

A hot-spot of native terrestrial isopods in an urban area in the Carpathians, Herculane Spa: an emergence of the past into the present

(Crustacea, Isopoda)

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Understanding urban biodiversity is an important task in the context of nowadays rapid urban development. Urban expansion affects even areas with high biodiversity; the native fauna related to natural habitats is more sensitive to urbanization. We intended to observe the effect of an old town upon a group of soil-dwelling invertebrates, namely terrestrial isopods, in an area with a unique biodiversity, Herculane Spa, located in southwestern Romania. We identified 23 terrestrial isopod species in this small resort town with geothermal waters. This number is larger than in many regions of Romania, including other urban areas. More than a quarter of the species are endemic to the region or to the Carpathians in general. This fact confirms the biogeographical peculiarity of the Mehedinți Mountains. The old part of the town, built for touristic purposes, has a richer isopod fauna with native and endemic species. Forest and wetland species were also found in this area and in the periurban area. The newly built residential areas have fewer species, generally eurytopic. The presence of the local endemic species *Trachelipus trilobatus* is limited to natural regions and their vicinity, which proves its native origin. The town, with its special history, and its surrounding natural areas, shelters the native fauna of the region, despite the recent aggressive urban development. The resort areas are more important for the native fauna than the residential ones.

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Introduction

The Mehedinți Mountains are of high biogeographic importance, being the region of the south-eastern Carpathians which shelters the largest number of range-restricted endemic plant species (Hurdu et al. 2016). This biogeographic importance is true for the fauna also. The region has special biodiversity compared to other regions of Romania, sheltering many endemic, relict, or southern species (e.g. Pop 1980, Vojnits & Szabo 1988, Tăbăcaru et al. 2002-2003, Falniowski et al 2009, Cameron et al. 2016). This applies for terrestrial isopods too, a biogeographic indicator group (e.g. Tăbăcaru & Giurginca 2013), with many species endemic to the region (e.g. Radu 1985, Tăbăcaru & Giurginca 2013, Tomescu et al. 2015). The most remarkable species is *Trachelipus trilobatus*, which is endemic only to the vicinity of Herculane Spa (e.g. Tomescu et al. 2015), a town situated in Mehedinți Mountains region, which dates back to the Roman times, more than 1900 years ago (e.g. Spânu 2012, Aluculesei & Nistoreanu 2014). Due to the numerous thermal springs (e.g. Povară et al. 2008), the town is an important touristic resort, with a developmental period in the Roman times, one in the Habsburg Empire times, and one in the recent years (e.g. Spânu 2012, Aluculesei & Nistoreanu 2014). Despite the presence of the town, *T. trilobatus* was mentioned only in natural habitats, in caves and in forests with limestone slopes and gorges (Tomescu et al. 2015). These habitats indicate that *T. trilobatus* is a native relict species, which has evolved in the region (Tomescu et al. 2015). However, *T. trilobatus* is considered to have a Mediterranean trait (Radu 1958). Thus, we were wondering if the species could have been introduced to the region, because it is not present in the areas outside of Herculane Spa (Tomescu et al. 2015). There are terrestrial isopods known to be introduced by recent construction in Romania (e.g. Ferenți & Covaciu-Marcov 2013). Also, urban areas represent a gateway for the introduction of non-native species (e.g. Vilisics & Hornung 2009, Horsák et al. 2016). But, in contrast to other urban areas, where urban green spaces play an important role for biodiversity (see in Aronson et al. 2017), Herculane Spa is situated inside a large natural area. Thus its fauna could be highly shaped by the region's native fauna. Therefore, considering the previous knowledge, we hypothesized that if *T. trilobatus* is a native species, it will not be present in the town, but only in the neighboring natural areas. We also aimed to verify if the native isopod fauna is present in the town, and the extent to which the town affects native species. Urbanization is known to have a negative effect on species characteristic for natural habitats (e.g. Magura et al. 2008, 2010, 2013,

Bogyó et al. 2015, Rzeszowski & Sterzyńska 2016). Our objectives were as follows: 1. to establish the composition of the terrestrial isopod fauna of the town; 2. to emphasize the town's influence upon the native fauna, especially upon *T. trilobatus*.

