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Article

New Records of Stoneflies (Insecta: Plecoptera) in National Park Shar Mountain (R. North Macedonia)

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Abstract
The collection of stoneflies in Shar Mountain resulted with records of four species (*Isoperla bosnica* Aubert, 1964, *Isoperla albanica* Aubert, 1964, *Nemoura uncinata* Despax, 1934, *Nemoura asceta* Murányi, 2007) for this mountain massif. From the mentioned species *Nemoura asceta* Murányi, 2007 and *Isoperla bosnica* Aubert, 1964 are endemic species to the Balkan Peninsula, while *Nemoura asceta* Murányi, 2007 is recorded for the first time in the country. These results highlight the importance of Shar Mountain as a stoneflies biodiversity hotspot, increasing the focus on the conservation status of these rare species.

Key words Stoneflies, new records, endemic species, Shar Mountain, R. North Macedonia.

Introduction

Stoneflies as aquatic insects play crucial roles in ecosystems as they act as indicators of water quality, providing food for predators, helping with energy flow and nutrient cycling (DeWalt & Ower, 2019).

Their life cycle involves distinct habitat preferences, so the larvae of stoneflies are mostly found in aquatic environments, such as streams, rivers, and springs and when they emerge as adults can be found around the aquatic ecosystem's vegetation (DeWalt et al., 2015)

The first investigations of stoneflies in R. North Macedonia were concentrated in the western parts of the country and dates back to the initial decades of the last century (Komárek, 1935; Šamal, 1935). Additionally, Šamal (1935) revised the stonefly collection by Komárek (1935) and confirmed the presence of 25 stonefly species in the country. However, this number changed when the Czech plecopterologist Raušer (1963) conducted thorough analysis of the stonefly collections of these two authors and showed that there are only 13 species of stoneflies for the Macedonian fauna.

The Plecoptera checklist of R. North Macedonia significantly expanded over the next two decades (Ikonomov 1969; 1970; 1971; 1972; 1973; 1974a; b; 1975, 1976a; b; 1977; 1978a; b; 1979; 1980a; b;1981; 1982; 1983a; b; c; d; 1986). These studies contributed in identifying 93 species across to 18 genera and 7 families (Taeniopterygidae, Nemouridae, Leuctridae, Capnidae, Perlodidae, Perlidae, and Chloroperlidae) within the country (Ikonomov, 1986). The stonefly investigations in R. North Macedonia have also continued over the last years (Murányi, 2007; Murányi et al. 2014; 2016; 2021; Graf et al., 2012; 2014) increasing the total number of species known from the country to 102. Data on the distribution of stoneflies were provided by Slavevska/Stamenkovic et al. (2016) in some rivers: such as Bregalnica, Orevovechka, and Lenishka rivers, and more recently, in Karadak Mountain Kozhuf Mountain, Sveta Voda and village Orebobej (Bilalli et al. 2023).

When it comes to the stonefly diversity of Shar Mountain, Ikonomov (1980) described two new species, *Isoperla breviptera,* Ikonomov 1980 and *Taeniopteryx fusca*, Ikonomov 1980 from Popova Shapka and Tserepashina, respectively. The following year the author reported presence of 41 taxa in the aquatic ecosystems of the central part of Shar Mountain (Ikonomov, 1981). Later, Memeti & Janeva, (1999) recorded three species in the river Pena, namely *Leuctra nigra* (Olivier, 1811), *Perla pallida* Guérin-Méneville, 1843, and *Isogenus nubecula* Newman, 1833, from which only the last species was new to the fauna of the R. North Macedonia. *Isogenus nubecula* Newman, 1833 was originally occurring at many localities in western and central Europe (Derka et al. 2002). In 1992, Zwick highlighted the widespread disappearance of *Isogenus nubecula* during the second half of the 20th century. Therefore, the finding of this species in our country can be considered a dubious record.

During the first two decades of the 21st centrury, *Isoperla citrina* Murányi, 2011 and *Nemoura anas* Murányi, 2007 were recorded also for the first time in R. North Macedonia (Murányi, 2007; Murányi et al. 2016). In the original description of *Leuctra dalmoni* by Vinçon & Murányi in 2007, this species was reported from Popova Sapka among the non-type materials. The latest data on the stonefly diversity of Shar Mountain was presented within the study for valorization of Sharr Mountain (UNEP, 2020), when the group of experts estimated the number of Plecoptera species on Shar Mountain to 57. Since then, the only investigations on aquatic insects conducted on this mountain included caddisflies (Hinić-Jordanovska et al. 2024), while new data on stoneflies have not yet been reported.

