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Research article

Ground beetle (Coleoptera: Carabidae) taxocoenoses from high-altitude *Pinus peuce* and *Pinus heldreichii* forests in Bulgaria

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Abstract: The sensitivity of ground beetles to changing habitat conditions makes them a good indicator of environmental change. The shift in their distribution could be used as an early warning signal for natural disturbances due to climate change. To reflect and monitor these changes, initial information is needed on carabid taxa in different high-altitude habitats, including the endemic and climate-sensitive tertiary relict coniferous forests of *Pinus peuce* (Macedonian pine) and *Pinus heldreichii* (Bosnian pine). Data on the carabid beetles in the different coniferous habitats in Bulgaria are scattered in various faunistic or taxonomic publications, and there is no exact information about their taxocoenoses in the Macedonian and Bosnian pine habitats. The present study aims to clarify the species composition and diversity patterns of ground beetles in these habitats in Bulgaria. Six sample sites were selected: five natural forest stands in the Rila Mts and Pirin Mts and one forest plantation in the Vitosha Mts. A total of 18 species and subspecies typical of forest habitats were recorded. Five of them were endemic to Bulgaria and five to the Balkan Peninsula. The most common species and the eudominant in the majority of the sample sites was *Calathus metallicus aeneus*. The exception was one of the Bosnian pine sample sites. Here, the dominant species was *Xenion ignitum*. The ground beetle species found in the studied forests were mostly zoophagous, mainly crevice or burrowing stratobionts in leaf litter, rocks and soil, a few epigeobionts, one botrobiont and one mixophytophagous geohrotobiont. The classification of the carabid taxocoenoses according to their qualitative composition showed two main clusters: the first being the carabids from the studied sites of the Vitosha and Rila Mts, and the second being the taxocoenoses from the Pirin Mts. The Bosnian pine habitats provide more favourable conditions for the high-altitude ground beetles, where they are in a state of equilibrium with higher species richness and evenness compared to those inhabiting the Macedonian pine forests. The forest communities of *Pinus peuce* and *Pinus heldreichii* in Bulgaria are of high conservation importance for the ground beetle and an even higher level of protection of these habitats is required.

Keywords: Balkan Peninsula, biodiversity, Bosnian pine, Bulgaria, endemics, ground beetles, Macedonian pine, relicts

Introduction

Carabid beetles are highly sensitive to changing habitat conditions and are good indicators of the ecosystem state (Niemelä, 2000; Rainio & Niemelä, 2003; Avgin & Luff, 2010; Koivula, 2011; Kotze et al., 2011). The species distribution of ground beetles at high altitudes depends mostly on the climate and soil conditions, as they respond to variations in temperature and moisture (Thiele, 1977; Fuller et al., 2008). In this context, the shifts in their distribution could be used as an early warning signal for natural disturbances due to climate change (Koivula, 2011; Zou Yu et al., 2014). To reflect on and monitor these changes, initial information on carabid species composition in different high-altitude habitats is needed. Such habitats of biogeographical and conservation importance are the coniferous forests dominated by tertiary relict Macedonian pine *Pinus peuce* (Balkan endemic) and Bosnian pine *Pinus heldreichii* (Balkan-Apennine endemic) in Bulgaria. Both species form specific monodominant or mixed (mainly the Macedonian pine, very limited the Bosnian pine) forest phytocoenoses (EUNIS code T39) between 1400–2200 m a.s.l., often at the timberline in the highest mountains in Bulgaria (Rusakova, 2015; Rusakova & Valchev, 2015). *P.*

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peuce forests are distributed mainly in the Rila and Pirin mountain ranges and in a very limited area in the Stara Planina Mountains, while the *P. heldreichii* forests are distributed in the Pirin and Slavyanka Mountains. These habitats are sensitive and under threat from climate change, logging and other anthropogenic pressures. In the Red Data Book of the Republic of Bulgaria, their status was assessed as 'Endangered' (for the Macedonian pine forests) and 'Vulnerable' (for the Bosnian pine forests) (Rusakova, 2015; Valchev & Rusakova, 2015). *P. peuce* and *P. heldreichii* forests in Bulgaria are protected by Bulgarian biodiversity law and are listed in Annex I of Habitat Directive 92/43/EEC (Code 95A0). A large territory of the forests in Bulgaria is situated in protected areas: Rila, Pirin and Central Balkan National Parks, including some Natural Reserves (Rila: Skakavitsa, Central Rila Reserve, Ibar; Pirin: Bayuvi Dupki-Dzhindzhiritsa, Yulen; Slavyanka: Ali Botush) and NATURA 2000 sites. Attempts have been made in the past to reforest deforested parts of various mountains with these tree species to protect them against erosion and snow drifts on high-altitude mountain roads. One of the oldest and largest forest plantations of *Pinus peuce* is located in Vitosha Mts, a natural park. The mountain was almost completely deforested by the beginning of the 20th century. The natural Macedonian pine forests in Vitosha were completely destroyed and reduced to only 30 trees (Penev, 1942). Then a large-scale afforestation was carried out (Biolchev, 1941). The planting of *P. peuce* was a successful attempt to reintroduce the species to the region.

