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WHAT IS NEW IN CROATIAN MAYFLY FAUNA?

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Here we present a first record of *Baetis vardarensis* Ikonomov, 1962 in Croatian freshwater habitats. The species was collected in February and March 2020 at two sites in the Sava River. With this record, Croatian mayfly species richness increased to a total of 85 species. In addition, one of the species previously known only from literature, *Kageronia fuscogrisea* (Retzius, 1783), was confirmed in Croatia. The species was collected in the Rečica River, in March 2020. These results confirm that our knowledge about mayfly fauna in different regions of Balkan Peninsula is still incomplete and is increasing with systematic studies.

Key words: Ephemeroptera, Baetidae, new records, Heptageniidae, species richness, Balkan Peninsula

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U ovom radu predstavljamo nalaz nove vrste vodencvijeta, *Baetis vardarensis* Ikonomov, 1962 u fauni Hrvatske. Ličinke su sakupljene tijekom veljače i ožujka 2020. godine na dvije lokacije u rijeci Savi, u sklopu redovitog monitoringa tekućica u Republici Hrvatskoj. Ovim nalazom broj vrsta vodencvjetova u Hrvatskoj je dosegao brojku od 85 vrsta. Tijekom ovog istraživanja potvđena je i prisutnost vrste *Kageronia fuscogrisea* (Retzius, 1783) u fauni Hrvatske, koja je do sada bila navedena samo u starijoj literaturi. Ličinke ove vrste su sakupljene tijekom ožujka 2021. godine na rijeci Rečici. Ovi rezultati potvrđuju da broj do sada zabilježenih vrsta vodencvjetova u Hrvatskoj, kao i u brojnim regijama Balkanskog poluotoka, još uvijek nije konačan te se povećava sa svakim sustavno provedenim istraživanjem.

Ključne riječi: Ephemeroptera, Baetidae, Heptageniidae, novi nalazi, bogatstvo vrsta, Balkanski poluotok

INTRODUCTION

The species rich mayfly fauna of freshwater ecosystems in the area of North and Central Europe is nowadays well-known, due to long-term systematic studies (e.g. Bauernfeind & Humpesch, 2001; Bauernfeind & Moog, 2000; Bauernfeind & Soldán, 2012; Sartori & Landolt, 1999). For instance, 140 mayfly species have been recorded in Slovakia (Derka, 2003), 113 species in Germany (Haybach & Malzacher, 2003). On the other hand, more detailed faunistic and especially ecological studies of mayflies in the Balkan Peninsula started only approximately two decades ago, and our knowledge is far from complete (e.g. Petrović *et al*., 2015; Vidinova, 2003; Vilenica *et al*., 2015; 2016; 2017, 2018a, b; 2019; 2020). Many regions are still rather poorly and unevenly investigated. For instance, in the Balkans, the highest number of mayfly species

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was recorded for Bulgaria (102, Vidinova, 2003), followed by that for Serbia (85, Petrović *et al.* 2015), Macedonia (80, Rimcheska, 2020), Slovenia (75, Zabric & Sartori, 1997), Greece (70, Bauernfeind, 2003), and Bosnia and Herzegovina (51, Bauernfeind & Soldán, 2012), reflecting the different durations and levels of systematic research. Therefore, discoveries of new species data can be expected with more frequent surveys.

In Croatia, mayfly-focused research started less than a decade ago (e.g. Vilenica *et al*., 2015, 2016, 2017; 2018a, b, 2019, 2020). Until then, published data on mayflies were largely parts of various limnological studies that focused mainly on benthic macroinvertebrates and their communities (e.g. Habdija & Primc, 1987; Habdija *et al.*, 1994, 2004; Matoničkin, 1987; Matoničkin & Pavletić, 1967). In most of these studies, the identification literature used was not cited, and voucher specimens are not available. Therefore, these identifications could not be verified. Only 51 mayfly species were recorded in Croatia during this period (Bauernfeind & Soldán, 2012; Ćuk *et al*., 2015; Kovács & Murányi, 2013, Wagner *et al.* 2011). Vilenica *et al*. (2015) published the first checklist of mayflies in Croatia and increased this number by 29 additional taxa. However, as a much higher sampling effort was devoted to the Dinaric Western Balkan ecoregion (ER 5; Illies, 1978), the Pannonian lowland assemblages remained rather neglected (ER 11). Vilenica *et al.* (2015) suggested that it was highly likely that rare, new and taxonomically interesting findings would be recorded in future studies. This proved to be true, and so far, further studies have resulted in 4 new species records (Vilenica *et al.*, 2016, 2019), showing a total of 84 mayfly species for the country.