The results obtained from this paper will constitute a significant contribution to the understanding of the stonefly diversity in Shar Mountain and in the whole country. Identifying aquatic ecosystems with high biological value, will once again highlight the natural importance and significance of the Shar Mountain area as a biodiversity hotspot.

Material and methods

Study area

Shar Mountain is the second-highest mountain range in R. North Macedonia, standing at 2747 m and it is situated in the northwest part of the country along the border with Kosovo, the mountain range covers an area of 1670 km^2 with nearly half (840 km^2) belonging to North Macedonia, additionally, it has a length of 80 km and is part of the Dinaride-Hellenide mountain belt. (Milevski, et al. 2020)

Data sampling and processing

The sampling of stoneflies was conducted from May 2021 to October 2023, using entomological net and beating sheet during the day, at 35 selected localities on Shar Mountain including lotic and lentic ecosystems. Out of the 35 sampling localities investigated, the species in this study were recorded at

only four localities, as shown in Figures 1 and 2. The collected material was labeled and preserved with 96% ethanol. The taxonomic identification of adult specimens was carried out using Leica/Wild Microscope M3Z Combi Stereo and corresponding identification keys (Aubert 1964, Despax, 1934, Murányi, 2007).

Table 1. Geographical coordinates of each location and the period of collection.

Code	Localities	Collecting period	Latitude	Longitude	Altitude
L1	Tributary of the river Pena, village Brodec	May 2021 - October 2023	42° 3' 35.21" N	20° 53' 2.34" E	998 m
L2	River of Bogovinje, village Bogovinje		41° 55' 33.58" N	20° 54' 26.98" E	564 m
L3	Stream on the way to Mazdracha		41° 54' 27.32" N	20° 49' 8.18" E	1329 m
L4	Stream after Lisec village		42° 0' 3.32" N	20° 54' 54.63" E	1172 m

