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New Records of Species *Taeniopteryx hubaulti* Aubert, 1946 and *Taeniopteryx schoenemundi* (Mertense, 1923) (Plecoptera: Taeniopterygidae) in Serbia

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ABSTRACT

Species *Taeniopteryx hubaulti* Aubert, 1946 and *T. schoenemundi* (Mertense, 1923) have been emphasized as endangered or vulnerable species in several European countries due to their local and limited distribution. More intensive research of these species on the territory of Serbia was conducted from 2011 to 2022. *Taeniopteryx hubaulti* was registered in seven localities. Five of these localities (Brusnička and Jerma rivers, the spring of the Raška River, and two localities on Grza River, spring and tufa barriers) were recorded as new sites of this species in Serbia. Two localities (Barska River and Lisinski Stream) had already been known from previous studies. The species *T. schoenemundi* was recorded in four new localities (Dulenska, Visočica, Temštica, and Trnavska rivers). Both species were found in waters with high oxygen concentration, relatively uniform low water temperature, and variable values of hardness, electrical conductivity, and pH values, with a small amount of inorganic nutrients. Considering the rare occurrence and prominent importance of these species as biological indicators of water quality and climate change, their findings have a great deductive value. This research represents an important contribution to the insight into the distribution of *T. hubaulti* and *T. schoenemundi* species.

Keywords: Stoneflies, distribution, endangered species, climate change, karst rivers.

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INTRODUCTION

Taeniopterygidae (early or winter stoneflies), a small family within the order Plecoptera, is an ecologically significant component of the entomofauna of lotic waters worldwide, characterized by a high degree of endemism and narrow ecological requirements (Koese, van Maanen, & Boumans, 2014; Fochetti, 2020a). The global diversity of the family Taeniopterygidae includes 104 known species classified into 12 genera, out of which 48 species inhabit European waters (Fochetti & de Figueroa, 2007; DeWalt & Ower, 2019). The genus *Taeniopteryx* Pictet, 1841, the only genus within the subfamily Taeniopteryginae, shows a holarctic distribution (Zwick, 2000; Zhiltzova, 2006) and comprises ten species in European freshwater (Guareschi, Ramos-Merchante, Ruiz-Delgado, & Mellado-Díaz, 2018). However, some of these species have become extinct from their habitats (Lock & Goethals, 2008, Kosterin, Akimbekova, & Dubatolov, 2017), while others are given the status endangered or vulnerable due to local and limited distribution (Lubini, Knisp, Sartori, Vicentini, & Wagner, 2012; Petrović, Simić, Milošević, Paunović, & Sivec, 2014; Tyufekchieva, Evtimova, & Murányi, 2019). The genus *Taeniopteryx* includes typical winter, cold-stenothermal species, with an early flight period from January to April, according to the altitude (Fochetti & Paolo, 1996; Zwick & Hohmann, 2003; Rupprecht, 2014). In European waters, larvae begin to appear in late September and mostly behave as shredders (Harper, Lauzon, & Harper, 1991; Lock & Goethals, 2008; Cummins, 2019).

In some countries, there are numerous studies on the distribution of species of the genus *Taeniopteryx* (Zhiltzova, 2006; Kovács & Murányi, 2008; Bojková, Soldán, Špaček, & Straka, 2011; Tyufekchieva et al, 2013). However, many authors believe that these species have not been adequately studied, blaming their typical winter life cycle and inaccessibility of habitats for it (Koese et al, 2014; Guareschi et al, 2018). Nevertheless, due to their extreme sensitivity to elevated temperatures, these species are useful indicators of climate change. In addition, they are considered as good bioindicators of environmental quality, as they respond to habitat change and pollution (Puig, Ubero-Pascal, Amore, & Fochetti, 2011; Guareschi et al, 2018; Fochetti, 2020b).

The diversity and distribution of the order Plecoptera in aquatic ecosystems on the territory of Serbia were recently studied by Petrović et al (2014). However, as a separate entity, the family Taeniopterygidae has not been the subject of scientific research yet. According to literature data, three species of the genus *Taeniopteryx* have been recorded in Serbia: *T. nebulosa* (Linnaeus, 1758), *T. hubaulti* Aubert, 1946, and *T. schoenemundi* (Mertense, 1923) (Petrović et al, 2014). Based on previous data on their distribution in aquatic ecosystems of Serbia, mutually isolated populations of the species *T. nebulosa* were recorded in the Timok Basin (the Trgoviški Timok) (Simić,1993) and the West Morava (the Lomnička River) (Konta, 1997), and the South Morava (the Vlasinska River) Basins (Paunović, Jakovčev-Todorović, Simić, Stojanović, & Petrović, 2006).

