Species composition and ecology of the Chironomidae family from the surface and groundwater habitats of Central Balkan National Park (Balkan Mountains, Bulgaria)

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Introduction

Central Balkan is the second largest National Park in Bulgaria and is an important protected area including valuable biodiversity of flora and fauna. The aquatic fauna of many of the rivers springing from the park's territories are well studied in their lowland sections. Yet data on the hydrofauna of the upper sections of these rivers is poorly studied giving only partial information regarding the hydrofauna

Regarding the park's biodiversity there is consequential data on the aquatic insects for several rivers springing from the "Central Balkan" National Park – for Tundzha River (Dimitrov, 1972; Russev et al., 1984; Yaneva & Russev, 1985; Borisova et al., 2013), and for the rivers Vit and Osam – (Russev, 1977; Yaneva, 1979; Yaneva, 1987, 1988; Russev et al., 1994). Records of aquatic Diptera within the borders of "Central Balkan" are given for the Chironomidae (Kowancki & Kownacka, 1973) and Simuliidae (Kovachev, 1969) families. Hubenov et al. (2000) gives a checklist of the adult stages of several Diptera families from "Central Balkan", from which 5 families (Limoniidae, Syrphidae Ephydridae, Empididae and Muscidae) are known to have species with aquatic and semi-aquatic larvae stages. Yet there is still no precise knowledge on the aquatic Diptera of the "Central Balkan" National park.

We studied the composition of aquatic Diptera from the surface and groundwater habitats of "Central Balkan" National Park with the aim to give an updated fauna checklist, as well as comment on the Diptera abundance, habitat preference and special distribution.

Material and Methods

Studied area

"Central Balkan" National Parks has a total area of 717 km^2 with 9 Preserves within the Park. From the National Park spring rivers from the Danube watershed including major Danube tributaries such as Vit, Osam, Rosica and Yantra and from the Aegean watershed, including major rivers such as Tundzha. Within the park, these are typical mountain rivers with mostly course substratum, boulders and rock bed sections, running through deciduous forests. Aquatic macroinvertebrates were collected from a total of 23 rivers (Tab. 1), 8 of which were sampled twice – during the summer and autumn period, and from 11 Mountain fountains (Tab. 2).

Table 1. Sampled rivers (2012 – 2016) on the territory of National Park "Central Balkan" with name of sampling sites, river basin date, geographical coordinates and altitude

Site №	Sampling site (River names)	River basin	Date	Geographical coordinates (N- latitude, E- longitude)	Altitude [m]
Rivers from the Northern slope of the Balkan Mountain ("Central Balkan" National Park)					
1	Mominska	Vit	2.07.2015	N42 43.790 E24 21.793	1303
2	Kostina	Vit	28.07.16	N42.82310° E24.33154°	705
3	Zelenika	Vit	30.07.16	N42.82068° E24.61865°	675
4	Zavodna	Vit	7.11.14 and 28.07.16	N42.81050° E24.37322°	730
5	Stara Ribaritsa	Vit	7.11.14 and 28.07.16	N42.78845° E24.42666°	872
6	Cherna	Vit	4.07.15	N42 48.061 E24 28.279	848
7	Golyam Zhidov Dol	Osam	8.11.14	N42 45.840 E24 44.913	778
8	Cherni Osam	Osam	8.11.14	N42 46.000 E24 44.351	764
9	Lyava Vidima	Rosica	9.11.14 and 28.07.16	N42.77753° E24.91950°	707
10	Praskalska	Rosica	9.11.14 and 28.07.16	N42.75707° E24.94217°	911
11	Bagarenshtitsa	Rosica	10.11.14 and 29.07.16	N42.80718° E25.07146°	638
12	Tsarvulshtitsa	Rosica	4.07.15	N42 47.832 E25 04.377	723
Rivers from the Southern slope of the Balkan Mountain ("Central Balkan" National Park)					
13	Vartopa	Topolnica	2.07.15 and 23.11.14	N42 48.605 E24 13.543	926
14	Korudere	Stryama	23.11.14 and 29.07.16	N42.75889° E24.57793°	865
15	Damladere	Stryama	23.11.14	N42 43.799 E24 21.762	1306
16	Korfiyska	Stryama	2.07.15	N42 45.323 E24 36.160	923
17	Malkata	Stryama	22.11.14	N42 43.099 E25 10.405	714
18	Byala	Stryama	23.11.14	N42 45.610 E24 34.652	872
19	Tundzha	Tundzha	21.11.14 and 29.07.16	N42 39.079 E24 54.523	1040
20	Tazha (1)	Tundzha	21.11.14	N42 39.966 E24 58.553	917
21	Tazha (2)	Tundzha	3.07.15	N42 40.059 E25 02.825	567
22	Kademliyska	Tundzha	3.07.15	N42 44.519 E25 02.573	1698
23	Gabrovnitsa	Tundzha	19.10.14	N42 39.490 E24 57.706	586

