Ecologica Montenegrina
ISSN 2336-9744 (online) | ISSN 2337-0173 (print)

Ecologica Montenegrina 13: 80-85 (2017)
This journal is available online at: www.biotaxa.org/em

Article

A first record of *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) from the Sava River, with notes on its ecological preferences and rarity of findings in the region

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Received 5 October 2017 │ Accepted by *V. Pešić*: 13 November 2017 │ Published online 15 November 2017.

Abstract

Ephoron virgo (Olivier, 1791) is an European burrowing filter-feeding mayfly species. Once common in large rivers, in XX century it became rare due to increased negative anthropogenic influences. In Serbia there was only one recent report of its presence, from the Danube River (Iron Gate area). The aim of this paper is to report on the first finding of *E. virgo* from the Sava River, and to discuss its scarce findings in the region. Three larval specimens were found in the Belgrade region in grab sample taken in 2014. In order to assess the development of the population in the region, a comprehensive investigation should be conducted.

Key words: burrowing mayfly, grab-sampling, rare species, large river, Belgrade.

Introduction

Ephoron virgo (Olivier, 1791) is one of only two European representatives of world wide distributed group Polymitarcyidae (pale burrowers). The species is considered as a Siberian (or Amur-Siberian) faunistic element, probably of polycentric origin, distributed throughout Palaearctic, with records in Northern Africa, Asia Minor, Europe (except British islands, while in Fennoscandia and the Baltics it has been replaced by the other species *Ephoron nigridorsum* (Tshernova, 1934)) and the Eastern Palaearctic (Bauernfeind & Soldan 2012). This large potamal and hiporhitral mayfly has univoltine life cycle, either of winter (overwinters in the nymphal stage) or summer (overwinters in the egg stage) type (Clifford, 1982). In western Palaearctic (Europe) the univoltine summer type seems to be more common, where the most of the lifetime (up to 9 months including winter) is spent in the egg with a dormant embryo. Larvae grow within 3-4 months, while as an adult it lives only a few hours during the night. Subimago emerges in late summer (from the end of the July to the middle of September) and the flight activity is positively correlated with the air temperature (Ibanez *et al*, 1991; Kureck 1996; Haybach *et al*, 2004; Kureck & Seredszus 2007). The adults are strongly

attracted by light traps (Bauernfeind & Soldan 2012). The larvae (body length 12 - 20 mm) are of the „burrowing“ type with pronounced mandibular tusks projecting far beyond anterior head margin, with numerous tubercles and prominent spines on the upper surface. They have frons with a pronounced, conical, protuberance mediodistally (longer and thiner than in *E. nigridorsum*) and frontoclypeus with a transversal row of long, bipectinate bristles. More morphological details are provided in literature (Nilsson, 1996; Oscoz *et al*, 2011; Bauernfeind & Soldan 2012). During their investigation of this species in Germany (the Rhine and Main rivers) Schleuter *et al* (1989) found that the larvae live in the littoral zone (down to a depth of 6 m) and prefer mixed substrate of coarse and fine material. The larvae construct U-shaped burrows and feed on small organic particles (seston) including bacteria and algae. and pumping water through their burrows and filtering small particles, they enhance aerobic microbial activity by oxygenating river sediments (Cid *et al*, 2008; Kureck *et al*, 2014).

E. virgo disappeared from many European rivers in the XX century, but returned in the late XX and the beginning of XXI century, with the improvement of water quality (Graf *et al*, 2015), becaming a symbol for the recovery of polluted rivers. In the Danube basin numerous are reported recent findings of this mayfly e.g. from the Danube (Slovakia: Elexová, 1998; Adamek *et al*, 2007; Hungary: Szaz *et al*, 2015; Bulgaria: Vidinova & Russev, 1997), and the Tisza (Hungary: Galdean, 1997; Kovacs *et al*, 2001). In Serbia, however, the findings of this unmistakable mayfly species are scarce. According to our knowledge the taxon was recorded only once, in the Danube (Iron Gate area) (Paunović *et al*, 2005). In the recent update of check-list of Ephemeroptera from Serbia (Petrović *et al*, 2015), the presence of this taxon was reported from „Danube small direct tributaries“ without any additional specifications.

Herein we report a new finding of *E. virgo* from Serbia which is the first record of this species from the one of the largest Danube tributaries, the Sava River.

Material and Methods

As a part of yearly investigation of large rivers in the Belgrade region, aquatic macroinvertebrates samples were taken on June 29th, 2014. at the sampling locality „Duboko“ situated on the right riverbank of the Sava River (25th rkm) (Fig. 1). Sampling was conducted by Van Veen grab sampler in littoral zone (5-8 m from the riverbank, an average water depth of 3-5 m). Samples were preserved in 95% ethanol and taken to the laboratory of the Department of Hydroecology and Water Protection of Institute for Biological Research „Siniša Stanković“ Belgrade for further processing. The main physico-chemical parameters of water were measured *in situ* (pH, T, conductivity and dissolved oxygen), while selected nutrient concentrations were measured in the laboratory (Table 1).

Table 1. Main physicochemical parameters at the Duboko locality (Belgrade area).