Species *T. schoenemundi* was recorded almost 30 years ago at only one site in the South Morava Basin (the Manojlovica River) (Simić, 1996); while *T. hubaulti* was registered in the West Morava Basin (the Barska River and the Lisinki Stream), in the area of the Kopaonik National Park (Konta, 1997; Petrović et al, 2014). In Serbia, the species of *T. hubaulti* and *T. nebulosa* are on the list of strictly protected species (Anonymous, 2010). Although *T. schoenemundi* is not included in the list of protected species by any Serbian regulative, efforts should be directed towards its conservation. Moreover, there are already reports about population decline in Germany and Italy or the extinction of local populations in Switzerland and Belgium (Lock & Goethals, 2008; Fochetti, 2020b). This paper provides data on new findings of species *T. hubaulti* and *T. schoenemundi* in the aquatic ecosystems of Serbia, together with key environmental factors affecting their biogeographical distribution in current aquatic ecosystems.

MATERIAL AND METHODS

Study area

The primary data source for this study was hydrobiological surveys in Serbia that were conducted every three years during different seasons from 2011 to 2022 in 71 localities in the Morava basin, including the West, South, and Great Morava rivers and their tributaries. The list and details about investigated localities can be found in Simić et al (2022). Additionally, this study included seasonal investigations of aquatic macroinvertebrates in 20 karst rivers of Serbia that were conducted over four years from 2019 to 2022. The study encompassed various lotic karst habitats (springs, mountainous rivers, streams, and tufa barriers). Within all investigated localities, target species were found in the following 11: Barska River, Lisinski Stream, spring of Raška River, Brusnička River, and Trnavska River (the West Morava basin); Dulenska River, Grza River (two localities: spring and tufa barriers) (the Great Morava basin); Temštica, Visočica, and Jerma rivers (the South Morava basin) (Fig. 1). Data for the geographical coordinates, altitude, and type of substrates of each locality are presented in Table 1.

Table 1. Sampling sites of species *Taeniopteryx hubaulti* and *T. schoenemundi* in Serbia.

Abbreviation	River/localites	Geographical coordinates	Altitude	Substrate
BA	Barska River	43°17'41.2"N 20°45'31.6"E	1346 m	cobbles, gravel, sand
LI	Lisinski Stream	43°16'54.4"N 20°45'02.0"E	1170 m	cobbles, gravel, sand
JE	Jerma River	42°57'06.2"N 22°36'37.7"E	554 m	large stones, cobbles, gravel, and sand
BR	Brusnička River	43°25'05.9"N 20°22'28.1"E	794 m	cobbles, gravel, and sand
GRS	Spring of Grza River	43°53'49.2" N 21°38'45.0"E	432 m	large stones, cobbles, gravel, moss, macrophytes
GRT	Tufa barriers of Grza River	43°53'48.4"N 21°38'42.8"E	411 m	mosses on tufa, gravel, macrophytes
RA	Spring of Raška River	43°06'57.4"N 20°22'13.7"E	739 m	large stones, cobbles, gravel, sand, moss
DU	Dulenska River	43°53'38.7"N 21°00'50.1"E	338 m	cobbles, gravel, sand
VI	Visočica River	43°09'23.9"N 22°48'27.2"E	694 m	cobbles, gravel, sand
TE	Temštica River	43°17'48.0"N 22°36'22.7"E	509 m	large stones, cobbles, gravel, sand
TR	Trnavska River	43°17'13.9"N 20°34'31.9"E	528 m	cobbles, gravel, sand