Materials and methods

Herculane Spa (44.8771°N, 022.4138°E) is a town situated in south-western Romania (Mândruț 2006), at approximately 20 km from the border with Serbia, in the western part of the Romanian Southern Carpathians, between the Mehedinți Mountains and the Cernei Mountains (Posea & Badea 1984) (Fig. 1). The Cerna River, a Danube tributary (e.g. Mândruț 2006), runs through the town. Because the mountains have very steep slopes, the town lies along the Cerna River, with a length of approximately 7 km and a width of less than 1 km. The lower part of the town is situated at 120 m altitude and the upper part at 180 m. Herculane Spa is a small town, with 5008 inhabitants in 2011 (see National Institute of Statistics 2011, <http://www.recensamantromania.ro/rezultate-2/>). The old town, which was initially a Roman resort and subsequently rebuilt in the Habsburg Empire's period (e.g. Spânu 2012, Aluculesei & Nistoreanu 2014), is represented by the upper part of Herculane Spa. It includes massive buildings with approximately 150-year-old hotels (Spânu 2012), many of which are abandoned nowadays. Older residential houses with green areas or gardens are also present. Beech forests typically surround this part of the town. The lower part of the town is made up of multi-story residential apartment buildings and new hotels constructed in the last 40-50 years, usually without green areas. Houses are newer in the lower part of town, many of which function as guest homes. The surrounding forests contain both beech (*Fagus sylvatica*) and oak (*Quercus* sp.) but also black locust (*Robinia pseudoacacia*) plantations.

Field sampling took place on 5th and 6th May 2016, spring being an optimal season for studying terrestrial isopods (Messina et al. 2016). Isopods were collected directly under various shelters. Samples were collected from 47 locations in the town and its surroundings. The sampling points were selected randomly. We made transects in the town from the upper to the lower sector. We planned our route while at the field, according to the town sectors' appearance and the existence of possible shelters for isopods. We did not find shelters for isopods in the recently arranged and well managed areas. The minimum distance between sampling points was approximately 20 m, with an average of 200 m, depending on the situation from the field. We did not collect multiple samples from the same microhabitat. We spent approximately 20 minutes in each location, similar to other studies (e.g. Ferenți et al. 2015, Ferenți & Covaciu-Marcov 2016). Thus, the time dedicated to sample collection was standardized. The same two persons collected each sample. The isopods were pre-

