# Do terrestrial isopods from Vâlsan River protected area reflect the region's peculiarities? Zoogeographic and conservative implications of a possible answer

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Keywords: endemic species, peculiarities, humid zones, Vâlsan, anthropic impact, protected area

## Abstract

Vâlsan River valley has proved to be a refuge for different animal groups. As a consequence of its importance, we investigated the terrestrial isopods from the area, using the direct collecting method, in the years 2010–2015 and encountered 21 species. Three of them, *Hyloniscus motasi*, *Trachelipus ater* and *Cylisticus transsilvanicus*, are endemic in Romania, underlining the region's zoogeographical peculiarities. We divided the region into three sections (upper, middle and lower), which differ as a result of their different human disturbance. The upper section, which is less affected and without villages, conserves the native isopod fauna better. It has the highest diversity and species richness, the endemic ones being present here. In the middle and lower sections, with settlements, the native terrestrial isopod species' habitats are more limited, the anthropic changes facilitating the advance of some anthropophilous species, even introduced ones. The presence of endemic species for Romania increases the conservative value of the region. The endemic species occupy restricted, natural, humid and forested habitats, underlining their conservative importance.

Profile

Protected area Vâlsan River valley Mountain range Southern Carpathians Country Romania

## Introduction

The zoogeographical importance of terrestrial isopods has been discussed in recent years (Tăbăcaru & Giurgincă 2013; Ferenți & Covaciu-Marcov 2014). They joined other groups in indicating the zoogeographic peculiarities of some regions (Ferenți et al. 2012, 2013a, b). The Vâlsan River basin natural protected area has biogeographical particularities, the endemic fish Romanichthys valsanicola surviving here (see in: Telcean et al. 2011). In addition, the region's particularities, explained by its past, were observed in other groups (Covaciu-Marcov et al. 2014). The Vâlsan basin as well as other Carpathian areas seem to be zones of refuge (see in: Varga 2010). In this context we hypothesized that terrestrial isopods can contribute to revealing the region's zoogeographical importance. Also, we assumed that species composition, richness and diversity change along an altitudinal and disturbance gradient. To our knowledge, detailed studies of isopods in the Vâlsan River basin have not been carried out, only one endemic species, Trachelipus ater, being mentioned in Vâlsan Gorge (Tomescu et al. 2015). This species was also reported in the neighbouring Arges River basin (Schmidt 1997). Information on terrestrial isopod assemblages in the Southern Carpathians are available for the Piatra Craiului Mountains (Giurgincă et al. 2006) and the Jiului Gorge (Tomescu et al. 2011). We aimed to analyse the terrestrial isopod fauna from the Vâlsan River basin, observing both its composition and particularities as well as its conservative role in a protected area.



Figure 1 – The upper section (Vâlsan Gorge) (top) and the middle section (below) of the Vâlsan River, situated in the Făgăraş Mountains, Southern Carpathians.  $\bigcirc$  Covaciu-Marcov Severus-Daniel



Figure 2 – The location of the study area in Romania and in the Arges River hydrographic basin.

## Materials and methods

## Study area

The Vâlsan River is a tributary of Arges River, situated in the Făgăraș Mountains, Southern Carpathians (Tufescu 1986). The upper river section crosses the Făgăraș Mountains and the Ghitu Mountains, the middle part flows through Muscelele Argeșului, which belongs to the Sub-Carpathians, and the lower course crosses Gruiurile Argeșului (Posea & Badea 1984). On leaving the mountain area, the river crosses the Vâlsan Gorge. The Vâlsan River, which starts in the Făgăraş Mountain at 2310 m a.s.l. (Ujvári 1972), flows into the Arges River at approximately 300 m altitude. We collected isopods from the river confluence with Arges through to an elevation of approximately 1 100 m a.s.l. The study area was divided into three sections: 1. the upper section, overlapping with the mountain area, down to the river's outflow from the gorge; 2. the middle section in the Sub-Carpathians; 3. the lower section, situated in the hilly area (Figure 2). In the upper section there are no settlements, human impact manifests itself in clearings and tourism (Covaciu-Marcov et al. 2014). In the other two sections numerous settlements are strung along the river (e.g. Telcean et al. 2011), the region being anthropogenically affected and heavily deforested (Badea 2011).