The information on the carabid beetle taxocoenoses in the Macedonian and Bosnian pine coniferous habitats in Bulgaria is scattered in various faunistic or taxonomic publications, often giving notes only about the altitude or forest belt of the three mountains. The known number of species of ground beetles that reach 1800–2000 m above sea level in the three mountains is as follows: 122 from Rila Mts, 97 from Pirin Mts, and 95 from Vitosha Mts (Guéorguiev & Guéorguiev, 1995). Sakalian and Guéorguiev (1997) listed the endemic species of the Pirin but did not specify the exact type of their habitat. The most recent information on high-altitude endemic carabids in the Rila and Pirin Mts was published by Donabauer (2020), but the material was collected mainly from the rocky part and *Pinus mugo* forests of the subalpine zone, and from one locality with *Picea abies* forest. A few studies provide characteristics of ground beetle assemblages in coniferous forests dominated by *Picea abies* and *Pinus silvestris*. Krusteva et al. (1995), using gradient analysis, determined the direct negative effect on carabids of lowering the temperature and decreasing the productivity of the near-ground vegetation layer under a dense spruce canopy in Vitosha Mts. A total of 20 species were found in the old spruce forest of the Mantaritsa Nature Reserve (Rhodope Mts) (Kostova, 2009; Teofilova, 2017). The eudominant species were *Molops dilatatus* Chaudoir, 1868, *Calathus metallicus aeneus* Putzeys, 1873, *Carabus violaceus azurescens* Dejean, 1826, and *Cychrus semigranosus balcanicus* Hopffgarten, 1881 (Kostova, 2009). From the *Pinus sylvestris*-dominated forest near the Trite Buki Hut, Osogovo Mts, Guéorguiev (1996, 1999) reported a low taxon diversity of six species. Guéorguiev et al. (2003) reported *Dromius agilis* (Fabricius, 1787) as a new species for Vitosha Mts, collected from *Picea abies* forest in the Bistrishko Branishte Nature Reserve. The present study aims to clarify the species composition and diversity patterns of carabid beetles in the old growth forests of endemic and relict *Pinus peuce* and *Pinus heldreichii* in Bulgaria.

Material and methods

The following representative sample sites for the study were chosen: in Rila Mts: under Malyovitsa Hut (northern slope) and above Treshtenik Hut (southern slope); in Pirin Mts: near Banderitsa Hut, under Vihren Hut (Northern Pirin: eastern slope of Vihren Peak) and near Yavorov Hut (Northern Pirin: western slope of the circus Razlozhki Suhodol); in Vitosha: under Aleko Hut (north slope) (Figs 1 and 2, Table 1). The material was collected monthly during the period of July–September 2020 and May–July 2021 in the selected sites by pitfall traps: 500 ml containers filled up to 1/3 with propylene glycol as a conservant, set in a line by 10 at each site. The traps were set deep in the forests at least 50 m from the borders with other types of forest, semi-open or open habitats to avoid the edge effect. To standardise the data, the number of ground beetles captured, reflecting the ground beetle activity density, was converted into abundance: the number of individuals captured per 100 nights of trapping. The collected material was deposited at Sofia University Zoological Collection (BFUS).

