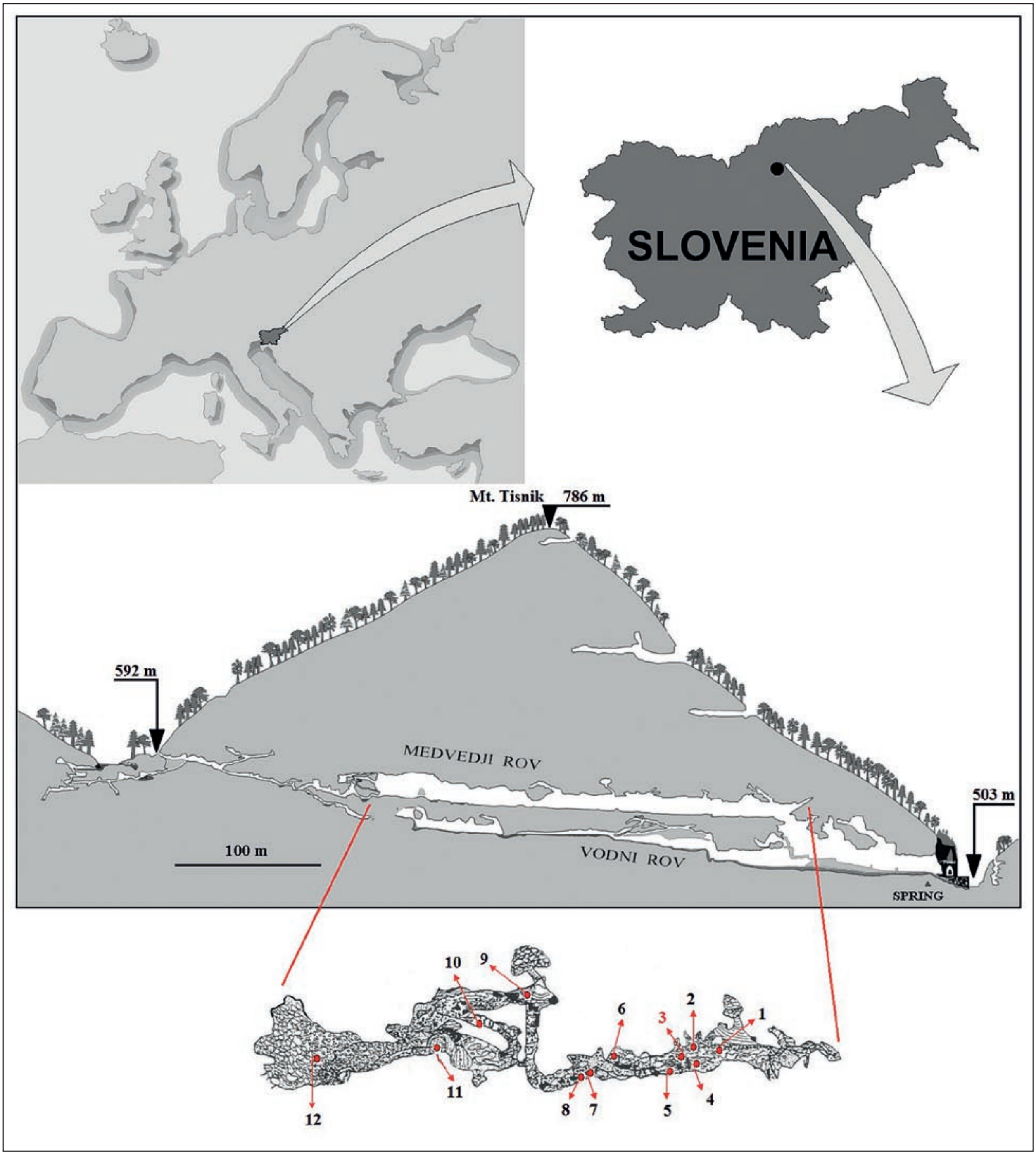


*Fig. 1: Geographical location and the longitudinal section of the Huda luknja cave system with the ground plan of the Medvedji rov passage; from Pipan et al. (2008), partly modified. Courtesy Acta Carsologica.*

2.45 % paraformaldehyde in a 0.1 M sodium cacodylate buffer (pH 7.3), at room temperature for 3 hrs, and at 4 °C for 12 hrs. The samples were washed in a 0.1 M sodium cacodylate buffer (pH 7.3), at room temperature for 3 hrs, postfixed with 2 % $OsO_4$, at room temperature for 2 hrs and dehydrated in a graded series of ethanol (50, 70, 90, 96, 100 %, each for 30 min at room temperature). Tissue samples were embedded in TAAB epoxy resin (Agar Scientific Ltd., Essex, England). Semi-thin sections (5 μm) were cut and stained with 0.5 % toluidine blue in aqueous solution. After staining, the tissue was analyzed with a Nikon Eclipse E800 microscope. 22 individuals were measured for their length, width and number of segments.