Many freshwater habitats in the Balkan Peninsula are subject to various anthropogenic pressures: rivers and streams are hydromorphologically modified, numerous hydroelectric power plants are built, and springs are often captured for drinking water supply. In addition, many habitats are exposed to various types of urban, pharmaceutical and agricultural pollution (Lucić *et al*., 2015; Previšić *et al*., 2020; Schwarz, 2012; Vilenica *et al*., 2020). Such activities significantly alter freshwater habitats and their connectivity, i.e. they change the flow regime, water quality, and habitat geomorphology, and enable the establishment of non-native or invasive species. This negatively impacts aquatic organisms, leading to changes in species diversity and composition, homogenization, and even extinction (e.g. Vilenica *et al*., 2019, 2020). Therefore, in order to properly protect habitats and their biota, it is essential to conduct a qualitative assessment and to have a good knowledge of the species that inhabit them.

MATERIALS AND METHODS

Study area

The territory of Croatia is included in two limno-ecological regions: the Dinaric Western Balkan Ecoregion (ER 5) and the Pannonian Lowland Ecoregion (ER 11) (Illies, 1978). The study area is located in the Croatian part of the ER11. The area is characterised by a temperate humid climate with warm summers (Cfb, Köppen classification). The average temperature of the warmest month is below 22 °C (Šegota & Filipčić, 2003), while the average annual air temperature is around 12 °C. The average annual precipitation is between 800 and 1100 mm (Zaninović *et al*., 2008).

Tab. 1. Environmental factors recorded at the studied localities.

Localities and their characteristics	Sava, Jankomir	Sava, Rugvica	Rečica
Date	20.03.2020.	25.02.2020.	10.03.2020.
Coordinates	X 450190, Y 5072319	X 478969, Y 5067424	X 434829, Y 5038250
Altitude (m a.s.l.)	120	100	105
Microhabitats	50% mesolithal (larger pebbles 6–20 cm), 50% microlithal (smaller pebbles 2–6 cm)	100% technolithal (artificial concrete tiles)	100% argyllal (mud, clay)
Anthropogenic pressures	waterfront	waterfront	-
Water temperature (°C)	12.0	9.6	11.7
Dissolved oxygen (mg/L)	9.97	9.75	9.11
Oxygen saturation (%)	92.5	86.4	84.4
Conductivity (µS/cm)	406	450	208
pH	8.24	8.33	7.75

Sampling and mayfly identification

The study sites are part of the national monitoring program. Macroinvertebrates, including mayfly nymphs, were collected according to the AQEM protocol (AQEM expert consortium 2002), using a benthos hand net (25 × 25 cm; mesh size = 500 µm). At each site, 20 subsamples proportional to the microhabitats present were collected and pooled into one composite sample. Substrates consisted mainly of fine sediment (sand, silt, mud), lithal (stones, gravel), and aquatic vegetation (submerged and emergent), and detailed substrate composition of the study sites are presented in Tab. 1.

At each locality, physico-chemical water parameters were measured (water temperature, dissolved oxygen concentration, oxygen saturation, conductivity, pH) (Tab. 1). Collected samples were stored in 96% alcohol and analysed in the laboratory.

Mayfly nymphs were identified using the relevant identification keys: Bauernfeind & Humpesch (2001) and Müller-Liebenau (1969). All voucher specimens are deposited in the Department of Biology, Faculty of Science, University of Zagreb, Croatia.

