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Diversity of Periphytic Chironomidae on Different Substrate Types in a Floodplain Aquatic Ecosystem

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Abstract: Different types of water bodies in lowland river floodplains represent vital biodiversity havens and encompass diverse microhabitats, which are essential for structuring different macroinvertebrate communities. Chironomidae larvae (Diptera) are an inseparable part of these communities, with their high richness and abundance. In three water body types within the Danube floodplain Kopački Rit in Croatia, over the course of four sampling campaigns, we recorded 51 chironomid taxa in periphyton on macrophytes, twigs, and glass slides. The most diverse were chironomid communities on macrophytes, whilst month-old periphyton on twigs supported the least taxa. *Cricotopus* gr. *sylvestris*, *Dicrotendipes lobiger*, *Dicrotendipes* spp., *Endochironomus albipennis*, *Glyptotendipes pallens* agg., *Polypedilum sordens* and *Polypedilum* spp. were present in all studied microhabitats. The type of substrate is a very important factor influencing Chironomidae diversity and abundance, which was evident in the presence and dominance of *Corynoneura* gr. *scutellata* and *Monopelopia tenuicalcar* in the dense macrophyte canopy epiphyton. Finding pristine floodplains such as Kopački Rit can be very challenging, as such areas are increasingly altered by human activities. Studies of resident species and the extent to which changes in the parent river influence floodplain communities are important for the protection and restoration of the floodplains.

Keywords: chironomid larvae; taxonomic diversity; substrate preference; Danube; floodplain

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1. Introduction

Riverine floodplains and different types of wetlands represent very dynamic and diverse habitats, created by prolonged interactions of water inflow from the parent river, ground water and the terrestrial area [1–3]. The hydrological connectivity of adjacent water bodies to the main river channel can be continuous or alternating, depending on the water level, as flooding occurs only during maximum river water level [1,2,4]. The lower reaches of the Danube and its major tributaries are representative of rivers typical for temperate regions of Europe, with wide meandering river channels that have the potential to create floodplains as natural water retention areas, as in the case of Kopački Rit [1,2]. Such floodplains are comprised of both deep and shallow lentic and lotic water bodies, which can be permanent or temporary. Aquatic and semi-aquatic habitats intermingle with forests, dry terrain and water meadows creating habitats suitable for many invertebrate and vertebrate species, providing shelter, food or spawning areas within these ecosystems [4–6]. Negative impacts on the river, e.g., pollution, riverbed destruction, and invasive species introduction, affect the whole watershed [7]. Floodplains around the world have different characteristics, and threats to their ecosystem can be from river regulation, drainage-basin alterations, deforestation, global climate change and extended drought periods [7]. In Europe, floodplain areas or specific segments can be given forms of protective status due to their uniqueness and sensitivity to anthropogenic activities—such as Nature or National Parks—even if they have been modified to some extent [7]. Organisations such as the Danube River Network of Protected Areas, the International Commission for the Protection of the Danube River, and the WWF have an important part in the protection process. Besides being biodiversity hotspots, floodplains provide a very broad spectrum of ecological services, making them even more important for researchers and the general public [7–9].

High biodiversity indicates the high functional diversity of organisms in the floodplain and complex trophic interactions, among others [10]. One of the key elements in the normal functioning of the floodplain food webs is macrozoobenthos, particularly the early life stages of insects. Among them, the prevalent taxonomic group often standing out in its abundance, species and functional diversity is the dipteran family Chironomidae [11–13]. Chironomid larvae are fascinating organisms, distributed across the globe and adapted to an array of different living conditions [11,14–16]. Even though most of the chironomid taxa are euryvalent [11,17], there are some very tolerant species subject to habitat degradation [11,13,17,18], while some have narrow ecological valences and are found only in pristine environments [15,19]. Previous studies have shown that the whole chironomid community responds to changes in their environment [11,13,17,18], which is why they are becoming one of the basic tools in water-quality assessment projects [17,18,20]. However, this is only one aspect of why hydrobiologists find this group interesting. Their ecological traits enable them to fill many niches and serve as different functional groups in aquatic ecosystems. Feeding on algae, detritus, microorganisms or other invertebrates, while at the same time being preyed upon by other aquatic insects, fish or waterfowl, they link different trophic levels [20–22].

Substrate type can influence the structure of Chironomidae communities, since the larvae often exploit the substrate by boring into plant or animal tissue, mining wood, burrowing into the sediment surface, or attaching themselves to a hard substrate [11,23,24]. Chironomid larvae or pupae inhabit not only sediment, but also periphytic communities developed on natural and artificial substrates [25–27]. Woody debris and aquatic macrophytes are common and ecologically important types of natural substrates in floodplain water bodies [28–30]. Aquatic macrophytes represent complex colonisation substrates for all aquatic invertebrates—including Chironomidae—but especially submerged macrophytes, providing refuge from predators, a source of food, and an oxygen-rich environment [31]. Hence, this substrate type is one of the most suitable for chironomids, supporting their high abundance and diversity [30,32]. Woody debris such as twigs, branches and tree trunks also provide important microhabitats for aquatic invertebrates, offering a food source and shelter from predators [28,29]. However, information about the chironomid communities on this substrate type is still limited, especially in aquatic ecosystems such as riverine floodplains.

The main objective of the present study was to assess the diversity of chironomid larvae communities from periphytic communities developed on different substrates with different structural complexities. Some authors found no significant differences between communities on artificial and natural substrates [33], so we additionally aimed to compare species composition and relative abundance of Chironomidae taxa to better understand the differences between the communities that form upon different types of substrate.