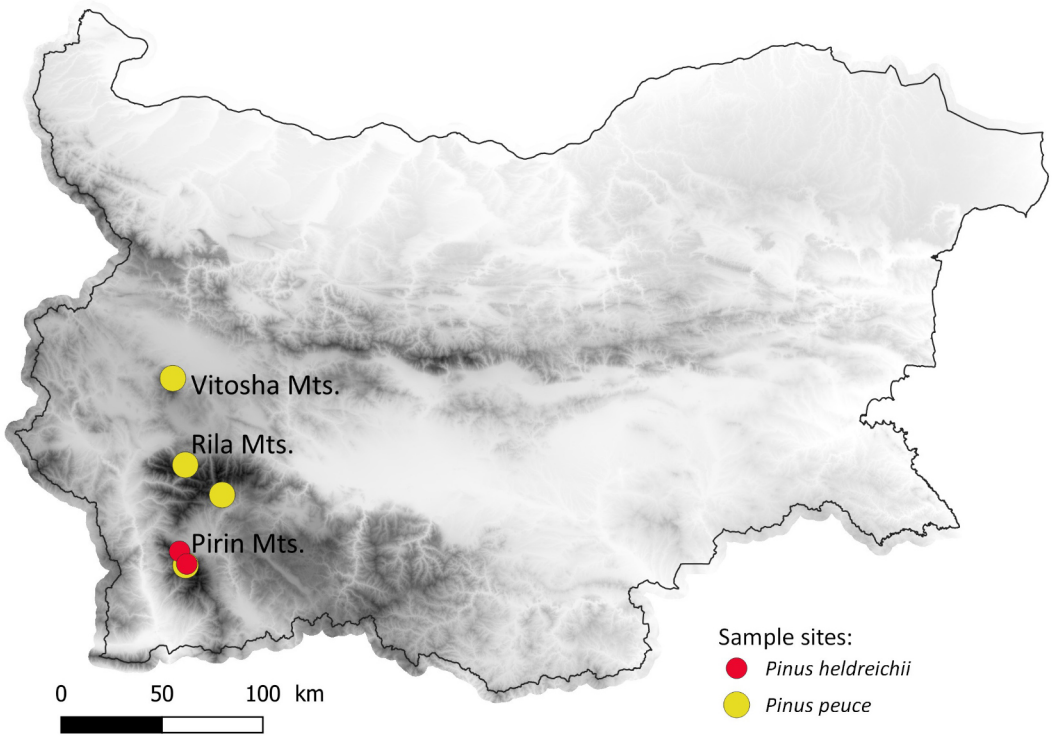

Fig. 1. Map of the studied sample sites with *Pinus peuce* and *Pinus heldreichii* forest stands in Bulgaria.

