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New distributional records of rare riffle beetles (Coleoptera: Elmidae) from the Balkan Peninsula

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Abstract: We present new data on rare riffle beetle findings (Coleoptera: Elmidae) from the Balkan Peninsula. Larvae and adult elmid beetles were collected during 2007-2018. The following Balkan countries were included in the study: Serbia, Bulgaria, Romania and Bosnia and Herzegovina. Riffle beetle fauna of the Balkan Peninsula is poorly explored due to a lack of data, particularly in southern and eastern areas. The main objective of the study was to add to the knowledge of this group of aquatic insects. New records of 7 riffle beetle species are provided, as follows: *Stenelmis puberula* Reitter, 1887, *Stenelmis consobrina consobrina* Dufour, 1835, *Potamophilus acuminatus* (Fabricius, 1792), *Elmis syriaca zoufali* (Reitter, 1910), *Elmis rioloides* (Kuwert, 1890), *Limnius intermedius* Fairmaire, 1881 and *Limnius opacus* Müller, 1806, with notes on their occurrence, habitats and co-occurrence with other riffle beetle taxa. Because of the scarcity of species findings, their disjunct distribution and specific ecological requirements, some riffle beetle species are becoming more vulnerable to pronounced human impact. Accordingly, adequate habitat protection is necessary for rare riffle beetle species and for their conservation on the Balkan Peninsula, particularly in small streams.

Keywords: riffle beetles; rare species; ecological preferences

INTRODUCTION

The Elmidae family, commonly known as riffle beetles, are found in freshwater streams all around the world. There are about 1400 known species, but probably many more have not yet been discovered. Riffle beetles are small (1-8 mm long) aquatic beetles that are most often found crawling on stones and other solid debris in fast-moving streams. A few species are found in slow-running streams or still waters. They have relatively long legs and both adults and larvae are well-sclerotized. The larvae and adults are fully-aquatic, extracting oxygen from the water around them [1-3]. Elmid beetles inhabit permanent running waters of different size with more or less exposed margins. In streams, both larvae and imagoes chiefly inhabit microhabitats with moss and stones. Morphological adaptations, such as long legs and claws and dense hairs allow riffle beetles to cling to the substrate in fast-running waters, while the larvae of some species prefer sandy bottoms. In lakes and slow-running sections of rivers, elmids are found in the littoral among stones or gravel, but may also cling to aquatic plants. The occurrence of elmids is negatively affected by both acidification and pollution [4].

The riffle beetle fauna of the Balkan Peninsula is poorly studied. Morphological characters among species are highly variable due to morphological plasticity. The species habitus, pronotum characteristics and proposed organism size make correct taxa identification difficult. The classical morphological approach

in taxa identification based on habitus, size and other characters can lead to misidentification. Thus the species-level identification can be reliably performed only on the basis of species-specific male genitalia (aedeagus) determination [5]. In the Balkan Peninsula, high ecophenotypic species variability is presented, particularly in the *Elmis* and *Limnius* genera. Also, in some cases, up to several congeneric species of these genera can co-occur in the same habitats [6].

The present report aims to add new faunistic data for rare elmid species to Balkan fauna as well as new ecological information.

MATERIALS AND METHODS

Sampling

The study was conducted at different sampling sites in Serbia, Bulgaria, Romania and Bosnia and Herzegovina from 2007 to 2018. The individual localities, collectors and numbers of captured specimens are presented for each species separately. Aquatic beetle samples were either obtained using a hand net (25x25 cm, 500 μm mesh size) or the specimens were manually collected. The multihabitat sampling procedure [7] and AQEM protocol [8] were applied. Sampling in Bulgaria was conducted using the adapted multihabitat methodology [9] based on both standardized methodology, EN 27828:1994/ISO 7828:1985, and the multihabitat approach used in AQEM/STAR methodology [10]. The samples were preserved using 70-90% ethyl alcohol and further processed in the laboratory.