In each section we investigated a large variety of habitats. The upper section contains the less disturbed zones, especially in the Vâlsan Gorge. In this area we investigated swamps, streams, a sulfurous spring, ponds, an abandoned mine, abandoned buildings, camping zones, alder, beech or beech / spruce forest. In the middle section the habitats become characteristic for a rural zone, including roadsides, buildings nearby, but also wet zones near the river and forests. The lower section is apparently anthropogenically affected in the same way as the middle section, the relief

Species	Preferred Habitat	Upper section		Middle section		Lower section		Total	
		<b>A</b> %	<b>f%</b>	<b>A</b> %	<b>f%</b>	<b>A</b> %	<b>f%</b>	<b>A</b> %	f%
Ligidium hypnorum	W	6.83	16.13	9.37	22.58	1.97	12.90	3.27	17.20
Ligidium germanicum	W	4.35	16.13	1.78	6.45	1.97	3.23	2.93	8.60
Hyloniscus riparius	W	2.48	9.68	9.82	32.26	20.69	32.26	9.61	24.73
Hyloniscus transsilvanicus	W	13.04	38.71	13.39	32.26	2.46	9.68	10.28	26.88
Hyloniscus motasi	Ś	5.28	3.23	-	-	-	-	2.26	1.07
Trichoniscus sp.	Ś	1.24	6.45	0.45	3.23	-	-	0.66	3.22
Mesoniscus graniger	C, EN	-	-	1.79	3.23	-	-	0.53	1.07
Haplophthalmus danicus	W	-	-	-	-	4.93	3.23	1.33	1.07
Haplophthalmus mengii	Mš	1.24	3.23	8.93	12.90	-	-	3.20	5.37
Cylisticus convexus	EU, SI	0.31	3.23	3.57	9.68	-	-	1.20	4.30
Cylisticus transsilvanicus	W, F	3.11	6.45	0.89	6.45	-	-	1.60	4.30
Protracheoniscus politus	F	1.86	12.90	4.91	9.68	4.43	6.45	3.47	9.67
Porcellium conspersum	W	12.11	12.90	-	-	-	-	5.20	4.30
Trachelipus ater	F	5.28	12.90	-	-	-	-	2.26	4.30
Trachelipus difficilis	F	10.56	22.58	1.78	9.68	0.99	6.45	5.34	12.90
Trachelipus arcuatus	F	27.95	45.16	26.33	51.61	31.53	58.06	28.43	51.61
Trachelipus rathkii	EU	2.79	12.90	3.12	12.90	4.93	3.23	3.47	9.67
Porcellionides pruinosus	EU, SI	-	-	-	-	3.44	9.68	0.93	3.22
Porcellio scaber	EU, SI	0.31	3.23	-	-	7.38	9.68	2.13	4.30
Porcellio spinicornis	SI?	-	-	-	-	0.49	3.23	0.13	1.07
Armadillidium vulgare	EU, SI	1.24	3.23	13.84	48.39	14.78	38.71	8.67	30.10
Species richness		17		14		13		21	
No. of endemic species		3		1		-		3	
Diversity (H)		2.31		2.21		2.03		2.50	

Table 1 – The percentage abundance (A), frequency of occurrence (f), species richness, number of endemic species and Shannon Wiever diversity of terrestrial isopod species in the Vâlsan River basin (W – wetland, C – cavernicolous, EN – endogeous, EU – euritopic, SI – sinanthropic, F – forest, ? – not established).

is softer. The investigated habitats here are streams, roadsides, ponds, beech/oak forest, etc. There are altitudinal differences between the three sections, the upper one ranges from 680 to  $1\,100$  m, the lower one from 320 to 470 m.