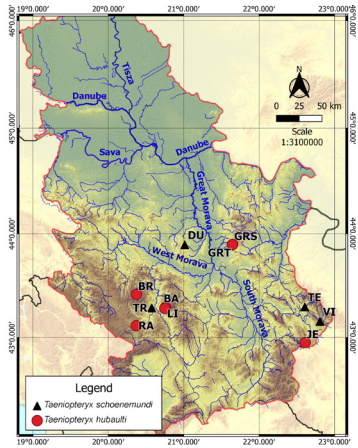

Figure 1. The geographical distribution of *Taeniopteryx hubaulti* and *T. schoenemundi* in Serbia: (●) *T. hubaulti*: the Jerma River (JE), the Brusnička River (BR), the spring of the Raška River (RA), and the spring and tufa barriers of the Grza River (GRS and GRT), the Barska River (BA), the Lisinki Stream (LI); (▲) *T. schoenemundi*: the Dulenska River (DU), the Visočica River (VI), the Temštica River (TE) and the Trnavska River (TR).

Sample collection and processing

At each site, six subsamples of macrozoobenthos were collected from the most common substrate types with a 0.0625 m^2 Surber sampler of 250 mm mesh, according to the standard EN 10970 (2012). The subsamples were merged into a single sample. The samples were fixed with 4% formaldehyde solution and stored at the Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia. From the entire macrozoobenthos sample, genus *Taeniopteryx* specimens were separated for further identification and taxonomic studies. Collected specimens were analyzed under a NIKON SMZ 800 stereomicroscope with a MOTIC camera and Nikon Eclipse E100 microscope. The material was identified according to established entomological keys (Aubert, 1959; Hynes, 1967; Raušer, 1980; Zwick, 2004). Quantitative estimation of macrozoobenthos was based on numerical counting, i.e., units per square meter (ind. per m^2). Simultaneously with collecting benthic invertebrate samples in the field, the following physical and chemical parameters were measured: water temperature (°C), pH (1-14), oxygen concentration (mg L^{-1}), saturation (%), conductivity (μs cm^{-1}), nitrate, ammonia and phosphate concentration (mg L^{-1}) and water hardness (CaCO3 mg L^{-1}) according to EN ISO 5667 (2017).

RESULTS

New records of *T. hubaulti* larvae on the territory of Serbia were recorded at the following localities: the Jerma River (the South Morava Basin), the Brusnička River, the spring of the Raška River (the West Morava), and the Grza River (spring and tufa barriers) (the Great Morava). During these investigations, two previously recorded

findings in the area of NP Kopaonik (the Barska River and the Lisinski Stream) were confirmed (Fig. 1). During the research, new findings of *T. schoenemundi* were recorded in four new localities: the Dulenska River (the Great Morava Basin), Visočica and Temštica rivers (the South Morava), and the Trnavska River (the West Morava).

The springs of karst rivers had higher number of individuals of *T. hubaulti* in comparison to other study sites. In the spring of Grza River 45 and 24 ind./m^2 were recorded, while 21 and 16 ind./m^2 were recorded in the spring of Raška River. Similarly, in the locality on the Jerma River 16 ind./m^2 were detected. In other localities, small populations were recorded with less than 11 ind./m^2 (Table 2). The number of *T. schoenemundi* individuals found at investigated sites ranged from 3 to 16 individuals per m^2 (Table 3). In a comprehensive study of the genus *Taeniopteryx* performed in this work, several preferences according to substrate type were observed. The high number of individuals of *T. hubaulti* in springs can be attributed to a greater variety of substrates. These localities are characterized by substrate with stones, cobbles, and specialized vegetation, especially mosses. The types of substrate in other localities consisted mostly of stones, cobbles, gravel, and, less frequently, tufa with mosses (Table 1). The highest number of individuals of *T. schoenemundi* was on the Temštica River (16 ind./per m^2), which is characterized by a substrate consisting of large stones, cobbles, and gravel. In general, based on our results, *T. schoenemundi* prefers mineral substrate without vegetation (Table 1).

Table 2. The number of individuals per m^2 and environmental parameters of *Taeniopteryx hubaulti* localities in Serbia.

