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Ephemeroptera, Plecoptera and Trichoptera (Insecta) of Mountain Tributaries of the Struma River: Diversity in Relation to Environmental Parameters and Zoogeographic Features

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Abstract: The study presents results on diversity and geographical distribution of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) in eight mountain tributaries of Struma River. The EPT benthic fauna was studied for the first time in the Otovitsa and Mochura Rivers. The research was performed in August 2020 at nine localities within the Struma River Basin. During the study, a total of 19 mayfly, 18 stonefly and 20 caddisfly taxa were found. The dominant taxa were of the families Heptageniidae (Ephemeroptera), Nemouridae (Plecoptera) and Limnephilidae (Trichoptera). Ordination analyses indicated that the EPT fauna at the locality in the Mochura River was the least similar to the others, owing to the high altitude and prevalence of stone substratum. The recorded EPT taxa belong to six zoogeographical categories (Holarctic, Palaearctic, European, Mediterranean, Pontic and Endemic) with overall dominance of taxa of European and Mediterranean complexes (ca. 67% of all species and subspecies). *Leuctra hirsuta* Bogoescu & Tabacaru, 1960 and *Ecdyonurus (H.) epeorides* Demoulin, 1955 are Balkan endemic species, while *Odontocerum hellenicum* Malicky, 1972 is considered a subendemic species.

Key words: mayflies, stoneflies, caddisflies, Struma River tributaries, Bulgaria

Introduction

Lotic ecosystems are a global priority (Milner et al. 2001), given that the decline in species diversity in freshwater ecosystems is five times than that of terrestrial ecosystems and three times than that of coastal marine ecosystems (Riccardi & Rasmussen 1999, Saunders et al. 2002). A number of studies have discussed the importance of various physical and chemical factors (Lock & Coethals 2011, Krepski et al. 2014, Adu 2016, Soufi et al. 2018) and altitude (Barquin & Death 2006, 2011) as substantial parameters that have a significant effect on the formation of macrozoobenthos in river coenoses.

The ecological preferences of freshwater organisms are used to assess climate change and its impact on the composition and structure of benthic communities in lakes and rivers (Durance & Ormerog 2007). In this regard, invertebrate benthic fauna is one of the key communities, which reflect

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changes in the aquatic ecosystems because of natural and anthropogenic impacts on surface waters. The immature stages of Ephemeroptera, Plecoptera and Trichoptera (EPT) are some of the most sensitive macroinvertebrate orders that have a certain range of tolerance and very particular requirements to the environmental conditions (Tierno de Figueroa et al. 2010, Pirvu & Pacioglu 2012, Tyufekchieva et al. 2013, Zedkova et al. 2015, Suhaila & Che Salamah 2017).

The studied rivers within the catchment of the Struma River (South-West Bulgaria) occupy the westernmost parts of the Aegean Sea Basin. They drain the Pirin and Rila Mountains (Hristova 2021) and are characterised as medium-sized rivers. Published data on EPT fauna of the Pirin and Rila National Parks and the main course of the Struma River are presented in Kumanski (1997a, b), Hubenov et al. (2000), Vidinova et al. (2000, 2006) and Vidinova & Russev (1997, 2009). Studies regarding the ecological and saprobiological condition of the rivers in this region of Bulgaria also include information on mayflies, stoneflies and caddisflies as important components of macrozoobenthic communities: e.g. in Sakelarieva et al. (2008) for Blagoevgradska Bistritsa River, Moskova et al. (2009) and Moskova & Uzunov (2011) for Rilska and Iliyna Rivers and Stoyanova et al. (2014) for Luda River. Tyufekchieva & Rimcheska (2019) provide data on the composition and zoogeographic characteristics of stoneflies from montane streams in the Aegean Sea Basin, including tributaries of Struma River.

Here, we present new data on the diversity and zoogeography of the EPT communities inhabiting the mountainous tributaries of the Struma River, South-West Bulgaria. The aims of the present paper were: (1) to study the species diversity and distribution of EPT taxa of mountain tributaries of Struma River in Bulgaria; (2) to analyse the physical and chemical water parameters in the tributaries; (3) to identify the main factors affecting the diversity and distribution of EPT taxa; (4) to characterise their zoogeographic affiliation.

Materials and Methods

Study area

Nine localities representative of eight mountain tributaries of the Struma River, South-West Bulgaria were sampled in August 2020 (Fig. 1). The sites are situated in the Rila and Pirin Mountains. Data for the geographical coordinates and altitude of each locality are presented in Table 1.

Sample collection and processing

The multi-habitat approach for macrozoobenthos sampling was applied following Cheshmedjiev et al. (2011). After sample processing in the laboratory, the specimens of larvae of EPT taxa were separated from the other organisms and preserved in 70% ethanol. The abundance of the taxa was presented as a number of specimens per sample. The nomenclature and systematics followed Bauernfeind & Soldan (2012) for Ephemeroptera, Muranyi (2008) and DeWalt et al. (2018) for Plecoptera and Kumanski (1985, 1988), Holzenthal (2011) and Morse (2018) for Trichoptera. Zoogeographical observations were taken based on our own faunistic results and literature data. Zoogeographical complexes were assigned according to Puthz (1978), Bauernfeind & Soldan (2012) and Belfiore & Thomas (2017) for Ephemeroptera, Muranyi (2008) and Dewalt et al. (2018) for Plecoptera and Kumanski (1976, 1985, 1988) and Malicky (2017) for Trichoptera.

Standard physical and chemical water parameters (water temperature, dissolved oxygen, pH and electrical conductivity) were measured *in situ* using portable Windaus Labortechnik Package and HANNA multi-parameter instrument.

