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On the presence of the endemic earthworm *Dendrobaena rhodopensis* (Černosvitov, 1937) in the Balkan Peninsula: biogeographical consideration and conservation status

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Abstract. The purpose of this paper is to summarize all occurrences of the endemic earthworm *Dendrobaena rhodopensis* in the Balkan Peninsula, by compiling bibliographic data and data from our own collecting, in order to present its currently known distribution. During the last 70 years, this species has been recorded from 17 localities in Bulgaria, while it is sporadically present in Montenegro and Serbia. Until now, the northernmost findings of the species have been in the Serbian part of the Western Stara Planina Mts., while its southernmost occurrence was reported from Lefkonas near Serres (Greece). Based on all records on the Balkan Peninsula, it was possible, for the first time, to present graphically the extent of occurrence (EOO) for *D. rhodopensis*. Considering that this species is mostly characteristic of the mountain ranges in the Balkans, we present its possible movement routes throughout the Balkan Peninsula. Current analysis based on the IUCN (2017) Red List Categories shows that *D. rhodopensis* belongs to the endangered category (B2: b iii, iv, v and c iii, iv) on a global level. The presented assessment may be considered as a baseline for further research and re-evaluation.

Key Words: *Dendrobaena rhodopensis*, Balkan Peninsula, earthworms, Rila Mt., distribution

Introduction

Dendrobaena rhodopensis (Černosvitov, 1937) is a broad range Balkan endemic earthworm species (Trakić et al. 2016). It was first described from Bulgaria, Rila Mts. (Kostenec) (Černosvitov, 1937). Later on, it has been found in several localities in Bulgaria (Plisko 1963, Šapkarev 1986, Zicsi & Csuzdi 1986, Delchev et al. 1998, Valchovski et al. 2018a, b), in Montenegro (Karaman & Stojanović 1995, Stojanović & Karaman 2003) and in Serbia (Stojanović et al. 2008, 2013, 2018) (Table 1). One specimen was reported by Šapkarev (1972) from Greece (near the village Lefkonas). This finding has been confirmed by Zicsi & Michalis (1981) and Szederjesi et al. (2017). Therefore, we have included also this record in our discussion.

D. rhodopensis is strictly a mountain species. All individuals have been found at high altitudes from 1098 to 2518 m.a.s.l. (Table 1). These data, together with our new record, indicate that the habitat of the species *D. rhodopensis* is limited to the forest soil of the high mountains.

A recent study on the endemic earthworms (Trakić et al. 2016) reported interesting results concerning the distribution of the endemics in the Balkan Peninsula and on its possible explanation. Unfortunately, there is a lack of knowledge concerning the conservation status of these species. Endemic species are more exposed to threats, therefore the evaluation of their conservation status is crucial in preventing their extinction. On a global scale, the World Conservation Union (IUCN) provides categories which attempt to classify species according to their likelihood of extinction within a given period. Estimations of extinction risk are calculated using extent of occurrence (EOO) or area of occupancy (AOO), which is based on geographic range size. Up to now, only a few attempts exist for assessing the preliminary conservation status for earthworms on the Balkans (Stojanović & Karaman 2006, Stojanović et al. 2008, 2017, Milutinović et al. 2013, 2015). Unfortunately, none of the earthworm species has a legally protected status in the Balkan countries.

On the other side, according to Sutherland (2000) many invertebrates are classified as data-deficient due to the insufficient information for such sensible classification as suggested by the World Conservation Union (IUCN). Moreover, Cardoso et al. (2011) indicated that only 0.3% of the described terrestrial invertebrates have been classified based on the IUCN categories and criteria. Consequently, there is an urgent need to increase the conservation assessment of invertebrates.

The purpose of this paper is to summarize all occurrences of *D. rhodopensis* in the Balkan Peninsula, by compiling bibliographic data and data from our own collecting, in order to present its current known distribution. In addition, important remarks are provided on the current conservation status (according to the 2017 IUCN threat categories and criteria) of *D. rhodopensis* on a global level. An updated distribution map is also drawn.

Material and Methods

Study area

Rila Mt. is the highest mountain range of Bulgaria and the Balkan Peninsula, with its highest peak being Musala (2925 m). Rila is a dome-shaped horst mountain, part of the Balkans' oldest land, the Macedono–Thracian Massif. More than one-third of the mountain is occupied by the Rila National Park in the south-west of the country, established in 1992. The national park overlaps with the territory of four nature reserves: Parangalitsa, Central Rila Reserve, Ibar and Skakavitsa.