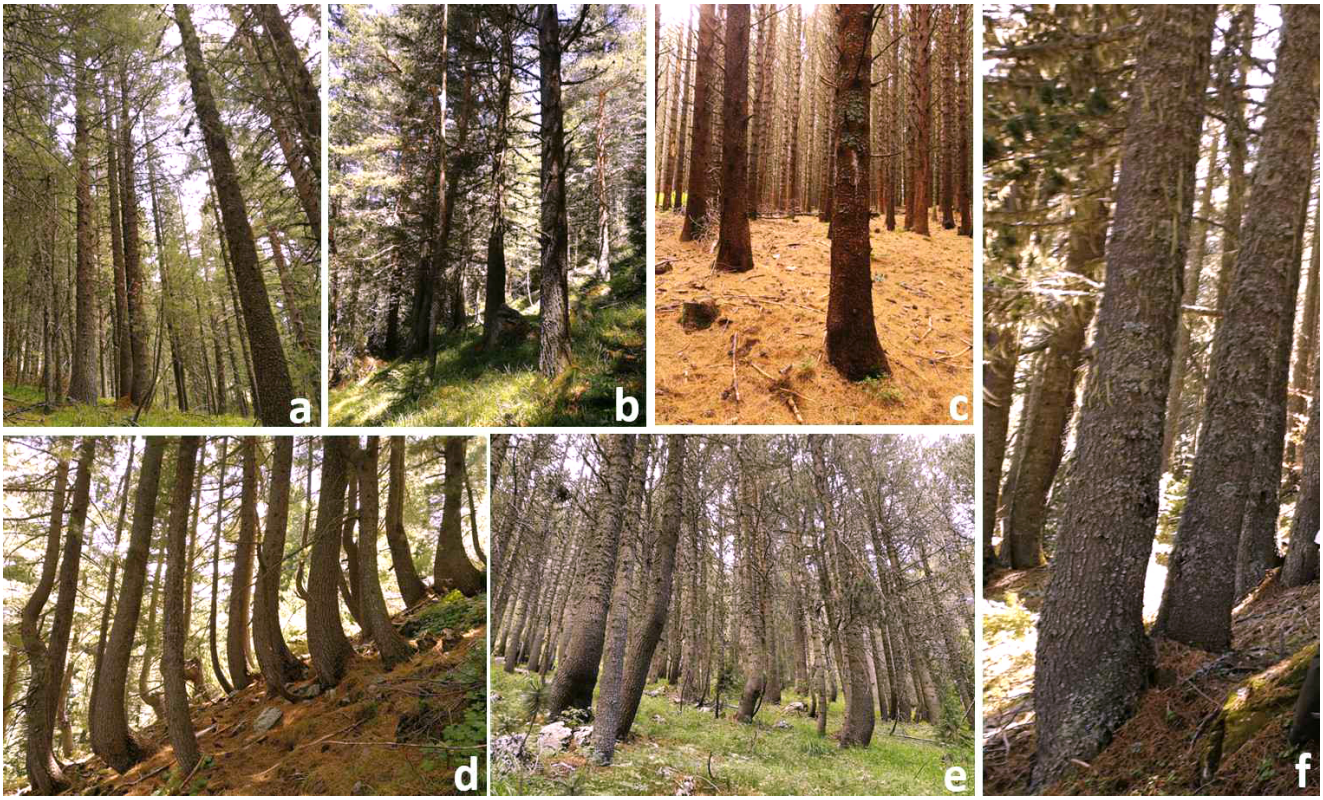

Fig. 2. Sample sites: *Pinus peuce* forests – (a) Treshtenik Hut, Rila Mts; (b) Malyovitsa Hut, Rila Mts; (c) Aleko Hut, Vitosha Mts; (d) Vihren Hut, Pirin Mts; *Pinus heldreichii* forests – (e) Banderitsa Hut, Pirin Mts; (f) Yavorov Hut, Pirin Mts.

Table 1. Studied sample sites and some of their main characteristics (accessed at Forest Database of the Bulgarian Forest Executive Agency).

No.	Mountain	Sample site	Coordinates	Altitude (m)	Forest-forming tree species	Age (years)	Inclination (°)	Substrate base
1	Rila	Malyovitsa Hut	42.208961° 23.390046°	1760	*Pinus peuce* 50% : *Pinus sylvestris* 30% : *Picea abies* 20% : *Abies alba* single trees	100	42	Silicate
2		Treshtenik Hut	42.082165° 23.618036°	1915	*Pinus peuce* 80% : *Pinus sylvestris* 10% : *Picea abies* 10%	90	28	Silicate
3	Pirin	Vihren Hut	41.761884° 23.416967°	1969	*Pinus peuce* 80% : *Pinus sylvestris* 10% : *Picea abies* 10%	140	33	Carbonate rocks
4		Banderitsa Hut	41.768033° 23.423944°	1876	*Pinus heldreichii* 80% : *Pinus sylvestris* 10% : *Picea abies* 10%	140	35	Carbonate rocks
5		Yavorov Hut	41.823000° 23.377267°	1716	*Pinus heldreichii* 60% : *Pinus sylvestris* 40%	100	32	Carbonate rocks
6	Vitosha	Aleko Hut	42.591967° 23.292617°	1814	*Pinus peuce* 90% : *Picea abies* 10% (culture)	80	25	Silicate

The following indices were used to analyse α-diversity in the studied habitats:, Pielou's evennes, Hill's diversity and Simpson's concentration of dominance (λ). Taxonomic similarity was calculated using Sørensen's index and represented graphically by a dendogram using the unweighted pair group method with arithmetic mean (UPGMA) (Sokal & Michener, 1958). PRIMER 7 (PRIMER-e, Quest Research Limited) software was used for the analyses and QGIS for generating a map.

Results

A total of 692 carabid beetle specimens were collected from *Pinus peuce* and *Pinus heldreichii* forests during the field studies in Rila, Pirin and Vitosha Mts. Eighteen species and subspecies typical of forest habitats were recorded. Five of them were endemic to Bulgaria and five to the Balkan Peninsula (Table 2).

As expected, the estimated number of ground beetle species was relatively low. However, the carabids were more abundant and diverse in *P. heldreichii* forests than in natural *P. peuce* forests. A species common to all sites was *Calathus metallicus aeneus*. The species of ground beetles found in the studied forests were predators, mainly crevice or burrowing stratobionts in the leaf litter, rocks and soil, a few epigeobionts of the genera *Calosoma, Carabus* and *Cyhrus,* one botrobiont (*Laemostenus terricola*) and one mixophytophagous geohrotobiont (*Harpalus rufipalpis*). Classification of the life forms followed Sharova (1981).