## Sampling methods

The study was carried out in the years 2010–2015. Sampling was done directly at different representative points in the area. In the six study years we spent 19 days in the field. From each section (upper, middle, lower) we collected samples from 31 locations. Sampling was performed in two ways. In all the 93 investigated points the samples were collected directly with tweezers under the available shelters. We spent 20–30 minutes on each sample. In forests we used the litter sieve. The isopods were conserved and determined in the laboratory using the specialty literature for diagnosis (Radu 1983, 1985) and present nomenclature (Schmalfuss 2003).

## **Statistics**

To estimate if the sample size was sufficient, the individual rarefaction was calculated, using Past.3x free software (Hammer et al. 2001). The data were analysed comparatively, taking into account the percentage abundance, frequency, diversity and species richness from each section. The similarity between section was estimated with the Jaccard index. For the significance of the differences between sections we used the Mann-Whitney U test (Zar 1999) calculated with Past.3x (Hammer et al. 2001). With the same software we performed a correspondence analysis on the species on different sections.

## Results

In Vâlsan River basin we identified 21 terrestrial isopod species (Table 1). The only species which could not be identified belonged to Trichoniscus genus, of which we captured only five females. In total we captured 749 individuals, 42.99% of them in the upper section, 29.90% in the middle section and 27.10% in the lower one. The relatively small number of individuals is due to the study's faunistic objective and to the direct method used. After performing the rarefaction analysis we found that we had collected enough samples for estimating the region's species diversity (the minimum number of necessary samples was 28 in the upper and lower sections and 30 in the middle one). There are differences between the number of individuals / sample between the sections, 10.38 in the upper section, 7.22 in the middle section and 6.54 in the lower one. The most important species, both by percentage abundance and frequency of occurrence, was Trachelipus arcuatus (Table 1). The Vâlsan River area has a diverse terrestrial isopod fauna, with a Shannon-Weaver index of H = 2.50.

Both the terrestrial isopod species composition and their ratio differ depending on the section. The upper



Figure 3 – Correspondence analysis between the investigated species composition from the three sections (Lh – L. hypnorum, Lg – L. germanicum, Hr – H. riparius, Ht – H. transsilvanicus, Hmo – H. motasi, Trich – Trichoniscus sp., Mg – M. graniger, Hd – H. danicus, Hm – H. mengii, Cc – C. convexus, Ct – C. transsilvanicus, Pp – P. politus, Pc – P. conspersum, Ta – T. ater, Td – T. difficilis, Tarc – T. arcuatus, Tr – T. rathkii, Ppr – P. pruinosus, Psc – P. scaber, Psp – P. spinicornis, Avu – A. vulgare)

section presents the highest species richness (Table 1). The same situation can also be observed for diversity (Table 1). The species overlap between assemblages is relatively reduced; according to the Jaccard index it is 72.22% between the upper and middle sections, and 50% between the lower and the other two sections. In the upper section species tied to natural habitats (forests, wetlands, floodplain forests) prevail, in the middle and lower sections species with anthropophilous tendency are present (Table 1, Figure 3). With the lower altitude the number of individuals of the Ligidium genus and Hyloniscus transsilvanicus decrease. Some sylvan species are present exclusively, or in an obviously higher number, in the higher section (e.g. Porcellium conspersum, Trachelipus ater, Trachelipus difficilis). Species with anthropogenic tendency appear exclusively or in a high number in the lower section (Porcellionides pruinosus, Porcellium scaber, Porcellium spinicornis, Armadillidium vulgare). The differences between sections were not significant according to the Mann-Whitney U test (p > 0.05).