Data analyses

The dominant analysis (frequency of occurrence – pF %, frequency of dominance – DF %, range of dominance – DT %) was done after De Vries (1937) and Kozhova (1970). Principal components analysis (PCA), a form of indirect gradient analysis, was used to explore the inherent patterns within environmental river variables and identify the key gradients of variation, reducing the dimensionality and arranging sites along a number of key axes. Redundancy analysis (RDA) (Van den Wollenberg 1977) was used to examine relationships between species composition and driving environmental factors. RDA can be considered as a multivariate form of regression analysis whereby the response data are modelled as a function of one or more ordination axes that are constrained to be linear combinations of the environmental variables, depending on the extent of species turnover along the ordination axes. We applied Detrended Correspondence Analysis (DCA), using the option "detrending-by-segment", which allowed the gradient length of species variance along orthogonal axes to be quantified in terms of standard deviation units. The first axis of our data had a length of 3.35, suggesting modest unimodality according Ter Braak & Šmilauer (2002). Therefore, we used linear direct analysis. In the latter analysis, we used abundances of the EPT

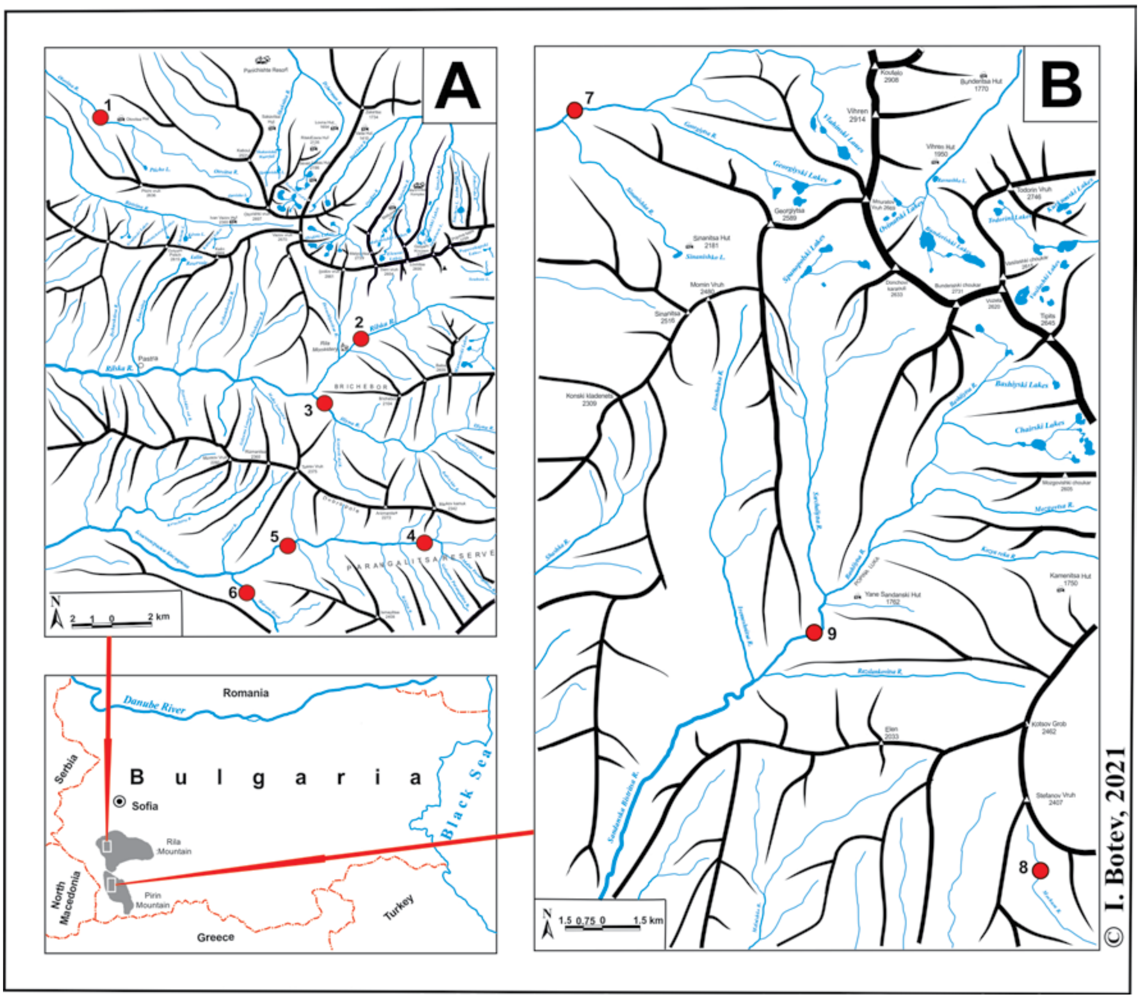

Fig. 1. Map of the sampling sites. **A.** Rila Mountain sites. **B**. Pirin Mountain sites. Sampling site numbers correspond to those in Table 1.

Table 1. Geographic coordinates and altitude of the sampling sites.

Site No	River	Latitude (N)	Longitude (E)	Altitude (m)
1	Otovitsa	42.23703	23.21361	1292
2	Rilska	42.13980	23.34915	1170
3	Iliyna	42.11056	23.32272	1025
4	Blagoevgradska Bistritsa 1	42.04059	23.36880	1471
5	Blagoevgradska Bistritsa 2	42.03098	23.27414	896
6	Slavova	42.02724	23.27337	913
7	Vlahinska	41.73778	23.22970	480
8	Mochura	41.62000	23.45251	1653
9	Sandanska Bistritsa	41.66738	23.38585	1124

identified to the species and subspecies level (39 taxa). All analyses were undertaken using the software statistical package CANOCO 4.5 for Windows (Ter Braak & Šmilauer 2002). The cluster analysis was performed using Bray-Curtis similarities for 37 taxa (with a defined zoogeographic affiliation) and six zoogeographical complexes with PRIMER-E v.6 (Clarke & Gorley 2006).