2. Materials and Methods

2.1. Study Area

Kopački Rit is one of the largest preserved Danube floodplain landscapes with a total surface area of 231 km^2, situated in the eastern part of Croatia. The Danube borders the east side of the floodplain, from 1383 to 1410 river km, while the river Drava creates the southern border from 0 to 15 river km (Figure 1). Kopački Rit has been protected as a Nature Park since 1999, but it was first declared and protected as an ecologically important area in the 1960s [6]. Furthermore, one part of the park is listed as a Special

Zoological Reserve. The floodplain is on the Important Bird Areas List and is recognized as an important Ramsar and Natura 2000 area; it also conjoins the Mura–Drava–Danube Biosphere Reserve. A multitude of floodplain water bodies within the park (e.g., lakes, ponds, channels) are changing and transforming under the influence of the Danube—its water level and other characteristics [6,34]. Water from the Danube enters Kopački Rit through several channels, but the main avenue is situated in the southern part of the floodplain in the Special Zoological Reserve, through the channels Hulovo and Čonakut, filling along the way Kopačko Lake. Lake Sakadaš, the furthest point from the main river channel, is the deepest lake (6 m in average) and the point of departure for scientific and tourist boats (Figure 1). On the other side of the embankment surrounding the main floodplain area, there is a network of channels and canals, ponds and fisheries that support diverse communities of flora and fauna.

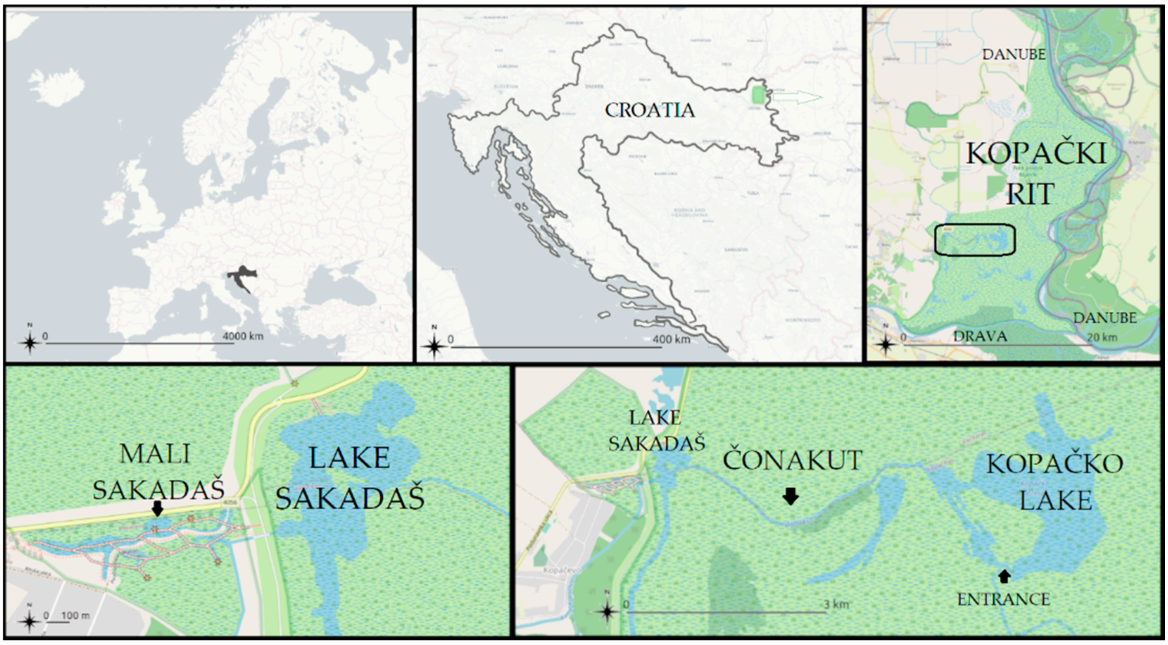


Figure 1. Research locations in the floodplain area of Kopački Rit Nature Park. Top middle, green shape: the geographical position of Kopački Rit in Croatia; top right, green colour: the floodplain area of the Danube; black rectangle: sampling area enlarged on the bottom right; left and right, blue colour: water bodies, green depicts the surrounding semi-aquatic and terrestrial area; water bodies are labelled in black letters.

2.2. Sampling Strategies

Communities of Chironomidae larvae have been studied through various projects, sampling campaigns or *in situ* experiments. Different studies, of which the results are presented here, applied different standard sampling techniques, depending on the substrate and habitat type. Periphytic communities have been studied on an artificial substrate (glass slides in 2008 and 2009) and natural substrates (twigs in 2011 and 2012, and macrophytes in 2013 and 2016).

To collect the data on the community structure and colonisation dynamics of periphytic chironomids on an artificial substrate, glass slides for periphyton development were immersed from April until August in Lake Sakadaš at a depth of 25 cm. The slides were sampled after the first seven days of exposure and afterwards every 14 days. On each sampling date three slides were taken for chironomid analysis and *in situ* placed in bottles with 4% formaldehyde. For a detailed description see Vidaković et al. [35].

Epixylon was studied on willow twigs placed in Lake Sakadaš as part of the *in situ* experiment which included the immersion of twigs (length of 10 cm, diameter 1 cm) to a depth of 20–25 cm, for 5 weeks during three different seasons: summer, late autumn, and spring of the following year. The experiment constructions were placed at three sites in Lake Sakadaš. For invertebrate community analysis, three twigs were sampled and preserved in 4% formaldehyde. For a detailed description see Mihaljević et al. [36].

