

Diversity and Distributional Patterns of Stoneflies (Insecta: Plecoptera) in the Aquatic Ecosystems of Serbia (Central Balkan Peninsula)

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Abstract: This work contributes to the knowledge of the fauna of Plecoptera (Insecta) of the Central Balkan Peninsula. A total of 90 species analysed in this study belong to 17 genera and 7 families. The largest number of stonefly species was recorded within the Južna and Zapadna Morava River Basins and the rivers belonging to the Aegean Sea Basin (Pčinja, Dragovištica and Lepenac), while the lowest taxa richness was observed within the rivers Kolubara and Velika Morava, as well as from the Danube River. Based on the IUCN criteria, 10 species were classified as important from conservational point of view: *Marthamea vitripennis*, *Protonemura lateralis* and *Taeniopteryx hubaulti* as Critically Endangered, *Dinocras megalcephala*, *Nemoura cambrica*, *Protonemura meyeri*, *Protonemura praecox* and *Taeniopteryx nebulosa* as Endangered, and *Amphinemura sulcicollis* and *Perlodes microcephalus* as Vulnerable.

Keywords: Plecoptera, Serbia, diversity, distribution, freshwater ecosystems

Introduction

Various anthropogenic activities severely disturb the freshwater ecosystems and thus endanger them. Data regarding the diversity and distribution of indicator groups reveal valuable information for conservation and environmental assessment approaches. Stoneflies (Plecoptera), as a significant ecological component of running water ecosystems (FOCHETTI, TIerno DE FIGUEROA 2008), in general are known to be intolerant to variation in their environmental conditions (ZWICK 1980, FOCHETTI, TIerno DE FIGUEROA 2006). Because of this characteristic, Plecoptera are used separately or together with other indicator groups (Ephemeroptera and Trichoptera) to calculate metrics applied to the majority of bioassessment programs (AQEM 2002, BARBOUR *et al.* 1999). On

the other hand, looking through the prism of conservation, the stonefly sensitivity to variation in abiotic factors may lead to the local or global extinction of taxa. In this regard, it seems that Plecoptera is one of the most endangered groups of aquatic insects (FOCHETTI, TIerno DE FIGUEROA 2006).

The global species diversity of the insect order Plecoptera is represented by more than 3497 described species distributed across 16 families and 286 genera (FOCHETTI, TIerno DE FIGUEROA 2008). The majority of studies so far has been conducted in North America and Europe, where 650 (STARK, BAUMANN 2005) and 571 (GRAF *et al.* 2009) species have been recorded, respectively. Other parts of the world have not been well studied; therefore, the esti-

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mates of the species richness in these regions are not representative.

Since Plecoptera show a high percentage of endemism (FOCHETTI, TIerno DE FIGUEROA 2006), the regional surveys of the stonefly fauna have both local and global importance. Although the taxonomic papers and regional catalogues report the real state of the stonefly fauna in Europe (ZWICK 1980, GRAFF *et al.* 1995, GRAFF *et al.* 2009, MURÁNYI 2007, MURÁNYI 2011), little information on the distribution and zoogeography of this group on the Balkan Peninsula is available at present (MURÁNYI 2008). This is especially true for Serbia, where previous research on the stonefly fauna was conducted on particular rivers only (DAUTI 1986, FILIPOVIĆ 1954, 1968, 1969, KONTA 1997, MARKOVIĆ 1995, 1998, MARKOVIĆ *et al.* 1998, SIMIĆ 1993, 1995, SIMIĆ, SIMIĆ 1999, 2003, PAUNOVIĆ *et al.* 2006, ŽIVIĆ *et al.* 2001, 2005, DAUTI *et al.* 2007). Since the lotic systems of Serbia are characterised by a wide range of elevation stream orders and habitat diversity (RADOVIĆ *et al.* 1995), and these systems belong to the drainage basins of three seas (the Black Sea, Adriatic Sea and Aegean Sea), high species richness of the Plecoptera group can be expected.

For the purpose of extending the knowledge on the stonefly species community, the diversity and distribution patterns of the Plecoptera were investigated for eight drainage basins that encompass the entire diversity of Serbia. We also present here the first checklist of Plecoptera from the Central Balkan Peninsula, with information about their distribution and IUCN Red List categories at a regional level.

Material and Methods

Study area

The Plecoptera review presented in this work covers the territory of the Republic of Serbia. Serbia is located in the central area of the Balkan Peninsula. All Serbian lotic systems are part of the drainage basins of three seas: the Black Sea, Adriatic Sea and Aegean Sea.