Results

EPT taxa composition

Totally, 57 EPT taxa (19 mayflies, 18 stoneflies and 20 caddisflies) of 20 families were recorded in the studied mountain tributaries of the Struma River (Table 2).

The dominant taxa belonged to the families Heptageniidae (Ephemeroptera), Nemouridae

Table 2. Taxonomic composition, zoogeographical complexes and dominant analysis of the EPT taxa from the mountain tributaries of the Struma River. For the numbers of localities (sample sites), see Table 1.

Taxa	Code	Localities	Zoogeographical complex	pF %	dF %	DT %
Ephemeroptera						
Baetidae						
Baetis (Baetis) alpinus (Pictet, 1843)	*Baetalp*	3,4,5,6,7,8,9	Mediterranean	77.78	33.33	42.86
Baetis (B.) fuscatus (Linnaeus, 1761)	*Baetfus*	1,7	Palearctic	22.22		
Baetis (B.) melanonyx (Pictet, 1843)	*Baetmel*	1,2,3,4,5,7,9	Mediterranean	77.78	22.22	28.57
Baetis (Nigrobaetis) muticus (Linnaeus, 1758)	*Baetmut*	5,7,8	Palaearctic	33.33		
Baetis (Rhodobaetis) rhodani (Pictet, 1843)	*Baetrho*	1,2,3,4,5,6,7,8,9	Palaearctic	100.00	44.44	44.44
Baetis sp.				22.22		
Ephemeridae						
Ephemera danica Müller, 1764	*Ephedan*	5,6,7	Palaearctic	33.33		
Ephemerellidae						
Ephemerella ignita (Poda, 1761)	*Epheign*	4,5,7,8	Palaearctic	44.44		
Ephemerella mucronata (Bengtsson, 1909)	*Ephemuc*	8	Holarctic	11.11		
Heptageniidae						
Ecdyonurus (Helvetoraeticus) epeorides Demoulin, 1955	*EcdHepe*	1,2,4,5,7,9	Endemic	77.78	22.22	28.57
Ecdyonurus (H.) helveticus (Eaton, 1883)	*EcdHhel*	2,4,7	Mediterranean	33.33		
Ecdyonurus (H.) carpathicus Sowa, 1973	*EcdHcar*	7	Mediterranean	11.11		
Ecdyonurus sp.		3,4,6		33.33		
Ecdyonurus (Helvetoraeticus) sp.		1,4,5,6,7,8,9		77.78	11.11	14.29
Rhithrogena gr. *hybrida*	*Rhithyb*	8	Mediterranean	11.11		
Rhithrogena sp.		1,2,7,9		44.44		
Epeorus assimilis Eaton, 1885	*Epeoass*	1,2,4,5,8,9	Mediterranean	66.67		
Leptophlebiidae						
Habrophlebia lauta Eaton, 1884	*Habrlau*	5,6	Pontic	22.22		
Habroleptoides confusa Sartori & Jacob, 1986	*Habrcon*	1,4,5,6,8	Mediterranean	55.56	22.22	40.00
Plecoptera						
Leuctridae						
Leuctra hirsuta Bogoescu & Tabacaru, 1960	*Leuchir*	3,8,9	Endemic	33.33		
Leuctra hippopus Kempny, 1899	*Leuchip*	2	Holarctic	11.11		
Leuctra major Brinck, 1949	*Leucmaj*	7	European	11.11		
Leuctra inermis Kempny, 1899	*Leucine*	8	European	11.11		
Leuctra sp.		6,8		22.22		
Nemouridae						
Nemoura uncinata Despax, 1934	*Nemounc*	8	European	11.11		
Nemoura sp.		1,4		22.22		
Protonemura intricata intricata (Ris, 1902)	*Protint*	5	European	11.11		
Protonemura praecox praecox (Morton, 1894)	*Protpra*	2,7	Mediterranean	33.33	11.11	33.33
Protonemura montana Kimmins, 1941	*Protmon*	8	European	11.11	11.11	100.00

Table 2. Continuation.

Taxa	Code	Localities	Zoogeographical complex	pF %	dF %	DT %
Protonemura meyeri (Pictet, 1841)	*Protmey*	1	European	11.11		
Protonemura sp.		3,6		22.22		
Perlodidae						
Perlodes intricatus (Pictet, 1841)	*Perlint*	1,2,8,9	European	44.44		
Isoperla grammatica (Poda, 1761)	*Isopgra*	8	European	11.11		
Perlidae						
Perla marginata (Panzer, 1799)	*Perlmar*	3,4,5,6,7,9	European	66.67	22.22	33.33
Perla pallida Guérin-Méneville, 1838	*Perlpal*	8	Mediterranean	11.11		
Dinocras cephalotes (Curtis, 1827)	*Dinceph*	3,7,9	European	33.33		
Chloroperlidae						
Siphonoperla neglecta (Rostock, 1881)	*Siphneg*	8	European	11.11		
Trichoptera						
Glossosomatidae						
Glossosoma sp.		4,5,7		33.33		
Goeridae						
Silo sp.		8		11.11		
Rhyacophilidae						
Rhyacophila (s. str.) gr. *nubila*	*Rhyanub*	3,9		22.22		
Rhyacophila (s. str.) sp.		1,2,3,9		44.44		
Rhyacophila (Hyporhyacophila) gr. *tristis*	*Rhyatri*	3,4,6		44.44		
Rhyacophila (Hyporhyacophila) sp.		6,7,8		33.33		
Hydropsychidae						
Hydropsyche cf. *incognita* Pitsch, 1993	*Hydroinco*	7	European	11.11		
Hydropsyche cf. *tabacarui* Botosaneanu, 1960	*Hydrotab*	3,5,6,9	European	44.44		
Sericostomatidae						
Sericostoma flavicorne/ personatum	*Serifla*	1,3	Palearctic	22.22		
Oecismus monedula monedula	*Oecimon*	1,7	European	22.22		
Limnephilidae						
Potamophylax sp.		2,3,7,8,9		55.56		
Drusus discolor (Rambur, 1842)	*Drusdis*	8	European	11.11	11.11	100.00
Drusus sp.		8		11.11		
Drusinae gen. sp.		8		11.11		
Halesus sp.		4,5		22.22		
Philopotamidae						
Philopotamus montanus (Donovan, 1813)	*Philmon*	1,3,4,5,6,7,8,9	European	88.89	11.11	12.50
Odontoceridae						
Odontocerum hellenicum Malicky, 1972	*Odonhel*	3,9	Subendemic	22.22		
Odontocerum sp.		4		11.11		
Chaetopterygini						
Chaetopterygini gen. sp.		1,3,8,9		44.44		
Polycentropodidae						
Polycentropodidae gen. sp.		1		11.11		

