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Non-biting Midges (Diptera: Chironomidae) from Continental Salt Marshes in Serbia

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ABSTRACT

Continental salt marshes represent specific inland saltwater bodies with unique ecological characteristics a fact that is mirrored by specific flora and fauna. However, there is still limited data on their biota in Serbia, which is especially true for aquatic macroinvertebrates, including chironomids (Diptera: Chironomidae). Here we investigated diversity and seasonal variations of chironomid community in six salt marshes distributed in the northern and southern part of Serbia. We recorded a total of 25 taxa, of which two are new for the Serbian chironomid fauna. Most of the recorded species are common in freshwaters and six of them are halotolerant. Chironomid community structure fluctuated in relation to the seasons. The highest diversity was recorded in the spring and summer months for the majority of studied salt marshes. Low diversity and dominance of halotolerant taxa were recorded in Pannonian salt marshes in the northern part of the country, suggesting higher salinity of these habitats comparing to the southern ones. The present findings represent the first inventory of chironomid larvae for these unique and highly endangered habitats in Serbia, which can help to fill the gaps in the knowledge of distributional ranges of halotolerant and halophilic species and provide baseline for future surveys.

Keywords: chironomids, taxa richness, saline lakes, soda pans, community structure.

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INTRODUCTION

Continental salt marshes and soda pans represent specific inland saltwater bodies with unique ecological characteristics, which makes them one of the most vulnerable aquatic habitats in Europe (Boros, Escedi, & Oláh, 2014). In Europe, the Carpathian basin is particularly rich in these types of habitats, with natural salt marshes found in Austria, Hungary, and Serbia. In the last two centuries these saline habitats were severely threatened by different anthropogenic activities, mainly by cultivation for agricultural purposes, which led to significant loss of these habitats (Boros et al., 2014). Therefore, the habitat type "Pannonian salt steppes and salt marshes" is listed in Annex I of the EU Habitat Directive (93/43/EEC) and is part of the Natura 2000 Network (Vidaković et al., 2019). In Serbia, the Pannonian salt marshes are located in the northern part of the country, Vojvodina Province. It is estimated that there are no more than 10 natural representatives of these habitat types in Serbia (Tóth et al., 2014) and only three of them are legally protected (Gavrilović et al., 2018). Besides these types of salt marshes, a few isolated continental salt marshes are patchily distributed in the valleys of the Južna Morava and Toplica rivers in southern Serbia (Zlatković, Zlatković, Ranđelović, Jenačković, & Amidžić, 2014). In phytogeographical sense they differ from salt marshes in Pannonian region. Also, saline water bodies in southern Serbia are much smaller than Pannonian salt marshes and soda lakes. Saline habitats in southern Serbia are highly threatened by different anthropogenic activities (e.g., agricultural production, draining, waste disposal) and most of them are included in protected areas of local importance (Zlatković, Jenačković, & Ranđelović, 2019).

Continental salt marshes encompass various types of lentic water bodies ranging from small stagnant waters to large lakes, but the common feature of all types of these saline water bodies is their shallow depth. Besides the shallowness, some of the common features of the continental salt marshes are high conductivity with mainly Na^{+}-HCO_3^{-} ionic dominance, high water pH value (9-10), high seasonal water-level fluctuations, and high daily and seasonal temperature variations (Lengyel et al., 2016). Some other environmental parameters, such as salinity, can also show strong annual variations, changing over hypo-, meso- and hypersaline limits (Boros et al., 2014). Thus, continental salt marshes are inhabited by unique flora and fauna that can tolerate extreme environmental conditions which gives high conservation value to these habitats (Gavrilović et al., 2018). Surveys of biodiversity and the seasonal change of resident biota could be of a vital importance for management and protection of continental saltmarshes. Nevertheless, salt marshes have not been studied extensively in Serbia, and therefore, there is still limited data on their biota. Previous studies on Pannonian salt marshes in Vojvodina Province have majorly focused on the diversity of diatoms and crustaceans (Lukić et al., 2012; Gavrilović et al., 2018; Vidaković et al., 2019). Salt marshes in the southern part of Serbia have been extensively studied only in terms of their floristic composition (Zlatković et al., 2014), with scarce published data on avifauna in Lalinačka salt marsh (Nikolić & Ilić, 2021). Macroinvertebrates are important component of animal diversity in continental salt marshes and soda

lakes, with unique species compositions (Boda, Móra, & Csabai, 2019). However, macroinvertebrate communities of the continental salt marshes in Europe have been generally poorly studied (Boda et al., 2019). In Serbia, except some information on aquatic species of Coleoptera in Pannonian salt marshes in the Vojvodina Province (Gavrilović et al., 2018), there is no published data on any other groups of aquatic macroinvertebrates in salt marshes.