A taxonomically interesting specimen of the genus *Tapinopterus* was found in the sample site of *Pinus peuce* near the Vihren Hut (Pirin Mts), which does not fit the descriptions of any of the known Bulgarian taxa. Further study of this genus will be necessary after the collection of more material and the publication of the expected revision of the group by Giachino, Picciau, Vailati and Casale (Gueorguiev, personal communication).

In three of the Macedonian pine sample sites – Pirin – Vihren Hut, Rila – Malyovitsa Hut and Rila – Treshchenik Hut, there was one strongly dominant species: *C. metallicus aeneus,* represented respectively by 85%, 81% and 67% of all collected specimens (Figs 3 and 4). In the fourth, the Macedonian pine forest plantation in Vitosha Mts, two species dominated almost equally: *P. rhilensis* (46%) and *C. metallicus* (43%). In the Bosnian pine forests of the

Table 2. List of the Carabidae species recorded from the high-altitude Macedonian and Bosnian pine forests in Bulgaria. *Bulgarian endemics; **Balkan endemics.

Species/Coniferous community	Rila Mts		Pirin Mts			Vitosha Mts
	Malyovitsa Hut	Treshtenik Hut	Vihren Hut	Yavorov Hut	Banderitsa Hut	Aleko Hut
	Pinus peuce	*Pinus peuce*	*Pinus peuce*	*Pinus heldreichii*	*Pinus heldreichii*	*Pinus peuce* (culture)
***Calathus metallicus aeneus* Putzeys, 1873	+	+	+	+	+	+
Calosoma inquisitor Linnaeus, 1758					+	
Carabus coriaceus cerisyi Dejean, 1826				+	+	
Carabus hortensis Linnaeus, 1758					+	+
***Carabus violaceus azurescens* Dejean, 1826	+			+	+	+
***Cyhrus semigranosus balcanicus* Hopffgarten, 1883						+
Harpalus rufipalpis Sturm, 1818		+				
Laemostenus terricola (Herbst, 1784)				+		
Leistus spinibarbis rufipes Chaudoir, 1843						+
**Molops alpestris rhilensis* Apfelbeck, 1904					+	
***Molops dilatatus dilatatus* Chaudoir, 1868					+	
**Molops rhodopensis kourili* Maran, 1942			+	+	+	
Notiophilus biguttatus (Fabricius, 1779)	+	+				+
**Pterostichus rhilensis* Rottenberg, 1874	+					+
**Tapinopterus balcanicus balcanicus* Ganglbauer, 1892		+	+		+	
Tapinopterus sp.			+			
**Trechus rhodopeius* Jeannel, 1921		+				+
***Xenion ignitum* (Kraatz, 1875)				+	+	

Pirin Mts, a high degree of evenness of the species abundance was found to exist, with a very slight predominance of *X. ignitum* in the Pirin – Yavorov Hut (43%) and *C. metallicus* in the Pirin – Banderitsa Hut (42%). Of all the coniferous sites studied, *C. coriaceus, C. hortensis* and *X. ignitum* (usually frequent species for the altitude) were only recorded in the Bosnian pine forests of Pirin and never in the Macedonian pine forests.

The ground beetles from the Bosnian pine forests in Pirin and from the forest plantation of Macedonian pine in Vitosha had the highest species richness, while those from the Macedonian pine forests in Rila – Malyovitsa Hut and Pirin – Vihren Hut had the lowest (Table 3). The Macedonian pine plantation in Vitosha was characterised by the highest ground beetle activity density, and the natural forest of Macedonian pine in Rila – Malyovitsa Hut and Bosnian pine in