River/localties	**BA**	**LS**	**JE**	**BR**	**GRS**		**GRT**	**RA**	
Date	16.08.'11.	17.08.'11.	02.11.'11.	15.10.'12.	24.10.'20.	25.02.'22.	24.10.'20.	31.10.'21.	17.02.'22.
Number of individuals per m^2	11	3	16	5	45	24	3	16	21
Water temperature (°C)	12	13.1	8.2	9.4	10	8.9	10.2	10.7	4.6
Conductivity (μS cm^{-1})	80	120	390	710	500	480	490	360	170
Hardness (mg L^{-1})	40	60	200	350	230	240	240	180	80
pH	6.7	7.92	6.90	7.86	6.88	7.24	7.56	7.39	7.44
O_2 (mg L^{-1})	9.40	9.10	11.10	10.98	10.16	-	9.92	10.29	-
O_2 (%)	91.5	95.5	105.7	84.3	101.2	-	99.1	100.8	-
NO_3 (mg L^{-1})	5.6	6	3	<3	<3	5	3.5	3.2	<3
PO_4 (mg L^{-1})	0.08	0.09	0.10	0.10	0.08	<0.06	0.22	0.07	<0.06
NH_4 (mg L^{-1})	0.3	0.24	0.07	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03

Table 3. The number of individuals per m^2 and environmental parameters of *Taeniopteryx schoenemundi* localities in Serbia.

River/localites	**DU**	**VI**		**TE**		**TR**
Date	29.10.'11.	02.11.'11.	25.12.'13.	22.10.'21.	22.03.'22	16.02.'22.
Number of individuals per m^2	3	11	5	16	5	8
Water temperature (°C)	5.9	7.8	6	5.8	4.9	6.2
Conductivity (μS cm^{-1}).	460	240	180	80	100	400
Hardness (mg L^{-1})	230	130	90	40	50	190
pH	9.09	7.78	8.11	7.63	8.04	7.68
O_2 (mg L^{-1})	11.36	11.31	9.93	10.71	-	11.43
O_2 (%)	95.6	105.4	100.8	96.5	-	103.5
NO_3 (mg L^{-1})	6.7	<3	<3	4.9	<3	4.5
PO_4 (mg L^{-1})	0.29	0.10	0.12	0.14	<0.06	0.06
NH_4 (mg L^{-1})	0.09	<0.03	<0.03	<0.03	0.05	<0.03

Findings of *T. hubaulti* species were recorded at altitudes ranging from 411 m a.s.l. (the Grza River - tufa barriers) to 1346 m a.s.l. (the Barska River), and *T. schoenemundi* species at altitudes from 338 m a.s.l. (the Dulenska River) to 694 m a.s.l. (the Visočica River). *Taeniopteryx hubaulti* and *T. schoenemundi* species were recorded in waters with relatively uniform high values of oxygen concentration (> 9.1 mg L^{-1}) and low temperature at all localities (< 13.1 °C). However, values of hardness, electrical conductivity, and pH varied significantly. In all localities where both species were recorded, the amounts of inorganic nutrients were small (Table 2 and 3). During our investigations, both species, as typically cold stenotherms, usually occur during autumn and winter. Specifically, the species *T. schoenemundi* was observed to occur from October to April, while the species *T. hubaulti* was observed to occur earlier, from mid-August to March (Table 2 and 3). These observations of earlier occurrence of species *T. hubaulti* were recorded at higher altitudes in the watercourses of the Kopaonik National Park (Barska River and Lisinski Stream).

CONCLUSIONS AND DISCUSSION

The main environmental factors determining the distribution of the abundance of *Taeniopteryx* species in rivers are altitude, type of substrate, oxygen saturation, the solubility of oxygen in water, and temperature (Tyufekchieva et al, 2013).

Species *T. hubaulti* is a western Palearctic species with fragmented and localized areal in European freshwaters (Zhiltzova, 2006). According to recent studies, *T. hubaulti* is widely spread across Central Europe (Bojková et al, 2011), while there are only a few findings in eastern and southern Europe, including the Balkan Peninsula (Tyufekchieva et al, 2019; Ridl et al, 2018). *Taeniopteryx hubaulti* inhabits clean and cold streams in mountainous and sub-mountainous regions in the rhithral zones (Bojková et al, 2011; Graf et al, 2022). Previous studies on Serbian freshwater systems confirmed the distribution of *T. hubaulti* in regions of upper and middle rhithral, altitudes between 1000 and 1500 m a.s.l. (Petrović, 2014). However, in our research, the species was found at a lower altitude, in a zone between 400 and 800 m a.s.l., similar to the latest findings in Macedonia by Slavenska-Stamenković et al (2016). Studies conducted in the National Park Plitvice Lakes, located in the karst part of northwestern Dinarides (in the mountainous part of Croatia), recorded the presence of *T. hubaulti* on several locations between 390 and 720 m a.s.l. (Korana River, springs, and rhithral of the Bjela and the Crna rivers) (Popijač & Sivec, 2009; Ridl et al, 2018). The species was not recorded on the tufa barriers of the previously mentioned rivers. In Bulgaria, Tyufekchieva et al (2013) described the findings of this species above 1000 m a.s.l. This species is widely studied in the mountainous parts of the Czech Republic, between 320 and 900 m a.s.l. (Bojková et al, 2011). In Switzerland, it was recorded at an altitude between 700 and 1400 m a.s.l. (Lubini, 2012), whilst in Spain and France between 1400 and 1600 m a.s.l. (Puig, 1984; Guareschi et al, 2018).