Fig. 2. Number (N) and abundance (S) of the EPT taxa. For site numbers, see Table 1.

(Plecoptera) and Limnephilidae (Trichoptera). The most frequent (with frequency of occurrence higher than 50 %) were the mayflies *Baetis rhodani* (at all nine stations), *B. melanonyx*, *B. alpinus*, *Ecdyonurus (H.) epeorides*, *E. (Helvetoraeticus)* sp., *Epeorus assimilis*, *Habroleptoides confusa*, the stonefly *Perla marginata*, the caddisflies *Potamophylax* sp. and *Philopotamus montanus*. The commonly occurring taxa (pF= 20–50 %) were 27, while 20 taxa had values of pF between 10 and 20 %. The highest ranges of dominance were determined for the stonefly *Protonemura montana* and caddisfly *Drusus discolor* (Table 2). These species had low values of frequency of occurrence and dominance, as they dominated in the Mochura River only. The highest was the number of EPT taxa in the Mochura River (27), Vlahinska River (22) and Sandanska Bistritsa River (21), and the lowest – in the Rilska River (11; Fig. 2). The highest abundance was recorded in the Mochura River (900 specimens) and the lowest – in the Slavova River (55 specimens). The most abundant species *P. montana* (with 200 individuals) and *E.* (*H.*) *epeorides* (180 ind.) were found in the Mochura River. *Habroleptoides confusa* was the most abundant species (104 ind.) recorded from the Otovitsa River. Twenty taxa were found at only one of each of the nine localities.

EPT taxa diversity in relation to environmental parameters

The study localities were situated between 480 and 1653 m a.s.l. At all sites, the water temperature was below 18°C. The pH values varied from neutral to alkaline ranges, i.e. from 7.81 to 9.01. The lowest conductivity was registered at the station in the Blagoevgradska Bistritsa River, Kartalska Polyana (27.2 $\mu S.cm^{-1}$) and the highest at the station in the Vlahinska River (109.5 $\mu S.cm^{-1}$). The maximum concentration of dissolved oxygen (10 $mg.dm^{-3}$) was measured at the same locality.

Principal components analysis based on correlation matrix, following centering and standardisation was used to summarise the major patterns of variation within environmental data. The first two principal components (λ_1=0.568, λ_2=0.225) cumulatively explained 79.4 % of total variance (Fig. 3). Summary statistics for the physical and chemical water parameters and altitude are presented in Table 3. Variable scores for Axes 1 to 3 are included in this table with highest absolute scores indicating strength correlation between these axes and each of the determinants. The primary gradient (Axis 1) accounted for 56.8% of the variance in environmental data and differentiated between sites where conductivity and water temperature were high – Vlahinska River (7), 109.5 $\mu S.cm^{-1}$; Blagoevgradska Bistritsa River (5), 17.1°C and those where these determinants had relatively lower values but the altitude was high – Mochura River (8) 1653 m a.s.l. The second main axis of variation (accounting for 22.5% of the variance) was associated with dissolved oxygen and pH. Along this axis grouped sites where these parameters were high – Rilska River (2), 9.5 $mg.dm^{-3}$ and 9.01; Blagoevgradska Bistritsa River (4), 9.5 $mg.dm^{-3}$ and 8.82 and Mochura Rver (8) which had the lowest concentration of dissolved oxygen 7.0 $mg.dm^{-3}$.

A redundancy analysis was undertaken to examine which factors were responsible for variation in environmental data across the survey sites. The ordi-