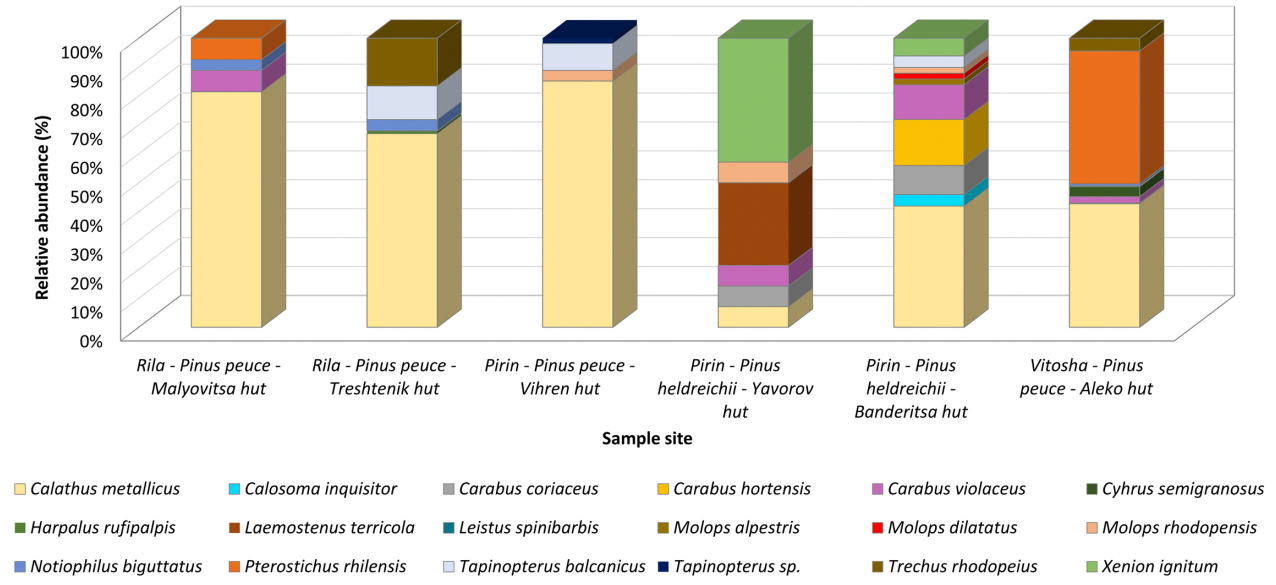

Fig. 3. Species composition and abundance of the ground beetles in the studied coniferous habitats.

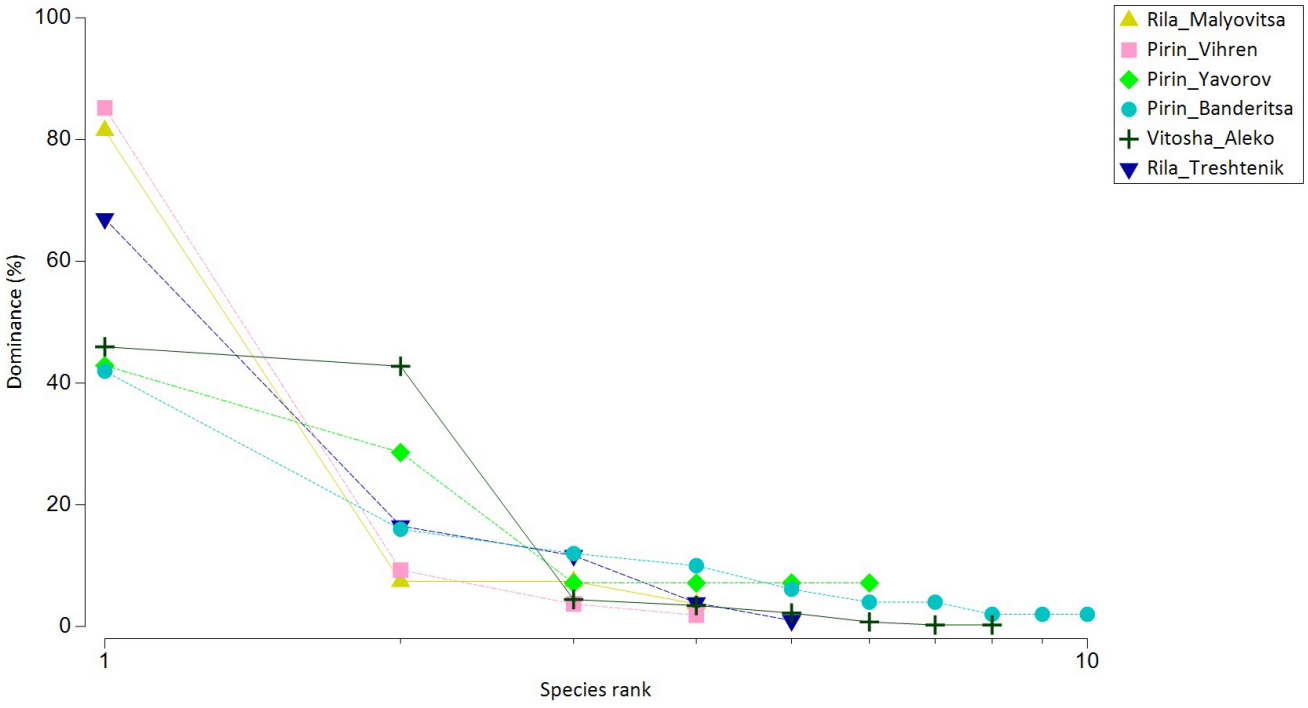

Fig. 4. Rank structure of the ground beetle taxocoenoses in the studied coniferous habitats.