Taxa identification

Elmid specimens were identified according to the main literature sources [11-13]. Identification was done using stereomicroscopes (Leica MS 5 and Carl Zeiss SteREO Discovery V8). Data on species distribution is given according to [14]. All photographs were created using an AxioCam ICc5 camera and edited with imaging software (ZEN2, Carl Zeiss Microscopy). All specimens are stored in the collection of the National Laboratory of the Serbian Environmental Protection Agency in Belgrade.

RESULTS

The study contains new distributional data on 7 rare riffle beetle species from the Balkan Peninsula; these are as follows: *Stenelmis puberula*, *S. consobrina consobrina, Potamophilus acuminatus, Elmis syriaca zoufali, E. rioloides, Limnius intermedius* and *L. opacus*. The habitus of some species is provided, with the main goal of distinguishing the genus level, particularly relevant for the identification of larval instars. For *L. intermedius* and *L. opacus*, photographs of male genitalia are given, as the main diagnostic character of the species. For the distribution results, the findings for larvae are abbreviated as Lv. and for imagoes as Im. respectively.

Considering the world distribution of the taxa, a question mark (?) means doubtfully present taxa (probably misidentification). Certain country names are followed by a reference that refers to either a questionable records, to unpublished data (e.g. theses, papers in preparation, etc.) and little known or rarely cited publications, or to recently published papers. Some country names are in brackets when the respective species almost certainly occur but have not yet been officially recorded and no specimens are known [14].

New data are provided for the following species.

Stenelmis puberula Reitter, 1887

Material examined: Bulgaria – June 19, 2017, Studena River (Yantra River Basin) at the bridge of Hadži Dimitrovo Village, N 43°29'36", E 25°28'14", 44 m a.s.l., 4 Lv., leg. P. Pandakov. Distribution: Afghanistan [15], Armenia, Bosnia and Herzegovina, China (Xinjiang)? [15], Georgia, Iran, Israel, Lebanon, Russia, Slovakia, Syria, Turkey, Turkmenistan, Ukraine. This is the first record of this species in Bulgaria; the habitus of the larva (lateral and ventral view) is provided (Fig. 1A). Notes: Poorly known biology and feeding preference. At the Studena River in co-occurrence with *P. acuminatus, E. syriaca zoufali, E. maugetii* and *L. volckmari.* The Studena River is a small river in northern Bulgaria, a left tributary to the Yantra River (Danube River Basin). The river is heavily modified by human activity; a large part of the wetlands in the river valley is drained and artificially dried or transformed into fish ponds and dams. A specific characteristic of the

river is that its water never becomes transparent due to the dissolving ability of the bottom substrate, loamy soils and loess [16].

***Stenelmis consobrina consobrina* Dufour, 1835**

Material examined: Bosnia and Herzegovina – September 1, 2008, Derventa/Ukrina River (confluence to the Sava River), N 45°04'54", E 17°57'38", 91 m a.s.l., 2 Im., leg. P. Mitrović; September 18, 2009, the Una River/downstream of Kozarska Dubica, N 45°5'33", E 17°29'47", 95 m a.s.l., 1 Im., 1 Lv., leg. P. Mitrović. Distribution: Algeria, Armenia, Bosnia and Herzegovina, Croatia, Czech Republic, France, Georgia, Germany, Greece, Israel, Italy, Morocco, Palestine (West Bank), Russia, Spain, Switzerland, Syria, Tunisia, Turkey, Turkmenistan. Notes: Poorly known feeding preference. In the Czech Republic it occurred in lower parts of streams, perhaps with a preference for wood and shore rootlets similar to *E. obscura* or *Macronychus quadrituberculatus* [6]. In the Derventa/Ukrina River, it co-occurred with *Esolus parallelepipedus* and *Oulimnius tuberculatus*. In the zoobenthic sample, Chironomidae and Tubificidae spp. taxa were dominant, and a small number of *Asellus aquaticus* occurred, indicating high organic pollution of the water. The habitus of *S. consobrina consobrina* larva (lateral view) and imago (dorsal view) are shown (Fig. 1B and Fig. 1C, respectively). At the Kozarska Dubica/Una River, it co-occurred with *L. volckmari* and *O. tuberculatus*.