Additionally, from February 26th till June 25th 2007, seven earthworms were kept in a refrigerator at 8 °C to provide information about their behavior. The specimens were kept in a 1.5 dL vessel with 2 cm deep water from the trickle, and with a piece of folded toilet paper, half of it projecting above the water surface. No food was added. The worms were inspected once a week (14 observations in total) for their placement selection in or outside the water, and their gregariousness, controlling at the same time their condition to cease the observation immediately, if any sign of disturbance would appear.

Descriptive statistics was used to evaluate the physical and chemical characteristics of the drips, as well as the main morphological characteristics of the worms. A t-test for independent samples was used in testing the differences between the drip with and those without *Dendrodrilus* for each physical and chemical parameter (Legendre & Legendre 2012). The program SPSS 21.0 for Windows was used in the statistical procedures.

## RESULTS

During one-year monthly sampling, we found *Dendrodrilus* specimens in only one of the 12 trickles sampled (No. 3 in Fig. 1) from April till October with an abundance peak in June, while they were absent during winter. Trickle No. 3 (hereinafter *Dendrodrilus* drip) and two other trickles (No. 6, 10) had similar permanent discharge slightly varying throughout the year (ca. 0.05–0.1 dL $sec^{-1}$), but they differed in other parameters (details in Pipan *et al.* 2008). Ca- and total hardness (TH), and conductivity (con.) in the *Dendrodrilus* drip were constantly significantly higher with respect to other drips (Ca: $t=16.10$, TH: $t=17.49$, con.: $t=6.33$, $df=276$, $p<0.0001$) (Fig. 2) in every month of the worm's activity in the hypogean habitat. In other parameters these differences either alternated between the *Dendrodrilus* drip and the other drips (temperature, $K^+$, $Ca^{2+}$, $SO_4^{2-}$), or did not differ significantly (pH, $Na^+$, $Mg^{2+}$). Besides, in the *Dendrodrilus* drip, $NO_3^-$ values drastically increased from August till October, and $Cl^-$ values in September and October (Fig. 2); these values were both significantly different from the other drips ($NO_3^-$ $t=4.84$, $Cl^-$ $t=4.61$, $df=276$, $p<0.0001$).

In the relatively pure water collected in the vessel during continuous percolation, all specimens were alive. During dry periods, a few of them died because the water in the vessel was polluted with fresh bat droppings. Those earthworms that escaped and crawled up the vessel walls above the water survived. After 2006 we collected nine juveniles from the *Dendrodrilus* drip. On May 16, 2009, for the first time, two individuals–one of them adult–were found in a little pool eroded by trickling water in a guano pile a few meters from the trickle, and one juvenile was observed on June 17, 2009 (Fig. 3). Only in this guano accumulation *Dendrodrilus* were found.

In the trickle, 22 individuals measured 15.0±1.7 (12.1–17.0) mm in length and were 1.8±0.2 (1.6–2.0) mm wide, counting 96 (79–105) segments. The adult worm found on 29.5.2007 was 28.0 mm long and had 99 segments.

In the refrigerator, all specimens crept into the submerged, tightly folded paper and were permanently crowded together, with the exception of one or two individuals 1–3 cm away. Whenever the paper was turned upside down, i.e., the immersed earthworms were turned to the air, they crawled into the lower, immersed folds within a minute. While stretching parts of their body, the earthworms elongated these parts 4–5-times their entire length and became correspondingly thinner. They fed on the paper, and all survived four months without any evident disturbance or damage. All specimens were fully fed and of normal appearance with simple, low and wide typhlosole (Fig. 4).