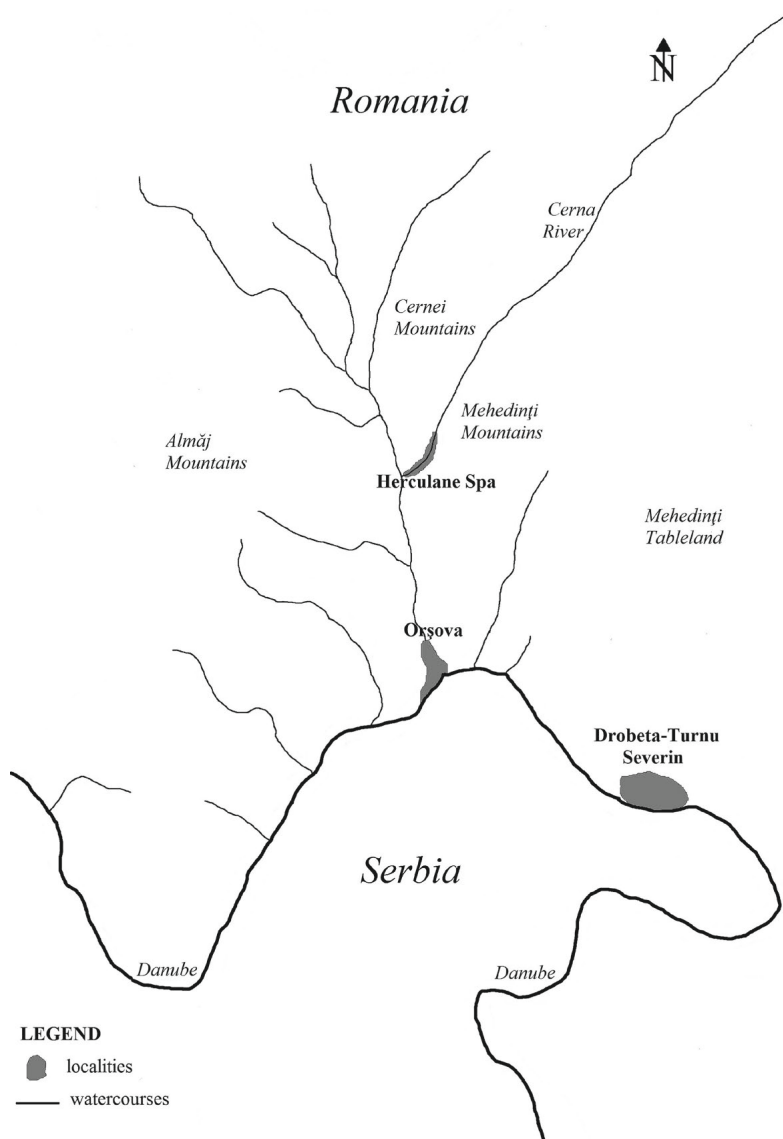


Fig. 1. The Herculane Spa region in southwestern Romania.

served in test tubes with alcohol and identified with the help of literature (e.g. Radu 1983, 1985, Farkas & Vilisics 2013). The studied material was deposited in the last author's personal collection. The local endemic species *T. trilobatus* was identified while on the field. Considering the town sectors' different appearance and history, the samples were treated according to the town sections: 1. the upper town (the tourist resort with 150-year-old massive buildings) and 2. the lower town (the residential area with 40-50-year-old blocks of flats). The same samples were also treated according to their habitat of origin: 1. new buildings (recent and standardized resi-

dential blocks and guest houses); 2. old buildings (large classical constructions and old living houses) and 3. periurban area (natural and semi-natural wooded habitats, tangent to the town). The forests are closely attached to the whole locality, both in the upper and lower town. This is a consequence of the town's position on a valley between steep slopes, which extend to the town's outskirts, the forest reaching the old and new buildings' area. However, there are more native forests near the upper town, usually beech, and black locust plantations near the lower town. This is also a reflection of the different anthropogenic disturbances between the

two areas of the town. The isopod species were grouped according to their zoogeographic and ecological status, since global species richness is not considered a comprehensive urbanization indicator (Magura et al. 2013). From the zoogeographical point of view, the species were divided into the following categories: local endemic species, south-western Carpathian endemic species, Carpathian endemic species, and largely distributed species. The following ecological categories were established: eurytopic, wetlands, endogeic, myrmecophilous, and forest species. The zoogeographical and ecological categories were established according to the species distribution range and ecological demands presented in the literature (e.g. Radu 1983, 1985, Schmalfuss 2003, Tăbăcaru & Giurginca 2013, Tomescu et al. 2015).

Statistical analysis was performed on the entire sample set and on each subset, i.e. the town sections (lower and upper town), and the habitats of origin (old and new buildings, and the periurban area). As the samples were collected using the direct method, the standardization was achieved by spending equal time on the field and by having the same two persons as collectors. The number of individuals or species per sample was provided, according to the town sections or habitats of origin. In order to verify our first objective we made a list of species, indicating the percentage abundance and the frequency of occurrence for each species. For our second objective (which focuses on the relation between the town parts and terrestrial isopod species) we used both quantitative and qualitative approach. For quantitative comparisons we estimated species richness and abundance from each town part. We also estimated the species diversity with the Shannon Weaver index (H) for both the total sample set and for the groups from different habitats or parts of the town. The Mann-Whitney-U-test was used to compare the effects of the town parts and habitat types on terrestrial isopod species abundance. For qualitative approach we focused on species composition – town part relationship. After analyzing the species composition of each subset, we estimated the similarity between the terrestrial isopod assemblages from different town parts, using the Jaccard index. Also, we applied correspondence analysis (CA) to model the species' affinity to the different sections of the town or habitat of origin. The Shannon-Weaver index, Mann-Whitney-U-test, Jaccard index and the CA analysis were performed by the free software PAST 3 (Hammer et al. 2001).