Table 2. Sampled fountains (July, 2016) on the territory of National Park "Central Balkan" with name of sampling sites, river basin date, flow rate geographical coordinates and altitude

Site №	Sampling site (River names)	River basin	Date	Flow rate [l/s]	Geographical coordinates (N- latitude, E- longitude)	Altitude [m]
24	Fountain near site 4; scraped from the inside of the wooden spout	Vit	30.7.16	1l/26s	N42.81711° E24.37205°	695
25	Wetted rocks under site 24	Vit	30.7.16			
26	Pipe on the trail to site 5 (filtered water)	Vit	30.7.16	1l/24s	N42.79050° E24.42264°	836
27a	Fountain near Svinska River (trough)	Vit	30.7.16	1l/19s	N42.81943° E24.31128°	838
27b	Fountain near Svinska River (filtered water)	Vit	30.7.16			
28a	Fountain near site 3 (trough)	Vit	30.7.16	1l/7s	N42.83725° E24.62437°	595
28b	Fountain near site 3 (filtered water)	Vit	30.7.16			
29	Fountain near site 5 (filtrated water)	Vit	30.7.16	1l/54s	N42.80120° E24.41994°	752
30	Fountain № 1 near site 10 (trough)	Rosica	28.7.16	1l/4s	-	
31a	Fountain № 2 near site 10 (trough)	Rosica	28.7.16	1l/9s	N42.75764° E24.94204°	878
31b	Fountain № 2 near site 10 (filtrated water)	Rosica	28.7.16			
32	Fountain near site 11 (filtered water)	Rosica	30.7.16	1l/22s	N42.80785° E25.07161°	639
33a	Fountain near site 14 (trough)	Stryama	31.7.16	1l/23s	N42.75836° E24.57763°	864
33b	Fountain near site 14 (filtered water)	Stryama	31.7.16			
34	Fountain near site 18 (filtered water)	Stryama	31.7.16	1l/60s	N42.62922° E24.95179°	644

a.  b. 

Figure 1. Studied fountains in the territory of "Central Balkan" National Park: 1a. sites 24 (wooden spout) and 25 (wetted rocks) near Zavodna River; 1b. site 33(a and b) near Korudere River with placed water filtering net.

Sampling

Samples was carried out during October and November 2014, and July 2015, 2016. A total of 3 habitats were studied – river sections were sampled for reobiotic larvae, fountain troughs for diptera inhabiting small aquatic and semi-aquatic habitats, and filtered outflowing water for diptera associated with groundwater (Fig. 1). For each river a single site, representative for the catchment area, was chosen. The sampling was carried out with hand held, 30 x 30 cm with mesh size 500μm (EN ISO 10870:2012) with an approach in accordance to the "multi-habitat sampling" method, standard EN ISO 16150:2012 adapted for Bulgarian conditions (Cheshmejiev et al. 2011). Through washing of sediment particles and submerged vegetation ten subsamples were gathered from different microhabitats (different substratum, ripples, pools, water mosses, etc.) and collected in a single sample. Fountain outflowing water was filtered using portable nets which were attached to the spouts of the fountains and leaf for 2 to 6 hours. Fountain troughs were scraped for water invertebrates with a round hand net (mesh size, diameter). All samples from fountains are only qualitative, while river samples area relatively quantitative.

On site each samples were fixed with 70% alcohol. In the laboratory the macroinvertebrates were sorted in "the basic hydro-biologic groups" according to Uzunov et al. (2010). For the river samples since using the "multi-habitat sampling" method, a relatively same amount of macroinvertebrates was collected, the abundance in each sample was treated as relative abundance, recalculated as number of individuals per square meter. Portion of the Diptera larvae from the whole macrozoobentos sample were also calculated.