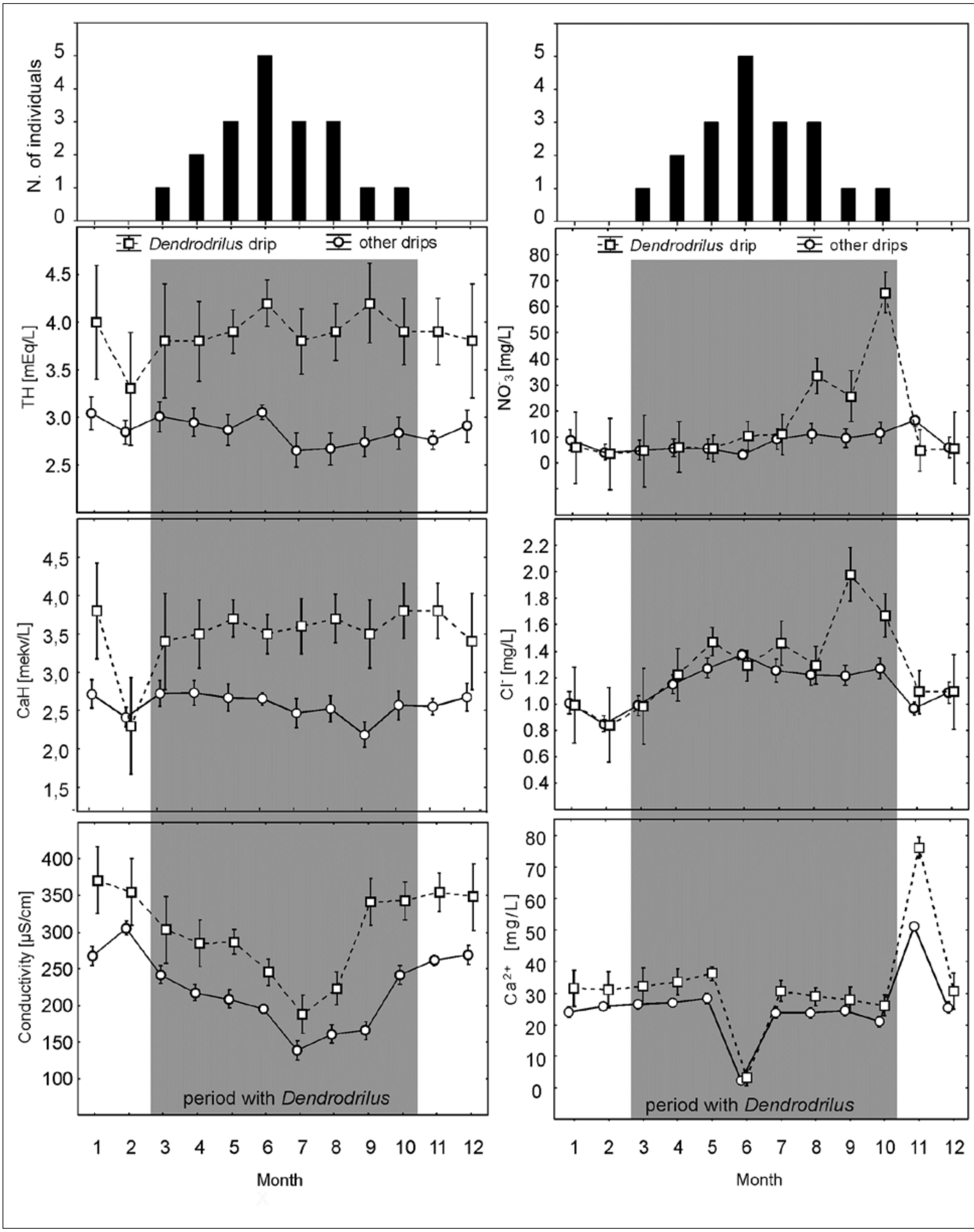


*Fig. 2: Total (TH) and calcium hardness (CaH), conductivity, nitrate ($NO_3^-$), chloride ($Cl^-$) and calcium ($Ca^{2+}$) in the* Dendrodrilus *drip vs. other drips.*

*Fig. 3: Pits made by dripping water in a bat guano pile, where an adult* Dendrodrilus rubidus tenuis *was found.*

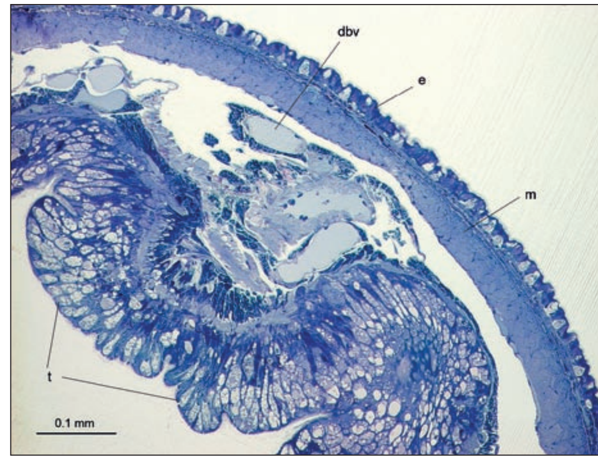


*Fig. 4:* Dendrodrilus rubidus tenuis, *typhlosole in the 40th segment of a representative specimen. e epidermis, m longitudinal muscle layer, dbv dorsal blood vesel, t typhlosole.*