Epiphytic chironomids were sampled in two different types of macrophyte associations. One was a dense, thick layer of floating macrophytes, *Salvinia natans*, *Spirodela polyrhiza*, *Lemna* sp. and, sporadically, submerged *Ceratophyllum demersum*, formed and sampled alongside three locations: the entrance to Kopačko Lake, the Čonakut channel and the entrance to Lake Sakadaš (listed as epi I). The second association type was sampled in the Mali Sakadaš pond. It represents a typical pond macrophyte association and includes different contributions of *Nymphoides peltata*, *Nymphaea alba*, *S. natans*, *Typha* sp., *Hippuris* sp., *C. demersum* and *Utricularia vulgaris* (listed as epi II). Apart from Lake Sakadaš, during the epi I research there were three sampling sites at each location. Macrophytes were sampled within a surface area of 50 × 50 cm, marked by a wooden frame. Triplicate samples were carefully removed from the water to avoid loss of organisms and preserved in 96% ethanol.

2.3. Periphyton

The starting point of the laboratory work was specific for each type of periphyton. In the experiment with the artificial substrate, periphyton was scraped from both sides of the glass slides and collected in a beaker. A similar procedure was applied for epixylon, namely cleaning the surface of the whole twig. Macrophytes were thoroughly rinsed and cleaned on a sieve over white trays to ensure that all organisms were collected. In all samples, the removed remains were rinsed above a sieve and prepared for the separation and isolation of larvae from the rest of the periphyton under stereoscopic microscopes (Carl Zeiss Jena, Olympus SZX9). Chironomidae larvae were prepared for identification either in the form of temporary native slides—in a drop of ethanol—or as a permanent slide mounted in Berlese medium. A microscope (Olympus BX51) and the following identification keys were used to identify the species and genera of Chironomidae: Schmid [37]; Vallenduuk and Moller Pillot [38]; Bitušík [39]; Bitušík and Hamerlík [40]; Andersen et al. [14]; and Vallenduuk [13].

2.4. Statistical Analysis

After the data on Chironomidae community structure were collected, applying diverse methods to enable comparison between different communities, we calculated, for each sample, relative abundances as the number of individuals of a given taxon divided by the total number of individuals collected in the sample. PRIMER 6 software [41] was applied for multivariate statistical analyses. Non-metric multidimensional scaling (NMDS) was used to present the relations between the chironomid communities from different substrates and analysis of similarity (ANOSIM) was applied to identify the significance of differences between substrates. These methods were applied to the Bray–Curtis similarity matrix based on the square root-transformed relative abundance data. The contribution of Chironomidae taxa to the average dissimilarity between groups was assessed using the SIMPER analysis. For every sample in each substrate type, we calculated the following diversity indices as a biotic metric: species richness (S), Shannon index (H'), and Simpson index (1-lambda). To test whether chironomid communities of different substrate types (epi I, epi II, twigs, and glass slides) differed in S, H' and 1-lambda, Kruskal–Wallis tests followed by Mann–Whitney tests were applied. These analyses were performed using SPSS version 19.0 software.

3. Results

3.1. Diversity of Periphytic Chironomidae

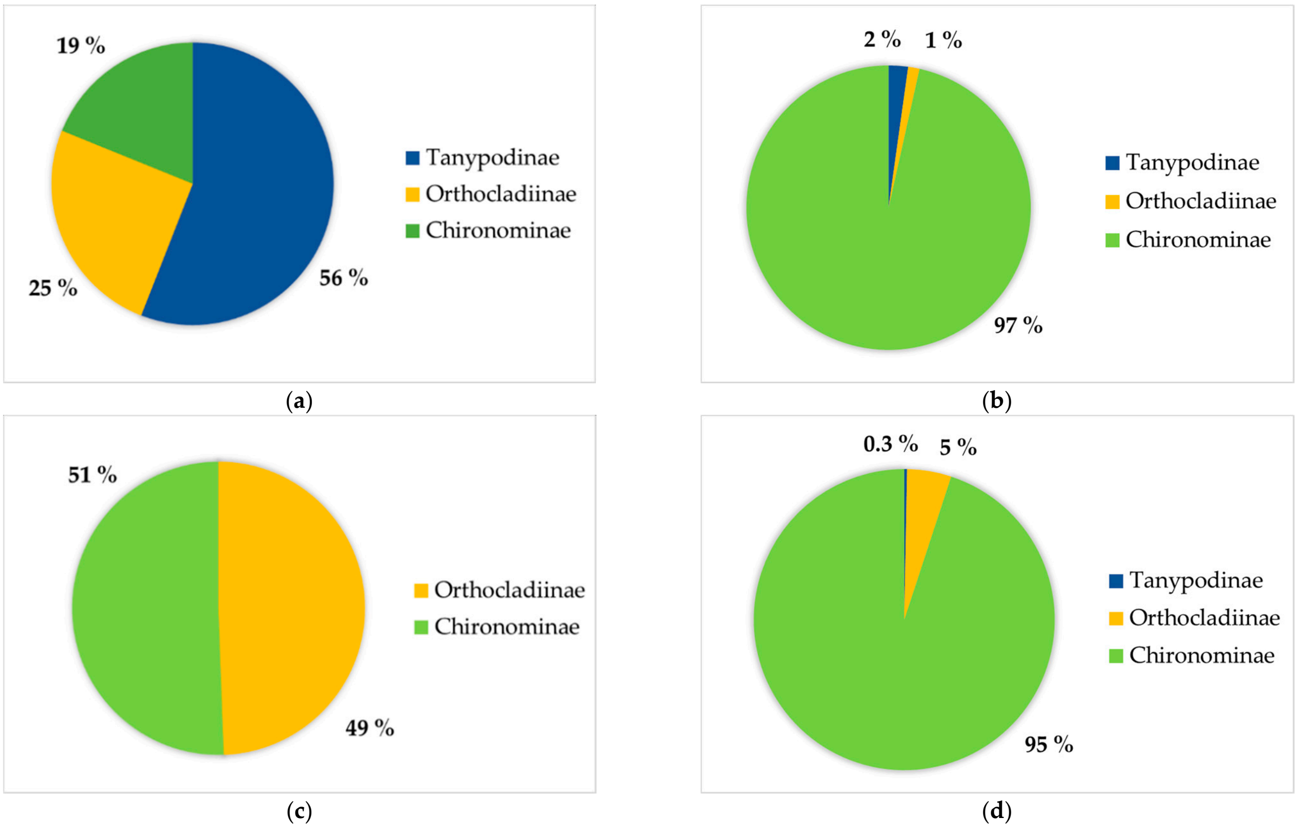
In all sampled communities, 51 Chironomidae taxa were recorded, belonging to three subfamilies: Tanypodinae, Orthocladiinae and Chironominae (Table 1). In the epixylon, only Orthocladiinae and Chironominae larvae were recorded. In the periphyton on the glass slides, Tanypodinae represented less than 1% of the community and these larvae were too young to be identified even to the genus level (Figure 2, Table 1). The most diverse was tribe Chironomini, including 32 different taxa belonging to eight genera. The Tanytarsini tribe was represented with only two genera, *Paratanytarsus* and *Tanytarsus*. Five genera and the *Cricotopus*/*Orthocladius* taxon represented the Orthocladiinae subfamily (Table 1).