The study was carried out in the Parangalitza Reserve, which is situated on the southwestern side of the Rila Mountain. It is entirely included in the Rila National Park. The reserve was established in 1933, and it was declared a biosphere reserve in 1977. It covers an area of 1509 ha from 1400 to 2485 m.a.s.l. and includes about 400 ha of the oldest conifer forest in Bulgaria. Other habitats include subalpine and alpine vegetation.

Methods

Data on the distribution of the species were obtained from the literature and from fieldwork. As far as possible, all published and

Table 1. Geographic information on a new locality of *Dendrobaena rhodopensis* and the data from literature.

Country	Locality	No. of individuals	Altitude	Coordinates	Autor and year of notice
Bulgaria	Kostenets (Rilo-Rodopi Mts.)	9 exp.	?	42°18′N, 23°52′E	Černosvitov 1937
	Monastery of Rila Mt.	2 exp.	1147 m	42°08′N, 23°20′E	Šapkarev 1986
	Borovec-Musala	3 exp.	1800 m	42°16′N, 23°36′E	Šapkarev 1986
	Teteven (Balkan Mts.)	1 exp.	1800 m	42°55′N, 24°16′E	Plisko 1963, Delchev et al. 1998
	Vitosha	2 exp.	2290 m	42°34′N, 23°17′E	Plisko 1963
	Rila Mt.- Maliovitsa	6 exp.	1600 m	42°17′N, 23°36′E	Plisko 1963, Delchev et al. 1998
	Borovec-Musala	12 exp.	1500 m	42°16′N, 23°36′E	Zicsi & Csuzdi 1986
	Borovec-Musala	6 exp.	1900 m	42°16′N, 23°36′E	Zicsi & Csuzdi 1986
	Borovec-Musala	13 exp.	2100 m	42°16′N, 23°36′E	Zicsi & Csuzdi 1986
	Kloster Rila Mt.	6 exp.	1300 m	42°08′N, 23°20′E	Zicsi & Csuzdi 1986
	Kloster Rila Mt.	27 exp.	1100 m	42°08′N, 23°20′E	Zicsi & Csuzdi 1986
	Kloster Rila Mt	10 exp.	1600 m	42°08′N, 23°20′E	Zicsi & Csuzdi 1986
	Kloster Rila Mt.	8 exp.	1100 m	42°08′N, 23°20′E	Zicsi & Csuzdi 1986
	Kloster Rila Mt.	1 exp.	1100m	42°08′N, 23°20′E	Zicsi & Csuzdi 1986
	Rila, Maritsa basin	2 exp.	2518 m	42º09′N, 23º37′E	Valchovski et al. 2018.
	Rila, Struma basin	2 exp.	1950 m	-	Valchovski et al. 2018
	Rila Mt. Parangalitza Reserve	1 exp.	1700 m	42°11′N, 23°35′E	author's data 2009
Greece	Lefkonas	1 exp.	-	41°06′N 23°29′E	Šapkarev 1972
Montenegro	Biogradska gora Mt.	2 exp.	1090 m	42°53′N 19°36′E	Stojanović & Karaman 2003
	Žabljak-Durmitor Mt.	1 exp.	1450 m	43°09′N, 19°07′E	Karaman & Stojanović 1995
Serbia	Stara Planina Mts.	1exp	1363 m	43°09′N, 22°35′E	Stojanović et al. 2013

unpublished data presently known have been included. Earthworms were collected throughout the territory from various habitats, which included forest communities and mountain pastures. The habitats were situated near 1700 m.a.s.l. a large number of earthworm genera and species were found, but in this paper we have only analyzed data relating to the endemic *D. rhodopensis*.

The data from several authors (Plisko 1963, Šapkarev 1972, 1986, Zicsi & Csuzdi 1986, Karaman & Stojanović 1995, Delchev et al. 1998, Stojanović et al. 2008) were used to complete the distribution map of *D. rhodopensis* for the whole Balkan Peninsula (Figure 1).

The specimens were collected by digging, turning over rocks, debris and logs, and hand sorting, complemented with the diluted formaldehyde method. The earthworms were killed in 70% ethanol, fixed in 4% formalin solution and stored in 90% ethanol. Identification of the species was done in accordance to Blakemore (2004), Mršić (1991), Zicsi (1982), Šapkarev (1978), Csuzdi & Zicsi (2003).