Non-biting midges or mime midges (Diptera: Chironomidae) are a widely distributed insect family which represents the most diverse and often the most abundant group of macroinvertebrates in both lotic and lentic ecosystems (Ferrington, 2008). This group also plays an important role in ecosystem functioning due to their intermediate position in the food-web and their key role in detritus processing in freshwaters (da Silva & Henry, 2018). The absence of fish in continental salt marshes allows development of diverse chironomid assemblages that represent a mixture of halobiont and halophil species, but also eurytopic species with wide geographical distribution can be abundant (Boda et al., 2019). Previous research on chironomid communities on the Balkan Peninsula has been conducted majorly in different freshwater ecosystems (Milošević, Simić, Todosijević, & Stojković, 2011; Płóciennik & Pešić, 2012; Bitušík & Trnkova, 2019; Popović et al., 2016, 2022; Dorić, Koh, & Mihaljević, 2020; Gadawski et al., 2022; Čerba et al., 2023; Ergović, Koh, Čerba, Mihaljević, & Hamerlík, 2023) with some records on chironomid species in coastal regions in Montenegro and Croatia (Płóciennik, Gadawski, & Kazimierczak, 2012; Čerba et al., 2020). To date, chironomid fauna in continental salt marshes on Balkan Peninsula has not been investigated. Moreover, chironomid fauna in Serbia is still not explored sufficiently. To date only three extensive faunistic studies on chironomid communities in Serbia have been published (Janković, 1978, 1985; Milošević et al., 2011). Besides these faunistic studies, the data on chironomid fauna comes from several ecological studies on chironomids (Milošević, Simić, Stojković, & Živić, 2012; Milošević et al., 2013, 2018; Popović et al., 2016, 2022). Faunistic studies could be beneficial for conservation of salt marshes by providing baseline for monitoring of habitat and diversity changes as well as for future surveys (Scheibler & Ciocco, 2011). Additionally, they contribute to the knowledge of species distributions which is important baseline for defining effective conservation measures. This is especially true for halotolerant, halobiont and halophilic species as data on their distribution is still relatively scarce (Boda et al., 2019).

The aim of the present study is to contribute to the knowledge on diversity and distribution of chironomid larvae in Serbia and to the knowledge on animal diversity of continental salt marshes. Specifically, we aimed to explore seasonal variations of diversity and chironomid larvae community structure across different saline habitats and to compare saline habitats from southern and Pannonian region in Serbia in terms of chironomid community.

MATERIAL AND METHODS

Study sites

The study encompasses a total of six salt marshes (study sites), of which three are Pannonian salt marshes in the northern part of Serbia declared as protected areas: Special nature reserve “Slano Kopovo” (SK), Special nature reserve “Okanj bara” (OK) and Nature Park “Rusanda” (RU; Fig. 1). These three salt marshes are paleo-meanders of the Tisa River situated on its left bank about 15 km northwest of the city of Zrenjanin (Fig. 1). The maximum depth of these saline lakes does not exceed 1.5 m, while the sizes of surface area are around 1.5 km^2 (Figs. 2a-c). The remaining three study sites are small salt marshes located in the south-eastern part of the country, in the valleys of the Južna Morava and Toplica rivers (Fig. 1). Two of them belong to the Lalinac salt marsh area in the vicinity of the village of Lalinac near the city of Niš (Lalinačka salt marsh, LA and Oblačinska salt marsh, OB; Fig. 1), while the remaining one belongs to the Bresničić salt marsh area in the vicinity of the village of Bresničić near the city of Prokuplje (Bresničićka salt marsh, BR; Fig. 1). These salt marshes are small water bodies whose surface areas do not exceed 0.02 km^2 (Figs. 2d-f). Two of them are declared as protected areas: Natural monument “Lalinačka slatina” and Natural monument “Bresničićka slatina”. Flora and vegetation of all study sites are characterized by the presence of rare and endemic plant species. Some of the characteristic protected plants in Pannonian salt marshes are succulent halophytes *Salicornia europea*, *Sueda maritima* and *Salsola soda*, while salt marshes in southern Serbia are characterized by the presence of endemic species such as *Stachys milanii*, *Camphorosma monspeliaca* and *Allium guttatum* subsp. *dalmaticum* and the absence of succulent halophytes (Zlatković et al., 2014; Luković & Šilc, 2021).