The majority of the Serbian freshwater streams (92%) belongs to the Danube River (Black Sea) drainage basin (81 700 km²) (GAVRILOVIĆ, DUKIĆ 2002). The entire basin is drained by only one river, the Danube River, which flows into the Black Sea. The rest of the large rivers in Serbia (*e.g.* the Sava, Velika, Zapadna and Južna Morava, Kolubara, Timok and Drina) also belong to this basin.

The second large drainage basin is that of the Adriatic Sea, which is drained by one river, the White Drin River. It is situated in western Kosovo and Metohija and covers an area of 4732 km² or 5.36%

of the territory of Serbia (GAVRILOVIĆ, DUKIĆ 2002). The White Drin in Albania meets the Black Drin to create the Drin River, which flows into the Adriatic Sea. These rivers have not been investigated recently due to the current political situation in Serbia.

Finally, in the southern part of Serbia, the Aegean Sea basin covers an area of at least 1926 km², which is approximately 3% of the territory of Serbia. It is drained by the Lepenac, Pčinja and Dragovištica rivers.

The aquatic ecosystems where the Plecoptera species has been found were classified into one of the three drainage areas existing on the territory of Serbia. In addition, the aquatic ecosystems were divided according to the size of their catchment area (near or above 4000 km²) into the following: the Danube River, the Kolubara, Velika, Zapadna and Južna Morava, Timok, Drina, and the rivers belonging to the Aegean Sea basin (the Pčinja, Lepenac, and Dragovištica).

ILLIES (1978) divides the territory of Serbia into 5 ecoregions: ecoregion 5 – Dinaric Western Balkans; ecoregion 6 - Hellenic Western Balkans; ecoregion 7 - Eastern Balkans; ecoregion 10 - Carpathians; and 11 - Pannonian Lowland. Ecoregions 5, 6, 7, and 11 cover large areas of the country, while ecoregion 10 covers only a restricted area in Northeast Serbia. Even though ecoregion 10 is restricted to a narrow area, the influence of the Carpathians is evident in Northeast Serbia (PAUNOVIĆ *et al.* 2012).

Sampling and data analysis

In order to prepare a regional catalogue of Plecoptera and estimate the species richness and distribution patterns in each drainage basin, we used an informative database (BAES *ex situ*), <http://baes.pmf.kg.ac.rs> (SIMIĆ *et al.* 2006). BAES *ex situ* was developed as a product of the biodiversity project “*Ex situ* biodiversity protection of the aquatic ecosystems of Serbia”, which covered almost all surface waters in Serbia.

In addition to the literature data, the BAES *ex situ* database includes data obtained in our field research during the period 2003-2011. The literature data encompass 146 ecosystems where species of Plecoptera have been recorded at 745 sites. The field campaigns conducted from 2003 to 2011 involved a network of 248 sampling sites within 69 ecosystems.

The database comprises information on the taxa regarding the taxonomy of the species, the ecosystem in which the species were found, the year of the particular record, frequency of the records, abundance and other data. All Plecoptera species were recorded in the period 1950-2011. The material from

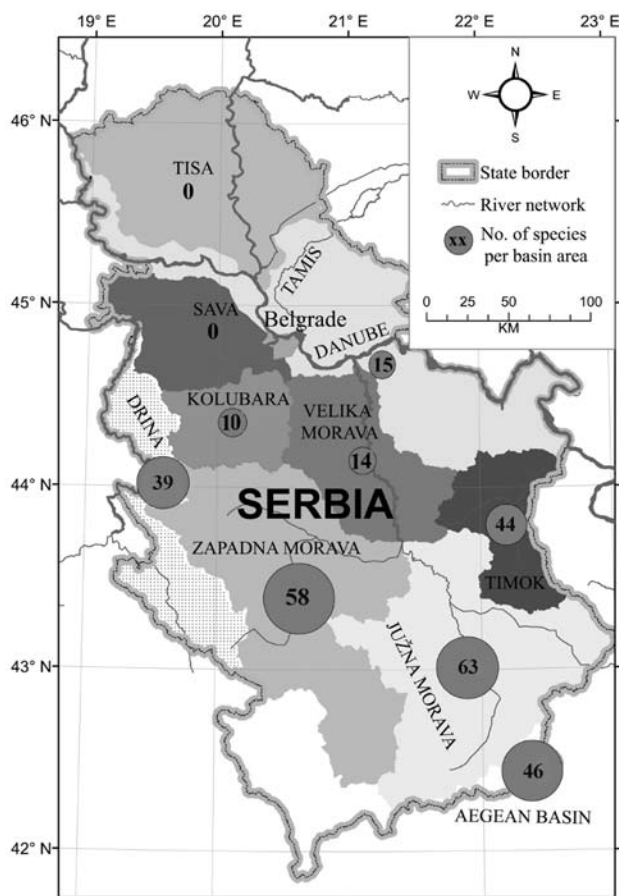


Fig. 1. The number of stonefly species per catchment area (larger basins with an area size near or above 4000 km²); the size of the circles corresponds to the number of taxa

the Plecoptera collection at the Slovenian Museum of Natural History in Ljubljana was used along with the data from the database.