Table 1. Relative abundance of Chironomidae taxa in periphyton on all substrate types.

Taxa/Substrate	**Epiphyton I**		**Epiphyton II**		**Twigs**		**Glass Slides**	
	Range	**Average (N = 25)**	**Range**	**Average (N = 9)**	**Range**	**Average (N = 24)**	**Range**	**Average (N = 19)**
Tanypodinae								
Ablabesmyia (*Ablabesmyia*) *longistyla* Fittkau, 1962	0–1.96	0.29	0–0.46	0.05				
Ablabesmyia (*Ablabesmyia*) *monilis* agg.	0–1.39	0.06	0–2.16	0.32				
Ablabesmyia spp.	0–0.32	0.01	0–0.46	0.05				
Conchapelopia agg.			0–0.01	0.001				
Monopelopia tenuicalcar (Kieffer, 1918)	27.81–79.49	55.96						
Tanypodinae non det.			0–11.70	1.78			0–5.59	0.29
Orthocladiinae								
Chaetocladius spp.	0–3.97	0.58						
Corynoneura gr. *scutellata*	0–45.77	19.49					0–0.74	0.04
Corynoneura spp.			0–1.49	0.23				
Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)	0–0.20	0.01						
Cricotopus (*Isocladius*) *intersectus* agg.	0–14.81	0.99			0–100	25.05	0–5.90	0.50
Cricotopus (*Isocladius*) gr. *sylvestris*	0–18.17	4.20	0–3.68	1.01	0–100	11.36	0–8.07	1.60
Cricotopus spp.	0–0.65	0.03			0–14.29	1.92	0–0.74	0.08
Cricotopus/Orthocladius spp.					0–58.33	4.63		
Nanocladius gr. *dichromus*	0–0.65	0.03						
Psectrocladius (*Psectrocladius*) *limbatellus* (Holmgren, 1869)			0–0.27	0.04				
Psectrocladius (*Psectrocladius*) gr. *sordidellus*					0–1.59	0.07		
Orthocladiinae non det.					0–100	6.39	0–50	3.00
Chironominae								
Chironomus (*Chironomus*) *annularis* agg.			0–0.42	0.05				
Chironomus (*Chironomus*) *luridus* Strenzke, 1959			0–1.60	0.57				
Chironomus (*Chironomus*) *plumosus* agg.			0–2.93	0.44			0–2.63	0.14
Chironomus (*Chironomus*) *tentans* Fabricius, 1805			0–0.91	0.10				
Chironomus (*Lobochironomus*) *dorsalis* Meigen, 1818			0–17.07	3.73				
Chironomus spp.	0–2.47	0.44	0–29.07	13.56				
Dicrotendipes lobiger (Kieffer, 1921)	0–2.30	0.12	3.25–21.64	10.33	0–3.13	0.25	0–10.81	0.57
Dicrotendipes modestus (Say, 1823)			0–7.33	1.27				
Dicrotendipes nervosus (Staeger, 1839)	0–1.85	0.14			0–50	6.07	0–56.72	9.76