Pirin – Yavorov Hut were characterised by the lowest activity density. Diversity indices showed that the carabid taxocoenose of *P. heldreichii* forests had the highest diversity and evenness of species. The *P. peuce* forest in Pirin – Vihren is characterised by the lowest diversity of ground beetles, low evenness and the presence of highly dominant species.

The classification of the carabid taxocoenoses by qualitative composition showed two main clusters: the first are ground beetles from the studied sites of

Table 3. Indices of α-diversity of the carabid taxocoenoses in the studied coniferous habitats.

Index	Rila Malyovitsa	Rila Treshtenik	Pirin Vihren	Pirin Yavorov	Pirin Banderitsa	Vitosha Aleko
Taxa number	4	5	4	6	10	8
Beetle number per 100 trap/days	6	11	11	5	7	45
Pielou's evenness	0.49	0.61	0.40	0.82	0.78	0.54
Hill's diversity N2	0.44	2.04	1.36	3.50	4.27	2.52
Simpson's dominance: λ'	0.62	0.44	0.71	0.12	0.11	0.38

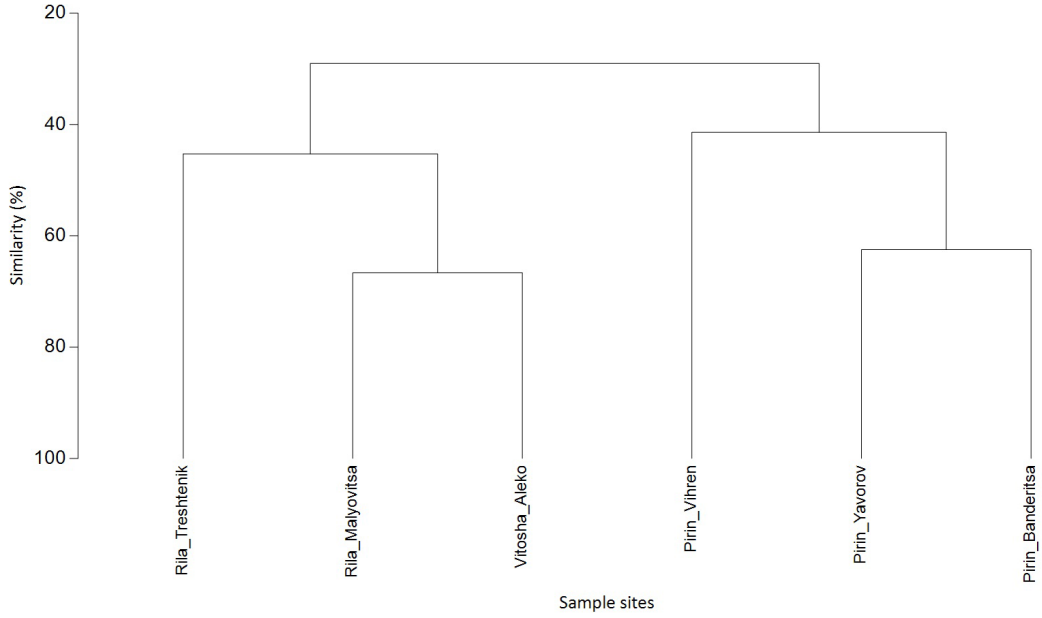

Fig. 5. Dendrogram of the taxonomic similarity of the ground beetles in the studied coniferous habitats. Sørensen index of similarity. UPGMA.

Vitosha Mts and Rila Mts (with the highest similarity being between Vitosha – Aleko Hut and Rila – Malovytsa Hut 67%) and the second one was formed by the taxocoenoses from Pirin Mts (with the highest similarity being between Pirin – Yavorov Hut and Pirin – Banderitsa Hut 63%) (Fig. 5).