In this study, the samples of adults and larvae of Plecoptera were collected from the rivers' basins in the period 2003-2011. These samples were taken manually, by entomological nets, by Surber sampler with 250 µm mesh or by an Ekman dredge. The collected material was fixed in 4% formaldehyde and 75% ethanol. The material was determined at the Institute of Biology and Ecology (University of Kragujevac, Serbia). Plecoptera were identified to the genus or species level, according to the established keys (HYNES 1967, SOWA 1970, KIS 1974, RAUJBER 1980, SIVEC 1980, GRAF, SCHMIDT-KLOIBER 2003, ZWICK 2004). The nomenclature and systematics follow DeWALT *et al.* (2013).

In order to determine the level of threat, we used the IUCN Red List Categories and Criteria, Version 2013.1 (IUCN 2013). The conservation status was estimated for those taxa for which precise and accurate data were available, and whose population status was revised.

Since BAES *ex situ* was compiled from studies with unknown or different sampling methods, we used presence/absence data to create Sørensen's similarity matrix. To analyse the faunistic similarity of Plecoptera between the drainage basins, the Sørensen similarity index and the group average clustering method were used. The analysis was performed by using PC-ORD 4.0 Windows software (McCUNE, MEFFORD 1999).

Results

A total of 90 species analysed in this study were distributed into 17 genera and 7 families. The most diverse family was Nemouridae, represented by 30 species, followed by Leuctridae and Perlodidae (19 species each).

The highest species diversity was recorded within the genera *Leuctra* and *Isoperla* with 19 and 14 species, respectively, while the rest of the genera were represented by fewer species.

The distribution pattern of the Plecoptera species is presented in Table 1 and Fig. 1. On comparing the species richness in each of the river basins, the highest values were observed in the rivers Južna Morava (63 taxa) and Zapadna Morava (58 taxa), the rivers belonging to the Aegean Sea basin (the Pčinja, Lepenac and Dragovištica) (46 taxa), the Timok River (44) and the Drina River (39 taxa). In contrast, the Danube River (15 taxa), and the rivers Velika Morava (14 taxa) and Kolubara (10 taxa) were characterised by a significant decrease in the number of the recorded species. In the Sava and Tisa rivers, no Plecoptera species were found.

Out of the total number of species, the following 21 species were found at a single site: *Siphonoperla transsylvanica* (Kis, 1963); *Leuctra autumnalis* Aubert, 1948; *Leuctra moselyi* Morton, 1929; *Nemoura avicularis* Morton, 1894; *Nemoura fusca* Kis 1963; *Nemoura longicauda* Kis, 1964; *Nemoura minima* Aubert, 1946; *Nemoura monticola* Рауљер, 1965; *Nemoura uncinata* Despax, 1934; *Arcynopteryx dichroa* (McLachlan, 1872); *Isoperla graeca* Aubert, 1956; *Isoperla bosnica* Aubert, 1964; *Isoperla oxylepis* (Despax, 1936); *Isoperla pawlowski* Wojtas, 1961; *Isoperla sudetica* (Kolenati, 1859); *Brachyptera braueri* (Klapálek, 1900); *Brachyptera helenica* Aubert, 1956; *Brachyptera graeca* Berthélemy, 1971; *Brachyptera trifasciata* (Pictet, 1832); *Taeniopteryx hubaulti* Aubert, 1946; and *Taeniopteryx schoenemundi* Mertens, 1923.

Based on the IUCN criteria (Table 2), 10 species were assessed and classified into one of the three categories: Critically Endangered: *Marthamea vitripennis*

Table 1. Species of the order Plecoptera in the Republic of Serbia: checklist and distribution of species recorded within the river basins during the period 1950-1995.