Results

Composition of the terrestrial isopod fauna

We collected 449 terrestrial isopod individuals belonging to 23 species in Herculane Spa: *Ligidium germanicum* Verhoeff, 1901, *Ligidium intermedium* Radu, 1950, *Hyloniscus riparius* (C. Koch, 1838), *Hyloniscus transsilvanicus* (Verhoeff, 1901), *Trichoniscus*

inferus Verhoeff, 1908, *Haplophthalmus danicus* Budde-Lund, 1880, *Haplophthalmus mengii* (Zaddach, 1844), *Buddelundiella cataractae* Verhoeff, 1930, *Platyarthrus hoffmannseggii* Brandt, 1833, *Cylisticus convexus* (De Geer, 1778), *Protracheoniscus politus* (C. Koch, 1841), *Porcellium collicola* (Verhoeff, 1907), *Trachelipus arcuatus* (Budde-Lund, 1885), *Trachelipus nodulosus* (C. Koch, 1838), *Trachelipus difficilis* (Radu, 1950), *Trachelipus trilobatus* (Stein, 1859), *Trachelipus pleonglandulatus* Radu, 1950, *Porcellionides pruinosus* (Brandt, 1833), *Porcellio scaber* Latreille, 1804, *Porcellio spinicornis* Say, 1818, *Armadillidium vulgare* (Latreille, 1804), *Armadillidium versicolor* Stein, 1859, *Armadillidium carniolense* Verhoeff, 1901. The highest number of species per sampling point (9) was registered in the old town. We found only one species in eight of the sampling points. The most abundant species was *C. convexus*, followed by *H. danicus* and *A. vulgare*. *C. convexus* had the highest frequency of occurrence, followed by *A. vulgare* and *H. riparius* (Table 1). The general number of individuals per sample was 9.55, and the number of species per sample was 0.48. The total species diversity was $H = 2.54$.

The town's effect upon the terrestrial isopod fauna

C. convexus registered the highest percentage abundance and frequency of occurrence both in the lower and upper town (Table 1). Examples of other abundant species include *A. vulgare* and *P. scaber* in the lower-town, and *H. danicus* and *B. cataractae* in the upper-town. This was also observed in samples divided by habitat of origin. *C. convexus* ranked first in the new buildings region, followed by *A. vulgare* and *P. scaber*. In the old buildings region, the most abundant species were *H. danicus* and *B. cataractae*. Finally, in the periurban area, *H. danicus* and *P. collicola* were the most abundant species (Table 1).

There were variations in species richness or diversity both by the town zones or habitat types (Table 2). In the upper town, the species richness, the number of individuals per sample, the number of species per sample, and the species diversity were higher. Higher species richness, diversity and species per sample were observed in the periurban area. The new buildings shelter fewer species than the old ones (Table 2). However, abundance was not significantly affected nor by the town area (lower town – upper town: $p=0.595$), neither by habitat types (old buildings – periurban: $p=0.052$, new buildings – periurban: $p=0.068$, old buildings – new buildings: $p=0.762$).

