

Distributional patterns of aquatic Oligochaeta communities (Annelida: Clitellata) in running waters in Serbia

Ana D. Atanacković*, Katarina S. Zorić, Jelena M. Tomović, Božica M. Vasiljević and Momir M. Paunović

Department for Hydroecology and Water protection, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia

*Corresponding author: adjordjevic@ibiss.bg.ac.rs

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Abstract: The study of aquatic Oligochaeta in freshwaters in Serbia contributes to the oligochaete fauna inventory of the Balkans. Based on our results and literature review, 97 species (45 genera from 8 families) are listed in the running waters of Serbia. From the list of 61 species recorded during our investigation, 12 were noted for the first time. The ecological analysis of Oligochaeta communities showed the largest participation of potamal and rhithral species. As regards current preferences, rheolimnophilous species predominated, and with respect to feeding type, collectors prevailed. Most of the species are tolerant to moderate and high organic load. According to microhabitat preferences, three types of rivers stood out, as follows: (1) pelophilous (dominance of *L. hoffmeisteri*); (2) psammophilous (dominance of *S. heringianus*); (3) phytophilous (dominance of *N. bretscheri*). Analyses of the relation to different types of waterbodies showed regularity in the distribution of oligochaetes, with a higher species richness in main watercourses (types 1 and 2), and a lower species richness in tributaries (types 3, 4 and 6). According to the dominant taxa, the running waters of Serbia can be classified into four groups which are characterized by the prevalence of naidids (naidins and tubificins), enchytraeids and lumbriculids. Our results show that community composition is a good indicator of the horizontal distribution of Oligochaeta.

Keywords: Oligochaeta; aquatic worms; distribution; typology; freshwaters

INTRODUCTION

The high diversity of aquatic oligochaetes is a reflection of the generally high biological diversity in Serbia [1] that is attributed to heterogeneous climatic and edaphic factors and paleogeographic historical circumstances. This has affected the wildlife of the Balkan Peninsula and Serbia, which covers a significant part of Europe's ecoregions: the Dinaric western Balkan, Eastern Balkan and Hungarian lowlands/Pannonian Plain [2]).

Previous investigations of Oligochaeta in Serbia have been discussed [3,4], as well as their communities in different types of freshwaters (reservoirs, ponds, canals and rivers) in the Pannonian Plain ecoregion [5]. Examination of oligochaetes from two large lowland rivers that flow through Serbia, the Danube and Sava, has been conducted [6-15]. However, hilly and mountainous rivers south of the Danube corridor (Dinaric western Balkan and Eastern Balkan ecoregions) have only been investigated sporadically [16-26]. During previous

research of the rivers and canals of the territory north of the Danube River (Pannonian Plain ecoregion), 65 species were recorded, with 13 of them recorded only in that ecoregion [5]. Previous investigations of hilly and mountainous regions (the Dinaric western and Eastern Balkan ecoregions) listed 72 taxa with 27 taxa found only in these ecoregions [9-13,16-19,26,27].

The aim of our work was to provide an updated checklist of Oligochaeta communities from different types of running waters in Serbia, as well as observations on the ecological status of river courses.

MATERIALS AND METHODS

Study area

In total, 115 rivers in Serbia were investigated in the period 2004-2012 (383 samples from 186 locations/sites; Supplementary Fig. S1A, Supplementary Table S1). All

ivers were divided into ten basin areas (Supplementary Fig. S1B) as follows: Danube (D), Sava (S), Kolubara (K), Velika Morava (VM), Ibar (I), Lim (L), Zapadna Morava (ZM), Južna Morava (JM), Timok (T), Drina (Dr), and one more type of waterbody – artificial canals in the Belgrade region (Ch).

Sample collection

Samples were collected using a combination of the kick and sweep and multihabitat sampling technique according to European Standards (EN) [28] using a FBA hand net (mesh sizes 950, 500 and 250 μm), with a Surber net (mesh size 250 μm), benthological dredge and Ekman and Van Veen grab samplers. Additionally, during the Joint Danube Survey 2, samples were collected by air-lift sampling and the multicorer technique [29]. The samples were pooled, and the material was preserved in formaldehyde (4%) or ethyl alcohol (70%).

Material processing

For species determination, appropriate identification keys were used [30-32]. Taxa from families Enchytraeidae and Lumbricidae were not all determined to the species level. The frequency of occurrence (F) for each species in oligochaete assemblages was calculated using the formula: $F = 100 \times n/N$, where n is the number of samples in which a taxon was found, and N is the total number of samples. The oligochaetes were classified into 5 distribution classes using a modified scale by Nijboer et al. [33] as follows: very common ($F > 12$), moderately common ($F = 4-12$), common ($F = 1.5-4$), moderately rare ($F = 0.5-1.5$) and rare ($F = 0-0.5$).

The work discusses the main ecological features of the recorded community (the characterization of the species with regard to saprobic preference, feeding, current and substrate type and horizontal distribution – general river zonation), with respect to autecological data taken from AQEM [34] and Hörner et al. [35]; ASTERICS software 3.1.1. [34] was used to calculate the relation of functional groups within the community. Further, Oligochaeta fauna was analyzed using the following classification of waterbodies [36] which was based on abiotic typology descriptors (altitude, geological substrates, catchment area and substrate type): type 1 – large lowland rivers, with a dominance

of fine substrate; type 2 – large rivers, except the rivers of the Pannonian Plain, with a dominance of small- to medium-sized substrates; type 3 – minor and middle water courses, altitudes from 200 to 500 m, with a dominance of a hard substrate; type 4 – minor and middle water courses, to altitudes above 500 m, with a dominance of a hard substrate; type 5 – watercourses from the Pannonian Plain area, except the rivers included in type 1; type 6 – minor water courses outside the Pannonian Plain area not included in types 3 and 4; artificial waterbodies (AWB); lakes; reservoirs. Watercourses from the Pannonian Plain area (Type 5) were not investigated during our research.

Data analysis

Similarities in oligochaete communities within the investigated river basins were determined using Pearson's correlation coefficient, the complete linkage method, whereby the disagreement percentage was used as a measure of the distance based on which the clusters formed. We used the complete linkage method, also referred to as the furthest neighbor or maximum method. Input data were 11 variables/own cluster (river basins). The clusters were then sequentially combined into larger clusters until all elements ended up in the same cluster. The order of merging objects into groups (clusters) was established based on of the most distant elements. Analysis was performed using the software package StatSoft Inc. 2001. Statistica for Windows, version 6.0. (<http://www.statsoft.com>). To test species variability and applicability to oligochaetes for distinguishing between the main types of Serbian waterbodies (according to the national legislative [36]), discriminant analysis (DA) [37,38] was applied. An input matrix consisting of 66 oligochaete taxa from 234 samples. Analysis was performed by the FLORA software [39].