Stonefly Taxa	River Basins							
	Danube	Kolu- bara	Velika Morava	Južna Morava	Zapadna Morava	Timok	Drina	Aegean Basin
Capniidae								
<i>Capnia bifrons</i> (Newman, 1839)	-	+	+	+	+	+	+	+
<i>Capnia vidua</i> Klapálek, 1904	-	-	-	+	+	+	-	+
Chloroperlidae								
<i>Siphonoperla neglecta</i> (Rostock, 1888)	+	-	-	+	+	+	+	+
<i>Siphonoperla torrentium</i> (Pictet, 1841)	+	-	-	-	+	+	-	+
<i>Siphonoperla transsylvanica</i> (Kis, 1963)*	-	-	-	-	+	-	-	-
<i>Chloroperla tripunctata</i> (Scopoli, 1763)	-	-	-	+	+	-	-	-
Leuctridae								
<i>Leuctra albida</i> Kempny, 1899*	-	-	-	-	-	-	+	-
<i>Leuctra aurita</i> Navás, 1919	-	-	-	+	-	-	-	+
<i>Leuctra autumnalis</i> Aubert, 1948*	-	-	-	+	-	-	-	-
<i>Leuctra braueri</i> Kempny, 1898	+	-	-	+	+	-	+	+
<i>Leuctra bronislawi</i> Sowa, 1970	+	-	-	+	+	+	+	+
<i>Leuctra cingulata</i> Kempny, 1899*	-	-	+	+	-	+	+	-
<i>Leuctra digitata</i> Kempny, 1899	+	-	+	+	+	+	+	-
<i>Leuctra fusca</i> (Linnaeus, 1758)	-	+	+	+	+	+	+	+
<i>Leuctra hippopoides</i> Kačanski & Zwick, 1970	-	-	-	-	+	-	+	+
<i>Leuctra hippopus</i> Kempny, 1899	+	+	+	+	+	+	+	+
<i>Leuctra hirsuta</i> Bogescu & T abacaru, 1960	-	-	+	+	+	+	+	+
<i>Leuctra inermis</i> Kempny, 1899	-	-	-	+	+	+	+	+
<i>Leuctra major</i> Brinck, 1949*	-	-	-	+	+	+	-	+
<i>Leuctra moselyi</i> Morton, 1929	-	-	-	-	-	-	+	-
<i>Leuctra nigra</i> (Olivier, 1811)	+	-	-	+	+	+	+	+
<i>Leuctra prima</i> Kempny, 1899	-	-	-	+	+	-	-	+
<i>Leuctra pseudohippopus</i> Raušer, 1965	-	-	-	-	+	-	-	+
<i>Leuctra pseudosignifera</i> Aubert, 1954	+	-	-	+	+	+	+	+
<i>Leuctra quadrimaculata</i> Kis, 1963	-	-	-	-	+	-	+	+
Nemouridae								
<i>Amphinemura standfussi</i> (Ris, 1902)	-	+	-	+	+	-	-	-
<i>Amphinemura sulcicollis</i> (Stephens, 1836)	-	+	+	+	-	+	+	+
<i>Amphinemura triangularis</i> (Ris, 1902)	-	-	+	+	+	+	+	+
<i>Nemoura avicularis</i> Morton, 1894*	-	-	-	-	-	+	-	-
<i>Nemoura cambrica</i> Stephens, 1836	+	-	-	+	+	+	-	-