All the gathered Diptera were identified to lowest possible taxonomic level. Larva of Chironomidae, Simuliidae and Dixidae were mounded in permanent slides using Canadian balsam or Swan solution (15 g arabic gum, 50 g chloral hydrate, 3 g glucose monohydrate, 20 ml distillated water and 5 ml glacial acetic acid). For the Chironomidae family several keys were used (Schimd 1993; Celohin 2000; Moller Pillot 2009; 2013a; 2013b; Vallenduk & Moller Pillot 2013; Bitušík & Hamerlik 2014). For the Simuliidae family the Czechoslovakian key of Knoz (1965) and fauna of the Soviet Union of Rubtsov (1990) were used. For obtaining most recent taxonomic nomenclature of the family the world list of Adler & Crosskey (2008) was used. Larvae from the Dixidae family were

identified using Celohin (2000). The rest of the Dipetra families were observed under stereomicroscope (Micros Austria with magnification 10 to 30 times). For their identification families the keys of Rozkošný (1980) and Celohin (2000) were used. All specimens are stored in the Diptera collection of the "Laboratory of hydrological monitoring and saprobiology" for further analysis or revision.

Results and Discussions

The aquatic Diptera taxa list of "Central Balkan" National park includes a total of 84 taxa from 14 families (Annex 1). Since for the most families species identification is not possible using only larva, in many cases genus and above genus taxa are given.

Taxonomic composition

Both Bulgarian species of the Athericidae family (*Atherix ibis* and *Ibisia marginata*) were common along most of the rivers of "Central Balkan"; in five sites both species were present. The species *A. ibis* was rare for the rivers of the National Park and in some countries is proposed as a vulnerable species (Bulánková & Ďuričková, 2009). For the Blephariceridae family two taxa were recorded – the species *Blepharicera fasciata*, a common species for Europe and *Liponeura* sp. Within the genus *Liponeura* there are species endemic for Bulgaria such as *Liponeura komareki* Vimmer, 1916. At this point species identification of the *Liponeura* larvae was not possible and the material is stored further revision.

The Chironomidae family, found in almost all the sites, showed highest biodiversity of all the aquatic Diptera. Of the 51 reported taxa 15 were previously found by Kowancki & Kownacka (1973). (…) species are new for the National park and three taxa are first records for the Bulgarian fauna – *Paramerina divisa* (Walker, 1856), *Microtendipes rydalensis* (Edwards 1929) and *Phaenopsectra* sp.. *Paramerina divisa* was found in three sites on both sides of the Balkan Mountain. The species was identified using Vallenduk & Moller Pillot (2013). *M. rydalensis* was found in three sites, all from the northern slope of "Central Balkan". The larva was distinguished from other species by the pectin epipharyngis (7-9 teeth) and the three media teeth on the mentum (Moller Pillot, 2013b). *Phaenopsectra* sp. was found only in Tundzha River. For Europe there are two species of this genus, both found throughout most of Europe with an unclear taxonomy (Moller Pillot, 2013b). Of the genus *Tvetenia* two taxa were reported for the park, distinguished by the presence (*Tvetenia calvescens* agg.) or absence (*Tvetenia* sp.) of a notch on the media tooth. There are at least three species of the genus *Micropsectra* for "Central Balkan", but further reviewing of the gathered material plus gathering of adults and/or exuvia is needed for a more detailed identification. The taxa *Metriocnemus (Metriocnemus) hygropetricus* agg. refers to a species aggregate in the key of Moller Pillot (2009) which includes *M. (M.) albolineatus* (Meigen, 1818) and *M. (M.) eurynotus* (Holmgren, 1883), more over *M. (M.) eurynotus* (Holmgren, 1883) on its own probably includes at least two undistinguished species (Moller Pillot 2013a). These taxa has been previously reported in high altitude springs near Botev peak (Kowancki & Kownacka, 1973).

There is very limited knowledge for the Dixidae in Bulgaria. It seems that the only reported species is *Dixella amphibia* (De Geer, 1776) (Cooper & Rapp, 1944). This is the first report for *Dixa submaculata* and *D. nebulosa* for the Bulgarian fauna. Both species are

found thought out most of Europe (Wagner & Cobo, 2001). The third taxa, *Dixa sp.* is of interest, since the larva doesn't remind any of the species keyed in Celohin (2000). Further studies are needed to evaluate the biodiversity of this family in the Balkan Mountain.