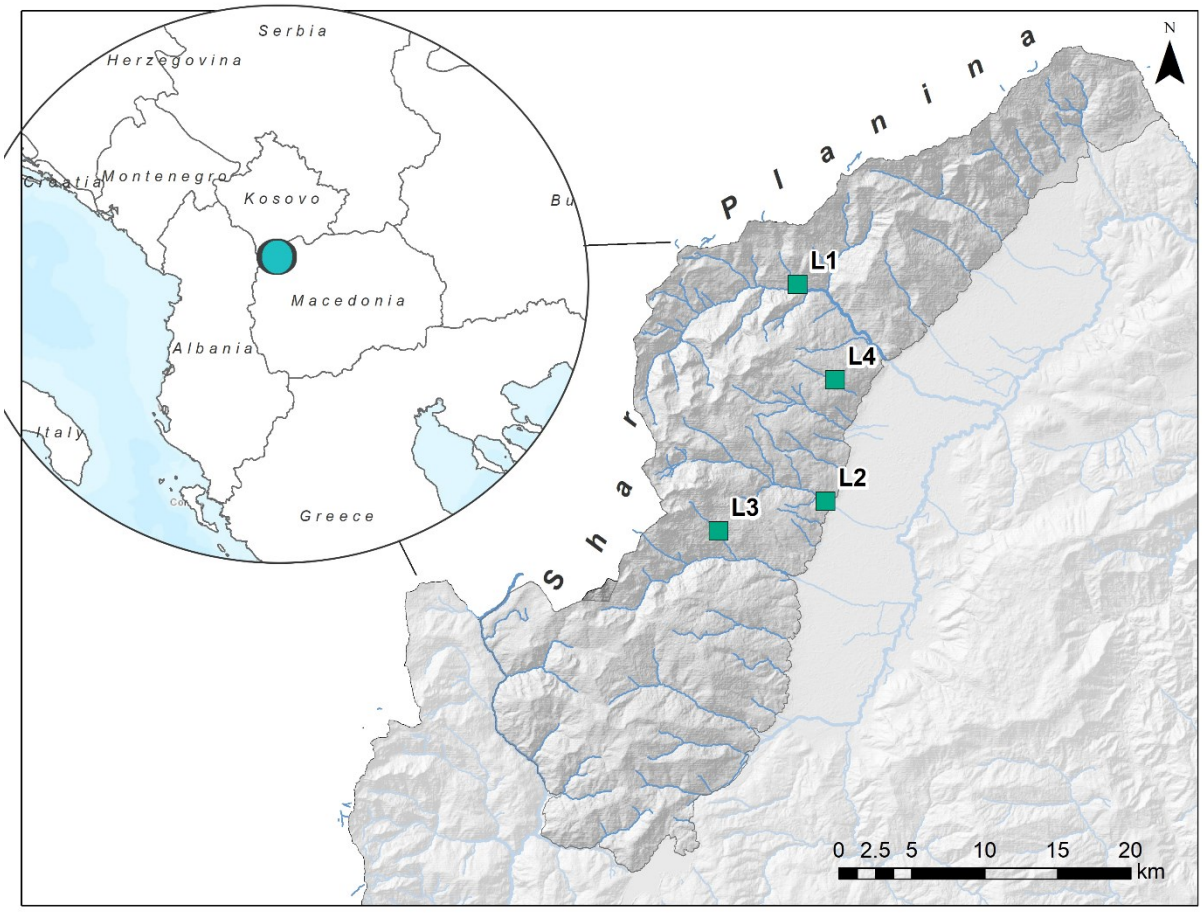

Figure 1. Map of the sampling localities (L1 - L4) in Shar Mountain (R. of North Macedonia). Map created by Daniela Jovanovska, PhD.

Figure 2. Sampling localities from where the newly recorded species were collected: a) L1 - Tributary of the river Pena, v. Brodec, b) L2- River of Bogovinje, v. Bogovinje, c) L3- Stream on the way to Mazdracha, d) L4 – Stream after Lisec village.

Results and Discussion

The collection of stoneflies, from the four localities selected in this paper resulted, among others, in the recording of four Plecoptera species from the National Park Shar Mountain in R. of North Macedonia. Among these, three species are new records for the range (*Isoperla bosnica* Aubert, 1964, *Isoperla albanica* Aubert, 1964 and *Nemoura asceta* Murányi, 2007, two of them are Balkan endemics: *Isoperla bosnica* Aubert, 1964 and *Nemoura asceta* Murányi, 2007. Additionally, ***Nemoura asceta*** Murányi, 2007 is a new record for the country. Two of the newly identified species belong to the Perlodidae family and the other two species belong to the Nemouridae family. The distribution data and taxonomic ranking of three new species from Shar Mountain are given in Table 2.

Table 2. Taxonomic ranking and distribution of stoneflies in four localities on Shar Mountain. Abbreviations: m - male specimen(s); f - female specimen(s); BSH– Beating sheet; EN – entomological net.

Phylum: Arthropoda		
Class: Insecta		
Order: Plecoptera		
Family: Perlodidae		
Genus: Isoperla Banks, 1906		
1.	Species: *Isoperla bosnica* Aubert, 1964	Material examined: L2, (EN), 24.04.2023, 2 m
2.	Species: *Isoperla albanica* Aubert, 1964	Material examined: L3 Stream on the way to Mazdracha, (EN), 18.06.2021, 3 f, 2 m
Family: Nemouridae		
Genus: Nemoura Latreille, 1796		
3.	Species: *Nemoura uncinata* Despax, 1934	Material examined: L1, (BSH), 08.05.2021, 2 f
4.	Species: *Nemoura asceta* Murányi, 2007	Material examined: L4, (BSH), 07.06.2023, 1 f, 1 m

Figure 3. ***Nemoura asceta*** Murányi, 2007 (dorsal view). First record for R. North Macedonia. © Ignac Sivec.

The Balkan endemic species *Isoperla bosnica* Aubert, 1964 presents a taxon with a quite controversial taxonomic history. Namely, the species was firstly classified into the *rivulorum* group right after its description from material collected in Trnovo, Bosnia and Herzegovina (Aubert, 1964), but only three years later was transferred to the *goertzi* group (Consiglio, 1967). However, because of the shape and distribution of the scales in the heavy penial armor, Murányi (2011) considered this species more appropriate for the *oxylepsis* group. Despite the constant transfer between groups, the name of this species was also in question as it was considered a synonym of *Isoperla oxylepis balcanica* Raušer, 1962 (Sivec, 1980a). This triggered doubts in the further investigations when Ikonomov (1986) for the same specimens collected from Mavrovska river (750-830 m.a.s.l) and Boshavica river (150 m.a.s.l) gave two names, *Isoperla bosnica* Aubert, 1964 and *Isoperla oxylepis balcanica* Raušer, 1962 making this distribution data confusing. Bearing this in mind, some authors (Murányi, 2011) discuss that it is not clear if these specimens refer to *Isoperla bosnica* or *Isoperla oxylepis balcanica* and consequently, it is not clear whether both or only one of these species inhabits in R. North Macedonia. This study

dismisses previous doubts and reports the presence of *Isoperla bosnica* in R. North Macedonia. The species was previously known from few countries on the Balkan Peninsula as Bosnia and Herzegovina (Aubert, 1964), Montenegro (Murányi, 2011), and Croatia (Helbec, et al. 2022). The notation of the species in R. North Macedonia extends its distribution to the southern part of the Balkan Peninsula. Comparing the nature of the habitat with the collections by previous researchers (Aubert, 1964, Murányi, 2011, Helbec et al. 2022), the collection of the species *Isoperla bosnica* in a sub-mountainous habitat (546 m.a.s.l., L2, Table 1) further confirms its preference for large streams and rivers.