RESULTS

Community structure and distribution

During our investigation, 61 taxa from seven families were recorded of which 56 taxa were identified to the species level and 12 taxa were recorded for the first time in the running waters of Serbia (Table 1). In terms of species richness, the most dominant was the family

Table 1. Species list from Serbian watercourses with distributions and frequencies of species recorded during our investigations from 2004 to 2012.

Species	F _Σ	Catchment area	Type of waterbody
Ordo TUBIFICIDA			
Fam. NAIDIDAE			
Subfamily Naidinae			
<i>Amphichaeta leydigi</i> Tauber, 1879			
<i>A. rostrifera</i> Akinschina, 1984			
<i>Aulophorus furcatus</i> (Oken, 1815)	0.85	D, S	1, 3
<i>Chaetogaster crystallinus</i> Vejdovsky, 1883			
<i>Chaetogaster diaphanus</i> (Gruithuisen, 1828)	0.42	S	1
<i>Chaetogaster diastrophus</i> (Gruithuisen, 1828)			
<i>Ch. langi</i> Bretscher, 1896	1.27	JM, T	2, 3
<i>Ch. limnaei</i> (von Baer 1827)	0.42	K	3
<i>Dero digitata</i> Müller, 1773			
<i>Dero dorsalis</i> Ferronière, 1899	0.42	Ch	AWB
<i>Dero nivea</i> Aiyer, 1929			
<i>D. obtusa</i> d'Udekem, 1835	1.27	D	1
<i>Nais alpina</i> Sperber, 1948			
<i>Nais barbata</i> (Müller, 1773)	2.12	D, S, JM, I	1, 2, 3, 4, 6
<i>N. behningi</i> Michaelsen, 1923	3.39	VM, ZM, L, I, Dr	1, 2, 3, 4, 6
<i>N. bretscheri</i> Michaelsen, 1899	30.51	D, S, K, VM, ZM, JM, T, L, I, Ch	in all
<i>N. communis</i> Piguet, 1906	1.27	D, VM, ZM,	1, 2, 3
<i>N. christinae</i> Kasprzak, 1973			
<i>N. elinguis</i> Müller 1773	27.12	D, S, K, VM, ZM, JM, T, L, I	1, 2, 3, 4, 6
<i>N. pardalis</i> Piguet, 1906	0.85	D	1
<i>N. pseudobtusa</i> Piguet, 1906	1.27	K, ZM	3, 6
<i>N. simplex</i> Piguet 1906	0.42	ZM	6
<i>N. stolci</i> Hrabě, 1981			
<i>N. variabilis</i> Piguet, 1906	1.27	K, ZM, I	3, 6
<i>Ophidonais serpentina</i> (Müller, 1773)	7.20	D, K, VM, ZM, JM, Dr	1, 2, 3, 6
<i>Piguetiella blanci</i> Piguet, 1906			
<i>Paranais frici</i> (Hrabě, 1941)	3.39	D, S, K, I	1, 2, 3
<i>Specaria josinae</i> (Vejdovsky, 1883)			
<i>Stylaria fossularis</i> Leidy, 1852			
<i>Stylaria lacustris</i> (Linnaeus, 1767)	8.90	D, S, VM, JM, T, I, Ch	1, 2, 3, AWB
<i>Uncinai uncinata</i> (Ørsted, 1842)	0.42	S	1
<i>Vejdovskyella comata</i> (Vejdovský, 1883)	1.27	D, S, ZM, Ch	1, 3, AWB
<i>V. intermedia</i> Bretscher, 1896 *	0.42	D	1
Subfamily Pristininae			
<i>Pristina aequiseta</i> Bourne 1891			
<i>P. bilobata</i> (Bretscher, 1903)			
<i>P. breviseta</i> Bourne, 1891			
<i>P. foreli</i> (Piguet, 1906)			
<i>P. longiseta</i> Ehrenberg, 1828			
<i>P. menoni</i> (Aiyer, 1929)			
<i>P. rosea</i> (Piguet, 1906)	2.96	ZM, JM, T, L	2, 3, 4
Subfamily Rhyacodrilinae			
<i>Bothrioneurum vejdoskyanum</i> Štolc, 1888 *	1.69	D, I	1, 2
<i>Branchiura sowerbyi</i> Beddard, 1892	16.95	D, S, K, VM, ZM, JM	1, 2, 3
Subfamily Tubificinae			