Based on the data from all sites listed in Table 1, it was possible, for the first time, to graphically display the extent of the currently known occurrence (EOO) for *D. rhodopensis* (Figure 1). EOO is defined by IUCN (2017) as the area that contains all sites of present occurrence of a taxon. The area of occupancy (AOO) is defined as the area inside the extent of occurrence that is occupied by the species. In order to determine EOO and AOO of *D. rhodopensis*, a bibliographical survey was carried out.

To determine the conservation status of *D. rhodopensis*, we have applied an analysis based on the IUCN (2017) Red List Categories. Given the lack of basic data on population monitoring for this species, only criterion on geographic distribution can currently be used to assign a conservation status to *D. rhodopensis*. Moreover, according to Lewis & Senior (2011), criterion B proved to be the most important for invertebrates. The IUCN Red List measures range size, either in the form of EOO to evaluate the spatial spread of extinction risk (Criterion B1), or in the form of AOO (Criterion B2), which measures actual range size. Species may qualify as at-risk under either criterion.

Results

Until now, *D. rhodopensis* has been known from 17 localities in Bulgaria (Plisko 1963, Delchev et al. 1998, Zicsi & Csuzdi 1986, Valchovski et al. 2018a, b), two localities in northern (Karaman & Stojanović 1995, Stojanović & Karaman 2003) and eastern Montenegro (Stojanović & Karaman 2003) and only one locality in Serbia (Stojanović et al. 2008, 2013).

Unfortunately, despite our intensive searches in Serbia for the last 20 years (Stojanović 1996, Karaman & Stojanović 1998, 2002, Stojanović & Karaman 2005, Stojanović et al. 2008, Milutinović 2014, Stojanović & Milutinović 2014, Milutinović et al. 2015, Sekulić 2017, Stojanović et al. 2017, 2018), only one specimen of *D. rhodopensis* was found in the Serbian part of the Western Stara Planina Mts. (Stojanović et al. 2013) (Table 1). Also, after recent investigations in the various parts of Bulgaria (Valchovski 2012, 2014, Szederjesi 2013, Valchovski & Szederjesi 2016, Valchovski et al. 2018a), this species was found only on Rila Mt. (Valchovski et al. 2018a). However, additional distribution data from the authors' earlier unpublished investigations represent new records of *D. rhodopensis* in the Rila Mt. and in Bulgaria after four years (Table 1). Also, *D. rhodopensis* has not been registered in any recently conducted surveys in Serbia (Stojanović et al. 2018), Montenegro (Stojanović & Milutinović 2013), in Croatia (Hackenberger & Hackenberger 2013), Albania (Szederjesi & Csuzdi 2012a, 2015) or in Greece (Szederjesi & Csuzdi 2012b, Szederjesi 2015). Additional data on its distribution in Bulgaria are based on fieldwork carried out in the mountain range, in southwestern Bulgaria, from where only one specimen was recorded.

***Dendrobaena rhodopensis* (Černosvitov 1937)**

Eisenia rhodopensis Černosvitov, 1937: 82.

Dendrobaena rhodopensis: Plisko 1963: 436, Zicsi & Michalis 1981: 263, Zicsi 1982: 437, Šapkarev 1986: 80, Zicsi & Csuzdi 1986: 117, Mršić 1991: 617, Valchovski 2012: 93, Csuzdi 2012: 97, Stojanović et al. 2013: 638, Trakić et al. 2016: 263, Szederjesi 2017: 93, Szederjesi et al. 2017: 66, Valchovski et al. 2018: 3.

Diagnosis. The length of body 20 mm and 2 mm in diameter, consisting of 105 segments. Anterior part of the body rosy. The prostomium epilobous. Dorsal pores absent. The distance between the setae aa=bc, cd>ab. Glandular papillae surround setae *ab* on segments 15 and 16. The pores of the spermathecae lie near the median line. The male aperture with a glandular atrium is on the 15th segment. The clitellum extends from segments ½ 27, 28 to 32, ½ 33 and the tubercula pubertatis present from segments 29 to 31. Three pairs of seminal vesicles in the 9th, 11th and 12th segments and two pairs of spermathecae in the 9th and 10th segments. Spermathecal pores in intersegments 9/10 and 10/11. The calciferous glands and lateral tubercles situated in the 10th to 12th segments. The longitudinal muscles of a pennate type.