Four taxa from four genera of the Empididae family were reported in "Central Balkan". In only one site (Tundzha River) two taxa were found together – *Chelifera* sp. and *Hemerodromia* sp. A number of species of the Empidae families have been reported by Hubenov et al. (2000) all of which belonging to the Empinae subfamily while the empidids from our samples belong to the Hemerodrominae and Clinocerinae subfamilies, know to include species with aquatic and semi-aquatic larvae stages (Celohin 2000).

The Limoniidae family was very difficult to identify using only larva – we report only three taxa, but jugging on the larva morphology there are at least six more species in our samples. Hubenov et al. (2000) report the species *Hexatoma (Hexatoma) bicolor* (Meigen, 1818) in the territory of the Dzhendema Preserve from which spring Tazha and Tundzha Rivers (sites 19 and 21) in which we found larvae of *Hexatoma* sp..

Of the large family of the Muscidae we found only single individuals of two taxa in a single fountain, site 26 (a and b) – *Limnophora* sp. from the trough of the fountain and Cf *Lispe* sp. from the filtered water sample. Hubenov et al. (2000) report *Limnophora riparia* (Fallen, 1824) in the territory of Kozya stena Preserve, the boarder of which is roughly 17 km away from site 26. Both taxa were identified using Celohin (2000), but since there are tens of species and genera with unknown larvae stages; we strongly note that a review of the material and further study on this family in the Balkan Mountain is needed.

Of the Rhagionidae family the genus *Chrysopilus* sp. has typically aquatic larvae (Celohin 2000; Krivosheina 2007). The individuals found in river habitats (site 5) probably belong to this genus, but the specimens found in fountains could be larvae of semi-aquatic or terrestrial species. This is why further studies are needed to evaluate the biodiversity of this family in the Balkan Mointain. Of the Stratiomyidae family two taxa were found – one individual of a typically aquatic taxa (*Oxycera* sp.) was found on the wetted rock just after a mountain fountain near Zavodna river (site 25) and *Beris* sp. (site 23) which have semi-aquatic larva stage (Nartshuk 2009). For Tundzha River Borisova et al. (2013) report *Beris* sp. and Stratiomydae g. sp. from a river section near the boarders of the "Central Balkan" National Park.

The last six taxa were identified only to family level – Psychodidae, Tipulidae, Thaumaleidae, Ceratopogonidae, Pediciidae, and Tabanidae. The individuals of the Pediciidae family found in fountain troughs (Pediciidae g. sp. 2) were very different from the individuals found in river habitats (Pediciidae g. sp. 1) and most probably the larvae belong to different species with semi-aquatic and aquatic stages, respectively. We suppose that there are at least several species of the Psychodidae, Ceratopogonidae and Tipulidae families, while the rest probably include one, or two species. For these groups furthers studies on the imago can enrich the knowledge of the biodiversity of the water habitats in the Balkan Mountain.

Special Distribution

The two taxa of the Athericidae family were found on both slopes of the Balkan Mountain, in all the studied watershed, and *A. ibis* and *I. marginata* can be considered as common species for the park. The Blephariceridae family was rarer and while *Liponeura*

sp. was found on both slopes (in three watersheds), *B. fasciata* was recorded only in rivers from the northern slope of the Balkan. In some rivers the two taxa were found together (for sites 3 and 11).

For the Chironomidae family, among the most common taxa were *Brillia bifida*, *Micropsectra* sp. *Parametriocnemus stylatus*, *Tvetenia calvescens* agg. and *Tvetenia* sp. A total of 18 taxa were found only in single sites and can be considered rare for the National Park – *Phaenopsectra* sp., *Pseudochironomus prasinatus*, *Pseudodiamesa nivosa*, *Nilotanypus dubius*, etc. Only some taxa were confined to single watersheds *Limnophyes* sp. *Tanytarsus* sp. and *Paratanytarsus* sp for Vit River watersheds. It is interesting to note that *Tanytarsus* sp. was found in all three habitats near Stara Ribaritsa River (the adjacent fountain trough, its filtered outflow and the river itself) showing a clear confinement to this stream within the National Park. Some species were found in several watersheds but only on one slope of the Balkan, like *Potthastia longimanus* (found in the Vit and Stryama watersheds both flowing on the northern slope). However most of the chiros showed no restrictions in their distribution and for the taxa found only in single sites we believe there are probably more widely distributed, although rare for the Balkan Mountain water habitats. A more précised identification using adults and/or exuvia would give a more accurate image of the distribution of the Chironomidae family.