Table 1. Continued

Stonefly Taxa	River Basins							
	Danube	Kolu- bara	Velika Morava	Južna Morava	Zapadna Morava	Timok	Drina	Aegean Basin
<i>Nemoura carpathica</i> Illies, 1963*	-	-	-	+	-	+	-	-
<i>Nemoura cinerea</i> (Retzius, 1783)	+	-	+	+	+	+	+	+
<i>Nemoura flexuosa</i> Aubert, 1949	-	-	-	+	-	+	-	-
<i>Nemoura fusca</i> Kis, 1963*	-	-	-	-	-	+	-	-
<i>Nemoura longicauda</i> Kis, 1964*	-	-	-	+	-	-	-	-
<i>Nemoura marginata</i> Pictet, 1835	-	-	-	+	+	-	+	+
<i>Nemoura minima</i> Aubert, 1946*	-	-	-	-	-	-	+	-
<i>Nemoura monticola</i> Raušer, 1965*	-	-	-	+	-	-	-	-
<i>Nemoura uncinata</i> Despax, 1934*	-	-	-	-	-	+	-	-
<i>Nemoura subtilis</i> Klapálek, 1896*	-	-	-	+	-	-	-	+
<i>Nemoura zwicki</i> Sivec, 1980*	-	-	-	+	-	+	-	-
<i>Nemurella pictetii</i> (Klapálek, 1900)	-	-	-	+	+	+	+	+
<i>Protonemura aestiva</i> Kis, 1965*	-	-	-	+	-	+	-	-
<i>Protonemura auberti</i> Illies, 1954	-	-	-	-	+	+	+	-
<i>Protonemura autumnalis</i> Raušer, 1956	-	-	+	+	-	-	+	+
<i>Protonemura beaumonti</i> (Aubert, 1956)*	-	-	-	+	-	+	-	-
<i>Protonemura brevistyla</i> (Ris, 1902)	-	-	-	+	-	-	-	+
<i>Protonemura hrabei</i> Raušer, 1956	-	-	+	+	+	+	+	+
<i>Protonemura illiesi</i> Kis, 1963*	-	-	-	-	+	+	-	-
<i>Protonemura intricata</i> (Ris, 1902)	+	+	-	+	+	+	+	+
<i>Protonemura lateralis</i> (Pictet, 1836)	-	-	-	+	+	-	-	-
<i>Protonemura meyeri</i> (Pictet, 1841)	-	+	+	+	-	-	+	-
<i>Protonemura montana</i> Kimmins, 1941	-	-	-	+	+	-	+	+
<i>Protonemura nitida</i> (Pictet, 1836)	-	-	-	+	+	-	+	+
<i>Protonemura praecox</i> (Morton, 1894)	-	-	-	-	+	+	-	+
Perlidae								
<i>Dinocras megacephala</i> (Klapálek, 1907)	-	-	-	-	+	-	+	+
<i>Marthamea vitripennis</i> (Burmeister, 1839)	-	-	-	+	+	-	+	-
<i>Perla abdominalis</i> Guérin- Méneville, 1838	-	+	-	+	+	+	+	+
<i>Perla illiesi</i> Braasch & Joost, 1971	-	-	-	-	+	-	+	-
<i>Perla marginata</i> (Panzer, 1799)	-	-	-	+	+	-	+	+
<i>Perla grandis</i> Rambur, 1842	-	-	+	+	+	-	-	-
<i>Perla pallida</i> Guérin- Méneville, 1838	+	-	-	+	+	-	+	+
Perlodidae								
<i>Arcynopteryx dichroa</i> (McLachlan, 1872)*	-	-	-	-	-	+	-	-
<i>Isogenus nubecula</i> Newman, 1833	-	-	-	+	+	+	-	+
<i>Isoperla belai</i> Illies, 1963*	+	-	-	-	+	+	-	+

Table 1. Continued

Stonefly Taxa	River Basins							
	Danube	Kolu- bara	Velika Morava	Južna Morava	Zapadna Morava	Timok	Drina	Aegean Basin
<i>Isoperla bosnica</i> Aubert, 1964*	-	-	-	-	+	-	-	-
<i>Isoperla buresi</i> Raušer, 1962	-	-	-	-	-	+	-	+
<i>Isoperla difformis</i> (Klapálek, 1909)	-	-	-	+	-	-	-	+
<i>Isoperla goertzi</i> Illies, 1952	-	-	-	+	+	-	-	-
<i>Isoperla gramatica</i> (Poda, 1761)	-	+	+	+	+	+	-	+
<i>Isoperla graeca</i> Aubert, 1956*	-	-	-	-	-	-	+	-
<i>Isoperla submontana</i> Raušer, 1965*	-	-	-	-	+	+	-	-
<i>Isoperla obscura</i> (Zetterstedt, 1840)	-	-	-	-	+	-	-	-
<i>Isoperla oxylepis</i> (Despax, 1936)	-	-	-	+	-	-	-	-
<i>Isoperla pawlowski</i> Wojtas, 1961*	-	-	-	-	+	-	-	-
<i>Isoperla rivulorum</i> (Pictet, 1841)	-	-	-	+	+	-	-	-
<i>Isoperla sudetica</i> (Kolenati, 1859)*	-	-	-	-	-	-	-	+
<i>Isoperla tripartita</i> Illies, 1954	+	-	-	+	+	+	+	+
<i>Perlodes dispar</i> (Rambur, 1842)	+	-	-	+	-	-	-	-
<i>Perlodes intricatus</i> (Pictet, 1841)	-	-	-	+	+	-	-	+
<i>Perlodes microcephalus</i> (Pictet, 1833)	-	-	-	+	+	+	+	+
Taeniopterygidae								
<i>Brachyptera braueri</i> (Klapálek, 1900)*	-	-	-	-	+	-	-	-
<i>Brachyptera helenica</i> Aubert, 1956*	-	-	-	+	-	-	-	-
<i>Brachyptera graeca</i> Berthélemy, 1971*	-	-	-	+	-	-	-	-
<i>Brachyptera risi</i> (Morton, 1896)	-	+	-	+	+	+	+	+
<i>Brachyptera seticornis</i> (Klapálek, 1902)	-	-	-	+	+	+	-	+
<i>Brachyptera trifasciata</i> (Pictet, 1832)*	-	-	-	+	-	-	-	-
<i>Taeniopteryx hubaulti</i> Aubert, 1946	-	-	-	-	+	-	-	-
<i>Taeniopteryx nebulosa</i> (Linnaeus, 1758)	-	-	-	+	+	+	-	-
<i>Taeniopteryx schoenemundi</i> (Mertens, 1923)*	-	-	-	+	-	-	-	-
Total number of species	15	10	14	63	58	44	39	46