According to the Jaccard index, the similarity in species composition between the upper and lower

town was 0.521. The similarity between the new buildings region and the old buildings or periurban region was 0.5. Yet, the similarity between the old buildings region and the periurban area was 0.391. The local endemic species *T. trilobatus* was present only in the upper town, in the periurban area, and near some old buildings, but still at the limit of the

periurban area (Table 1). The eurytopic species like *A. vulgare* and *T. nodulosus* seem to have an affinity for the lower town, while *H. danicus* – for the upper town. *P. collicola* and *P. politus* seem to be linked to the periurban zone. *A. vulgare* and *P. scaber* are associated with the new buildings region, while *H. mengii*, *T. inferus* and *B. cataractae* with the old buildings

Table 1. Terrestrial isopod species, zoogeographical (Z) and ecological categories (E), the percentage abundance (A %) and frequency of occurrence (f%) in the different zones and habitat types in Herculane Spa. LD, largely distributed species; C, Carpathian endemic species; SW, south-western Carpathian endemic species; L, local endemic species; w, wetlands species; en, endogeous species; m, myrmecophilous species; eu, eurytopic species; f, forest species).

Species	Z	E	Total		Town zones				Habitat types					
					Lower town		Upper town		New buildings		Old buildings		Periurban area	
			A %	f %	A %	f %	A %	f %	A %	f %	A %	f %	A %	f %
<i>L. germanicum</i>	LD	w	1.11	2.13	–	–	1.82	4.35	–	–	–	–	3.73	6.67
<i>L. intermedium</i>	C	w	1.55	2.12	–	–	2.55	4.35	–	–	–	–	5.22	6.67
<i>H. riparius</i>	LD	w	5.79	29.7	9.2	33.33	3.64	26.09	8.66	21.05	4.26	38.46	5.22	33.33
<i>H. transsilvanicus</i>	C	w	1.33	4.25	0.57	4.17	1.82	4.35	–	–	–	–	4.48	13.33
<i>T. inferus</i>	SW	en	1.33	2.12	–	–	2.18	4.35	–	–	3.19	7.69	–	–
<i>H. danicus</i>	LD	w	13.5	14.89	1.72	8.33	21.09	21.74	2.36	10.53	22.34	30.77	11.94	6.67
<i>H. mengii</i>	LD	w	1.55	2.12	–	–	2.55	4.35	–	–	3.72	7.69	–	–
<i>B. cataractae</i>	LD	en	5.79	2.12	–	–	9.45	4.35	–	–	13.83	7.69	–	–
<i>P. hoffmannseggii</i>	LD	m	2.22	6.38	5.75	12.5	–	–	2.36	10.53	–	–	5.22	6.67
<i>C. convexus</i>	LD	eu	28.06	65.95	25.29	62.5	29.82	69.57	27.56	68.42	36.7	69.23	16.42	60
<i>P. politus</i>	LD	f	1.55	8.51	1.72	8.33	1.45	8.7	–	–	–	–	5.22	26.67
<i>P. collicola</i>	LD	w	3.11	4.25	8.05	8.33	–	–	–	–	–	–	10.45	13.33
<i>T. arcuatus</i>	LD	f	2.22	6.38	1.15	4.17	2.91	8.7	1.57	5.26	–	–	5.97	13.33
<i>T. nodulosus</i>	LD	eu	3.34	12.76	8.62	25	–	–	9.45	26.32	–	–	2.24	6.67
<i>T. difficilis</i>	C	f	0.66	4.25	1.72	8.33	–	–	–	–	–	–	2.24	13.33
<i>T. trilobatus</i>	L	f	1.33	4.25	–	–	2.18	8.7	–	–	0.53	7.69	3.73	6.67
<i>T. pleoglandulatus</i>	SW	f	0.89	4.25	–	–	1.45	8.7	–	–	–	–	2.99	13.33
<i>P. pruinusosus</i>	LD	eu	1.55	8.51	1.72	8.33	1.45	8.7	4.72	15.79	0.53	7.69	–	–
<i>P. scaber</i>	LD	eu	8.24	21.27	10.92	29.17	6.55	13.04	19.69	31.58	5.85	23.08	0.75	6.67
<i>P. spinicornis</i>	LD	eu	1.11	10.63	1.15	8.33	1.09	13.04	2.36	15.79	0.53	7.69	0.75	6.67
<i>A. vulgare</i>	LD	eu	9.35	40.42	16.67	58.33	4.73	21.74	20.47	57.89	4.79	30.77	5.22	26.67
<i>A. versicolor</i>	LD	eu	2	10.63	3.45	8.33	1.09	13.04	0.79	5.26	1.06	15.38	4.48	13.33
<i>A. carniolense</i>	LD	f	2.22	8.51	2.3	4.17	2.18	13.04	–	–	2.66	15.38	3.73	13.33