In our research, *T. hubaulti* was found in various biotopes such as springs, streams, small and medium-sized rivers with stones, and tufa barriers. According to

recent studies, the species was recorded in the area of the springs and the upper flows of streams (epirhithral and metarhithral), in turbulent and fast-flowing water with few specialized macrophytes on mineral substrates, mainly consisting of stones, cobbles, and gravel (Guareschi et al., 2018; Ridl et al., 2018). In our research, the type of substrate in investigeted habitats (stones, cobbles, and gravel with or without specialized vegetation) directly correlates with findings in similar studies (Tyufekchieva et al, 2013; Ridl et al, 2018). The exception is the habitats of tufa barriers for which there is no literature evidence.

Although widespread throughout Central Europe and the Balkans, populations of *T. schoenemundi* count only a few specimens (Angersbach, Stein, & Wolf, 2010). In the east, the distribution area extends to Poland, Slovakia, and Romania, while in the south and the west, it occurs in the Iberian Peninsula, Italy, the southwestern France (Berthelemy & Whytton Da Terra, 1980; Vincon & Pardo, 2004; Enting, 2005; Graf et al, 2022). This species was found in a wide range of altitudes, from 100 to 1900 m with the largest number of finds in the hyporhithral and epipotamal water zones, with rocky and gravelly bases (Angersbach et al, 2010; Manko, 2011; Tyufekchieva et al, 2013). However, based on the results obtained in our research, it can be concluded that the species, in addition to hyporhithrals (the Dulenska River), also inhabit metarhithral, being supported by Tyufekchieva et al. (2013) research.

To this date, the presence of the species *T. schoenemundi,* on the territory of Serbia has been recorded only in the Manojlovica River, in the South Morava Basin (Simić, 1996). Simić (1996) and Paunović et al (2006) stated the presence of *Taeniopteryx* sp. in the area of Vlasina, the landscape of exceptional features.

On the territory of Serbia, both investigated species were found in waters with high oxygen concentration above 9 mg L^{-1} and oxygen saturation above 80%, which is in correlation with the research from other areas. In Bulgaria, the studied species were not found in waters with dissolved oxygen below 7 mg L^{-1} and 70% saturation (Tyufekchieva et al, 2013). In Croatia, Ridl et al (2018) found *T. hubaulti* species in waters with oxygen concentration from 7.6 to 14.1 mg L^{-1}, and saturation between 65 and 121%. In the Netherlands, *T. schoenemundi* was recorded in waters where oxygen conditions always remain relatively high, i.e., above 8 mg L^{-1} (Koese et al, 2014).

Almost all authors state that *T. hubaulti* prefers cold, clean waters of the rhithral type (Tyufekchieva et al, 2013; Schmidt-Kloiber & Hering, 2015; Graf et al, 2022). According to our research, the maximum recorded water temperature at which *T. hubaulti* was found was 13.1 °C. According to the literature, the best growth is achieved at temperatures below 10 °C (Graf et al, 2022), which corresponds to some findings. Koese et al (2014) states that the limiting factor for the survival of *T. schoenemundi* is 20 °C. While *T. hubaulti* is a typical indicator species of oligosaprobic waters, *T. schoenemundi* can tolerate mildly polluted waters and live in β-mesosaprobic waters corresponding to individual findings in epipotamal water zones (Tyufekchieva et al, 2013).