nis (Burmeister, 1839), *Protonemura lateralis* (Pictet, 1836), and *Taeniopteryx hubaulti* Aubert, 1946; Endangered: *Dinocras megacephala* (Klapálek, 1907), *Nemoura cambrica* Stephens, 1836 *Protonemura meyeri* (Pictet, 1841), *Protonemura praecox* (Morton, 1894), and *Taeniopteryx nebulosa* (Linnaeus, 1758); and Vulnerable: *Amphinemura sulcicollis* (Stephens, 1836) and *Perlodes microcephalus* (Pictet, 1833). The Official Gazette, No. 5/2010, promulgated 7 species as strictly protected and 2 species as protected in Serbia (Table 2) (ANONYMOUS 2010a, b).

According to the cluster analysis, three groups of drainage basins, characterised by their distinctive stonefly fauna, can be distinguished (Fig. 2).

Discussion

In comparison with the neighbouring countries in the Balkan Peninsula, the recorded diversity of Plecoptera in Serbia can be characterised as intermediate to high. The highest number of species was listed for Bulgaria (108 taxa; HUBENOV 1998, TYUFEKCHIEVA *et al.* 2013), followed by Slovenia (100 species; SIVEC 2001), Macedonia (93 species; SMITH, SMITH 2003), and Bosnia and Herzegovina (73 taxa; KAČANSKI 1976). The lowest species diversity was recorded in Montenegro where the presence of 57 stonefly species was reported (MURANYI 2008), while in Croatia the recorded taxa were 50 (POPIJAČ,

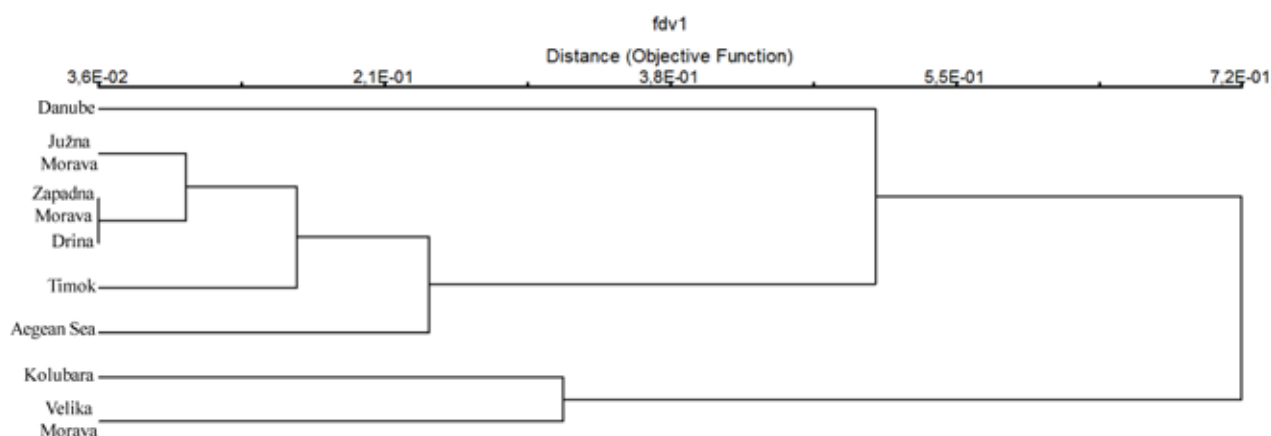


Fig. 2. Cluster analysis of similarity between drainage basins based on composition of stonefly fauna

Table 2. Preliminary Red List of Plecoptera in Serbia and the level of official protection in this country (ANONYMOUS 2010a,b). Abbreviations: CR, Critically Endangered; EN, Endangered; VU, Vulnerable.