Table 1. continued

<i>Aulodrilus limnobius</i> (Bretscher, 1899)	1.27	D	1
<i>A. pigueti</i> Kowalewski, 1914			
<i>A. pluriseta</i> (Piguet, 1906)			
<i>Embolocephalus velutinus</i> (Grube, 1879)	5.08	D, K, JM, T, I, L	1, 2, 3, 4
<i>Ilyodrilus templetoni</i> (Southern, 1909)			
<i>Isochaetides suspectus</i> Sokolskaya, 1964			
<i>Isochaetides michaelsoni</i> (Lastočkin, 1937)	6.36	D	1
<i>Limnodrilus claparedianus</i> Ratzel 1868	27.12	D, S, K, VM, ZM, JM, L, I, Ch	in all
<i>L. hoffmeisteri</i> Claparède, 1862	51.27	D, S, K, VM, ZM, JM, T, L, I, Dr, Ch	in all
<i>L. profundicola</i> (Verrill, 1871)	6.36	D, S, K, ZM, L	1, 3, 4, 6
<i>L. udekemianus</i> Claparède, 1862	16.53	D, S, K, VM, ZM, I, Dr, Ch	1, 2, 3, 6, AWB
<i>Peipsidrilus pusillus</i> Timm, 1977			
<i>Potamothis bavaricus</i> (Oschmann, 1913)	1.69	D	1
<i>P. danubialis</i> (Hrabě, 1941) *	2.54	D, K	1, 2
<i>P. hammoniensis</i> (Michaelson, 1901)	24.15	D, S, K, VM, ZM, JM, T, L, I, Dr, Ch	in all
<i>P. heuscheri</i> (Bretscher, 1900) *	0.42	D	1
<i>P. isochaetus</i> (Hrabě, 1941)	2.12	D, ZM	1, 6
<i>P. moldaviensis</i> Vejdovský & Mrázek, 1902 *	3.39	D	1
<i>P. vejdovskyi</i> (Hrabě, 1941)	2.97	D, I	1, 2
<i>Psammoryctides albicola</i> (Michaelson, 1901)	10.59	D, S, VM, ZM, L, I, Dr	1, 2, 3
<i>Ps. barbatus</i> (Grube, 1861)	7.20	D, S, ZM, Dr	1, 2, 3
<i>Ps. deserticola</i> (Grimm, 1877)			
<i>Ps. moravicus</i> (Hrabě, 1934)	2.54	D	1
<i>Spirosperma ferox</i> Eisen, 1879	0.42	L	4
<i>Tubifex ignothus</i> (Stolc, 1886)			
<i>T. montanus</i> Kowalewski, 1919			
<i>T. nerthus</i> Michaelson, 1908			
<i>T. newaensis</i> (Michaelson, 1903)			
<i>T. tubifex</i> Müller 1774	21.61	D, S, K, VM, ZM, JM, L, I, Dr, Ch	in all
<i>Tubificoides benedii</i> (d'Udekem, 1855)			
Subfamily Phallodrilinae			
<i>Thalassodrilus prostatus</i> (Knöllner, 1935)			
Ordo ENCHYTRAEIDA			
Fam. PROPAPPIDAE			
<i>Proppapus volki</i> Michaelson, 1905	5.51	VM, ZM, JM, L, I	2, 3, 4, 6
Fam. ENCHYTRAEIDAE			
<i>Achaeta</i> sp. *	0.42	T	2
<i>Cognettia sphagnetorum</i> (Vejdovský 1877) *	0.42	I	2
<i>Enchytraeus albidus</i> Henle, 1837	1.27	D, ZM, Dr	2, 3
<i>E. buchholzii</i> Vejdovský, 1879 *	2.12	D, K, ZM, JM, L	2, 3, 4
<i>E. christenseni</i> Dozsa-Farkas, 1992 *	0.85	S	1
<i>Fridericia</i> sp.	0.42	L	6
<i>Fridericia perrieri</i> (Vejdovský, 1878)			
<i>Henlea ventriculosa</i> (Udekem, 1854) *	9.75	D, S, K, VM, T, L, I	1, 2, 3, 4, 6
<i>Marionina argentea</i> (Michaelson, 1889) *	2.12	JM, L, I	2, 3, 4
<i>Mesenchytraeus</i> sp.			
Ordo HAPLOTAXIDA			
Fam. ACANTHODRILIDAE			
<i>Microscolex</i> sp.			
Fam. HAPLOTAXIDAE			

Table 1. continued

<i>Haplotaxis gordioides</i> Hartmann, 1821	1.69	K, T, I	2, 3
Ordo LUMBRICULIDA			
Fam. LUMBRICULIDAE			
<i>Lamprodrilus</i> sp.			
<i>Lumbriculus variegatus</i> (Müller 1774)	2.12	K, ZM, L	3, 6
<i>Rhynchelmis limosella</i> Hoffmeister, 1843	0.42	VM	2
<i>Stylodrilus lemami</i> (Grube, 1879)			
<i>Stylodrilus heringianus</i> Claparède, 1862	33.05	D, S, K, VM, ZM, JM, T, L, I	1, 2, 3, 4, 6
<i>Stylodrilus parvus</i> (Hrabe & Cernosvitov, 1927)			
<i>Tatriella slovenica</i> Hrabe, 1936			
<i>Trichodrilus</i> sp.			
<i>Trichodrilus strandi</i> Hrabe, 1936			
Ordo CRASSICLITELLATA			
Subordo LUMBRICINA			
Fam. LUMBRICIDAE			
<i>Eiseniella tetraedra</i> (Savigny, 1826)	16.53	D, S, K, VM, ZM, L, I, Dr, Ch	in all
Fam. CRIODRILIDAE			
<i>Criodrilus lacuum</i> Hoffmeister, 1845	4.24	D, ZM	1, 4

Literature data – species in **blue** were recorded north of the Danube; species in **red** were recorded south of the Danube; * taxa noted for the first time in the running waters of Serbia.

Naididae (Tubificinae and Naidinae subfamilies), which was recorded in all investigated river basins. Other families (Enchytraeidae, Lumbriculidae and Lumbricidae) were recorded but with a significantly lower number of species, while the families Propappidae, Haplotaxidae and Criodrilidae were typically represented by one species each.

Generally, the most frequent species in Oligochaeta assemblages was *Limnodrilus hoffmeisteri* ($F=51.27$) followed by *Stylodrilus heringianus* ($F=33.05$). On the other hand, 12 taxa were recorded at only one location at the lowest frequencies ($F=0-0.5$): *Chaetogaster diaphanus*, *Ch. limnaei*, *Dero dorsalis*, *Nais simplex*, *Uncinaiis uncinata*, *Vejdovskyella intermedia*, *Potamothrrix heuscheri*, *Spirosperma ferox*, *Achaeta* sp., *Cognettia sphagnetorum* complex, *Fridericia* sp. and *Rhynchelmis limosella*).

In the Sava River, a naidid species, *Paranais frici*, was recorded with a high frequency of occurrence (Table 1), while *Chaetogaster langi* was found only in the Južna Morava and Timok basins. *Embolocephalus*

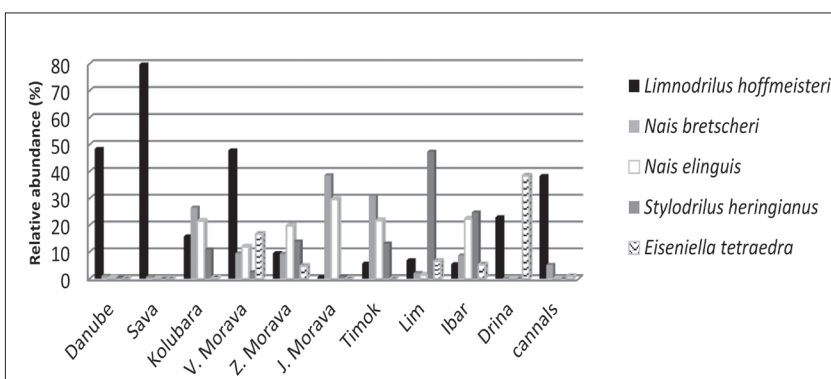


Fig. 1. The relative abundance (%) of dominant taxa in river basins.

velutinus, *Haplotaxis gordioides* and *Pristina rosea* were characteristic inhabitants of the Timok catchment area. The species *Vejdovskyella comata* and *Dero dorsalis* were found in canals in the Belgrade area.