## Discussion

The terrestrial isopod fauna from the Vâlsan section includes three species endemic in Romania, *Hyloniscus motasi, T. ater* and *Cylisticus transsilvanicus.* Their presence confirms the region's special characteristics also from the isopod's perspective, particuliarities proved by the endemic fish *Romanichthys valsanicola* (e.g. Telcean et al. 2011). *T. ater* is present in the central part of the Southern Carpathians in afforested areas with a gorge aspect, Vâlsan River basin being the eastern limit of its distribution range (Tomescu et al. 2015). According to older data, *C. transsilvanicus* and the synonymized C. major (see in: Schmalfuss 2003) are present in the eastern Apuseni Mountains, towards Transylvania (Radu 1951, 1985). Afterwards it was found in Bucharest (Giurgincă 2006), in eastern Transylvania (Giurgincă & Vănoaică 2006-2007) and recently in north-western Romania, a discovery which seemed to explain its formation and distribution (Ferenți et al. 2013a). The identification of C. transsilvanicus in Vâlsan River basin, however, seems to link different parts of its apparently isolated distribution range and raises again the issue of its formation and distribution, which presently seems to be scattered. From what we saw both previously (Ferenți et al. 2013a) and in the Vâlsan area, the species is truly linked to natural areas. In the Vâlsan River basin C. transsilvanicus occur in humid sections close to the river covered with natural beech forest. We have observed many individuals near a sulphurous spring, but the species was also found in forests under fallen logs or barks. Unlike for the above species, there are very few data about H. motasi. Under the name of Ropaloniscus motasi it was reported once in north-western Romania in the Oas Mountains (Radu, 1983) and subsequently considered to belong to the genus Hyloniscus (see in: Schmalfuss 2003). We have found it in a humid zone at approximately 4 m from the Vâlsan River. H. motasi individuals were present under fallen stones from a forest road's protective wall to 10 cm depth in the soil. H. motasi's identification in the Vâlsan Valley greatly expands its previously known distribution range, raising the issue of reconsidering the species' distribution range limits.

In terms of numbers of terrestrial isopod species, the Vâlsan River basin resembles other mountain areas in Romania, where samples were also collected directly (Giurgincă et al 2006; Tomescu et al. 2011; Ferenți et al. 2013c; Ianc & Ferenți 2014). We encountered the same number of species as in the Pădurea Craiului Mountains (Ianc & Ferenți 2014). Although the species composition between the two regions differs partially, in both areas sylvan and wetland species prevail. Compared to the Oaş Mountains (Ferenți et al. 2013c), there are more isopod species in the Vâlsan River basin. From Oas many anthropophilous species are absent; if we exclude them from the Vâlsan section, the two areas would have roughly the same number of species. The Oaş Mountains thus conserve the native fauna more uniformly, which in the Vâlsan valley was maintained especially in the upper section, probably due to the intense forest clearings in the past (Badea 2011). Compared with the Jiului Gorge (Tomescu et al. 2011), the Vâlsan section presents seven more species. From Jiului Gorge the small-sized species of Haplophthalmus genus is missing (Tomescu et al. 2011). Due to their larger distribution in the country (e.g. Giurgincă 2006; Ferenți et al. 2012; Bodin et al. 2013; Ianc & Ferenți 2014), the differences are probably coincidental. The terrestrial isopod fauna of Vâlsan seems richer than the one from the Piatra Craiului Mountains, where only six species were identified, but

where mainly caves were investigated (Giurgincă et al. 2006). Compared to other countries, relatively few species (only 10) were encountered in the karstic area from Aggtelek National Park, Hungary (Vilisics et al. 2011), of which we found only five in the Vâlsan River basin. This fact indicates the regional differences that appear when comparing different geographical units. These differences are even more obvious in comparison with some mountain areas in Greece, where only one species is also found in the Vâlsan Valley (Sfenthourakis et al. 2012).