RESULTS AND DISCUSSION

As part of the national monitoring of lotic habitats in the Pannonian Lowland ecoregion (Illies, 1978), which included small, medium and large rivers, we recorded a new species for the Croatian mayfly fauna; *Baetis vardarensis* Ikonomov, 1962. The nymphs were collected on 25th February 2020 along the Sava River in Rugvica (X 478969, Y 5067424), and on 20th March 2020 on the same river but at the site in Jankomir (X 450190, Y 5072319) (Figs. 1 and 2). This record increased the number of mayfly species in Croatia, which now counts 85 species in total (Vilenica *et al.*, 2015, 2016, 2019, Wagner *et al*., 2011). The current species list is presented in Tab. 2.

Baetis vardarensis (Fig. 3a) is a widespread species, recorded from the Iberian Peninsula to the Caucasus (Bauernfeind & Soldán, 2012). The species' range is considered to overlap with the morphologically similar species from the same species

Fig. 1. Map of Croatia, with localities where *Baetis vardarensis* and *Kageronia fuscogrisea* were recorded.

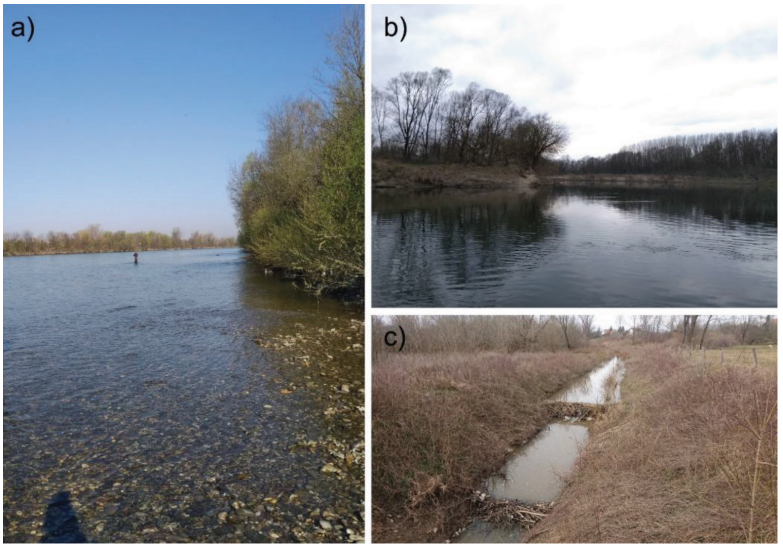

Fig. 2. Habitats of *Baetis vardarensis*: a) Sava River, Jankomir and b) Sava River, Rugvica. Habitat of *Kageronia fuscogrisea*: c) Rečica River.