The highest species richness was observed in the Danube basin (37 species), followed by the Zapadna Morava, Ibar and Kolubara basins (27, 24 and 23 species, respectively), while the lowest was recorded in the Drina basin and in canals in the Belgrade region (10 species). The relative abundance (%) of dominant species in river basins (species with the highest percentage participation) is presented in Fig. 1.

Table 2. Species richness in river basins.

Families	Basins																			
	Danube		Sava		Kolubara		Velika Morava		Zapadna Morava		Južna Morava		Timok	Lim		Ibar		Drina		Cannals
Naidinae	11	3	8	2	4	8	5	4		10	7	6	5	3	3	3	5	-	2	4
Pristininae	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Tubificinae	20	4	9	4	7	6	7	2	1	10	6	5	3	3	7	7	5	6	2	5
Propappidae	-	-	-	-	-	-	1	-	-	1	1	1	-	1	-	-	1	-	-	-
Enchytraeidae	1	2	2	-	-	2	1	-	-	2	2	1	2	2	4	3	2	1	-	-
Haplotaxidae	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	1	-	-	-
Lumbriculidae	1	1	1	-	1	2	2	1	1	2	1	1	1	1	2	1	1	1	-	-
Lumbricidae	1	-	1	-	-	1	1	1	-	1	-	-	-	1	1	-	1	1	1	1
Criodrilidae	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
No. of species	35	10	21	6	12	20	17	8	2	28	17	14	12	11	17	14	16	9	5	10
No. of species in the basin	37		21		23		18		28		17		12	20		24		11		10

□ No. of species in main watercourses; □ No. of species in tributaries

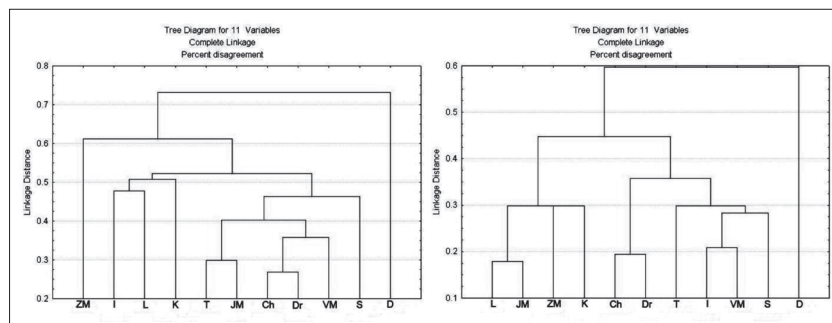


Fig. 2. Cluster analysis of the river basins based on correlation matrices A – quantitative data; B – qualitative data.

Higher species richness in main watercourses and a lower richness in tributaries were observed in almost all investigated river basins (Table 2). Discrepancies from this pattern were noted in tributaries of Zapadna Morava River (Đetinja – 9 species, Katušnica – 7 species, Dičina and Skrapež – 6 species each, Rasina and V. Rzav – 5 species each), while in the main watercourses only two species were recorded.

The performed cluster analyses of the river basins investigated, based on correlation matrices, revealed the complete separation of the Danube oligochaete community with regard to both quantitative and qualitative data (Fig. 2A, B), with linkage distances of 70% and 60%,

respectively. With regard to quantitative data, we observed separation of the Zapadna Morava basin as well, with a linkage distance of 60%. The greatest similarity in oligochaete fauna was between the Drina basin and canals in the Belgrade region (linkage distance 25.5%), Timok and Južna Morava basins (linkage distance 30%) and Velika Morava, Drina and the canals in the Belgrade region (linkage distance 35%). Regarding qualitative data, separation

of two main clusters was observed (linkage distance 45%). One group consists of basins: Lim, Južna Morava, Zapadna Morava and Kolubara, while the second included the Timok, Ibar, Velika Morava and Sava basins. The third cluster included the Drina basin and canals in the Belgrade region and were separated by a linkage distance of 20%.

Analyses of the main ecological features of Oligochaeta communities

The results of analyses of the main ecological features of oligochaete communities in the investigated river basins is presented in Table 3. According to Hörner et

Table 3. Functional traits of Oligochaeta communities.