Locality. Parangalitza Park (42º11'N, 23º35'E), beech forest, 1700 m.a.s.l., May 2009.

Analysis of the spatial distribution of *D. rhodopensis* indicates that the species has a limited geographical range in which the EOO is approximately 2000 km² (Figure 1) while AOO is around 120km². Unfortunately, this species has not been found in the Balkans since 2009. Our analysis based on the IUCN (2017) Red List Categories shows that *D. rhodopensis* belongs to the Endangered category on global level.

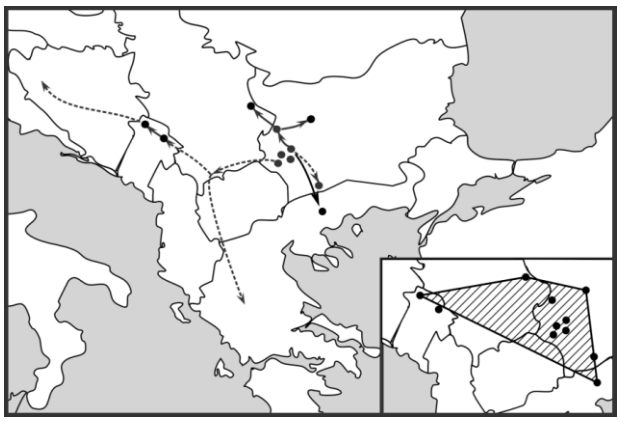

Figure 1. Spatial distribution of *Dendrobaena rhodopensis* on the Balkans and the current estimated EOO (solid line - probable moving route, dashed lines - possible routes).

Discussion

Biogeographical consideration

The genus *Dendrobaena* is the dominant faunal component of the earthworm fauna of the Balkan Peninsula. This is not surprising because one of the distributions centers of the genus *Dendrobaena* occupies the Carpatho-Balkan area (Omodeo & Rota 1991, 1999, 2008, Csuzdi et al. 2006, Szederjesi et al. 2014). Omodeo & Rota (2008) consider that the paleogeographic scenario can explain diversity and distribution of the genus *Dendrobaena.* Namely, they pointed out that at the end of the Oligocene and in the beginning of the Miocene, today's Anatolia was connected with the Balkans into a unique land mass. This situation contributed to the expansion of this species from the Balkans to the area of Anatolia at that time. Based on the number of endemic species from the genus *Dendrobaena* in the Balkans and Anatolia, it is clear that these are the two most important centers of diversity of this genus. Therefore, the presence and distribution of *D. rhodopensis* in the Balkans is undoubtedly the result of the so-called isolation effect and climate change in the Paleocene and Eocene when the mainland was represented by a system of smaller or larger islands that changed its contours during geological history (Mršić 1991).

The central occurrence of *D. rhodopensis* is in the Balkans, while its southernmost distribution limit is Lefkonas, Greece. The species also occurs in the central part of the Stara Planina Mts. (Plisko 1963, Stojanović et al. 2013), and the Serbian part of the Western Stara Planina Mts. represents the northernmost border of its presently known distribution.

The high number of locations from various parts of Rila Mt. and the pattern of the present distribution of *D. rhodopensis* prove the important role of the Rila Mt. area. Taking into account all distribution data reported for *D. rhodopensis*, it is possible to assume that the area of Rila Mt. is the area of origin of the species in the Balkans, and a distribution center as well.

Having in mind the fact that the easternmost point of its distribution is in Bulgaria, we suppose that its possible migration from Rila Mt. as a center could have occurred via three probable migratory routes: towards the north (the Stara Planina Mts.), the south (Pirin Mt.) and the southeast (the Rodope Massif). It is probable that *D. rhodopensis* is also present across the Rhodope Mountains although it has not been recorded in this region up to this very moment. This theory is supported by the connection of the Rila Mt. with the Rhodopes in the east through the Yundola (1375 m) and the Avramovo (1295 m) saddles. It is clear that the north direction could go across Vitosha Mt. reaching the western Stara Planina Mts. (in the Serbian part) and the central Stara Planina Mts. (on the Bulgarian side). Teteven in the central part of the Stara Planina Mts. (Bulgaria) represents the sector of the eastern limit of the species' natural range. *D. rhodopensis* spreads to the west as well, by a natural gateway from the region of Rila Mt. to one part of the Dinaric Alps (Bjelasica Mt. and Durmitor Mt. in Montenegro, Table 1). The Dinaric Alps are a south-eastern division of the Eastern Alps and they extend from the Julian Alps in the northwest down to the Šar-Koram Massif, where the mountain direction changes to north-south and reaches the Pindus Mts. in Greece.