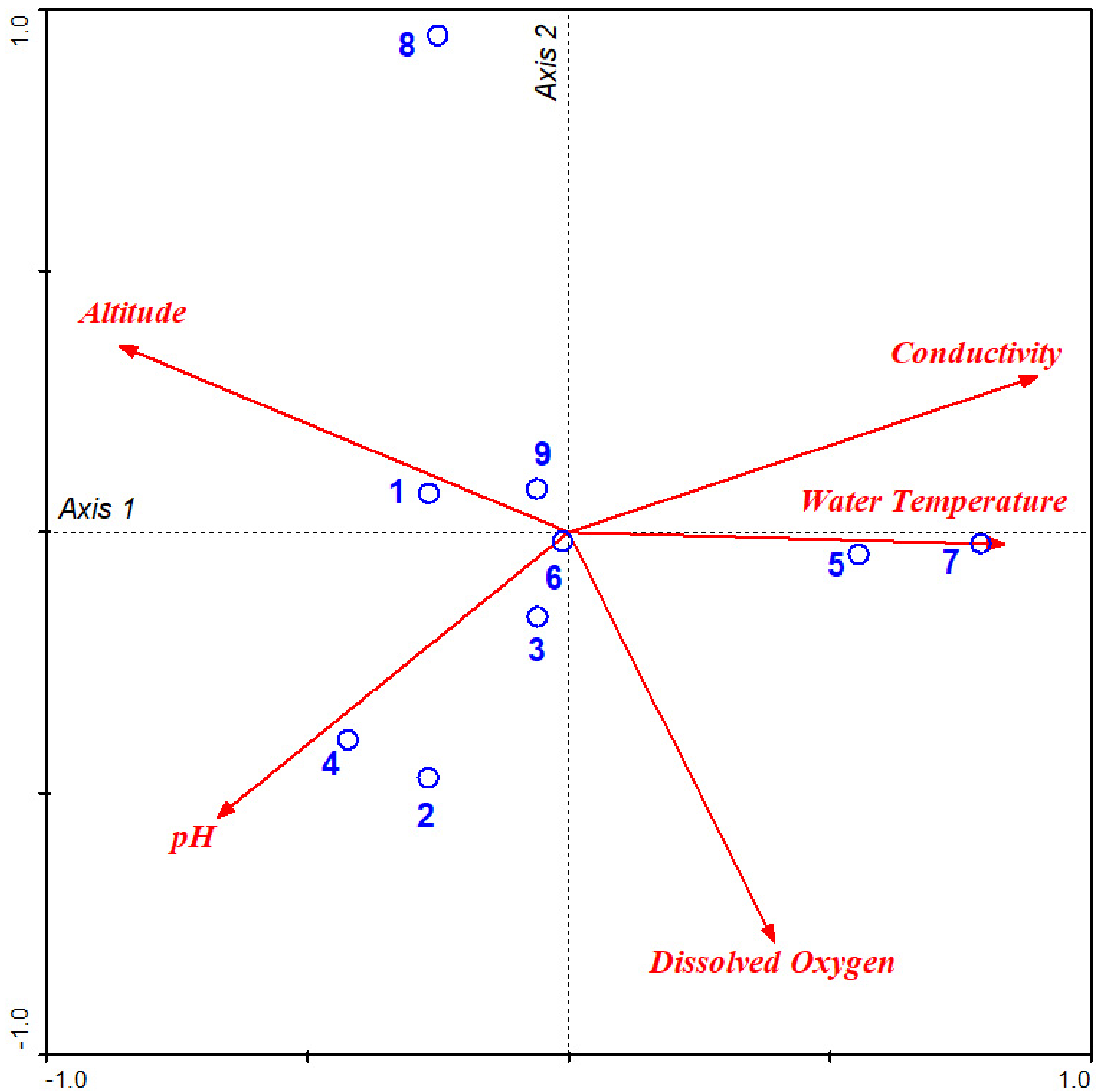

Fig. 3. Correlation biplot based on PCA of altitude, physical and chemical water parameters of nine localities of the studied river tributaries. For site numbers see Table 1.

Table 3. Summary statistics of physical and chemical parameters, altitude and scores of PCA axes 1 to 3 for each environmental determinant with the higher scores highlighted in bold.

Variable	Min	Max	Mean	AX1	AX2	AX3
Water temperature, ºC	10.70	17.1	13.20	**0.8341**	-0.0225	0.3387
Conductivity, μS.cm^{-1}	27.20	109.5	58.30	**0.8962**	0.2976	-0.0142
pH	7.81	9.01	8.58	**-0.6718**	**-0.5449**	0.4802
Dissolved oxygen, mg.dm^{-3}	7.00	10.00	8.79	0.3936	**-0.7835**	-0.4762
Altitude, m a.s.l.	480	1653	1113	**-0.8584**	-0.3560	-0.2798

nation of 39 species from 9 sites is shown in Fig. 4.

The eigenvalues (λ_1=0 0.319; λ_2= 0.133) of the first two axes as well as species-environmental correlations (0.9968; 0.9602) for these two axes denoted a good separation of the species along these axes. They accounted for 58.1 % cumulative percentage variance of the species data and 65.0 % cumulative percentage variance of species-environment relations. Monte Carlo unrestricted permutation tests (499 permutations) of axis 1 indicated that it was significant (P=0.04). The subsequent forward selection testing showed that the most important factor were conductivity and altitude, which alone accounted for 22 % and 19 %, respectively (Ter