Year: 2014	**June**	**Annual Average**
pH	8.6	8.36
cond (µS)	/	381.14
O_2 (mg/L)	9.65	9.39
O_2 (%)	108.6	93.1
NO_2 (mg/L)	0.06	0.06
NO_3(mg/L)	1.46	2.44
NH_4 (mg/L)	0.534	1

The investigated river stretch could be described as a free flowing, less impacted by hydromorphological degradation than other sectors of the Sava River and relatively natural in respect to riparian vegetation characteristics, with greenbelt of *Populus* and *Salix* trees on the bank. In the close vicinity settlements of

Umka (downstream) and Barič (upstream) are situated, while upstream there are small townObrenovac (with TP plant Obrenovac) and mouth of the Kolubara River (the right Sava tributary). As the main treats to the locality communal and industrial wastewaters and influence of TP Obrenovac could be singled out. The Kolubara River, which flows throughout one of the larger Serbian coal-mining areas, could deliver additional coal-based minning pollution. River bottom/sediment near the bank (3 to 5 m depth) consists mainly of mud and clay substrate.

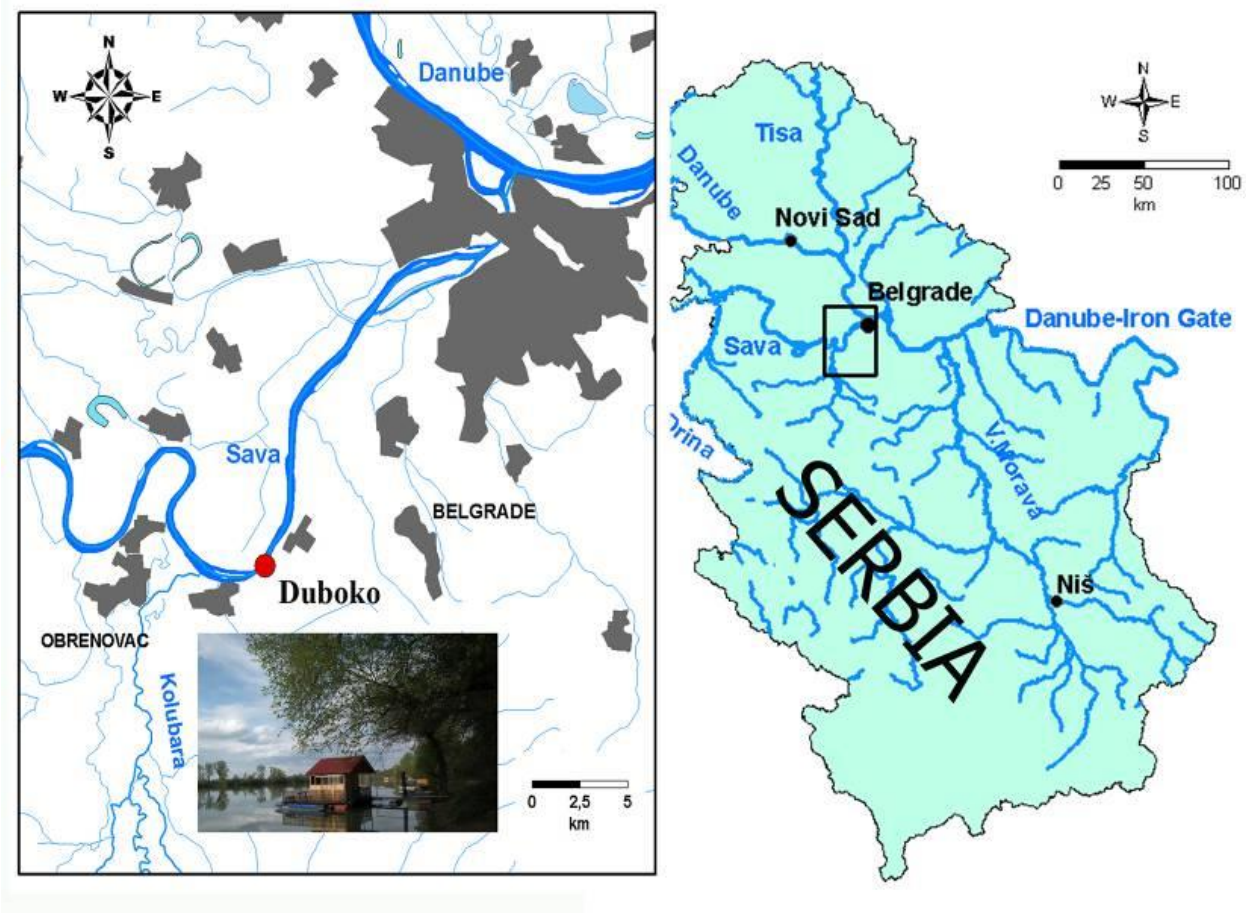

Figure 1. The Duboko locality on the Sava River (Belgrade Region); 44°39'44.12"N, 20°17'2.31"E; 80 m a.s.l. (based on Google Earth).