The second species *Isoperla albanica* Aubert, 1964 recorded for the first time in Shar Mountain is a rare species distributed in several countries of central Europe (Graf, 1999, Krno, 2000, Hohmann & Küttner 2020), and the Balkan peninsula (Kaćanski, 1976, Sivec, 1980b, Bilalli et al., 2023) and was originally described from a stream in the Skala Bicajt, Republic of Albania (Aubert, 1964). After several decades in the same country, Murányi (2011) expanded the known distribution range of this species to Korab Mountain. The only locality from which this species was reported in North Macedonia (Ikonomov, 1986) is the Mavrovska river (Bistra Mountain) at an altitude of 1060 m. The results presented in our study show that this species is also distributed in Shar Mountain, recorded from locality 3 (stream on the way to Mazdracha), a locality characterized by interesting and diverse riparian vegetation (Figure 2, c). Additionally, the species was collected on 18 June 2021, aligning with the collection time period noted by previous researchers (Aubert, 1964, Murányi, 2011).

Nemoura uncinata was previously reported from the Shar Mountain by Ikonomov (1973) under the name *Nemoura fulviceps* Klapálek, 1902 in several localities: stream at Popova Shapka; near the village Lisec; on the edge of the forest; spring under Ceripashina; and spring near village Lisec. The true identity of *Nemoura fulviceps* was resolved by Zwick (1982). All previous records from the Balkans actually refer to *Nemoura uncinata*, although they still appear as *Nemoura fulviceps* in Ikonomov (1986). The collection of *Nemoura uncinata* at locality 1 (tributary of the river Pena, near the village of Brodec) resulted in only two female individuals, and based on the fact that in the Balkan peninsula there are several similar females of the genus *Nemoura*, identification based solely on morphological characteristics is highly doubtful and controversial. As a result, the identification of these individuals has been further verified using DNA barcoding, the results of which are yet to be published. In recent years, this species was recorded from the southern parts of the R. of North Macedonia by Murányi et al., (2016) and Tyufekchieva & Rimcheska, (2019). It was reported from Nizhepole on Pelister Mountain, (1375 m.a.s.l.) (Muranyi et al., 2016), and from Klinoshtitsa River, upstream Dobarsko v., Dvorishka (Cironska) River, and tributary to Lebnitsa River, after Dobri Laki v. (Tyufekchieva & Rimcheska, 2019). During our investigation presented in this paper the species was recorded on the sampling site L1 which is placed in 1329 m and perfectly corresponds with the locality in the Nizhepole that is with the same altitude, satisfying the habitat preferences of this species: moderate-altitude mountain streams. The species is widely distributed across Europe (Tixier & Guérold 2005, Darilmaz, et al. 2016, Karaouzas et al.2016, Cíbik, et al. 2020, Fochetti 2020, Bilalli et al. 2023, DeWalt et al., 2024).

The endemic Balkan species *Nemoura asceta* is recorded for the first time for R. of North Macedonia at sampling site 4: a stream after Lisec village at the beginning of June (07.06.2023), at altitude of 1172 m.a.s.l. *Nemoura asceta* was originally described by Murányi (2007), in Albania - Shkodër Country at a high elevation (1622 m) in late May, and subsequently was found in the side torrent of Sheu River at low elevation (270 m) around mid-April, suggesting that the species is adaptable to diverse environmental conditions and has a wide temporal activity span within its habitat. The species was also found in the European part of Turkey, specifically in Tekirdağ, on Tekir Mountain (Darilmaz et al., 2016), and more recently was recorded in Kosovo and Serbia (Bilalli, et al. 2020; 2023).

The results of this study with the records of four rare species of stoneflies (including the endemics: *Isoperla bosnica* Aubert, 1964 and *Nemoura asceta* Murányi, 2007) once again indicate the presence of favorable conditions and suitable habitats in Shar Mountain obtaining populations of these rare species. With the results of this paper, the total number of stoneflies in Shar Mountain increases in 60. Recognizing the importance of these insects, as bioindicators of good water quality, these results can also initiate further conservation initiatives for endemic species in Shar Mountain. Furthermore, Shar Mountain, although designated as National Park in the country, is still under strong pressures by several anthropogenic activities. Therefore, this survey on stonefly species on river of Bogovinje, stream on the way to Mazdracha and stream after Lisec village, accompanied by the newest results on rare and

endemic caddisfly species on this mountain (Hinić-Jordanovska et al., 2024) underscores the need for designating priority areas for protection within the National Park Shar Mountain.

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