Discussion

The results obtained showed an expected low number of taxa of the family Carabidae since the number of carabid taxa decreases with increasing altitude (Krusteva et al., 1995; Guéorguiev & Sakalian, 1997). In addition, carabids are often more numerous and species-rich in open habitats than in forests (Halme & Niemelä 1993; Koivula, 2011). Although the taxonomic diversity was low, the results showed a specificity of the ground beetle complexes in the Macedonian and Bosnian pine communities. The species found are characteristic of the high altitudes of the three mountains, but the coenoses they form are unique. The plant communities, with their different canopies, undergrowth, leaf litter layers and moisture levels, are the main reasons for the ground beetle arrangement (Kostova, 2009). Due to the particular sensitivity of carabids to soil moisture (Ludwiczak et al., 2020), major reasons for differences in the species composition at even closely spaced stationary sites, such as those of Macedonian and Bosnian pine in the Pirin Mountains, are the differences in the soil drainage due to the slope, the soil layer thickness, and

the substrate rock beneath. The two main clusters in the taxonomic similarity analysis also showed the relationship of the carabid assemblages to the substrate type – the first representing the taxocenoses from Rila and Vitosha on a silicate substrate and the second representing the taxocenoses from Pirin on a carbonate substrate. In addition to the substrate and habitat type, the similarity of the taxa from Rila – Malyovitsa and Vitosha – Aleko may also be related to the origin of the seedlings for the Vitosha culture, which are very likely to be from Rila Mts (Georgiev, personal communication). Bosnian pine forests stand on a thicker layer of soil, which is well drained but not as dry and rocky, and have richer undergrowth than Macedonian pine forests. They provide more favourable conditions for communities of high-altitude ground beetles, where they are in a state of equilibrium with higher species richness and evenness than those of Macedonian pine. The low number of carabid species and the presence of a highly dominant species in the Macedonian pine communities are probably due to the unfavourable environmental conditions caused by the high slope and thin soil layer, leading to rapid loss of surface moisture after snowmelt and rain. This is especially true for Macedonian pine forests on carbonate bedrock, such as the Pirin – Vihren sample site (Dimitrov, 2005). In Vitosha, the old forest plantation of *Pinus peuce* is located on a relatively thicker layer of soil and provides, although not by much, a higher species richness of carabid beetles than the natural stands of the same tree species.

Calathus metallicus aeneus is dominant in most Bulgarian alpine and subalpine carabid taxocoenoses (Guéorguiev, 2007). It was confirmed as eudominant in most of the Macedonian and Bosnian pine habitats in our study; it was only in the Bosnian pine forest from the Yavorov Hut area that it was in lower abundance than *X. ignitum*. In the spruce forest in Mantaritsa Nature Reserve, the eudominants differ – *M. dilatatus* and *C. violaceus azurescens*. However, *C. metallicus aeneus, C. semigranosus balkanicus* and *X. ignitum* were also dominant but in significantly lower numbers (Kostova, 2009). With 9 common species, the ground beetle community of *P. heldreichii* of Pirin – Banderitsa Hut showed the closest taxonomic structure of the carabid beetle communities to that of the spruce forest in the Mantaritsa Nature Reserve, reported by Kostova (2009). In both habitats, there was also a high evenness of the ground beetle species. Hill's index of diversity showed almost the same value in both coenoses: N2 = 4.25 for the Bosnian pine forest and N2 = 4.78 for the spruce forest. The life forms' structure of the ground beetle taxocoenoses in the Macedonian and Bosnian pine forests is typical of forest ecosystems with prevailing stratobionts and epigeobionts, both in terms of species number and abundance (Sharova, 1981; Solodovnikov, 2008; Kostova, 2009).

In the Balkans, mountains higher than 2000 m above sea level serve as both refugia and centres of ongoing speciation for the carabid beetles (Guéorguiev, 2007). As one of the typical high-altitude forest habitats in Rila, Pirin and Vitosha Mountains, the studied Bosnian and Macedonian pine endemic and relict forests contain a significant part of the high mountain diversity of endemic and relict ground beetle species in the Balkans. The Balkan and Bulgarian endemics comprised 56% of the total number of carabid beetle species found in the studied habitats. The great conservation significance of the *Pinus peuce* and *Pinus heldreichii* forest communities in Bulgaria for ground beetles is obvious. These communities are very vulnerable to environmental disturbances, including climate change. Both types of trees are adversely affected by drought conditions in the summer and cold winter. The growth of *P. peuce* is dependent on summer temperatures, while that of P. *heldreichii* is dependent on the amount of summer rainfall, with both tree species being negatively affected by dry summer and cold winter conditions (Panayotov et al., 2010). These factors must be relatively stable over time for forests to maintain the necessary microhabitat conditions for the existence of carabid taxocenoses in them. In addition, a major anthropogenic threat is the fragmentation and direct destruction of these Bulgarian forests by logging, as they are often subjects of investment interest (especially in Pirin and Rila Mts) and need even stricter protection.

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