For the time being, there are no data that deal with a possible movement route of this species from Rila Mt. towards the Dinarides. Taking into account that *D. rhodopensis* is a high mountain species, we can only suppose that it has been moving along mountain ranges of the Balkan Peninsula going from Rila Mt. across the Osogovo Mts. (which are a continuation of the Rila-Rhodopes mountain massif in Macedonia) and reaching the Šar Mts. across Jakupica Mt. On the other hand, on the basis of the fact that the Šar Mts. are a continuation between the Dinaric Alps and the Pindus mountain ranges, it is reasonable to assume that this species could move from the Šar Mts. towards the present westernmost limit comprising the two registered localities of Bjelasica Mt. and Durmitor Mt. (Karaman & Stojanović 1995).

Since this species has been found at higher altitudes (Table 1), we may just as well assume that in Montenegro *D. rhodopensis* could spread west across the Dinaric Alps and southeast to Šar Mts. We assume that it could also spread south along Šar Mts, while in Greece one of the possible routes is in the direction of the Pindus Mts.

Therefore, it is not impossible to expect *D. rhodopensis* to be found in the near future in the Dinarides, Šar Mts., and in the Pindus Mts., as well. In Figure 1, the solid line represents a probable moving route of this species on the basis of registered localities, while dashed lines represent possible routes of the species which are yet to be confirmed in further investigations.

Conservation status

Dendrobaena rhodopensis is an endemic species known from altogether 18 locations in the Balkan Peninsula. In Montenegro (Karaman & Stojanović 1995; Stojanović & Karaman 2003) and Serbia (Stojanović et al. 2013), it is clear that *D. rhodopensis* appears sporadically. The last finding in Serbia dates back to 1997 (1 exp.), while in Montenegro only two exemplars were registered in 1989 and in 1998. On the other side, in the area of the Rila Mt., 17 locations were registered with altogether 104 individuals of *D. rhodopensis*. The last findings, preceding our study, was in 2005. Since then, Bulgaria has been thoroughly investigated and many articles on the distribution of lumbricides in Bulgaria have been published, unfortunately, without finding *D. rhodopensis*. Based on our investigations, only one exemplar of *D. rhodopensis* was registered in 2009 in Bulgaria. We can only assume that this species still exists in the Rila Mt. area, but what has happened in the meantime and why it is now much harder to encounter it, so that in spite of the targeted searches, only one specimen of *D. rhodopensis* has been found after four years? Answers on such disturbing questions certainly are very complex and only the future investigations will be able to give explanations. For now, a reasonable explanation for this situation can be found in the various anthropogenic impacts. Unfortunately, the Rila Mt. area has changed drastically during the 20th century, due to urbanization and touristic development and. This situation is even more alarming when it is observed in the light of new invertebrate researches of the European fauna. Namely, according to Fontaine et al. (2007), in Europe, among the 62 extinct taxa, 51 were endemic to one, two or three countries. The recorded extinctions of narrow-range taxa occurred mainly in mountain ranges (Alps, Pyrenees, Balkans). Moreover, only 41 invertebrate taxa are Red Listed and protected in Europe. Therefore, our data on *D. rhodopensis* are extremely important to support the evaluation of the species conservation status and enhance knowledge about endemic and little-known earthworms in the Balkans. According to Stojanović et al. (2008), the preliminary status of *D. rhodopensis* was Endangered. However, based on our study and considering the all published information on *D. rhodopensis* we propose its inclusion within the category Endangered under the IUCN criterion B2 and subcriterias b (iii, iv,v) and c (iii, iv). We think that the information brought to light by the present study, although limited, can provide enough information to assign a conservation status to the species. This means that the presented status assessment of *D. rhodopensis* is based on the current information, and thus may be taken as baseline data for further research and re-evaluation. Moreover, our study also indicates that *D. rhodopensis* is an endemic species potentially in need for conservation or at least for monitoring for future negative changes.

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