The Dixidae family was on a whole rarely found. Our data suggests a special distribution of the species *D. nebulosa* and *D. submaculata*, but since both this species are cosmopolites for Europe, there are probably distributed in all of the studied watersheds.

The Empididae family was relatively common, found in less than half the sites. The taxa *Dolichochephala* sp. and *Hemerodromia* sp. were rare, found only on single sites, while *Wiedemannia* sp. and *Chelifera* sp. were more common, however only in the sites from the northern slope of the Balkan. For the southern slope, the Empididae family was reported only of Tudzha River, interestingly the only site were two taxa (*Chelifera* sp. *Hemerodromia* sp.) were found together. This suggests that this river exhibits specific conditions suitable for these aquatic larvae, but since the Empididae family is a rare element of the macrozoobenthos it is probably more widely distributed along the park's rivers.

Abundance

As a generally observed rule, for the mountain rivers of Bulgaria only the Chironomidae and Simuliidae families can show high abundance and dominance in the macorozoobethos community, while the rest of the Diptera family show considerably lower abundance. The abundance of a given species in the water is strictly related to the patterns of its life cycle. Since we examined only several sites during both summer and autumn period we don't have enough data to comment on the cyclic patterns of the different diptera larvae, but can only present rough data on their abundance.

The Athericidae family had high abundance in site 10 (for *A. ibis*) during the summer period and in sites 15 and 18 (for *I. marginata*). In Damladere River (site 15) *I. marginata* dominated the macrozoobenthos (31% of the total abundance) while in Byala River (site 18) the species was subdominant. For the rest of the sites the species exhibited low abundance. In Praskalska River (site 10) *A. ibis* showed very low abundance during the autumn period (2 ind. per m^2) in oppose to the high abundance during summer. For

Tundzha River (site 19) *I. marginata*, on the contrary, showed low abundance during the summer period and high abundance during the autumn. In other sites like 9 and 11, *I. marginata* had more or less the same relative abundance for the summer and autumn period.

Table 3. Relative abundance (RA) in ind/m^2 of the aquatic Diptera from rivers of the Balkan Mountain with portion from the whole macrozoobentos sample in % (value in font bold if the Diptera group is subdominant and in font bold and underlined if the group is dominant) and number of taxa (NT) for each sample.

Site №	1(a)	2(s)	3(s)	4(s)	4(a)	5(s)	5(a)	6(s)	7(a)	8(a)	9(s)	9(a)	10(s)
RA [ind/m^2]	48	369	164	204	6	90	7	319	86	42	82	34	216
Diptera %	8%	19	10%	17%	2%	12%	2%	**42%**	13%	12%	14%	13%	18%
NT	16	21	12	16	3	13	4	22	11	6	12	6	12
Site №	10(a)	11(s)	11(a)	12(s)	13(s)	13(a)	14(s)	14(a)	15(a)	16(s)	17(a)	18(a)	19(s)
RA [ind/m^2]	65	424	39	385	96	140	124	50	86	72	126	128	344
Diptera %	15%	30%	12%	**22%**	9%	8%	12%	7%	**42%**	7%	13%	**21%**	-
NT	11	18	6	20	14	13	9	7	8	17	15	3	26
Site №	19(a)	20(a)	21(s)	22(s)	23(a)								
RA [ind/m^2]	121	16	449	42	5								
Diptera %	9%	1%	**31%**	10%	1%								
NT	13	5	16	5	4								

The Chironomidae family showed great abundance in 8 sites (2, 4, 6, 10, 11, 12, 19 and 21). It should be noted that high chiro abundance was observed only in the summer period as opposed to the autumn period when the family although still present, showed considerably lower abundance. In sites 2, 4 and 11 the taxa *Tvetenia* sp. was dominant, while in site 19 *Polypedilum* gr. *convictum* dominated, with subdominant *Tvetenia calvescens* agg. For site 10 *Micropsectra* sp. was dominant. For the sites 6, 12 and 21 members of the Tanypodinae subfamily dominated – *M. nebulosa* for 12 and *Conchapeopia* sp. agg. for site 6 and 21. In the last to sites the chiros dominated in the macrozoobenthos community (33 and 29% respectively of the total abundance).