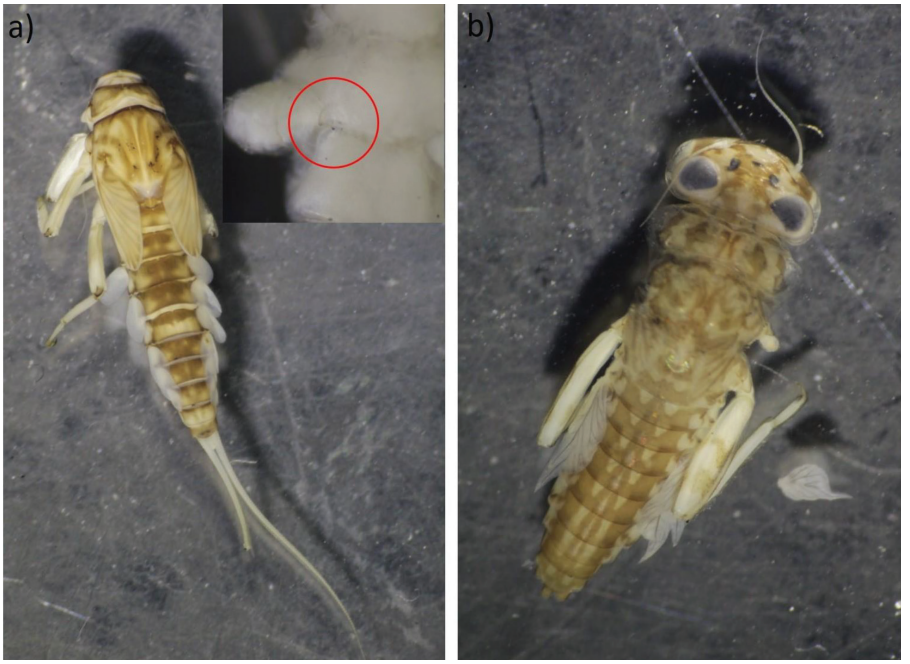

Fig. 3a) *Baetis vardarensis* (Inside the red circle is one of the ventral protuberances next to the coxae. Those are pointed in *B. vardarensis*, while in *Baetis lutheri* are rounded (Müller-Liebenau, 1974); b) *Kageronia fuscogrisea*. Both species are in dorsal view.

group, *Baetis lutheri* Müller-Liebenau, 1967. Although the two species are morphologically similar, there are certain differences such as the shape of the labrum bristles, the third segment of the labial palp, the bristles on the posterior margin of the femora, the length of the paracercus (Bauernfeind & Humpesch, 2001, Bauernfeind & Soldán, 2012; Müller-Liebenau, 1969).

The biology and ecology of *B. vardarensis* are still poorly known, but the nymphs of this rheophile species have previously been recorded in the epipotamal sections (lower reaches) of large rivers (Bauernfeind & Soldán, 2012; Buffagni *et al.*, 2009; 2020), where it usually replaces *B. lutheri*, which prefers rhithral sections (upper reaches) (Bauernfeind & Soldán, 2012; Haybach, 1998; 2006; Sartori & Landolt, 1999). In contrast to *B. lutheri*, which prefers macrophytes and gravel (Bauernfeind & Soldán, 2012; Vilenica *et al*., 2018b), *B. vardarensis* was most frequently recorded on lithal substrates (Buffagni *et al*., 2009; 2020), which was also the case at localities in the Sava River. More precisely, at the Sava River site at Jankomir the species was collected from small and large pebbles (2–20 cm) (i.e. meso- and microlithal), and at the Sava River in Rugvica from artificial substrate, concrete tiles (i.e. technolithal). Literature data show that sometimes it can also occur on fine to medium-sized gravel (akal) or macrophytes (Buffagni *et al*., 2009; 2020). Our findings of the species at 100–115 m a.s.l. confirm literature data showing that it occurs in the altitudinal range mainly up to the 800 m a.s.l. (Buffagni *et al*., 2009; 2020). Despite the previous research conducted on several large lowland rivers in Croatia, including the Sava River (Lucić *et*

Tab. 2. Updated checklist of Croatian mayflies. Legend: * – newly recorded species, ** – taxa whose presence in Croatia should be confirmed. Checklist is based on the recent literature (e.g. Vilenica *et al.* 2015, 2016, 2017, 2018a, b, 2019, 2020; Wagner *et al.*, 2011).