***Potamophilus acuminatus* (Fabricius, 1792)**

Material examined: Bulgaria – July 19, 2017, Studena River (Yantra River Basin) at the bridge of Hadži Dimitrovo Village, N 43°29'36", E 25°28'14", 44 m a.s.l., 2 Lv., leg. P. Pandakov; Romania – August 23, 2016, Nâdlac/Mureş River, N 46°09'05", E 20°43'07", 84 m a.s.l., 1 Lv., leg. I. Holló; Serbia – October 5, 2016, Draginac/Jadar River, N 44°30'18", E 19°24'48", 133 m a.s.l., 1 Lv., leg. S. Anđus. Distribution: Afghanistan, Albania (M. Hess, pers. comm.), Austria, Azerbaijan, Belarus, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Israel, Italy, Lebanon, Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Syria, Tunisia, Turkey, Turkmenistan, Ukraine. Notes: Xylophagous larvae feed on dead, partially decomposed wood submerged in water, preferring branches and logs with a diameter of ca. 8 cm [17-19]. Habitus of the larva (lateral and dorsal view) is given (Fig. 1D). In the investigated area, it prefers medium to mid-large rivers with a wide potamal zone and high oxygen content in the water. At the Draginac/Jadar River sampling site it co-occurred with *M. quadrituberculatus, Esolus angustatus* and *L. volckmari*.

***Elmis syriaca zoufali* (Reitter, 1910)**

Material examined: Bulgaria – July 19, 2017, Studena River (Yantra River Basin) at the bridge of Hadži Dimitrovo Village, N 43°29'36", E 25°28'14", 44 m a.s.l., 1 Im., leg. P. Pandakov; Serbia – October 26, 2015, Mrtvine/Gaberska Reka River, N 42°59'50", E 22°46'42, 574 m a.s.l., 1 Im., leg. B. Novaković; September 13, 2017, Mrtvine/Gaberska Reka River, N 42°59'50", E 22°46'42, 574 m a.s.l., 1 Im., leg. B. Novaković; September 18, 2018, Dimitrovgrad/Nišava River, N 43°00'27", E 22°49'01", 458 m a.s.l., 4 Im., leg. B. Novaković. Distribution: Albania, Armenia, Bosnia and Herzegovina, Bulgaria, Greece, (Montenegro), Serbia, Turkey. Notes: Poorly known biology and feeding preference. In the investigated area it prefers habitats with macrophytes, moss and epilithic algae; in small rivers in lowlands and foothills with medium or even slow currents. Habitus of the imago (dorsal view) is shown (Fig. 1E). At the Mrtvine/Gaberska Reka River it co-occurred with *E. maugetii, Riolus cupreus, R. cf. subviolaceus*, *L. volckmari* and *E. parallelepipedus*. At the Dimitrovgrad/Nišava River it co-occurred with *E. maugetii* and *R. cupreus*.

***Elmis rioloides* (Kuwert, 1890)**

Material examined: Serbia – September 8, 2011, Žagubica/Mlava River (0.5 km downstream of fishpond outlet), N 44°11'46.86", E 21°46'9.48", 310 m a.s.l., 1 Im., leg. I. Živić; September 13, 2017, the Monastery of Poganovo/Jerma River, N 42°58'43", E 22°38'04", 513 m a.s.l., 1 Im., leg. B. Novaković. Habitus of the imago (dorsal view) is provided (Fig. 1F). Distribution: Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia [20], Czech Republic, France, Germany, Greece, Hungary, Israel, Italy, Lebanon, Luxembourg, (Montenegro), Netherlands, Portugal, Romania, Russia, (Serbia), Slovakia, (Slovenia),