The Simuliidae family showed low abundance for the rivers of the Balkan Mountain with only the exception of Tsarvushtitsa River (site 12). There the family had the abundance of 181 ind./m^2, with *S. (S.)* Cf. *argyreatum* dominating in the family. The rest of the Diptera families showed low abundance and probably appear sporadically in the macrozoobenthos community. Our results show that in "Central Balkan" the Chironomidae family can reach high abundance in the summer period and therefore is an important element of the aquatic macroinvertibrate and as a potential source of food for the fish populations in these rivers. This is especially true for the Tanypodinae subfamily, larvae of which can reach large sizes.

Habitat preference

Six families showed clear tendency to inhabit only **river sections** – the Athericidae, Blephariceridae, Empididae, Psychodidae, Simuliidae and Tabanidae families. The Ceratopogonidae, Dixidae and Pedicidae families were also predominantly found in rivers.

Of the Chironomidae several taxa like *Tvetenia calvescens* agg. *Potthastia longimanus* and *Polypedilum* gr. *convictum* were occasionally found only in river sections. There are other chiro taxa found only in rivers but the material is from too few localities in order to show a clear habitat preference. Of the Limoniidae family the taxa *Hexatoma* sp. and *Antocha (A.) alpigena* were found only in rivers.

Seven families were found in both rivers and **fountain troughs**. In two sites the subfamily Forcipomyiinae g. sp. was recorded, which includes species with semi-aquatic and terrestrial larvae (Celohin, 2000). Of the Chironomidae some taxa like *Brillia bifida*, *Conchapelopia* agg., *Corynoneura* sp. and *Tvetenia* sp. were predominantly found in rivers and only exceptionally found in fountains. On the other hand taxa like *Microspectra* sp. *Prodiamesa olivacea* and *Macropelopia nebulosa* (Meigen, 1804) were a more common element in the sampled troughs. It seemed that *Pr. olivacea* was more common in fountain troughs that in mountain river sections, which can be related to the fact that the species preference slow flow with decomposing organic material (Moller Pillot 2013a). The Tipulidae family was one of the most commonly found in fountain troughs, however further studies are needed to evaluate whether terrestrial or truly aquatic Tipulidae inhabit this habitat. Families that were more really found in general along the waters of "Central Balkan: such as the Rhagionidae and Thaumaleidae were found both in rivers and in troughs.

Ceratopogonidae, Chironomidae, Limoniidae and Muscidae families were found in **filtered outflows.** The Muscidae family showed a tendency to "avoid" river habitats and the only found specimens were from a single fountain (site 27a and b). Of the Limoniidae family the taxa *Dicranomyia* sp. was found only in fountain troughs and filtered water. From the Chironomidae family several species where found in filtered outflow samples, with three species were found only in such samples (*Macropelopia notata*, and two taxa of the genus *Metriocnemus*). *M. notata* is known to inhabit spring water (Vallenduk & Moller Pillot 2013) and the majority of the species from the genus *Metriocnemus* inhabit terrestrial and semi-aquatic habitats (Moller Pillot 2013a). Another species of this genus *M. (M.) cavicola* which lives in association with wetted wood (Moller Pillot 2009; 2013a) was found only in site 24 on the inside of a wooden water spout (Fig. 1a). The species *Parametriocnemus stylatus* was a common element of the outflow samples, but was found in several rivers as well and showed a plasticity to live both habitats. Taxa like *Corynoneura* sp. and *Limnophyes* sp. also were common both in rivers and fountain outflows, but these taxa probably include more than one species. Other species like *Rheocricotopus (R.) effusus* were more common in filtered fountain water then in sampled rivers. In the sampled groundwater the most common chiro subfamily were the Orthocladinae. In several sites small predatory chiros such as *Zavrelimyia* sp. and *Larsia* sp. where also present and in their bodies head capsules of chiros where noticed, meaning that these taxa successfully feed on other diptera in the interstecial habitat.

Conclusion

In the waters of "Central Balkan" National Park, the aquatic diptera are presented in high biodiversity, utilizing different aquatic habitats and sometimes reaching significant abundance. Several of the reported species are first records for the Bulgarian fauna and we suspect there is still a lot of unknown and unreported species from the aquatic diptera

group. More data is needed, especially samples of pupa and/or imago stages to evaluate the biodiversity of this group. A more extensive number of records will also help truly understands the habitat preference of these species and their tendency to inhabit groundwater.