Metric	Danube	Sava	Kolubara	V. Morava	Z. Morava	J. Morava	Timok	Lim	Ibar	Drina	Channals
Zonation											
- [%] crenal	0.339589744	0.35671	1.121409091	0.960727273	2.037342105	1.467733333	1.719167	1.475820513	1.06028	0.4375	0.8785
- [%] hypocrenal	0.465512821	0.44019	3.937204545	2.049272727	3.797526316	1.5566	2.724667	5.470897436	4.82756	4.34375	0.97116667
- [%] epirhithral	3.996987179	7.39119	6.214340909	7.871272727	5.409052632	1.931866667	3.3845	6.527512821	6.79092	10.0405	5.41866667
- [%] metarhithral	4.280423077	7.70495	10.09363636	8.831181818	7.792184211	8.806933333	6.610167	11.37664103	11.84032	10.0405	6.16
- [%] hyporhithral	6.465948718	9.45729	11.95518182	9.887636364	9.057868421	8.839133333	9.240833	12.05251282	13.8518	11.1875	10.6593333
- [%] epipotamal	13.93607692	12.1463	13.89881818	12.98418182	10.48131579	11.68486667	11.80417	14.64666667	15.5544	13.272	15.121
- [%] metapotamal	14.67475641	12.5164	7.048	10.866	6.445394737	11.20633333	7.829667	6.539461538	6.80856	6.147	14.2321667
- [%] hypopotamal	8.842012821	9.75062	3.629113636	7.677545455	4.517315789	7.4816	2.914667	3.547153846	2.50744	2.75	8.9915
- [%] littoral	15.32567949	19.4734	11.77184091	16.81236364	9.425026316	12.5916	9.2295	11.82710256	11.31196	10.59375	18.6796667
- [%] profundal	11.67339744	17.4691	8.739027272	11.75727273	5.9625	2.48	4.663833	7.796102564	6.7894	7.125	17.142
- [%] no data available	19.99994872	3.29424	21.59134091	10.30272727	35.0745	31.9534	39.8785	18.74035897	18.65756	24.0625	1.746
Current preference											
- [%] Type LB	0	0	0	0	0	0	0	0	0	0	0
- [%] Type LP	2.273025641	5.35348	0.174818182	1.851818182	3.854	1.111133333	1.411333	3.663	0.09304	0	16.508
- [%] Type LR	30.44005128	17.2685	21.60702273	20.37190909	14.30913158	46.53713333	26.8175	21.14020513	10.58992	17.720625	40.8643333
- [%] Type RL	42.79152564	71.9396	56.08790909	57.52772727	41.31723684	19.85266667	29.9385	57.48576923	61.07184	12.279375	41.326
- [%] Type RP	4.321307692	2.56843	0.354522727	0.778272727	0.239236842	0.284933333	0	0	4.30724	0.625	0.376
- [%] Type RB	0	0	0	0	0	0	0	0	0	0	0
- [%] Type IN	6.365384615	0.36948	2.604886364	9.318	12.17165789	7.142466667	1.954	5.924384615	6.7098	39.6875	0.926
- [%] no data available	13.80875641	2.50057	19.17084091	10.15236364	28.10873684	25.07166667	39.8785	11.78664103	17.2314	29.6875	0
Microhabitat preference											
- [%] Type Pel	39.7499359	49.4967	27.60706818	40.71672727	25.51942105	20.46793333	7.987833	22.10684615	16.58132	19.617625	34.7888333
- [%] Type Arg	1.223551282	0.27243	0.776136364	0.653636364	0.856026316	0.064533333	0	2.773	1.4372	1.125	0.52383333
- [%] Type Psa	25.97555128	32.2209	22.556	29.37618182	23.81647368	26.10633333	8.685333	24.98084615	21.90316	20.72425	20.47
- [%] Type Aka	6.176410256	0.66133	8.750613636	5.135363636	9.572368421	4.354866667	3.016167	13.06494872	14.40308	17.09375	0.72233333
- [%] Type Lit	5.824871795	3.48133	4.07525	7.786181818	5.964342105	9.3076	1.544167	6.663230769	5.50168	15.53125	4.19983333
- [%] Type Pny	5.956089744	5.65648	20.663	13.796	20.06465789	33.78246667	33.89517	13.51394872	17.4996	8.59375	12.8868333
- [%] Type Pom	9.413846154	5.1709	7.559227273	0.443545455	6.574710526	3.425733333	4.8515	11.17369251	6.27768	5.595625	24.8398333
- [%] Type Oth	0.231961538	0.53948	0.488568182	1.107909091	0.617236842	0.196533333	0.141167	0.336025641	1.78028	3.90625	1.56883333
- [%] No data	5.447730769	2.50057	7.524272727	0.984727273	7.014657895	2.293866667	39.8785	5.387333333	14.36596	7.8125	0
Feeding types											
- [%] Grazers and scrapers	2.923692308	1.13719	12.40643182	7.435545455	13.34002632	19.12186667	24.19983	9.681794872	13.14992	3.125	4.3415
- [%] Gatherers/Collectors	96.46711538	96.2123	84.87038636	92.27054545	85.34418421	79.98926667	44.25483	87.84589744	73.09696	96.875	95.6585
- [%] Predators	0	0.32843	0.168181818	0	0	0	0	0	0	0	0
Saprobic Valence											
- xeno [%]	0.125923077	0.08348	2.815795455	1.099090909	1.948578947	0.088866667	1.005333	4.192307692	4.32956	5.15625	0.09266667
- oligo [%]	3.827615385	2.42157	8.561454545	3.270727273	7.400921053	6.038466667	7.081167	10.4975641	13.89384	6.30325	5.30183333
- beta-meso [%]	21.96170513	18.0122	23.48588636	21.973	20.70384211	24.282	19.22883	28.53784615	30.92652	30.887875	23.5606667
- alpha-meso [%]	29.89135897	32.1472	28.45111364	31.37027273	25.73778947	31.8792	26.18817	29.74235897	26.05076	28.397	34.1788333
- poly [%]	24.22815385	44.0413	15.45802273	31.98409091	9.218605263	5.758	6.618	8.289487179	6.14164	13.0055	35.1201667
- no data available [%]	19.96529487	3.29424	21.22770455	10.30272727	34.99028947	31.9534	39.8785	18.74035897	18.65756	16.25	1.746

al. [35] and the AQEM classification [34] with regard to the preferred zone within the river continuum (horizontal distribution), the greatest portion (28.40%) of the recorded species in a whole community for Serbia are potamal species that are characteristic to the lower stretches of a river, followed by taxa belonging to those of the rhithral type (24.65%). Potamal species were predominant in the oligochaete communities of the Danube (37.45%), Sava (34.41%), Velika Morava (31.52%) and Južna Morava (30.37%) river basins, and in the canals of the Belgrade region (38.34%), as well. In the Ibar (32.48%) and Drina (31.27%) river basins, rhithral species dominated. Participation of potamal and rhithral species in the communities of the Kolubara (24.56 and 28.26%), Zapadna Morava (21.44 and 22.26%) and Timok basins (22.55 and 19.23%) were similar.

With regard to flow preference, typical limnophilous and rheophilous species were recorded in a small percentage of the total number of recorded species (3.30% and 1.26%, respectively). Oligochaete fauna in Serbian running waters is characterized by the dominance of rheolimnophilous (44.69%) and limnorheophilous taxa (24.33%), which prefer slow-flowing streams and lentic zones in larger rivers. In most river basins, rheolimnophilous species had the largest percentage participation in the community (Table 3), while limnorheophilous species were dominant only in the Južna Morava and Drina basins (46.53% and 17.72%, respectively).