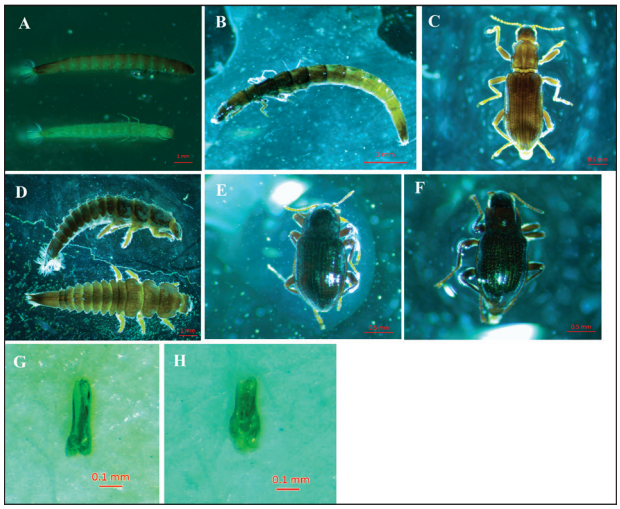

Fig. 1. Rare elmid species in the Balkans. **A** – *Stenelmis puberula* (larvae: lateral and ventral view), new for Bulgaria, found in the Studena River (Yantra River Basin); **B** – *S. consobrina consobrina* (larva: lateral view); **C** – *S. consobrina consobrina* (imago: dorsal view) from the Ukrina River (Bosnia and Herzegovina) which is characterized by high organic pollution; **D** – *Potamophilus acuminatus* (xylophagous larvae: lateral and dorsal view); **E** – *Elmis syriaca zoufali* (imago: dorsal view); **F** – *E. rioloides* (imago: dorsal view). In the Balkans, both species prefer habitats with a presence of macrophytes, moss and epilithic algae; **G** – *Limnius intermedius* (aedeagus, diagnostic character: ventral view); **H** – *L. opacus* (aedeagus, diagnostic character: ventral view).

Spain, Switzerland, Turkey. Notes: First confirmed species records in Serbia. Microphagous; in hilly and mountainous streams and rivers, it coexists at all altitudes with *E. maugetii*; on rocks, stones or mosses, between macrophytes [11,21,22]. In the investigated area, it preferred habitats with macrophytes, moss and epilithic algae, in small rivers in lowlands and foothills with medium or fast currents. At the Mlava River sampling site it co-occurred with *E. aenea*, *L. volckmari* and *E. parallelepipedus.*

Limnius intermedius Fairmaire, 1881

Material examined: Bosnia and Herzegovina – September 8, 2007, Novi Grad/Una River (downstream of Sana River mouth), N 45°01'00", E 16°21'51", 132 m a.s.l., 5 Im., leg. P. Mitrović; October 28, 2008, Velika Šnjegotina/Šnjegotina River, N 44°42'56.26", E 17°31'38.06", 247 m a.s.l., 1 Im., leg. P. Mitrović; Bulgaria – August 13, 2013, the Sivyak River Spring above the village of Ezeroto, NP "Balgarka", N 42°45'40.35", E 25°21'52.80", 780 m a.s.l., 56 Im., leg. P. Pandakov; Serbia – August 30, 2011, Milićе/Studenica River (0.5 km downstream the fishpond outlet), N 43°29'26", E 20°24'29", 627 m a.s.l., 1 Im., leg. I. Živić. Male genitalia (aedeagus), ventral view is provided, as a species diagnostic character (Fig. 1G). Distribution: Albania, Algeria, Armenia, Austria, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, France, (Germany), Greece, Hungary, Israel, Italy, Lebanon, Macedonia, (Montenegro), Morocco, Palestine (West Bank), Poland? [23], Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Tunisia, Turkey, Ukraine. Notes: Poorly known biology and feeding preference; similar habitats like *L. volckmari*, in small to moderately sized, rather warm streams; between rocks [11,21,22]. In the Balkans, it prefers small to medium unpolluted streams in submountain regions. At the Novi Grad/Una River it co-occurred with *O. tuberculatus*. At the Velika Šnjegotina/ Šnjegotina River, with *L. opacus*, and *L. volckmari.* At the Milićе/ Studenica River (0.5 km downstream of fishpond outlet) with *E. aenea, E. maugetii*, *L. opacus, L. volckmari* and *E. parallelepipedus.*