Family	Taxa
Ametropodidae	*Ametropus fragilis* Albarda, 1878
Ameletidae	*Ameletus inopinatus* Eaton, 1887 **
	Metreletus balcanicus (Ulmer, 1920) **
Siphlonuridae	*Siphlonurus aestivalis*(Eaton, 1903)
	Siphlonurus armatus (Eaton, 1870) **
	Siphlonurus croaticus Ulmer, 1920
	Siphlonurus lacustris (Eaton, 1870)
Baetidae	*Alainites muticus* (Linnaeus, 1758)
	Baetis alpinus (Pictet, 1843)
	Baetis buceratus Eaton, 1870
	Baetis fuscatus (Linnaeus, 1761)
	Baetis liebenauae Keffermüller, 1974
	Baetis lutheri Müller-Liebenau, 1967
	Baetis melanonyx (Pictet, 1843)
	Baetis cf. *nubecularis* (Eaton, 1898)
	Baetis pentaphlebodes Ujhelyi, 1966
	Baetis rhodani (Pictet, 1843)
	Baetis scambus Eaton, 1870
	Baetis tricolor Tshernova, 1928
	***Baetis vardarensis* Ikonomov, 1962 ***
	Baetis vernus Curtis, 1834
	Baetopus tenellus (Albarda, 1878)
	Nigrobaetis niger (Linnaeus, 1761)
	Centroptilum luteolum (Müller, 1776)
	Cloeon dipterum (Linnaeus, 1761)
	Cloeon simile Eaton, 1870
	Procloeon bifidum (Bengtsson, 1912)
	Procloeon nana (Bogoescu, 1951)
	Procloeon pennulatum (Eaton, 1870)
Caenidae	*Brachycercus harrisellus* Curtis, 1834
	Caenis beskidensis Sowa, 1973
	Caenis horaria (Linnaeus, 1758)
	Caenis luctuosa (Burmeister, 1839)
	Caenis macrura Stephens, 1835
	Caenis pusilla Navàs, 1913
	Caenis rivulorum Eaton, 1884
	Caenis robusta Eaton, 1884
Ephemerellidae	*Ephemerella mucronata* (Bengtsson, 1909)
	Eurylophella karelica Tiensuu, 1935
	Serratella ignita (Poda, 1761)
	Torleya major (Klapalek, 1905)

Family	Taxa
Ephemeridae	*Ephemera danica* Müller, 1764
	Ephemera glaucops Pictet, 1843
	Ephemera lineata Eaton, 1870
	Ephemera cf. *parnassiana* Demoulin, 1958 **
	Ephemera vulgata Linnaeus, 1758
	Ephemera zettana Kimmins, 1937
Palingeniidae	*Palingenia longicauda* (Olivier, 1791) **
Polymitarcyidae	*Ephoron virgo* (Olivier, 1791) **
Leptophlebiidae	*Choroterpes picteti* (Eaton, 1871) **
	Habroleptoides confusa Sartori & Jacob, 1986
	Habrophlebia fusca (Curtis, 1834)
	Habrophlebia lauta Eaton, 1884
	Leptophlebia marginata (Linnaeus, 1767)
	Leptophlebia vespertina (Linnaeus, 1758)
	Paraleptophlebia submarginata (Stephens, 1835)
	Paraleptophlebia werneri Ulmer, 1920
Oligoneuriidae	*Oligoneuriella rhenana* (Imhoff, 1852)
Potamanthidae	*Potamanthus luteus* (Linnaeus, 1767)
Heptageniidae	*Ecdyonurus aurantiacus* (Burmeister, 1839) **
	Ecdyonurus dispar (Curtis, 1834)
	Ecdyonurus insignis (Eaton, 1870)
	Ecdyonurus macani Thomas & Sowa, 1970
	Ecdyonurus siveci Hefti, Tomka & Zurwerra, 1986 **
	Ecdyonurus starmachi Sowa, 1971
	Ecdyonurus submontanus Landa, 1969
	Ecdyonurus torrentis Kimmins, 1942
	Ecdyonurus venosus (Fabricius, 1775)
	Ecdyonurus vitoshensis Jacob & Braasch, 1984
	Ecdyonurus zelleri (Eaton, 1885)
	Electrogena affinis (Eaton, 1883)
	Electrogena lateralis (Curtis, 1834)
	Electrogena mazedonica (Ikonomov, 1954) **
	Electrogena ujhelyii (Sowa, 1981)
	Epeorus assimilis Eaton, 1885
	Heptagenia coerulans Rostock, 1878
	Heptagenia flava Rostock, 1878
	Heptagenia longicauda (Stephens, 1835)
	Heptagenia sulphurea (Müller, 1776)
	Kageronia fuscogrisea (Retzius, 1783)
	Rhithrogena braaschi Jacob, 1974
	Rhithrogena gr. *diaphana*
	Rhithrogena germanica Eaton, 1885 **
	Rhithrogena iridina (Kolenati, 1839)
	Rhithrogena semicolorata (Curtis, 1834)