Table 2. The number of samples, species, individuals / sample, species / sample and Shannon-Weaver diversity index (H) in different town sectors and habitat types.

	Samples	Species	Individuals / sample	Species / sample	H
Total	47	23	9.55	0.48	2.54
Lower town	24	16	7.25	0.66	2.30
Upper town	23	19	11.95	0.82	2.32
New buildings	19	11	6.68	0.57	1.94
Old buildings	13	13	14.46	1	1.88
Periurban area	15	19	8.93	1.26	2.71

et al. 2012, 2013a,b, Ferentzi & Covaciu-Marcov 2015). A similar number of species was registered in the Valsan River basin, an important zoogeographical region (Ferentzi & Covaciu-Marcov 2016). The species richness at Herculane Spa is higher than any Romanian town studied before (Giurginca 2006, Bodin et al. 2013, Ferentzi et al. 2015, Herle et al. 2016, Giurginca et al. 2017). This number is even larger, considering that *Mesoniscus graniger* was also mentioned in the region's caves (Giurginca 2009). Compared to similarly-sized towns from western Romania (Bodin et al. 2013, Ferentzi et al. 2015, Herle et al. 2016), the number of species is sometimes more than double. The number is larger than in Bucharest (Giurginca et al. 2017), the biggest city in the country with a population of almost 2 million (Mândruț 2006). The number of species at Herculane Spa is larger compared to many European cities (Jedryczkowski 1981, Arndt & Mattern 1996, Riedel et al. 2009, Vilisics et al. 2012, Šatkauskienė et al. 2016). The number of terrestrial isopod species at Herculane Spa is surpassed by Budapest, the Hungarian capital, with a slightly smaller human population than Bucharest, but with many non-native species present in greenhouses and botanical gardens (Vilisics & Hornung 2009). The large number of species in Herculane Spa can be explained by the surrounding natural habitats. As a small town close to natural areas with high biodiversity, Herculane Spa enables terrestrial isopod species to survive in its many parts with mostly suburban appearance. Herculane Spa can be compared to a park in a large city, but a park surrounded by natural areas, not by urban infrastructure. Its history and status as a tourist resort gave it a different developmental direction compared to other urban areas. Thus, Herculane Spa is a special case among urban areas, due to the many surrounding natural areas, and because of its history. Therefore, its features are hardly similar to localities with different history and surroundings. Nevertheless, the endemic species are sheltered mainly in natural areas close to the town, and not in the town itself. Unlike Budapest (Vilisics & Hornung 2009), numerous non-native species are missing from Herculane Spa because the town is much smaller and lacks greenhouses and botanical gardens, where they could have been introduced. Nevertheless, some species can be considered non-native even if they are not exotic, especially those from the lower town. *P. hoffmannseggii*, a myrmecophilous species (Radu 1985) was found in the disturbed areas of the lower town. Its presence could be a consequence of its link to ants, a group which was frequently mentioned in urban areas (e.g. Pisarski 1982, Vepsäläinen et al. 2008). *P. pruinosus*, *A. vulgare*, and *P. scaber*, also linked to the lower town and new buildings, belong

to the same category and were recorded in the affected areas (e.g. Herle et al. 2016). *P. pruinosus* and *A. vulgare* are probably Mediterranean species, which have extended their range to most of Europe (e.g. Cochard et al. 2010).