Species	Preliminary Red List Serbia	Protected in Serbia
<i>Marthamea vitripennis</i>	CR	Strictly protected
<i>Protonemura lateralis</i>	CR	-
<i>Taeniopteryx hubaulti</i>	CR	Strictly protected
<i>Dinocras megacephala</i>	EN	Strictly protected
<i>Nemoura cambrica</i>	EN	Strictly protected
<i>Protonemura meyeri</i>	EN	Strictly protected
<i>Protonemura praecox</i>	EN	Strictly protected
<i>Taeniopteryx nebulosa</i>	EN	Strictly protected
<i>Amphinemura sulcicollis</i>	VU	Protected
<i>Perlodes microcephalus</i>	VU	Protected

SIVEC 2008, 2009a, b, c, 2010, 2011).

According to MURÁNYI (2008, 2011) and GRAF *et al.* (2009), some taxa (*Siphonoperla torrentium*, *S. transsylvanica*, *Leuctra fusca*, *Nemour cinerea*, *Protonemura intricata*, *Protonemura praecox*, *Isoperla tripartita*, *I. oxylepis*, and *I. graeca*) are considered as subspecies. Since this is not consistent with our results, it is necessary to conduct further studies which will determine the taxonomic status of these taxa using molecular analyses.

According to the long-term research, the diversity hot-spots of Plecoptera in Serbia are situated in submontane regions, which include the Južna and Zapadna Morava River basins and the rivers belonging to the Aegean Sea basin (the Pčinja, Lepenac and Dragovištica) (MARKOVIĆ 1980, SIMIĆ, SIMIĆ 2003, PAUNOVIĆ *et al.* 2006). In our study, the Drina River basin supported an unexpectedly low number of spe-

cies (39), which was presumably caused by a smaller density of the studied sites.

In line with the expectations, the lowest diversity was recorded in potamal rivers, such as the Kolubara, Velika Morava and the Danube River. The contrast in ecological and hydrological features between the lowland lotic systems, on the one hand and submontane rivers on the other hand, as well as the water quality degradation and physical alteration of streams and rivers have been the reasons for decreasing in the number of the Plecoptera species, particularly those inhabiting lowland rivers of industrialised countries.

According to the cluster analysis based on the Plecoptera community data, the most similar river basins were the Južna and Zapadna Morava with 40 species in common. These two basins belong to ecoregion 5 (Južna and Zapadna Morava) and ecoregion 7 (Južna Morava) and are characterised by a wide variety of habitats and very high species diversity of other aquatic macroinvertebrate groups (FILIPOVIĆ 1979, MARINKOVIĆ-GOSPODNETIĆ 1980, RADOVIĆ *et al.* 1995, MILOŠEVIĆ *et al.* 2013). The results of our study were consistent with the previous studies and showed that the highest Plecoptera diversity was recorded in the Južna and Zapadna Morava river basins.

Also, similarity was ascertained between the rivers Drina and Zapadna Morava, with 31 species in common. Such similarity could be expected since both of those areas belong to the same ecoregion 5. The rivers of the Aegean Sea (the Pčinja, Dragovištica and Lepenac), which belong to ecoregions 6 and 7, were distinguished as a separate group.

The Timok River basin as part of ecoregion 7 was also considered as a separate group. All streams flowing to this basin are from the Stara Planina Mountains, which is one of the diversity hot-spots on the Balkan Peninsula (RADOVIĆ *et al.* 1995). Previous studies also confirm this statement by

presenting a high diversity of aquatic macroinvertebrates with a qualitatively specific community in comparison to other basins (SIMIĆ 1993, 1995, ŽIVIĆ *et al.* 2005).

Finally, the Danube, Velika Morava and Kolubara rivers (from ecoregions 5, 7, 10 and 11) were represented in different groups by particular Plecoptera fauna, but with low species richness. This group is composed of lowland rivers, which is the cause of such a low Plecoptera diversity.

Since Plecoptera prefer cold, well-oxygenated waters, their distribution has become restricted, and due to the recent increases in the anthropogenic alteration of aquatic habitats, now they are a threatened macroinvertebrate group in Europe (RAVIZZA, NICOLAI 1983, ZWICK 1992, SÁNCHEZ-ORTEGA, TIERNO DE FIGUEROA 1996). TIERNO DE FIGUEROA *et al.* (2010) pointed out that at least 324 Plecoptera taxa in Europe can be characterised as threatened. Out of these, four species: *Leuctra moselyi* Morton, 1929; *Protonemura hrabei* Раульер, 1956, *Nemoura erratica* Claassen, 1936, and *Rhabdiopteryx navicula* Theischinger, 1974, have wide distribution, being present in three or more ecoregions. Despite such a distribution pattern, these species as cold stenoterm and rheobiont are considered as rare and threatened species (TIERNO DE FIGUEROA *et al.* 2010). In our study, two of these species were on our list, with *Leuctra moselyi* being recorded in one ecoregion, and *Protonemura hrabei* recorded in three ecoregions.