Of the 21 species in the Vâlsan River basin, only nine were present in all sections. Of these, four are praticolous, three sylvan and two euritopic species. The high number of species in the upper section is the consequence of the lower anthropogenic impact, but also of the fact that endemic species occur in higher sections. Three species are present only in high areas and low areas each, and just one, M. graniger, is present exclusively in the middle section. It is a mainly cavernicolous species, but endogeous populations were also registered (see in: Giurginca 2009). We found an endogeous population under stones deeply embedded in the soil, at 2-3 m from the Vâlsan River, like in other cases (Radu 1985; Giurginca 2009). According to literature data (Giurginca 2009) the species seems to have been found for the first time in the Vâlsan River basin and also in most parts of the Arges River basin. This range extension partly fills the gap between the M. graniger populations from the Eastern and Western Carpathians (see in: Giurginca 2009). Its exclusive identification in the middle section is probably coincidental, M. graniger having a much larger altitudinal distribution (Giurginca 2009), but because of its endogeous life it is more difficult to observe. If in the high area endemic species are characteristic, in the low one anthropophilous, non-native species like P. pruinosus (Cochard et al. 2010) are present. There are differences between the three sections in terms of percentage abundance and the frequency of occurrence of some species. Thus, for A. vulgare, a species frequent in disturbed areas (e.g. Bodin et al. 2013), the percentage abundance increases from the upper to lower sections. The situation is inversed in T. difficilis and Ligidium genus.

The differences of the terrestrial isopod fauna between the three sections can be explained both by the altitudinal differences and their different anthropogenic disturbance. Altitudinal differences in terrestrial isopods' distribution were previously reported in Romania (Tomescu et al. 2011) and other regions too (e.g. Sfenthourakis 1992; Lopes et al. 2005; Sfenthourakis et al. 2012). Sometimes, the diversity of the terrestrial isopod fauna decreased with increasing altitude (Sfenthourakis 1992), but in other cases this fact was not obvious (Sfenthourakis et al. 2012). In the Vâlsan Valley the most important area for isopods is the upper section. Still, in the case of other groups the higher mountainous region proved to be more unfavourable (Fabrizio & Pedrini 2007). Among others, this fact was attributed to the low temperatures at high altitudes (Fabrizio & Pedrini 2007). The thermal differences are also obvious at Vâlsan, approximately 5°C between the upper and lower sections (Stoenescu et al. 1966). Yet this difference does not reduce the terrestrial isopod assemblages from the upper area. But, unlike other regions with a poor upper section, the altitudinal differences at Vâlsan are smaller, not exceeding 800 m. Moreover, on Vâlsan there are no native species in the lower sections which are absent in the upper one because of the tough climatic conditions given by the altitude. On the contrary, the native species are distributed along the entire river basin, being well represented in the upper section. The changes of the isopod fauna along the three sections are therefore not determined by the altitude. The influx of anthropophilous species indicates the cause of these differences. The anthropogenic disturbance of the middle and lower sections had restricted the habitats of the native species and permitted the access of the anthropophilous ones. In the Vâlsan River basin the differences between the sections are recent and manmade. The human development of the region has limited the available surfaces for the native species, transforming them into favourable habitats of non-native, anthropophilous species. Probably the main cause that affected the lower sections was the intense clearing activity (Badea 2011). Forest conservation seems to guarantee the endemic species' survival here as well as in other cases (e.g. Pryke & Samways 2010).

The presence of three endemic species in Romania in the Vâlsan River basin confirms the region's peculiarities also in terms of terrestrial isopods. Isopod species seem to have taken refuge in the area, not only fish or amphibians (e.g. Telcean et al. 2011; Covaciu-Marcov et al. 2014). In the case of isopods, too, the endemic species with limited distribution range are linked to natural areas with restrictive conditions. This fact does not confirm only the specificity of the Vâlsan River basin but also the possibility to use terrestrial isopods as zoogeographical indicators (Tăbăcaru & Giurgincă 2013; Ferenți & Covaciu-Marcov 2014). In terms of conservation, the need to protect the natural forests and humid habitats becomes obvious, as does stopping the alteration of natural landscapes. Unfortunately not a single terrestrial isopod species is protected in Romania, not even the endemic species (OUG 57/2007). The protection of rare and endemic terrestrial isopod species can currently only be realized by preserving their habitats. The story told by the terrestrial isopods highlights the conservative importance of the Vâlsan River basin as a reservoir of endemic species which still survive here.

## **Acknowledgements**

Our study was made with the support of Freies Europa Weltanschauung Foundation, the custodian of the

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