The majority of identified species in Serbian oligochaete fauna (51.92%) prefers substrate types typical for large lowland rivers such as pelal and psammal. The dominance of pelophilous species was observed

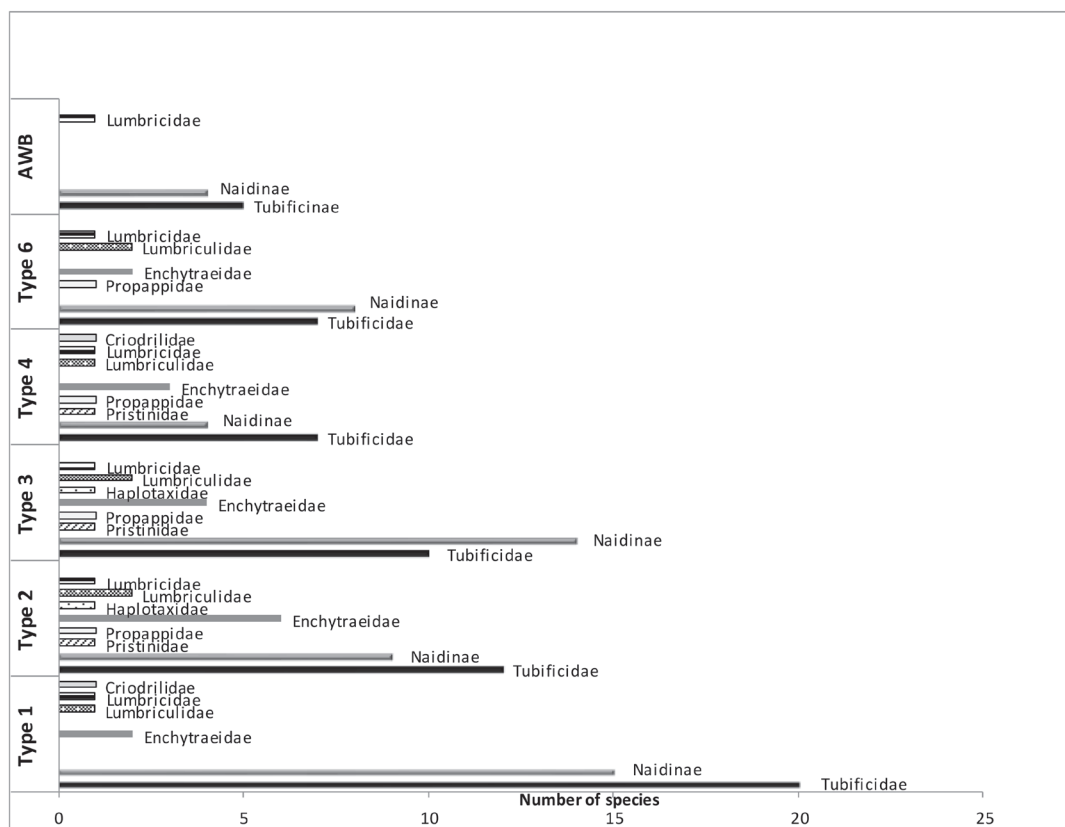


Fig. 3. Number of taxa in different types of waterbodies.

in the Danube (39.75%), Sava (49.50%) and Velika Morava (40.72%) river basins, as well as in canals of the Belgrade region (34.79%), while psammophilous species dominated in the Lim (24.98%) and Ibar (21.91%) river basins. Of the total number of recorded species, 16.96% were characterized as phytophilous (preferring macrophytes, algae and mosses), dominating in the communities of Južna Morava (33.78%) and Timok (33.90%) basins. Kolubara and Zapadna Morava had a similar percentage-participation of pelophilous, psammophilous and phytophilous species (Table 3).

Collectors, which collect particles of organic matter from the riverbed, predominated in the oligochaete community in Serbian running waters. Their participation in all investigated river basins ranged from 44.25% in the Timok river basin to 96.47% in the canals of the Belgrade region. Species characterized as scrapers contribute to a smaller degree (10.08% of all recorded species), while only 0.05% of the total numbers of species were predatory.

Taking into consideration all recorded species in the investigated rivers, the majority could be considered as tolerant to a high organic load. Thus, according to the ecological classification of the taxa with regard to saprobic conditions (saprobic valence) [35], 29.46% of the identified species in the entire oligochaete community belong to the alpha-mesosaprobic group, while 23.96% of the taxa could be characterized as beta-mesosaprobic. Species adapted to a high organic load (polysaprobic) were represented by 18.17% of the total number of taxa; however, the percentage participation of those species was high in the Sava (44.04%) and Velika Morava basins (31.98%) and in the canals of the Belgrade region (35.12%). The percentage participation of xeno- and oligosaprobic species in different waterbodies was as follows: type 1 – 3.70%, type 2 – 8.15%, type 3 – 12.35%, type 4 – 11.15%, type 6 – 13.84%.

The saprobic index (according to AQEM [34]) of all investigated river basins ranged from 3.13 (artificial

waterbodies – AWB) to 2.35 (minor water courses of type 6). Increased saprobic indices were observed in some minor water courses of type 3 (Kolubara river basin: Jablanica – 3.45, Lepenica – 3.43, 3.22; Zapadna Morava river basin: Đetinja – 2.8, Gaberska – 2.8, Skrapež – 2.83, Rasina – 2.97, Gruža – 3.45, Veliki Rzav – 2.97) and type 4 (Lim river basin: Mileševka – 2.88, Uvac – 2.85, 3.28, 3.57; Drina river basin: Crni Rzav – 3.46).

Observed patterns in relation to abiotic typology

The highest species richness was recorded in large lowland rivers with a dominance of fine sediment (type 1 waterbody) with 40 species, followed by type 2 and 3 waterbodies (33 species each), while the lowest was in artificial waterbodies (10 species) as expected (Fig. 3). Tubificins were dominant in large lowland rivers and in artificial waterbodies; in oligochaete assemblages from minor and medium water courses (up to an altitude of 500 m), naidins were dominant. However, a significant number of species (19, with a dominance of tubificins) has also been observed in watercourses at an altitude above 500 m (type 4).

Discriminant analysis (DA) based on community composition is presented in Fig. 4. The bivariate space of the first two axes covers 75.8% of the input data variability (DA axis 1 – 62.8%, DA axis 2 – 13 %). The distinction of samples from large lowland rivers (type 1) and other waterbodies was obvious. Similarities between waterbody types 1 and 2, according to abiotic typology descriptors (the same altitude and substrate type), were not as clear as expected. Statistically significant differences were not observed among other waterbody types. Oligochaete assemblages collected from water types 3, 4, 6 and artificial waterbodies are grouped together despite high heterogeneity (different typology descriptors), probably because of the unexpectedly high numbers of tubificid species.