Table 1. *Cont.*

Taxa/Substrate	Epiphyton I		Epiphyton II		Twigs		Glass Slides	
	Range	Average (N = 25)	Range	Average (N = 9)	Range	Average (N = 24)	Range	Average (N = 19)
Dicrotendipes notatus (Meigen, 1818)			0–0.21	0.02				
Dicrotendipes pulsus (Walker, 1856)	0–0.95	0.06			0–20	0.97	0–0.57	0.03
Dicrotendipes spp.	0–5.56	0.22	0–2.71	1.33	0–2.38	0.10	0–2.63	0.14
Endochironomus albipennis (Meigen, 1830)	0–15.82	1.33	0–2	0.61	0–4	0.53	0–59.86	6.36
Endochironomus tendens (Fabricius, 1775)	0–5.37	0.77	1.22–9.81	4.73				
Glyptotendipes (*Glyptotendipes*) *barbipes* (Staeger, 1839)	0–4.18	0.22						
Glyptotendipes (*Glyptotendipes*) *pallens* agg.	0–10.54	2.33	4.27–30.96	15.10	0–43.75	13.82	0–61.20	24.74
Glyptotendipes (*Glyptotendipes*) *paripes* (Edwards, 1929)			0–0.54	0.06				
Glyptotendipes spp.	0–11.24	2.47	0–5.26	1.28				
Kiefferulus (*Kiefferulus*) *tendipediformis* (Goetghebuer, 1921)	0–10.74	3.54	0–2.92	1.43				
Parachironomus gr. *arcuatus*	0–1.03	0.05	0–3.24	0.36	0–60	15.20		
Parachironomus gr. *frequens*							0–10.69	0.56
Parachironomus varus (Goetghebuer, 1921)							0–100	15.56
Parachironomus spp.			0–31.10	8.92				
Paratendipes nudisquama (Edwards, 1929)	0–0.32	0.01						
Paratendipes spp.	0–0.17	0.01						
Polypedilum (*Pentapedilum*) *sordens* (van der Wulp, 1875)	0–7.39	1.98	0–4.50	1.09	0–20	2.96	0–21.63	4.76
Polypedilum (*Pentapedilum*) *uncinatum* agg.	0–3.75	0.62	0–0.54	0.06				
Polypedilum (*Polypedilum*) *nubeculosum* (Meigen, 1804)	0–2.17	0.16					0–2.63	0.18
Polypedilum (*Polypedilum*) *pedestre* (Meigen, 1830)							0–18.07	3.13
Polypedilum (*Tripodura*) *scalaenum* (Schrank, 1803)	0–0.26	0.01						
Polypedilum (*Uresipedilum*) *cultellatum* Goetghebuer, 1931	0–2.25	0.16						
Polypedilum uncinatum agg./*cultellatum*			0–0.54	0.06				
Polypedilum spp.	0–14.46	2.65	0–3.73	0.70	0–50	2.51	0–50	3.20
Paratanytarsus spp.	0–5.37	0.99	2.26–23.13	8.81			0–50	5.15
Tanytarsus spp.	0–1.05	0.06	0–12.68	5.18				
Chironominae non det.			2.40–36.57	16.71	0–42.37	8.17	0–100	20.21



(**a**) (**b**)

(**c**) (**d**)

Figure 2. Percentage rate of Chironomidae subfamilies on each substrate type: (**a**) on macrophytes-epi I (epiphyton); (**b**) on macrophytes-epi II (epiphyton); (**c**) on willow twigs (epixylon); (**d**) on glass slides (periphyton).

The richest community type was the epiphyton with 33 chironomid taxa recorded in the first macrophyte study (epi I) and 31 taxa in the second (epi II) (Table 1). One of the important differences among these communities was evident within the subfamily Tanypodinae. *Monopelopia tenuicalcar* was very abundant in all samples and sites in epi I, whereas in epi II (macrophytes in Mali Sakadaš pond) it was not recorded at all, either on glass slides or twigs. In general, macrophytes were the best substrate for Tanypodinae larvae (Figure 2, Table 1). Many taxa were recorded only in the epiphyton, e.g., *Paratendipes* taxa in epi I, or most species of the *Chironomus* genus, which were mainly found in epi II (Table 1). In comparison to the diversity of Chironomidae larvae recorded on macrophytes, periphytic communities on glass slides and twigs were not as rich, comprising 18 and 14 different taxa, respectively. Both communities had high percentages of larvulae that could only be identified to the subfamily level (Table 1). The following species/species groups were recorded on all substrate types: *Cricotopus* gr. *sylvestris*, *Dicrotendipes lobiger*, *Endochironomus albipennis*, *Glyptotendipes pallens* agg., *Polypedilum sordens*, including *Dicrotendipes* spp. and *Polypedilum* spp. Larvae of *Polypedilum pedestre*, *Parachironomus* gr. *frequens* and *Parachironomus varus* were found only on glass slides, whereas *Psectrocladius* gr. *sordidellus* and *Cricotopus*/*Orthocladius* spp. were only characteristic for epixylon (Table 1).

According to the values of taxonomic diversity indices, the most diverse Chironomidae community was found on macrophytes, especially in epi II, while twigs and glass slides supported the lowest diversity (Figure 3).