Limnius opacus Müller, 1806

Material examined: Bosnia and Herzegovina – October 18, 2007, Kusonje/Spreča River (mouth to the Bosna River), N 44°22'07", E 18°59'22", 309 m a.s.l., 4 Im., leg. P. Mitrović; 05.IX 2008, the Sana River (mouth to the Una River), N 45°02'35", E 16°24'09", 130 m a.s.l., 1 Im., leg. P. Mitrović; October 28, 2008, Velika Šnjegotina/Šnjegotina River, N 44°42'56.26", E 17°31'38.06", 247 m a.s.l., 1 Im., leg. P. Mitrović; Serbia – August 30, 2011, Milićе/Studenica River (0.5 km downstream of the fishpond outlet), N 43°29'26", E 20°24'29", 627 m a.s.l., 1 Im., leg. I. Živić; October 14, 2015, Buci/Jastrebačka (Lomnička) Reka River, N 43°25'46, E 21°22'23", 505 m a.s.l., 1 Im., leg. J. Đuknić; October 20, 2017, Pašna Ravan/Trešnjica River, N 44°08'21.0", E 19°39'50.0", 888 m a.s.l., 2 Im., leg. B. Novaković. Male genitalia (aedeagus) is a species diagnostic character – its ventral view is provided (Fig. 1H). Distribution: Algeria, Armenia, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Israel, Italy, Lebanon, Luxembourg, (Montenegro), Morocco, Netherlands, Poland? [23], Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzer-

land, Turkey, Ukraine. Notes: Microphagous; streams and rivers in hills and lower mountains, coexists with *L. volckmari,* between stones [11,21,22]. In the investigated area, prefers cold hilly streams in submountain and mountain regions, sometimes with slow currents or even stagnant water. At the Kusonje/Spreča River it co-occurred with *L. volckmari* and *E. parallelepipedus*, at the Sana River (mouth to the Una River) with *L. volckmari, E. aenea, E. maugetii, E. parallelepipedus* and *O. tuberculatus*, at the Milića/Studenica River it co-occurred with *E. aenea, E. maugetii, L. intermedius, L. volckmari* and *E. parallelepipedus.* At the Buci/Jastrebačka Reka River it co-occurred with *E. maugetii* and *L. volckmari*, and at the Pašna Ravna/Trešnjica River with *L. volckmari*.

DISCUSSION

The present study provides new data on the distribution and ecology of rare elmid species in the Balkans, which could be useful in managing measures for their cross-border protection and conservation. *S. puberula* is new for the entomofauna of Bulgaria; the species was not previously listed for Bulgaria [14]. During our study, it was recorded at the Studena River. The species' recent distribution covers parts of Central Asia, the Middle East and only Bosnia and Herzegovina in the Balkans. Recently, *S. puberula* was recorded for the first time in Russia (Krasnodar Krai and the Republic of Adygea) and Abkhazia [24]. According to the ecological classification of the aquatic biota, adults and larvae of *Stenelmis* are typical lithorheophiles [24]. In the Caucasus and Transcaucasia, they live in various watercourses of the mountainous type, with considerable water discharge, strong currents and mainly stony substrates. These watercourses are usually small or middle-sized natural rivers, less often large rivers, and are not impacted by human activity [24], unlike the one we sampled in Bulgaria.

During our study, *S. consobrina consobrina* was found at two sampling sites in Bosnia and Herzegovina. The species is rare throughout most of its entire distribution range and extinct or critically endangered in central Europe [6]. In the Balkans, the species was predominantly recorded in small to mid-large perennial streams and rivers with fast currents, without moss and aquatic vegetation.

Based on our data, *P. acuminatus* was registered at three localities: in Romania, Bulgaria and Serbia. Its community with *S. consobrina* and *M. quadrituberculatus* is regarded as valuable and threatened assemblage of river insects (potamocoen zone) [17,25] due to the species' recent withdrawal.

In our study, *E. syriaca zoufali* was recorded at two sampling sites, in Serbia and in Bulgaria. The Balkans represent most of its world distribution [14]. The species is poorly known.

E. rioloides has not been reliably confirmed for Serbia in the latest edition of the catalogue [14]. This study confirmed its presence in Serbia.