Recognition of conservation and vulnerability of these species has been acknowledged by many countries, considering habitat quality change and high levels

of running water pollution (sewage, agricultural and industrial waste) and other human activities along with climate change as major factors causing decline and loss of their populations (Angersbach et al, 2010; Fochetti, 2020b). Based on previous findings on the territory of Serbia, species *T. hubaulti* has been marked as critically endangered (Petrović, 2014), and it is on the list of strictly protected species (Anonymous, 2010). *Taeniopteryx hubaulti* is classified as endangered in Bulgaria (Tyufekchieva et al, 2019), vulnerable in Switzerland (Lubini, 2012), while in Spain, it is considered as a moderately vulnerable and relict species (Puig et al, 2011; Guareschi et al, 2018). In the Czech Republic, it is considered by most authors as a vulnerable species (Bojková, Komprdová, Soldán, & Zahrádková, 2012; Bojková & Soldán, 2013). However, given a large number of new sites and the wider distribution in the Czech Republic, its vulnerability category has been put into question, and some authors have suggested that the Near Threatened category (NT) would better describe its current status (Bojková et al, 2011). In the Bulgarian Red List, *T. schoenemundi* is classified as vulnerable species (Tyufekchieva et al, 2019; Tyufekchiev & Rimcheska, 2019), while in Slovakia is critically endangered (Manko, 2011). In Germany, *T. schoenemundi* is considered an endangered species, with the threat of extinction in most parts of the country (Zwick, 1992; Enting, 2005). In Italy, sites of this species have been reduced to small, isolated relict populations threatened with extinction (Fochetti, 2020a). In most of Belgium and Switzerland, it is considered extinct because the rivers in which they were once recorded are now loaded with various types of pollution, resulting in the non-survival of sensitive Plecoptera species (Lock & Goethals, 2008; Lock & Oosterlynck, 2012; Lubini, 2012).

Some of these new habitats in Serbia are protected to various degrees. Localities on the Visočica and the Temštica rivers are located within the Stara Planina Nature Reserve, while the locality on the Brusnička River is part of the Golija Nature Park. The Barska River and the Lisinski Stream are in the area of National Park Kopaonik. The Jerma River belongs to the category of Special Nature Reserve. Species of the genus *Taeniopteryx* are on the Austrian list of sensitive macroinvertebrate taxa (Moog, 2002), as their biological characteristics and ecological preferences determine their vulnerability to freshwater ecosystem modification and climate change (Fochetti, 2020b; Graf et al, 2022). This point out their bioindicator significance. Since the studied species require specific ecological conditions, any direct or indirect negative anthropogenic influence may affect their survival in their microhabitats. This is especially true of the karst of the Grza River, which is in danger of pollution due to excessive and unplanned construction of recreational settlements and due to the so-called mass tourism. This also applies to the Brusnička River, where a small hydroelectric power plant was built. Future research will show whether these factors affect the population of species of the genus *Taeniopteryx*. During the systematic research of the rivers of NP Kopaonik, from 2016 to 2021 (during September), no records of the species *T. hubaulti* were made. However, we noticed various habitat modifications caused by intensive urbanization processes. Along with investigated species, it was discovered that some of these researched localities are habitats of many other critically endangered, endangered, and

vulnerable aquatic macroinvertebrate species (eg. *Thremma anomalum* (Mclachlan, 1876), *Helichopsyche bacescui* (Orghidan & Botosaneanu, 1953), *Drusus discolor* (Rambur, 1842), *Amphinemura sulcicoris* (Stephens, 1836)), which according to the local red list have conservation priority.

Insufficient literature data on these species could be explained by their winter life span or the inaccessibility of their habitats. It has been noted that the most frequent findings of these species were in the period from late October to mid-April (Zhiltzova et al, 2006; Tyufekchieva et al, 2013), which correlates with our research, with small deviations in the NP Kopaonik rivers. Therefore, future studies should include a larger number of study sites and microhabitats in higher lentic habitats, primarily during the winter months. The current study represents an important contribution to the evidence on the distribution of two species of the genus *Taeniopteryx*, but also their preferences for habitat and environmental factors in current aquatic ecosystems in Serbia. Due to their rare occurrence, these species are important biological indicators of water quality and climate change, and therefore their findings are always of great value. Further study analysis of the species would contribute to a better understanding of the real status and distribution of these species, which would precisely indicate its category of endangerment, and thus the appropriate necessary protection measures.

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