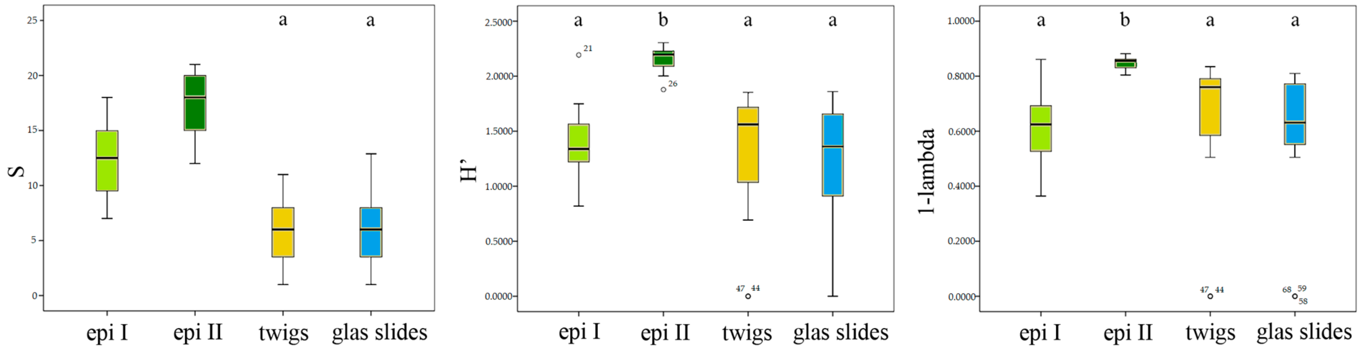


Figure 3. Boxplot representation of diversity indices (species (taxa) richness, S; Shannon index, H′; and Simpson, 1-lambda), across different substrate types. Epi I-macrophyte canopy epiphyton; epi II-pond macrophyte epiphyton; willow twigs; glass slides. Boxes which do not share a common letter are significantly different: a,b at $p < 0.05$ for S, H′, and 1-lambda. If letters are missing, all the boxes are significantly different.

Differences between communities on different substrates were evident in the number of recorded taxa, and the percentage rate of recurrent taxa differed among the substrates. *Corynoneura* gr. *scutellata* displayed a similar trend in appearance and percentage rate as the earlier mentioned *M. tenuicalcar*. *Cricotopus intersectus* agg. and *C.* gr. *sylvestris* from the Orthocladiinae subfamily and *G. pallens* agg. from tribe Chironomini were not only more frequently recorded, but they had a higher relative abundance (Table 1).

3.2. Statistical Analysis

All diversity indices differed between different substrate types (Figure 3). Species richness significantly varied among all substrates except for between twigs and glass slides (Mann–Whitney, $p < 0.05$). Epi II was significantly different in H′ and 1-lambda than all other substrate types (Mann–Whitney, $p < 0.05$).

Differences between the periphytic chironomid communities formed on different substrates were indicated by non-metric multidimensional scaling analysis and ordinated on the NMDS plot (Figure 4). Despite the relatively high stress, the analysis was considered robust by the PRIMER software, i.e., at stress <0.2 the two-dimensional ordination plot can still be considered useful. ANOSIM analysis confirmed the statistical significance of the differences between the communities from different substrate types (Global R = 0.728, $p < 0.001$). Results of the Pairwise tests are given in Table 2. Taxa that contributed the most to the differences among the substrates were indicated using SIMPER analysis (Table 3).

Table 2. Results of the ANOSIM analysis (R statistic values of pairwise tests) showing significant differences between chironomid communities from different substrate types. Results of the pairwise tests are all at $p = 0.001$, with the exception of glass slides vs. epi II at $p = 0.002$. Epi I-macrophyte canopy epiphyton; epi II-pond macrophyte epiphyton.

	Twigs	**Glass Slides**	**epi I**	**epi II**
Twigs				
Glass slides	0.392			
epi I	0.848	0.844		
epi II	0.602	0.360	0.999	

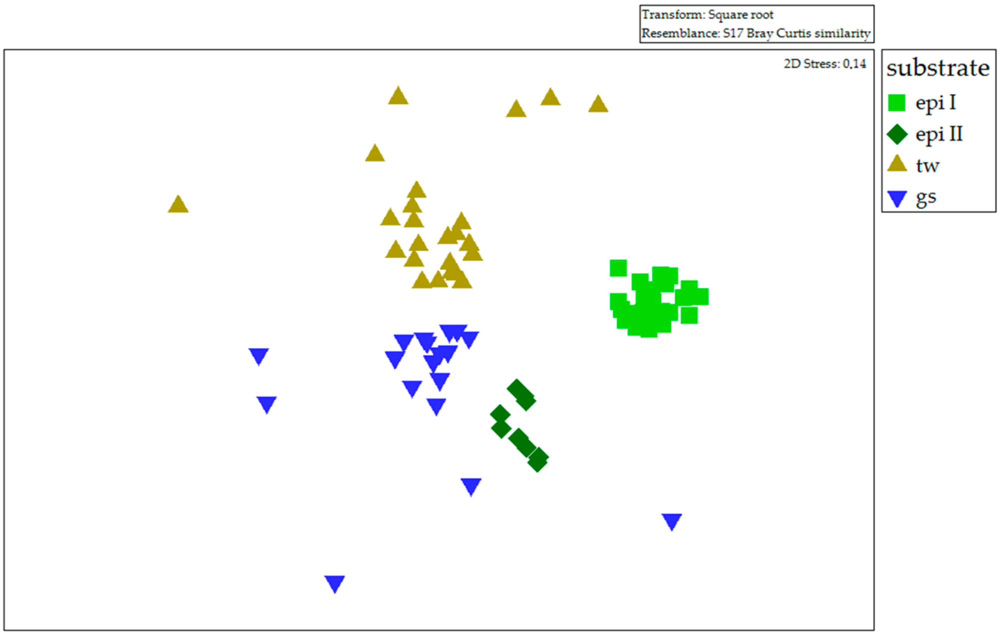


Figure 4. Non-metric multidimensional scaling plot of periphytic chironomid communities from different substrate types based on the relative abundance matrix data. Legend: epi I-macrophyte canopy epiphyton; epi II-pond macrophyte epiphyton; tw-willow twigs; gs-glass slides.

Table 3. Results of the SIMPER analysis showing the contribution of chironomid taxa to dissimilarities between substrate types: epi I-macrophyte canopy epiphyton; epi II-pond macrophyte epiphyton; tw, willow twigs; gs, glass slides.