al., 2015; Matoničkin *et al*., 1975; Mihaljević *et al*., 1998), the species has not been recorded. It is possible that it was overlooked if it was collected in very juvenile stages, or that it was mistaken for *B. lutheri*. As mentioned above, they are very similar at all stages (Bauernfeind & Soldán, 2012). Also, Petrović *et al.* (2015) did not record it at downstream sites of the Sava River in Serbia, but it was collected from the Kolubara River, one of the largest right tributaries of the Sava River. Nevertheless, the species was recorded at upstream sites of the Sava River in Slovenia (ARSO, 2011; Zabric & Sartori, 1997).

Many of the species' habitats across Europe are nowadays exposed to various anthropogenic pressures (e.g. Dolisy & Dohet, 2003; Kail *et al*., 2012). For instance, several threats have been identified only for the Sava River, such as habitat degradation (water regulation, impoundment, sedimentation), hydrological changes, disruption of longitudinal and lateral connectivity, desiccation of riparian ecosystems, organic and nutrient pollution, biological invasion (Lucić *et al.*, 2015). Baetidae is a mayfly family that shows a wide range of the sensitivity of the speces to organic pollution, with *B. vardarensis* being one of the more tolerant species (Dolisy & Dohet, 2003). Moreover, it was listed by Kail *et al.* (2012) as an indicator taxon for a high proportion of urban land use in the upper reaches of German low mountain rivers. Nevertheless, some of the well- studied countries, such as Germany, listed the species as endangered in their Red Lists due to multiple and increasing anthropogenic threats (Haybach & Malzacher, 2003), while recent studies show that it is extremely rare (Orendt *et al*., 2019). Therefore, it is important to continue monitoring the species and habitat quality.

As part of this survey, another interesting species was recorded on March 10, 2020: *Kageronia fuscogrisea* (Retzius, 1783) (Fig. 3b). The nymphs were collected at the Rečica River (X 434829, Y 5038250) (Figs. 1 and 2), before its confluence with the Kupa River. The species was previously mentioned only by Bauernfeind & Soldán (2012), who noted its presence in Croatia but without specifying the exact localities. Therefore, this record represents a confirmation of the species' occurrence in Croatian waters. The species has a Palaearctic distribution. Its broad range spreads from East Asia (far East Russia) westwards through the whole of Europe, and southwards from Macedonia to Great Britain and Ireland (Bauernfeind & Soldán, 2012). This limno-rheophilous species (Buffagni *et al.*, 2009; 2020) mostly occurs in lowland lakes and ponds, but it also inhabits slow-flowing streams (Bauernfeind & Soldán, 2012), as in our case. It has a high potamal preference, although it has been recorded from the epithithral to the metapotamal. Regarding the microhabitats, at Rečica River, fine sediments (argyllal, i.e. mud and clay) dominate. In previous studies, the species was mostly found on lithal substrates and macrophytes (Bauernfeind & Soldán, 2012; Buffagni *et al*., 2009; 2020). Our results confirmed that this is a typical lowland species, mostly found on sites below 300 m a.s.l. (Buffagni *et al*., 2009; 2020). Our records may contribute to the knowledge of habitat and microhabitat preferences of the species in Croatian freshwater habitats.

The Balkan Peninsula is considered a biodiversity hotspot (e.g. Griffiths *et al*., 2004; Ivković & Plant, 2015), encompassing a wide range of freshwater habitats where a very high number of rare and endemic species occur. Therefore, these habitats are of not only local and national but also global importance in terms of biodiversity conservation (Freyhof, 2012; Schwarz, 2012). At the same time, since these habi-

tats are highly threatened by anthropogenic activities, it is important to restore and protect them in order to conserve this diversity. Systematic studies such as this one not only increase our knowledge of the fauna of a given region, but also contribute to our knowledge of the ecology of species and their distribution patterns. Therefore, we would like to emphasize the importance of systematic studies and continuous monitoring of habitats and species, as this could provide a valuable background for further research and conservation practice.

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