Results and discussion

During processing of samples taken from the „Duboko“ locality at the Sava River near Belgrade, three larval specimens of *E. virgo* (Fig. 2) were found. Besides findings from the Danube (upstream the Iron Gate; Paunović *et al*, 2005), this finding represents the second confirmed report of this species from Serbia, and the first finding of this mayfly species from the Sava River Basin. Collected specimens are young larvae, about 7 mm in length and with very short wing pads (Fig. 2).This burrowing mayfly is considered as typical for a clay bottom of potamal rivers (Vidinova *et al*, 1997), but could be found also on the sand, gravel, mud and corophium mud (Ibanez *et al*, 1991; Vidinova *et al*, 1997). Our finding is in accordance with these literature data. The finding at some distance from shore (water depth 3 to 5 m), corresponds with similar reports from Spain (Ebro River), where it was found that the species prefer more stable, deeper river zones (Ibanez *et al*, 1991). Such more stable habitat is important in cases of often and/or extreme water-level fluctuations, such as was case with the Sava River in the 2014 with spring floods, and generally higher and more fluctuating water-level (Hydrological Yearbook, 2014).

Figure 2. *Ephoron virgo* specimen from the Duboko locality (the Sava River, Belgrade Region); the scale bar is 1 mm; photo taken using binocular magnifier Carl Zeiss, Stemi 2000-C and digital camera AxioCamERc 5s, Zeiss with ZEN 2011 Software.

As a majority of mayflies *E. virgo* has rather narrow abiotic valences/requirements, as being sensitive to various pollutants, particularly organic/eutrophic conditions (Oscoz *et al*, 2011). Due the pollution in European rivers from the middle of the XX century, a significant decline of its populations was recorded. This species is probably even more sensitive because of its burrowing life style in sediment, which, although being more stable environment than water-column, acts as a collector of various pollutants, especially heavy metals (Milenković *et al*, 2005). Various studies (e.g. Greve *et al*, 1999; van der Geest *et al*, 2000; Nguyen *et al*, 2012) pointed to sensitivity of this mayfly to some heavy metals (e.g. lead (Pb)) and thus applicability of the species in sediment toxicity assays. However, the sediment pollution rarely is only limiting factor affecting more sensitive insects/biota (de Haas & Kraak, 2008). Regarding other limiting factors, a more aerated environment preference (with 5.55 mg/l O_2 as lower threshold; Vidinova *et al*, 1997) of this potamal burrowing species, should be mentioned. Additionally, in case of short-living adults, problem could arise from light-pollution in urban areas. Szaz *et al* (2015) found in their investigation in Hungary particularly negative impact of lights on bridges. As the Duboko locality characterizes well aerated environment (Table 1), and with low organic pollution (except ammonia (NH_4)), it is clear that this locality meets the main abiotic (physic-chemical) requirements as the habitat for *E. virgo*. Moreover, as this site has relatively low light pollution it represents a favourable environment for adults of this mayfly as well.

Besides sensitivity to pollution, interactions with invasive species could influence this species. A large predatory amphipod *Dikerogammarus villosus* (Sowinsky, 1894) nowadays is one of dominant gammarids in the largest Serbian waterway - the Danube River (Paunović *et al*, 2007; Borza *et al*, 2015), and present in other larger rivers such as the Tisa (Zorić *et al*, 2015) and the lower parts of the Sava (Paunović *et al*, 2012) and the Velika Morava (Marković *et al*, 2015). Kureck *et al*. (2001) found a significant decrease of the *E. virgo* in presence of *D. villosus* because of predation (from Haas *et al*, 2002). Although spreading upstream the Sava River, *D. villosus* still has not reached such abundance and dominance as in the Danube, the Sava River could be considered as a more favourable environment for these mayflies. A competition for resources, particularly food, with aggressive and often in larger rivers very abundant invasive filtrators, such as bivalvs (e.g. *Corbicula fluminea* (Müller, 1774), *Sinanodonta woodiana* (Lea, 1834)) and crustaceans (e.g. *Chelicorophium curvispinum* (Sars, 1895)), could be another reason for declining of *E. virgo* (Kureck *et al*. 2014). The food competition often is considered as one of the most important factors limiting distribution of this species, although Kureck *et al* (2014) clearly stated importance of various environmental factors synergy.

Serbian large rivers (Danube, Sava, Tisa and Velika Morava), as potential habitats for *E. virgo* could be considered as a rather hostile and unfavourable environments in terms of previously discussed limitations. All these rivers, and particularly the Danube, are under heavy anthropogenic pressures (pollution, hydrological alterations, invasive species etc.). As more favourable habitats for this species remain a few

„ecological refugia“, such as the Duboko locality on the Sava River. In order to assess the development of the populations in the region, a comprehensive investigation should be conducted.

Acknowledgments

The investigation was supported partly by funding from the European Community's Seventh Framework Programme, Grant agreement 603629-ENV-2013-6.2.1 (Globaqua), and by the Ministry of Education and Science of the Republic of Serbia – Grants No. ON173025 and No. III 43002. We would like to express our gratitude to Roman J. Godunko (National Academy of Sciences of Ukraine, Kiev) for insightful comments that helped us to improve the manuscript.

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