	Contribution (%)
tw and gs	Average dissimilarity = 78.04
Cricotopus intersectus agg.	12.98
Chironominae non det.	10.37
Glyptotendipes pallens agg.	9.91
Monopelopia tenuicalcar	18.45
Corynoneura gr. *scutellata*	10.14
Cricotopus intersectus agg.	9.83
gs and epi I	Average dissimilarity = 88.53
Monopelopia tenuicalcar	18.79
Corynoneura gr. *scutellata*	10.24
Chironominae non det.	8.96
tw and epi II	Average dissimilarity = 82.64
Cricotopus intersectus agg.	9.39
Chironomus spp.	7.32
Parachironomus gr. *arcuatus*	6.7
gs and epi II	Average dissimilarity = 75.37
Chironomus spp.	8.13
Dicrotendipes lobiger	7.39
Glyptotendipes pallens agg.	7.13
epi I and epi II	Average dissimilarity = 79.28
Monopelopia tenuicalcar	16.03
Corynoneura gr. *scutellata*	8.82
Chironominae non det.	8.37

4. Discussion

The presented results, collected from several studies, allowed us to evaluate the different substrates and the mosaic of diversity in the aquatic communities that they support. Chironomidae larvae, as one of the most abundant, diverse and widely distributed invertebrate groups in aquatic systems of temperate regions, can adequately reflect that diversity [42]. Even though this study represents a “mosaic” of research results, it provides important data on chironomid taxa richness in a floodplain ecosystem.

Kopački Rit, as one of the largest preserved flooding areas of the Danube, harbours great biodiversity and consequently urges us to focus on its protection and conservation [6,32,35]. The park is a part of the Amazon of Europe UNESCO biosphere reserve, and although it is only a fragment of the reserve, it represents a very important component that encompasses a complex network of habitats and hundreds of species, creating a special ecosystem [6]. To better understand it, the study of the biology and ecology of the many communities inhabiting this area, especially aquatic ones, is urgently needed [9]. The main threats to the Kopački Rit floodplain are human activities on the Danube, e.g., pollution, hydromorphological degradation, and embankment. Deepening of the riverbed can lead to a lowering of the groundwater table, which influences at what water level floods enter the floodplain and reduces the overall amount of water available for the entire area [2,7]. Pollution of the Danube has already been detected in the vicinity of urban areas [6,7,18]. At what distance it dwindles and how it affects downstream areas and floodplains can be assessed by monitoring the changes in invertebrate communities, and the presence of tolerant chironomid species in benthic and epiphytic communities [18]. All in all, low biodiversity can indicate degradation of the floodplain ecosystem that motivates protection actions. The described changes and challenges correspond to global problems of floodplain protection and preservation [7]. Finding pristine floodplains such as Kopački Rit can be very challenging in Europe as well as worldwide, as these areas become increasingly altered by human activities. Furthermore, many flooding areas have been detached from the main river channel and have deteriorated over time. In the last decade, there has been much effort to revitalise and restore the already morphologically and hydrologically modified floodplains in Europe, particularly in the Danube and Drava watersheds [7,9]; thus, it is valuable to have data on the biodiversity of preserved ecosystems for comparison in assessment and monitoring projects. Studying the resident species and to what extent the changes in the parent river influence floodplain communities is also important for the protection of remaining intact floodplains.

Our sampling sites were located in the Kopački Rit Nature Park along the main water path of the flood- or flow-pulse from the Danube to the embankment. The connection of the floodplain to the parent river greatly influences all communities, their structure, stability and changes in diversity [43,44]. Concerning this, macrophytes have a varying dynamic of appearance in water bodies of the floodplain area, particularly in the channels leading from the Danube to Lake Sakadaš and in the lake itself. They are constantly present in the floodplain ponds or standing backwater. In the Čonakut channel, after several years, epiphytic communities developed in a dense canopy of floating and submerged macrophytes (epi I). In this community, *Monopelopia tenuicalcar*, which prefers substrates near the surface such as *Lemna* or *Azolla* [13,23], had a high relative abundance, even up to 80% in some samples, but this was not recorded in other communities, not even in epi II. Furthermore, the Orthocladiinae species group *Corynoneura* gr. *scutellata*, which also prefers this type of microhabitat [24] was quite abundant, thereby providing an adequate food source for *M. tenuicalcar*. In 2001 and 2002, in communities developed on macrophytes occurring in the Čonakut channel, Chironomidae larvae were the dominant taxonomic group contributing from 50 to 83% of the total invertebrate abundance [32]. Unfortunately, we do not have any data on the species composition from that research, which hinders a more detailed comparison and evaluation of the overall indicative values of epiphytic chironomids and supports the requirement for a better identification resolution in ecological studies. It also reflects the need to have as precise identification as possible. Epiphyton sampling activities in the Mali Sakadaš pond (epi II) did not meet all of our expectations regarding chironomid diversity on macrophytes, with a low relative abundance of *Cricotopus* species; however, a higher abundance of *Glyptotendipes pallens* agg., *Dicrotendipes*, *Paratanytarsus* and *Chironomus* species was as per other findings for epiphyton in eutrophic water bodies [30]. As Mali Sakadaš is an isolated pond, such differences could have been expected since epiphytic communities and macrophyte diversity and development depend on the connectivity and fluctuations of water level [44,45]. Moreover, Čerba et al. [21,46] previously recorded differences in Chironomidae epiphytic communities on two different submerged macrophytes, indicating the preferences of some taxa (e.g., *Cricotopus* gr. *sylvestris*, *Endochironomus albipennis*, *G. pallens* agg.) for specific macrophytes, including their architecture, tissue softness, the ability to hold more detritus, etc. [47]. It could then be anticipated that macrophytes that greatly differ in their leaf and stem architecture, or position related to the water surface described herein, display even greater differences in chironomid community composition. Our research confirmed a previously observed positive interrelationship of Chironomidae larvae abundance and diversity with macrophyte diversity, providing a spectrum of available food, microhabitats to inhabit, and shelter from predators [11].