DISCUSSION

Oligochaete fauna has been the subject of sporadic investigations in the past, particularly in hilly and mountainous regions. So far, a complete list of Oligochaeta fauna in running waters in Serbia has not been published. Based on the results of our study

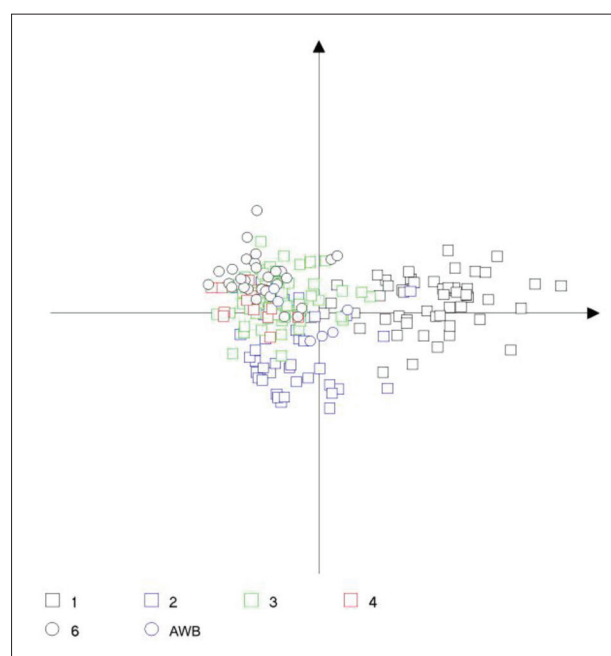


Fig. 4. DA biplot based on oligochaete taxa from different waterbody types. The waterbody types are numbered according to the Regulative of the Republic of Serbia [39] as follows: 1 – large lowland rivers; 2 – large rivers, except the rivers of the Pannonian Plain; 3 – minor and middle water courses up to altitudes from 200 to 500 m; 4 – minor and middle water courses up to an altitude above 500 m; 6 – minor water courses outside the Pannonian Plain area that are not included in types 3 and 4; AWB – artificial waterbodies.

and literature review, a total of 97 species (45 genera from 8 families) are recorded in the running waters of Serbia. It is assumed that the number of species is higher, because Enchytraeidae and Lumbricidae species were not determined with certainty due to their complicated identification. Most of the species in our investigation were common and cosmopolitan, with distributions typical for continental Europe. The diversity of oligochaete fauna in Serbia is in accordance with investigations in countries such as the Netherlands, Belgium, Germany [32], Poland [40] and Estonia [41]. Oligochaete fauna of Serbian rivers showed the greatest similarity with neighboring countries on the Balkan Peninsula, especially with Montenegro [42, 43], because the two countries belong to the same biogeographical territory (ecoregions 5 and 11) [2].

We did not confirm the presence of 39 species that were recorded in previous investigations. Species from the freshwaters of the Pannonian Plain could not be confirmed because we did not investigate this area

during our research. Some records, such as *Thalassodrilus prostatatus* (Knöllner, 1935) and *Fridericia perrieri* (Vejdovský, 1878) [5] are questionable, because these are marine species ([32]). Further, rare species such as *Tubifex montanus* Kowalewski, 1919 (known from the mountain waters in the Carpathians and the Near East, [31]), *Nais stolci* Hrabě, 1981 and *Trichodrilus strandi* Hrabě, 1936 in the Danube [12] are likely based on misidentification, as well as *Tubifex newaensis* (Michaelsen, 1903) found in the Sava River [13] and *Tatriella slovenica* Hrabě, 1939 [26] that was recorded in a hilly and mountainous region (Dinaric western Balkan and Eastern Balkan ecoregions). Other species from the literature that were not recorded during our research may have been missed due to the sampling and processing of the material, or possibly these results are testimony to the change of environmental factors that occurred in the examined areas.

The Danube represents the southern corridor for the spread of Ponto-Caspian species, and Serbia is considered as the center of biodiversity for the genera *Potamothrix*, *Psammoryctides* and *Isochaetides* [44]. Only two species recorded in Serbia are considered endemic [30, 44], *Potamothrix danubialis* (endemic for the Danube and Dnieper rivers) and *P. isochaetus* (endemic for the Danube and the Balkan Peninsula). These species are common in running waters and predominantly inhabit the Danube catchment area (the Danube, the Sava and their tributaries, river canals and the flooding area in the Pannonian Plain) [5,15].

As expected, the most important edicator species of the oligochaete communities was *Limnodrilus hoffmeisteri*, whose occurrence was typical for the Danube, Sava and Velika Morava catchment areas. However, the dominance of *Limnodrilus* sp. was recorded in some small- and medium-sized watercourses to/above an altitude of 500 m a.s.l. The explanation is that this “plastic” species inhabits a muddy substrate found as mosaic microhabitats in hilly and mountainous streams (along the shore in sectors where the current is slower).

The dominance of *Stygodrilus heringianus*, a second-most frequent species of oligochaete fauna, was characteristic of hilly and mountainous types of watercourses (above 400 m), especially in the Lim and Ibar basins. The dominance of the Lumbriculidae family was expected in these streams, as they possess medium- to

large-sized substrates (pebbles and stones from 6 to 40 cm) [45,46], and particularly of *S. heringianus* [47].

Oligochaete communities from hilly and mountainous streams of the Zapadna (Čemernica, Ljubišnica, Lučka River and Rasina) and Južna Morava (Nišava, Toplica and Gaberska River), Timok (Beli Timok, Crni Timok and Lenovačka River) and Kolubara (Tamnava, Beljanica, Turija, Dragobilj, Ribnica and Peštan) basins are distinguished by the frequency and abundance of naidids, especially of *Nais elinguis* and *N. bretscheri*. The rivers mentioned above belong to the same waterbody type. The dominance of naidins in these streams and streams from these regions was reported in previous investigations [16,17,19], suggesting that the presence of the massive growth of periphyton (green algae *Cladophora* sp., *Sphaerotilus natans*, Bacillariophyceae, Cyanophyceae, Dinophyceae, Chlorophyceae, Euglenophyceae, Protozoa, Rotatoria and Copepoda) had a significant effect on such oligochaete community structures. This conclusion is in accordance with Dumnicka [47] and Šporka [48] who determined the presence of periphyton as one of the important factors of community structure, given that it is used for feeding of detritivore species such as naidids.