After being considered as extinct in Western and Central Europe (ZWICK 1992), the species *Isogenus nubecula* (Newman, 1839) was again recorded in studies conducted within isolated and endangered populations in Hungary, Australia, and Slovakia (JUHÁSZ *et al.* 1998, KOVÁCS, AMBRUS 2001), and recently found in Slovenia as well (SIVEC, pers. comm.). This species is very seriously endangered or already extinct (Regionally Extinct, RE) in Croatia (POPIJAČ, SIVEC 2009c) and in Italy (FOCHETTI 1994). Vital populations have been discovered recently in the Rába River in Hungary and the Lafnitz River in Austria (KOVÁCS *et al.* 2004).

Data from our study extend the distribution area of *I. nubecula* to the south, where the presence of this species was recorded in four basins (the Južna and Zapadna Morava, the Timok River, and the rivers belonging to the Aegean Sea basin) and three ecoregions (5, 6 and 7) (SIMIĆ *et al.* 2006).

According to the Red List of Stoneflies (Plecoptera), the species of *Marthamea vitripennis* (Burmeister, 1839) is classified as Regionally Extinct (RE) in Croatia (POPIJAČ 2008), as well as in most of Europe (ZWICK 1984, 2004). The species was recorded in the Rába River in Hungary (KOVÁCS, AMBRUS

2000), and found again after one century in the Una River in Croatia (POPIJAČ, SIVEC 2011).

Three isolated subpopulations of the species *Marthamea vitripennis* were confirmed in Serbia (SIMIĆ *et al.* 2006). This species was categorised as Critically Endangered, since it has a very small distribution with a restricted area of occupancy (6.7 km²). The similar pattern of distribution was recorded for the threatened species *P. lateralis* and *T. hubaulti*. The species *D. megacephala*, *N. cambrica*, *P. meyeri* and *P. praecox* were categorised as Endangered with their areas of occupancy less than 150 km². Finally, the Vulnerable species *A. sulcicolis* and *P. microcephalus* cover an area of occupancy of 160 km².

After the last ice age some representatives of the Plecoptera species were reduced to small isolated populations, which is the case with *Perlodes microcephalus* (Pictet, 1833) (FOCHETTI, TIERNO DE FIGUEROA 2006). This species was distributed in the Južna and Zapadna Morava river basins, the Timok and Drina rivers, as well as the rivers belonging to the Aegean Sea basin. However, on repeating the survey, *Perlodes microcephalus* was not recorded in the Zapadna Morava river basin, where the same habitats were checked. According to the regional IUCN criteria and state policy, the species is a threatened vulnerable species (VU), included in the list of protected plant and animal species in the Republic of Serbia (SIMIĆ *et al.* 2006, ANONYMOUS 2010b).

The whole group of Plecoptera is exposed to a high risk of extinction. FOCHETTI (1994) claimed that out of 144 Plecoptera species, 22 have already become extinct in their aquatic habitats in Italy (*e.g.* the species *Brachyptera trifasciata* (Pictet, 1832), *Isoperla obscura* (Zetterstedt, 1840), and *Brachyptera braueri* (Klapálek, 1900)). Our data revealed a similar situation, where, after reevaluation, 29 species from previous taxonomic lists were not detected (see Table 1; SIMIĆ 1995, SIMIĆ *et al.* 2006).

In conclusion, the observed species diversity of Plecoptera in Serbia can be considered as high but with a decreasing trend. The studies conducted on Plecoptera fauna in Serbia in the period from 1950 to 1995 recorded the presence of 29 species that were not confirmed in later research.

In addition, 10 species of stonefly fauna are regionally threatened according to IUCN criteria. Three species (*Marthamea vitripennis*, *Protonemura lateralis* and *Taeniopteryx hubaulti*) are classified into the category Critically Endangered (CR); the species *Dinocras megacephala*, *Nemoura cambrica*, *Protonemura meyeri*, *Protonemura praecox*, and *Taeniopteryx nebulosa* should be regarded as Endangered (EN); and *Amphinemura sulcicolis* and *Perlodes microcephalus* are vulnerable (VU). In

Bulgaria the species *Taeniopteryx nebulosa* is classified as Critically Endangered, while *Taeniopteryx hubaulti* as Endangered (TYUFEKCHIEVA *et al.* 2013).

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Received: 14.04.2014

Accepted: 17.10.2014