Despite these differences, macrophytes harbour high chironomid diversity. They also influence other communities and the "health" of the entire floodplain ecosystem. Many fish species come from the main river channel to spawn or to find shelter and food in macrophyte-dominated habitats. Furthermore, firstly reported benthos-feeding fish have been found to feed primarily on the epiphytic Chironomidae larvae [48]. High chironomid taxa richness enables the sufficient colonization of various available microhabitats, depending on their specialties, and in turn caters to the different predatory fish that inhabit them. This does not only highlight the importance of macrophytes, but also the information on taxa diversity, which enables us to better understand the functioning of the relationships among different hydrobiocoenoses in the floodplain.

Another natural basis suited for periphyton development that is often available in floodplain water bodies are branches, tree trunks, or woody debris [49]. Depending on the duration of submergence and size of the wooden surface, epixylon includes various taxa [28] and chironomids can be the dominant invertebrate group [27]. Chironomidae larvae living in such communities can be either xylophagous or feed on algae, fungi or biofilm formed on the surface [11,50,51]. In our research we did not find true xylophagous or wood-boring taxa, which could be the consequence of the five-week immersion period of twigs. Nevertheless, Moller Pillot [24] lists decaying wood as one of the various feeding sources of *G. pallens* agg., as well as the utilisation of the woody microhabitat in self-made mines. Furthermore, *G. pallens* agg. can tolerate winter conditions better than many other species and is ubiquitous in floodplains [24]. During late autumn, besides the mentioned larvae, the chironomid epixylon community mostly included *C. intersectus* and gr. *sylvestris* representatives, previously described as good colonisers, cosmopolitan and pioneer species [11,16,46,52]. In other seasons, the submerged willow twigs represented an additional type of substrate in the lake, providing a temporary feeding and resting place for other chironomid larvae moving from surrounding microhabitats.

Even though glass slides are artificial substrates, they harboured a greater variety of Chironomidae than twigs. One of the reasons could be the presence of other invertebrate taxa in the developed periphyton that created a more suitable and heterogenous microhabitat, with bryozoans, sponges, or *Dreissena polymorpha* clusters [35]. The development of a complex autotrophic component [53] further augmented the colonisation of chironomid larvae as they are the main food source for many species, e.g., *E. albipennis*, *G. pallens* agg. and *C.* gr. *sylvestris* [54], including *Polypedilum pedestre* and *P. sordens* that feed on detritus, bacteria, diatoms, and other algae [24]. During this research, we found on more than one sampling occasion several larvae with the front part of their body in the mantle cavity of *D. polymorpha*. Since the larvae belonged to different nonparasitic species [55], we cannot state that this is a species-specific relationship, but rather a good example of how Chironomidae larvae successfully exploit available resources [56,57].

One of the important factors influencing the community structure is the life cycle dynamic, i.e., the number of generations per year and the diapause period [11,22,23,37], which can be partly influenced by environmental parameters such as temperature, as they can, in turn, influence the results if the sampling is conducted just after emergence or at the beginning of substrate colonisation. Early Chironomidae larvae stages, also known as larvulae, cannot be accurately identified to species or even genera level to give unambiguous results. Natural seasonal variability of the chironomid community is evident and a very important element to be taken into consideration when studying this group [58,59], and some of the observed differences between communities were undoubtedly a result of seasonal variability. Nevertheless, differences have been observed in the same season on different substrates.

The practical use of the knowledge on Chironomidae diversity in the floodplain would be a construction of biological metrics. Water framework directive (WFD) has regulations for bioassessment and monitoring procedures for lotic and lentic systems [18,60,61]. However, floodplains have different hydrological regimes, and in order to establish a practical chironomid-based assessment protocol it would be necessary to modify standard WFD protocols and biological indices to create specific ones for such ecosystem. Initial research to create a basic dataset would include the sampling of chironomids in all community types in different water bodies, as well as sampling in different seasons and at different water levels for comparison. Simultaneously, biotic and abiotic environmental parameters should be measured to assess the influence of environmental parameters on the community structure [18,60].

To conclude, we showed that the chironomid community's richness and diversity, as well as the relative abundance of Chironomidae taxa, significantly differed depending on the substrate type. As expected, the richest and most diverse community was found on macrophytes. Surprisingly, twigs supported lower taxa richness than an artificial substrate; however, this could be an artifact due to the short immersion period of the twigs. Even though chironomid larvae are considered simple opportunists, many taxa showed preference and adaptation to microhabitats with specific conditions and food availability, such as feeding in a bivalve mantle cavity; the colonisation of clean substrates; and abundant *C.* gr. *scutellata* and *M. tenuicalcar* larvae in dense macrophyte mats. Since floods are important for the accumulation and development of natural substrates such as macrophytes and wood remains in floodplain water bodies that support a high diversity of aquatic organisms (including Chironomidae), the protection of natural hydrological regimes is essential for biodiversity conservation in this unique and endangered aquatic ecosystem. Constant monitoring of diversity within floodplains can help us to better understand the changes of this ecosystem.

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