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Annex 1. List of the aquatic Diptera of the Mountain Rivers and fountains of "Centralen Balkan" National Park and "Balgarka" Nature Park; site codes as in Annex 1; habitat types –rivers (riv.), fountain troughs (f.), filtered water (fil.) and other habitats (oth.); * species new for the Bulgarian fauna.

Taxon	Sites code	Habitat type
Family Athericidae (2 taxa)		
Atherix ibis (Fabricus, 1798)	3, 6, 7, 10, 11, 13, 14, 16, 20, 23	riv.
Ibisia marginata (Fabricius, 1781)	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 21, 23	riv.
Family Blephariceridae (2 taxa)		
Blepharicera fasciata (Westwood, 1842)	3, 11	riv.
Liponeura sp.	3, 9, 11, 12, 22	riv.
Family Ceratopogonidae (2 taxa)		
Cf *Culicoides* sp.	31b	fil.
Forcipomyiinae g. sp.	19, 27a, 31a	riv. f.
Ceratopogonidae g. sp.	6, 7, 12, 13, 14, 16, 19, 21	riv.
Family Chironomidae (51 taxa)		
Boreoheptagyia legeri (Goetghebuer, 1933)	2, 6, 11, 16	riv.
Brillia bifida (Kieffer, 1909)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 17, 19, 22, 28a, 30	riv., f.
Brillia longifurca Kieffer, 1921	4, 6, 19, 21	riv.
Chaetocladius sp.	24, 27a	f. oth.
Conchapelopia sp. agg.	1, 6, 10, 12, 14, 31a	riv. f.
Conchapelopia sp.	2, 4, 5, 6, 10, 13, 19 21	riv.
Corynoneura sp.	2, 5, 7, 11, 13, 15, 16, 17, 26, 27b, 29, 30, 31b	riv. f. fil.
Cricotopus (Cricotopus) trifascia Edwards, 1929	4	riv.
Diamesa (Diamesa) latitarsis (Goetghebuer, 1921)	21	riv.
Diamesa (Diamesa) sp.	22	riv.
Epoicocladius ephemerae (Kieffer, 1924)	17	riv.
Eukiefferiella sp.	1, 5, 7, 9, 10, 11, 15, 17, 21, 22, 28a, 31b	riv. f. fil.
Heleniella sp.	1, 19, 24	riv. f. oth.
Krenosmittia sp.	16	riv.
Larsia sp.	3, 2, 9, 10, 21b	riv. fil.
Limnophyes sp.	2, 29	riv. f.
Macropelopia nebulosa (Meigen, 1804)	10, 11, 12, 19, 21, 27a, 33a	riv. f.
Macropelopia notata (Meigen, 1818)	31b	fil.
Macropelopia sp.	6	riv.
Metriocnemus (Metriocnemus) cavicola Kieffer, 1921	24	oth.
Metriocnemus (Metriocnemus) hygropetricus agg. (following Moller Pillot 2009)	28b	fil.
Metriocnemus (Metriocnemus) hirticollis agg. (following Moller Pillot 2009)	33b	fil.
Metriocnemus sp.	2, 9	riv.
Micropsectra sp.	2, 4, 5 6, 9, 10, 11, 12, 19, 21, 27a, 30, 33a	riv. f.
Microtendipes rydalensis (Edwards 1929)*	3, 9, 11	riv.
Nilotanypus dubius (Meigen, 1804)	6	riv.
Orthocladius sp.	10, 13, 21	riv.
Orthocladius (Symposiocladius) lignicola Kieffer, 1914	5	riv.
Paracladopelma laminatum agg.	12, 19	riv.
Parakiefferiella sp.	6, 27a	riv. f.
Paramerina divisa (Walker, 1856)*	12, 19, 21	riv.
Parametriocnemus stylatus (Spaerck, 1923)	2, 4, 6, 7, 10, 12, 14, 17, 19, 26, 27b, 28a, 31b, 32	riv. f. fil.