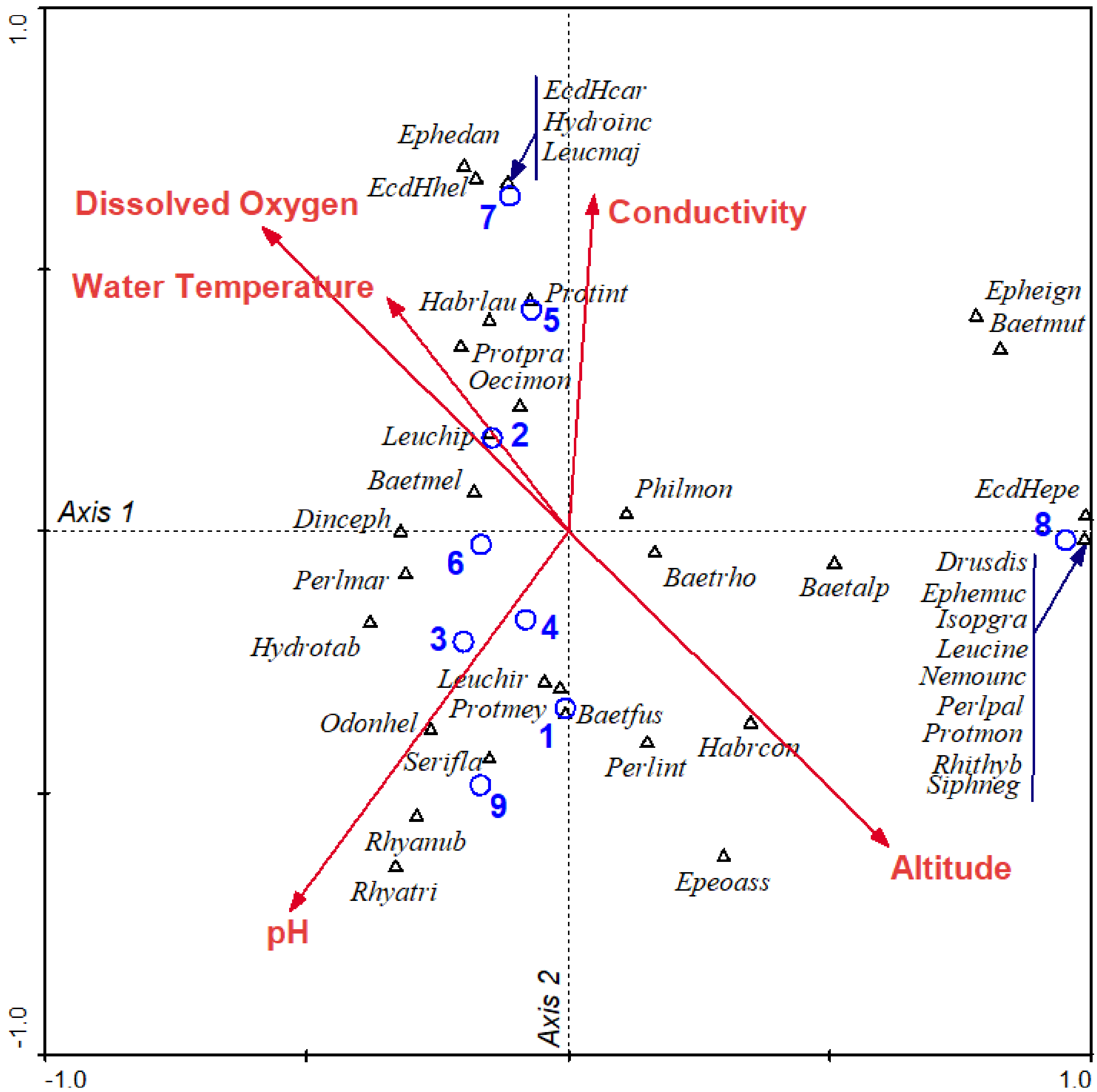

Fig. 4. Correlation triplot based on a redundancy analysis of benthic macroinvertebrate samples from the nine studied sites. For site numbers, see Table 1. For the species identification codes, see Table 2.

Braak & Šmilauer. 2002). Two main gradients were evident. The first, from top left to bottom right, was reflect by Axis 1, which was related to dissolved oxygen (intraset correlation 0.58) and altitude (intraset correlation 0.61). In contrasts, e. g., the site in Vlahinska River (7), with the highest concentration of dissolved oxygen and the constituent taxa *E. (H.) helveticus, E. danica, H.* cf. *incognita, L. major* and *E. (H.) carpathicus*, plotted on the top left of the diagram, together with the site in Mochura River (8). The latter had the lowest concentration of this parameter but the highest altitude among the studied sites. Furthermore, dissolved oxygen and altitude were strongly negatively correlated. This site (8), with its characteristic taxa, i.e. the mayflies *R.* gr. *hybrida*, *E. mucronata*, the stoneflies *L. inermis, I. grammatica, N. uncinata, P. pallida, S. neglecta, P. montana* and the caddisfly *D. discolor*, plotted on the right of the diagram. Axis 2 reflected the second gradient. It was related to conductivity (intraset correlation 0.64) and pH (intraset correlation -0,72) and separated the sites with high values of conductivity, i.e. Vlahinska (7) and Blagoevgradska Bistritsa Rivers (5), from the sites in Otovitsa (1), Iliyna (3) and Sandanska Bistritsa Rivers (9), which exhibited low values of conductivity but high pH. Taxa characteristic of the first two sites, plotted top centre may be associated with conductivity, while those plotted at the bottom, such as the caddisflies *R.* gr. *nubila*, *R.* gr. *tristis*, *S. flavicorne* and *O. hellenicum*, were characteristic of low conductivity and high pH. The mayflies *E. assimilis* and *H. confusa* and the stonefly

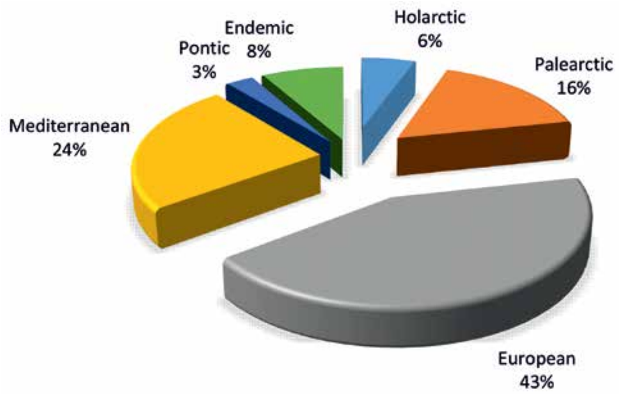

Fig. 5. Distribution of the EPT species according to zoogeographical complexes.

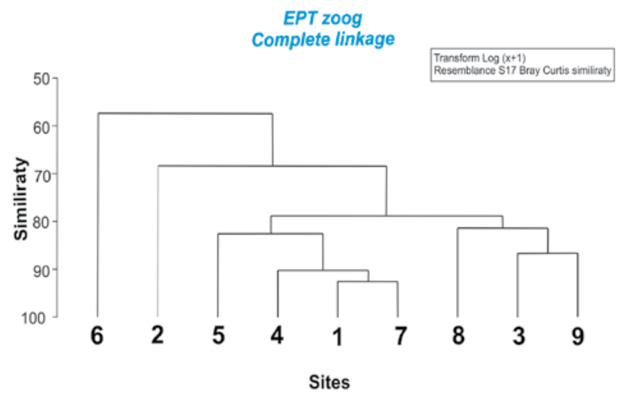

Fig. 6. Faunistic similarities between the studied nine river localities. For codes, see Table 1.

P. intricatus were positively correlated with the high altitude of the sampling localities in the Mochura and Otovitsa Rivers and negatively associated with the high water temperature.