L. intermedius was found at four sampling sites, two in Bosnia and Herzegovina, one in Bulgaria and one in Serbia. It scarcely co-occurred with the highly abundant *L. volckmari.* The species was abundant only in the Sivyak River spring zone, unimpacted by human activity and dominated by mesolithal, mosses and a lot of detritus in the water. The Sivyak River has relatively small seasonal water-level fluctuations, moderate currents and flows close to well-preserved deciduous forests.

L. opacus was found at six localities, three in Bosnia and Herzegovina and three in Serbia. As with the previous species, it often co-occurred with the more abundant *L. volckmari.*

Considering water pollution and riffle beetle occurrence, riffle beetles are regarded as excellent indicators of water quality [22], but recently some taxa, such as *S. puberula,* were found in more polluted watercourses [26,27]. According to the principle of the ecosystem approach in assessing the status of a water body as enshrined in the Water Framework Directive (60/2000/EC), the Studena River had a moderate to bad ecological potential, as determined by hydromorphological parameters and the Fish Based Index [28], and its state continues to deteriorate [16]. As a comparison, the streams where *S. puberula* was found in Slovakia were also small, but located close to human settlements. These slow-running streams were moderately organically polluted [26,27].

Similarly, *S. consobrina consobrina* was found in more polluted conditions in the Balkans, at the

Derventa/Ukrina River (Bosnia and Herzegovina), with a prevalence of α-β limnosaprobic conditions, characterized by high organic pollution as regards the benthic invertebrate community composition and structure, revealing a somewhat wider ecological tolerance of this species to pollution. The majority of its central European populations are restricted to unpolluted watercourses [6]. Its confirmed presence in sites with higher pollution, and at some sites in regulated watercourses degraded by hymo alterations, such as those from Bosnia and Herzegovina, indicates the species' wider ecological tolerance to organic pollution as well as its high potential to colonize more polluted and heavily modified streams and rivers. This could be regarded as the species' ability to adapt on a long-time scale.

Other species (*P. acuminatus, E. syriaca zoufali, E. rioloides, L. intermedius* and *L. opacus*) were predominantly found in streams and rivers with good ecological status/potential. At some of the sampling sites, the current was slow, but measured oxygen saturation in the water was still high, indicating that dissolved oxygen was the most important physicochemical parameter in terms of riffle beetle microhabitat occurrence, due to their specific type of respiration (plastron).

Regarding the feeding preference of the analyzed species, *E. rioloides* and *L. opacus* are microphagous species [11,21,22]. Wood debris in the water constitutes a specific microhabitat for xylobiont species, such as *P. acuminatus*, necessary for the development of its larval instars [17-19]. There are little literature data on the feeding preferences of *S. puberula*, *S. consobrina consobrina, E. syriaca zoufali* and *L. intermedius*.

The population decrease of some riffle beetles in the Balkans can be primarily explained as the result of hydromorphological changes, habitat fragmentation and water pollution (mainly organic), especially around human settlements. Habitat fragmentation and deterioration are particularly dangerous due to the low dispersal potential of the species [6,29] and the presence of brachypterous forms in mixed riffle beetle populations. Gravel and sand exploitation affect the spatial distribution of some species. Particularly vulnerable are potamal riffle beetles, such as *P. acuminatus*. The lack of habitats and habitat degradation could be the main reasons for the disappearance of riffle beetle species. These are the main threats affecting elmid populations.

A pronounced human impact (communal wastewater and agricultural runoff pollution) is particularly evident in small, wadeable lowland streams in the Balkans. Among riffle beetles, the most vulnerable are rheobiontic species inhabiting hyporhitral and potamal zones. According to the present study, *S. puberula, S. consobrina consobrina* and *P. acuminatus* were found to be most threatened by human interference in the Balkans, due to the lack of suitable habitats as well as specific ecological requirements. A specific threat for *P. acuminatus* is associated with the trophic requirements of larvae (xylophagous), so that even positive activities, such as increased water care (removal of decaying wood), could result in the total disappearance of the species from the area [19].

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