## DISCUSSION

Individuals of *Dd. r. tenuis* retrieved from the dripping water had drifted there from an unknown subterranean habitat above the sampling passage. This terrestrial species most likely preferred a substrate soaked with water. According to chemical analysis of the trickling water, this habitat has two specific properties that are distinct from the other seeps without earthworms. First, it is supplied by annually fluctuating, large quantities of fresh organic matter, the volume of which starts to rise in August, culminating in October. This is evident from the $NO_3^-$ and $Cl^-$ increases, which perfectly match the defecation and urination patterns of the progressively enlarging colony of the Bent-wing Bat, *Miniopterus schreibersii* (Kuhl, 1817), up to 2000 individuals. Most of these bats then suddenly moved to an unknown passage to overwinter and roost; most probably, the trickling water flows from there. Second, the particular drip is characterized by the highest values of Ca- and total hardness as well as conductivity. There could be a few reasons for higher values: 1. Water flowing from the sites with overwintering bats passes through narrow spaces with relatively high $CO_2$ pressure, which in turn enhances the water's dissolving capacity. 2. Longer residence time of the water caught underground for a long period in contact with the limestone bedrock provided higher saturation state, and hence higher values of both hardnesses and conductivity. 3. Presence of other ions such as $Cl^-$ and $NO_3^-$ caused higher equilibrium and consequently higher conductivity. Any of these or their combination could be the reason.

Theoretically, the hypogean characteristics of lumbricid species in carbonate rocks are their small size and a reduction in a number of organs, like testicles, spermathecae etc. (Dumnicka 1986; Dumnicka & Juberthie 1994), the hindrance or reduction of reproductive seasonality and its internal regulation (Rozen 2006), and, presumably, intensive activity of the calciferous glands (Robertson 1936, Canti & Pearce 2003). However, in this case, the reduction of the genital organs could also be due to the well-known parthenogenetic reproduction of *Dd. r. tenuis*. Besides, as a consequence of feeding on poor food resources, more extensive branching of the typhlosole may be expected in specialized hypogean species. Since fresh guano was deposited in the period when *Dendrodrilus* were carrying out their annual activity, they were able to exploit this resource to a limited extent. Later, the guano was exploited by moulds, while percolating water additionally washed out soluble compounds, leaving behind an energy-poor resource for the earthworms in spring. In this population of *Dd. r. tenuis*, small size and the large ratio of immature individuals are considered the direct consequence of malnutrition caused by such impoverishment of the substrate, while no hindrance in development, enlargement of the typhlosole and malformation of or damage to other organs was noticed. Moreover, surviving a four-month period of starvation in captivity without damage demonstrates that *Dd. r. tenuis* – reported to inhabit litter and organically enriched surface soil layers (Hendrix & Bohlen 2002) – is well adapted to poor food resources, as well. Additionally, the capacity for extreme elongation and narrowing may be an adaptation for living in systems consisting of narrow spaces, as can be expected in the rhizosphere and epikarst (Culver & Pipan 2009; Novak *et al.* 2012), or might be an adaptation to exploit more efficiently the water-solved oxygen. Their ability to crawl out of the water demonstrates that *Dd. r. tenuis* can leave an inappropriate habitat, and search for an appropriate microhabitat. Adult *Dd. r. tenuis* do not survive temperatures below 0 °C and do not overwinter in cold climates (Berman *et al.* 2010), while the adult found in May suggests that the species may overwinter in hypogean habitats. This is likely to be the case in Central Europe, where earthworms overwinter deeper in the soil. It is thus either death or overwintering that caused their absence from the samples during winter. All these denote that *Dd. r. tenuis* is a euryoecious rather than a specialized species, which helps in an understanding of its invasive character (cf. Global Invasive Species Database).

## CONCLUSIONS

Most likely, the reconstruction of this sampling site with *Dendrodrilus rubidus tenuis* is as follows: an undiscovered passage with bat guano accumulation somewhere above this particular passage is settled by the earthworms, from where water passes through narrow channels and drips into the passage under investigation. The narrow channels with percolating water function as merely a connecting habitat between the spacious habitat providing the guano and the sampling site in the gallery being investigated. *Dendrodrilus* shows a high tolerance to such energy-poor food resource, but no special adaptation to or damage from living in a subterranean habitat. Although this represents rather a harsh habitat for these earthworms, they can survive in such habitats with bat guano deposits and a permanent water supply.

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