Zoogeographical characteristics of EPT taxa

The species and subspecies of EPT of the studied localities were classified into six zoogeographical complexes (Table 2, Fig. 5). The EPT fauna was dominated by the European complex (43%). It included species and subspecies widely distributed in Europe and Bulgaria and was represented by 11 taxa of Plecoptera and five taxa of Trichoptera. The second place in the zoogeographical structure belonged to the taxa originating mainly and occurring in the Mediterranean complex (24%); these included the largest number of taxa of the order Ephemeroptera (seven) and two of the order Plecoptera. The species with the widest ranges were combined in the Holarctic (two species) and Palaearctic complexes (six species), with representatives of the order Ephemeroptera predominating (five species from the Palaearctic and one species from the Nearctic complexes). The order Plecoptera was not represented in the Palaearctic complex, while the order Trichoptera was not represented in the Holarctic and Mediterranean complexes. The Pontic complex included only the mayfly *H. lauta*. The endemic complex (8%) included *L. hirsuta* (Plecoptera), *E.* (*H.*) *epeorides* (Ephemeroptera) and *O. hellenicum* (Trichoptera).

The cluster analysis of similarities among localities (based on (sub) species only) identified the EPT fauna of Blagoevgradska Bistritsa (Kartalska polyana), Otovitsa and Vlahinska Rivers to be as similar as 90%, with high contribution of Mediterranean species (Fig. 6). We recorded similarities greater than 80% among the Mochura, Iliyna and Sandanska Bistritsa Rivers, mostly owing to the presence of endemic species. The localities in the Slavova and Rilska Rivers were the least similar to the rest of the river sections and to one another, with similarity lower than 70%.

Discussion

Totally, 57 taxa of Ephemeroptera, Plecoptera and Trichoptera were recorded in the nine studied mountain localities of tributaries of the Struma River in South-West Bulgaria. They were dominated by three families: Heptageniidae (Ephemeroptera), Nemouridae (Plecoptera) and Limnephilidae (Trichoptera), which are typical of many mountain cold river systems (Maiolini et. al. 2011, Kruitbos 2012, Potikha 2015). The mayflies *B. alpinus, B. rhodani*, *H. confusa* and the stoneflies *P. praecox praecox* and *P. marginata* had the highest frequency of occurrence and high range of dominance. *Perla marginata* is among the most spread stonefly in the semi-mountainous and mountainous river sections (Tyufekchieva & Rimcheska 2019). Our results confirm the findings of Moskova et al. (2009), who established a high frequency of occurrence of the same mayflies of family Baetidae (*B. alpinus* and *B. rhodani*) in the Rilska and Iliyna River in the summer of 2007. According to Sakelarieva et al. (2008), the same two species had a frequency of occurrence more than 50 % and dominated most often in the Blagoevgradska Bistritsa River in 2002–2003. From the same river during our study, we reported for the first time the caddisfly *Halesus* sp. The highest ranges of dominance were determined for the stonefly *P. montana* and the caddisfly *D. discolor*. These species had low values of frequency of occurrence and dominance parameters, as they were found and dominated at particular river locality only. These taxa have been previously identified by

Sakelarieva et al. (2008) but, during our survey, we found them only in the Mochura River.

The results of the PCA based on physical and chemical data and RDA of EPT data demonstrated that altitude, physical and chemical factors have a potential influence on the distribution of the mayflies, stoneflies and caddisflies in mountain river localities. Many studies have shown that altitude, dissolved oxygen (Quinn & Hickey 1990, Moskova et al. 2009, Lewin 2013, Tyufekchieva et al. 2013), pH (Hahn 2000, Lewin 2013), water temperature and conductivity (Bulankova 2001, Lewin 2013, Savic et al. 2017) as well as zoogeographical features (Hynes 1970, Krno 2003) are among the main structure-determining factors for the distribution and abundance of aquatic macroinvertebrates. Too extensive in scope and subject matter, the issue of vertical gradient as a factor in the structuring of fauna in lotic ecosystems (Marchant et al. 1995, Carter et al. 1996), has been largely related to and discussed in conjunction with water temperature (Macan 1962, Haidekker & Hering 2007). The increase of water temperature may affect aquatic organisms directly, thus optimal areas for some groups associated with a defined altitude might be limited (Domingues & Valdez 1992). This suggests that the vertical distribution of EPT taxa, and especially of the stonefly fauna, is an important factor for a future assessment in the context of climate change. In our study, altitude was the major factor influencing the total taxon richness of EPT assemblages. It should be noted that this is the first study of the EPT taxa in Mochura and Otovitsa Rivers. The Mochura River was characterised by the highest diversity (27 taxa) and abundance (900 specimens) of EPT taxa. Twelve species were recorded only at this locality, where the altitude was the highest and a negative correlation with water temperature and dissolved oxygen was established. That might be owing to the complementary influence of several factors. Such kind of rivers, because of their stable ecological characteristics and close connection to freshwater and groundwater, are able to support both sparse and more diverse fauna (Glazier 1991, Di Sabatino et al. 2003). The Otovitsa River was also characterised with high diversity and abundance (18 taxa and 331 specimens). The established dominant species have preferences of higher altitude (the elevation of the site is 1292 m a.s.l.) and lower conductivity (41.2 $\mu S.cm^{-1}$). The resemblance in EPT species among the rest of the localities was high, owing to the similar environmental conditions. Amid the species of the genus *Ecdyonurus*, there is a separation between species that occur at the highest (1653 m a.s.l., Mochura River) and lowest altitude (480 m a.s.l., Vlachinska River), like *E.* (*H.*) *epeorides* as compared to *E.* (*H.*) *carpathicus*. Among the species of the family Leptophlebiidae, there is a separation along the second axis between species that occur at the high values of water temperature (17.1°C) and the highest altitudes (1653 and 1292 m a.s.l.), like *H. lauta* as compared to *H. confusa.* The species of the genus *Rhyacophila* have a clear affinity for highly-oxygenated rivers (Pirvu & Pacioglu 2012) and are positively correlated with temperature (Kalaninova et al. 2014). During our studies, we established a positive correlation between two species of this genus and pH. The results of the RDA confirm that the species of the genus *Leuctra* (Plecoptera) are generalists and have no specific preferences towards the altitude, water temperature, electric conductivity and pH (Paril et al. 2014).