Paratanytarsus sp.	4, 5	riv.
Paratrissocladius excerptus (Walker, 1856)	10, 19	riv.
Parorthocladius nudipennis (Kieffer, 1908)	13, 21	riv.
Phaenopsectra sp.	19	riv.
Polypedilum Cf. *tritum* (Walker, 1856)	3, 6, 9, 12, 21	riv.
Polypedilum gr. *convictum*	2, 4, 5, 9, 10, 11, 19	riv.
Polypedilum gr. *laetum*	3	riv.
Polypedilum sp.	30	f.
Potthastia longimanus Kieffer, 1922	4, 10, 11, 12	riv.
Prodiamesa olivacea (Meigen, 1818)	2, 30, 33a	riv. f.
Pseudochironomus prasinatus (Staeger, 1839)	9	riv.
Pseudodiamesa nivosa (Goetghebuer, 1928)	1	riv.
Rheocricotopus (Rheocricotopus) effusus (Walker, 1856)	12, 19, 26, 27b, 31b	riv. fil.
Rheocricotopus (Rheocricotopus) fuscipes (Kieffer, 1909)	13, 17	riv.
Synorthocladius semivirens (Kieffer, 1909)	6, 19, 21	riv.
Tanytarsus sp.	5, 26, 28a	riv. fil.
Thienemanniella clavicornis agg.	2, 4, 10	riv.
Tvetenia calvescens agg.	1, 6, 9, 10, 11, 12, 15, 16, 19	riv.
Tvetenia sp.	1, 2, 3, 4, 5, 10, 11, 17, 20	riv. f.
Zavrelimyia sp.	17, 30	riv. f.
Orthocladinae g. sp.	8, 11, 18, 27a, 28b, 29, 30, 32	riv. f. fil.
Tanypodinae gen. sp.	1, 7, 16	riv.
Tanytarsini gen. sp.	1, 13, 16	riv.
Family Dixidae (3 taxa)		
Dixa nebulosa Meigen, 1830*	19	riv.
Dixa submaculata Edwards, 1920*	10, 24	riv. oth.
Dixa sp.	2, 6, 7, 11, 16	riv.
Family Empididae (4 taxa)		
Chelifera sp.	2, 10, 9, 11, 19	riv.
Dolichocephala sp.	4	riv.
Wiedemannia sp.	2, 5, 11, 12	riv.
Hemerodromia sp.	19	riv.
Family Limoniidae (3 taxa)		
Antocha (Orimargula) alpigena (Mik, 1883)	7	riv.
Dicranomyia sp.	33a, 34	f. fil.
Hexatoma sp.	1, 2, 6, 16, 19, 21	riv.
Limoniidae g. sp.	1, 4, 6, 7, 10, 12, 13, 15, 16, 19, 21	riv.
Muscidae (2 taxa)		
Limnophora sp.	27a	f.
Cf *Lispe* sp.	27b	fil.
Family Pediciidae		
Pediciidae g. sp. 1	1, 2, 4, 6, 10, 13 14, 15, 17, 19, 20, 21	riv.
Pediciidae g. sp. 2	33a	f.
Family Psychodidae		
Psychodidae g. sp.	2, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 19	riv.
Family Rhagionidae		
Cf *Chrysopilus* sp.	5, 27a	riv. f.
Family Simuliidae (9 taxa)		
Prosimulium sp. (*exuvia*)	21, 22	riv.
Simulium (Hellichiella) latipes (Meigen, 1804)	6, 16	riv.
Simulium (Nevermannia) angustatum (Rubtsov, 1956)	1	riv.
Simulium (Nevermannia) brevidens (Rubtsov, 1956)	13, 19	riv.
Simulium (Nevermannia) cryophilum (Rubtsov, 1959)	1, 10, 12, 13, 17, 23	riv.
Simulium (Simulium) Cf. *argyreatum* Meigen, 1838	1, 8, 9, 12, 13, 14, 16, 17, 18, 19, 20	riv.
Simulium (Simulium) trifasciatum Curtis, 1839	16	riv.
Simulium (Simulium) sp.	12	riv.

Simulium sp.	1, 13, 21, 22	riv.
Simuliidae g. sp.	8, 9, 12, 13, 15, 19, 20	riv.
Family Stratiomyidae		
Beris sp.	23	riv.
Oxycera sp.	24, 25	oth.
Family Tabanidae		
Tabanidae g. sp.	11, 19	riv.
Family Tipulidae		
Tipulidae g. sp.	1, 2, 3, 4, 6, 7, 9, 12, 10, 13, 14, 16, 17, 19, 24, 25, 27a, 28a, 30, 31a, 33a	riv. f.
Family Thaumaleidae		
Thaumalidae g. sp.	2, 10, 33a	riv. f.