Based on the percentage participation of dominant taxa in river basins, the running waters of Serbia could be classified into 4 groups dominated by tubificins, naidins, enchytraeids and lumbriculids. Hierarchical clustering with a complete linkage algorithm based on Pearson's correlations (complete linkage) confirmed that the Danube basin is distinguished by a high species diversity and dominance of *L. hoffmeisteri*. Lower river stretches of the Danube and Sava that flow through Serbian territory could be compared to lake ecosystems (with high depth, slow flow, lower oxygen concentration). These waterbodies represent typical potamal types, and as Paunović [27] showed, the slowing down of the river current significantly influenced the diversity and relative abundance of the macroinvertebrate fauna. According to our results, with respect to higher species richness in these river stretches and in the main watercourses of other river basins as well, we conclude that the qualitative compositions of oligochaete assemblages have a clear pattern – lower diversity in tributaries, and an increased diversity in the main flow.

Some discrepancies in the distribution of oligochaetes were detected in the Zapadna Morava river basin where the highest species richness was not recorded in the main watercourse but in some tributaries. The lower stretches of these tributaries are characterized by a slower river flow, more sediments and small substrate fractions within a harder type of substrate (coarse gravel, stones), with these conditions allowing habitation by cosmopolitan species [26]. On the other hand, the Zapadna Morava River (and its entire basin) is under considerable influence of organic pollution because the area is densely populated, with large towns in the region (Čačak, Kraljevo, Užice, Sevojno), from which it receives a large amount of various wastewaters, such as the urban and industrial discharges of Čačak [18,49]. Schenková and Helešić [50] noted that under these conditions, substrate type does not have a crucial role. The normal distribution of Oligochaeta can change in response to organic pollution, thus lower diversity in the polysaprobic zones of river stretches and higher diversity in oligosaprobic zones were recorded. The ability of oligochaetes to find suitable microhabitats within a harder type of substrate in conditions of increased pollution was confirmed.

The Kolubara basin represents the border between the Pannonian Plain and the Dinaric western Balkans (Ilić [2], modified by Paunović [27]). The following rivers of this basin, Gradac, Jablanica, Obnica, Ribnica and Lepenica, belong to the Dinaric western Balkans and their oligochaete fauna showed similarity with communities in the rivers from the Zapadna Morava catchment area. The Južna Morava River represents the eastern border of this ecoregion, and it is characterized by heterogeneous environmental conditions along the river stretch; thus, its oligochaete fauna is distinguished by not very common taxa, such as *Chaetogaster langi*, *Nais barbata*, *Pristina rosea* and *Proppapus volki*. This river possesses an oligochaete community similar to that in the Timok river basin, which belongs to the Eastern Balkans.

According to the analysis of functional traits, three groups of rivers in Serbia stood out based on microhabitat preference: a pelophilous group with a dominance of *L. hoffmeisteri* (Danube, Sava and Velika Morava basins), a psammophilous group with a dominance of *S. heringianus* (Lim and Ibar basins), and a phytophilous group with a dominance of *N. bretscheri* (Timok and Južna Morava basins). The pelophilous

group consists of frequent species such as *L. claparedeanus*, *B. sowerbyi*, *T. tubifex* and *P. hammoniensis*, preferring a slow river current and fine substrate, with acceptably increased levels of eutrophication. The psammophilous group (*S. heringianus*, *H. ventriculosa*, *N. bretscheri*, *N. elinguis* and *E. tetraedra*) is characteristic for habitats with lower water temperature and levels of eutrophication, harder substrates (sand, pebbles and stones) and faster currents.

The occurrence of some species is closely related to their adaptive characteristics, such as the ability to swim, feeding types and substrate preferences. Thus, special environmental conditions (e.g. the presence of detritus and macrophytes) influence the distribution of phytophilous *Ophidonais serpentina* (a resident on water plants) and *Stylaria lacustris* in the Danube basin.

Regarding saprobic conditions, we observed a dominance of alpha- and beta-mesosaprobic species of oligochaetes. With respect to the observation that an increase in organic pollution increases the abundance of specimens and reduces biodiversity of oligochaetes [51], we confirmed that the qualitative community composition is a good indicator of the horizontal distribution. Saprobic conditions (particularly related to oxygen regime) are important abiotic factors for the distribution of Oligochaeta; however, the substrate type can reduce the indicator value of some taxa, which is obvious for the Zapadna Morava, Lim and Drina catchment areas where the average values of saprobic indices of minor and medium water courses (altitudes above 500 m, type 4 waters) were increased due to the presence of tubificins with a saprobic valence higher than 3.5 (*L. hoffmeisteri* and *T. tubifex*). This issue has not been sufficiently elucidated in the literature because oligochaetes are considered *a priori* to be indicators of organic pollution [51-54].

The Oligochaeta in Serbia showed a typical vertical distribution characteristic for macroinvertebrates [55], with the greatest biodiversity observed in type 1 waterbodies (large lowland rivers), and diversity declining with increasing altitude (in minor and middle water courses, from 200 to 500 m a.s.l. and above 500 m a.s.l., types 3 and 4, respectively). The dominance of Tubificinae in types 1 and 2 watercourses was observed, while Naidinae were numerous in small- and medium-sized watercourses at higher altitudes and with larger

substrates. An increase in the number of xeno- and oligosaprobic taxa with increasing altitude was observed.

Therefore, Oligochaeta fauna clearly revealed the influence of communal and industrial inflow of wastewaters that were detected in the Zapadna Morava River case study, and the washing of nutrients from agricultural areas in streams at higher altitudes. Running waters in Serbia are under constant pressure because of urbanization. The greatest effect and changes in flow characteristics caused by water regulation are on large lowland rivers where oligochaetes are dominant participants in the macroinvertebrate community. This is especially obvious based on the results from the Danube and Sava rivers where the dominance of limno(rheo)philic taxa was recorded. The largest dams on the Danube (the Iron Gate dams I and II at rkm 943 and rkm 842, respectively) have significantly slowed down the river current and contributed to more intensive sedimentation in zones with a backwater effect, which extend to Belgrade [27], with consequences such as impounding, overheating, retention of organic matter and bed-load suspension. The construction of small hydropower plants predominates in small mountainous rivers, but data are incomplete as regards changes in composition of the macroinvertebrate community.

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Author contributions: MP collected the samples in the field. AA identified the studied species, initiated the research, interpreted the data and wrote the manuscript. KZ, JT and BV participated in the collection of samples in the field and in the processing of materials in the laboratory. All authors read and approved the final draft of the manuscript.

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Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/Atanckovic%20et%20al_5151_Supplementary%20Material.pdf