Our zoogeographical results demonstrate that the EPT fauna in the studied river stretches has been formed by autochthonous and as well as by allochthonous elements. Overall, the allochthonous elements of EPT originate from the European (especially Mid-and-South-European), Mediterranean and Palaearctic, and to a lesser degree from Pontic and Nearctic centres of speciation. Within the Palaearctic species, two subgroups were identified, including the Trans Palaearctic (*B. fuscatus, E. ignita* and *E. mucronata*) and West Palaearctic taxa (*B. muticus, B. rhodani* and *E. danica*) (Bauernfeind & Soldan 2012).

Plecoptra have been formed mainly during the Pleistocene and are of great interest for the zoogeographical studies (Zhiltsova 2010). In the present zoogeographical analysis, the European stonefly species complex is the best represented; it comprises ten species and one subspecies collected in summer. According to Tyufekchieva & Rimcheska (2019), the order Plecoptera of mountainous and semi-mountainous streams from Bulgaria and North Macedonia is defined mainly by European species (67%) in autumn and spring.

Malicky (1983) proposed that cold-water tolerant mountain caddisfly species might have survived the Pleistocene in perennial streams in Central Europe. The caddisfly *O. monedula* is a common inhabitant of small headwater streams in continental Europe (Dochet et al. 2008). After studying the genetic structure of European populations of *D. discolor*, it is suggested that instead of surviving in only the southern European refugia, it simply moved locally and to permanently-flowing mountain streams in Central Europe (Pauls 2004, Pauls et al. 2008). In comparison, the caddisfly fauna of Turkey is also strongly related to the European fauna (Sipahiler 2008). However, stoneflies and caddisflies are dominated by European species, while mayflies are dominated by Mediterranean species.

We recorded a high proportion of common EPT Mediterranean species in the Blagoevgradska Bistritsa (4) – 50%, Otovitsa – 33% and Vlachinska Rivers – 36%. The Mediterranean complex was represented by the seven taxa of the order Ephemeroptera and two of the order Plecoptera (European-Anatolian subspecies *P. praecox praecox* and Ponto-Caspian species *P. pallida*). According to Karaouzas et al. (2016), the Balkan records of the European species *P. marginata* probably refer to *P. pallida* but here we discuss the two species.

From a zoogeographical point of view, the Mochura (11% of endemic taxa), Iliyna (20%) and Sandanska Bistritsa (23%) Rivers were characterised with the highest diversity of endemic and subendemic taxa. Some of these Balkan endemics might be autochthonous for Bulgaria but fossil evidence is lacking to prove that. *Leuctra hirsuta* (Plecoptera) and *Ecdyonurus* (*H.) epeorides* (Ephemeroptera) are Balkan endemic species. The known localities of *Ecdyonurus (*H.*) epeorides* restrict its distribution on the South-Eastern Balkans (Vidinova 2003, Bauernfeind & Soldan 2012), although it has been reported also from Bosnia and Herzegovina (Buffagni et al. 2009). *Leuctra hirsuta* has been reported from Bulgaria (Tyufekchieva et al. 2019), North Macedonia, Bosnia and Herzegovina, Montenegro, Greece and Romania (Karaouzas et al. 2016). *Odontocerum hellenicum* (Trichoptera) is considered a subendemic of the Carpathians Mountains and the Balkan Peninsula (Graf et al. 2008). Based on this analysis, the so-called "biodiversity hotspots" can be identified (Hershkovitz et al. 2015). In this way, the potential aquatic ecosystems needing conservation are identified based on the concentration of endemic species and species with a certain conservation status (Eken et al. 2004, Lovejoy 2006). E.g., four species of stoneflies (*L. hirsuta*, *P. pallida*, *D. cephalotes* and *S. neglecta*) have been found at four of the localities and referred to the category Vulnerable (VU) (see Tyufekchieva et al. 2019). By contrast, the stonefly *P. marginata* was recorded at six localities overall. Similarly, among the mayflies, one species (*E.* (*H.*) *carpathicus*) was found at one site (the Mochura River) and one species (*B. rhodani*) at all nine localities. Amid the caddisflies, *D. discolor* was recorded at one site (the Mochura River) and *Ph. montanus* was found at eight localities. The highest number of endemic and rare species was recorded in the Sandanska Bistritsa and Mochura Rivers within the borders of the Pirin National Park and Iliyna River within the borders of the Rila National Park. Exploring the EPT biodiversity in the national park areas is important for assessing the ecological preferences of aquatic macroinvertebrates and understanding the formation of ecosystem structures without anthropogenic influence.

Conclusions

In the studied mountain tributaries of the Struma River, EPT benthic fauna is characterised by high taxonomic richness with a dominant representation of family Heptageniidae (Ephemeroptera), Nemouridae (Plecoptera) and Limnephilidae (Trichoptera). Altitude and water physical and chemical parameters such as water temperature, dissolved oxygen, pH and electrical conductivity have a potential influence on the EPT distribution in the mountain rivers. In general, EPT fauna of mountain rivers within the borders of the Rila and Pirin National Parks is heterogeneous in biogeographic composition; it is formed by endemic, subendemic and allochthonous elements (European, Mediterranean, Palaearctic, Pontic and Nearctic). The complex action of the local factors of the aquatic environment form specific conditions and, on a wider scale, characterise the zoogeographical features, which influence the diversity of EPT taxa in the studied mountain rivers.

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