B. cataractae seems to be an intractable case for the moment. It belongs to a family with representatives in the Mediterranean-south-west-European region (Radu 1983). Nevertheless, *B. cataractae* was introduced in many European areas (e.g. Verhoeff 1942, Korsós et al. 2002, Vilisics 2007, Cochard et al. 2010), usually surviving in urban areas, especially in indoor habitats (e.g. Korsós et al. 2002, Vilisics & Hornung 2009). This is the third record of this species in Romania, the previous two being in a well in the town of Mangalia, and in a cellar in Bucharest (see Radu 1983). The urban habitat was common to all three records, and thus it was inferred that the species was introduced along with urbanization. They also share some common traits, as they are situated in the warmest areas of Romania (Mândruț 2006). Herculane Spa and Mangalia shared a Roman past. Just like the two local endemic species (*T. trilobatus*, *T. inferus*), *B. cataractae* was encountered near an abandoned old building close to natural habitats at Herculane Spa. Therefore, it is difficult to establish which of the common traits between the Romanian records can explain the presence of this species here. Its exclusive link to the upper town suggests that *B. cataractae* was not recently introduced by urbanization. *B. cataractae* could have been favoured by geothermal waters at Herculane Spa, as it is encountered close to a thermal spring. This species was previously encountered in areas with thermal waters in Germany (Verhoeff 1942). Also, the numerous caves of the region could be beneficial for this species, which is considered troglomorphic (Bedeck et al. 2011). Nevertheless, *B. cataractae* populates a semi-natural habitat in a region with submediterranean climate, where other southern terrestrial isopods are present. For example, *A. carniolense* is a south-eastern European species, situated in Romania at its range limit (Schmalfuss 2003), which is relatively well represented in natural wooded and stony habitats in Herculane Spa.

The most common species in Herculane Spa was *C. convexus*. This is expected, since *C. convexus* is a eurytopic species, common to urban habitats, which is present even in strongly disturbed areas (e.g. Vilisics & Hornung 2009, Herle et al. 2016). Nevertheless, at Herculane Spa, *C. convexus* was encountered not only in the disturbed areas of the lower town, but in the entire region, including the natural wooded habitats from the periurban area. In the northern regions of Romania, the species is linked to disturbed or partially disturbed habitats

both inside and outside of urban areas (e.g. Ferentî et al. 2012, 2013a, Ferentî & Covaciu-Marcov 2015). In Herculane Spa it was observed everywhere. The populations from Herculane Spa seem to have a different past, when compared to other populations from the country. The ecological differences suggest that a group of populations found refuge in this area, from where they spread northwards, facilitated by the advancement of human disturbance.

The isopods from Herculane Spa confirm the fact that urbanization affects forest and wetlands species, their number decreasing along the rural-suburban-urban gradient (e.g. Magura et al. 2008, 2010, 2013, Bogyó et al. 2015). At Herculane Spa they are linked with the upper town, the periurban area, and eventually with the old buildings. The upper town is much older and it is more favorable to the native species. The contrasting age, architecture, and purpose of the town sectors pressured the native fauna in different ways. The upper town was built in the past as a tourist resort and is more natural, fitting in better with the landscape. In contrast, space was used to its maximum capacity in the lower town residential areas, because of the narrow valley; buildings are crowded, there are few green areas. This town plan affected the forest and wetland species and enabled the advancement of eurytopic, synanthropic or even non-native species. This fact was observed in the Vâlsan River basin, where the region with a lot of human settlements had the same effect on isopods (Ferentî & Covaciu-Marcov 2016).

In the Carpathian Mountains numerous endemic species were recorded in limestone karst areas (e.g. Varga 2010, Vilisics et al. 2011, Hurdu et al. 2016). Surrounded by limestone areas, there is no surprise that Herculane Spa is a hotspot for terrestrial isopods. It is an area with higher diversity than other regions of Romania, where species distribution is established by the region's history and geographical and ecological peculiarities, rather than by urbanization. Nevertheless, the complete explanation of the emergence of this complex terrestrial isopod fauna, with its many different pasts, seems to be a Herculean task.

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