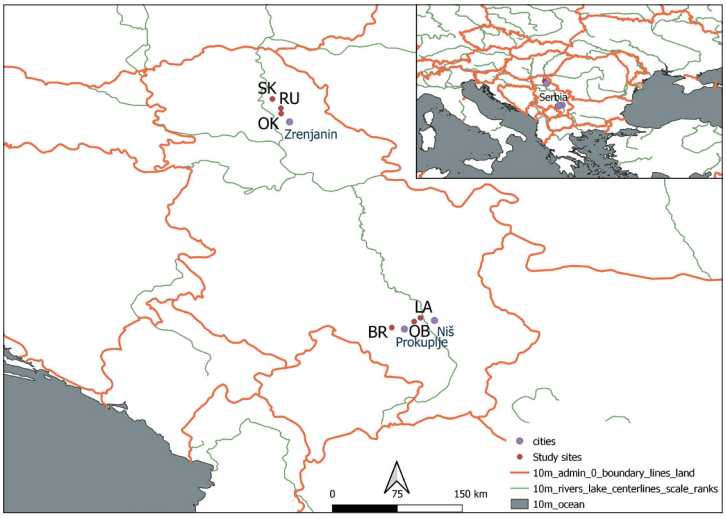

Figure 1. Map of the study sites: Lalinačka salt marsh, LA; Oblačinska salt marsh, OB; Bresničićka salt marsh, BR; Okanj bara, OK; Slano Kopovo, SK; Rusanda, RU.

Figure 2. Photographs of the study sites. a) Okanj bara, b) Slano Kopovo, c) Rusanda, d) Oblačinska salt marsh, e) Bresničićka salt marsh, f) Lalinačka salt marsh.

Sampling and analysis

Sampling campaign was performed from December 2016 to November 2017. Samples were collected at each salt marsh (hereinafter study site) every month except at Lalinačka salt marsh, which was not sampled during summer as it had dried up in late spring. Okanj bara, Slano Kopovo and Oblačinska salt marsh were sampled only once during summer before they completely dried up.

Given that not all study sites were sampled every month, the results below are presented for seasons. Benthic samples were collected by using a hand net of 250 μm mesh size. The net was dragged over an area of 0.0625 m^2 (opening size of a hand net) three times at the same point (i.e., removal sampling, Marchant & Hehir, 1999) in order

to obtain quantitative samples. A total of three subsamples were taken from the most common substrates at each study site, except at Oblačinska salt marsh where only one sample was taken due to the small size of this study site (Fig. 2d). The collected material was sorted out and preserved in 70% ethanol. Only 3rd and 4th instar larvae were used to assess chironomid community composition, and their identification to species or genus level was achieved using relevant taxonomic keys (Moller Pillot, 1984a, b, 2009; Schmid, 1993; Andersen, Cranston, & Epler, 2013; Cuppen, Tempelman, & van Haaren, 2015; Rossaro & Lencioni, 2015; Vallenduuk, 2017). The abundance of chironomids was expressed as the number of individuals per m^2. Chironomid diversity was expressed as taxa richness, S (number of taxa in a sample) and by calculating Shannon diversity, H (Shannon & Weaver, 1963). Diversity indices were calculated using the vegan package (Oksanen et al., 2022) in R version 4.3.1 (R Core Team, 2023).

RESULTS

Species richness and diversity of chironomid communities

Overall, 1953 specimens were collected and identified across all study sites and throughout all four seasons, belonging to 25 chironomid taxa from 19 genera and three subfamilies: Tanypodinae, Orthocladiinae and Chironominae (Table 1). The richest site was Bresničićka salt marsh where 17 taxa were recorded, followed by Oblačinska salt marsh with 11 taxa, and Lalinačka salt marsh with 9 taxa (Table 1). Lower species richness was recorded at study sites in the northern part of the country: 5 taxa at Slano Kopovo, 4 at Okanj bara, and 3 at Rusanda. Shannon diversity was the highest at Lalinačka salt marsh and Slano Kopovo since certain taxa dominated in the abundance at the remaining localities. According to the published data for chironomids in Serbia, among 25 chironomid taxa recorded in our study two were new for the chironomid fauna of Serbia: Microchironomus cf. deribae and Tanytarsus gr. *pallidicornis*.

Table 1. List of the recorded chironomid taxa across the study sites with their abundances (number of individuals per m2) per study site. Oblačinska salt marsh, OB; Bresničićka salt marsh, BR; Lalinačka salt marsh, LA; Okanj bara, OK; Slano Kopovo, SK; Rusanda, RU.

Taxa	OB	BR	LA	OK	SK	RU
Tanypodinae						
Macropelopia gr. *nebulosa*	21.88	12.25	5	0	0	0
Natarsia sp.	0	0	1	0	0	0
Procladius spp.	2.63	3.38	13.33	6	0.67	5.25
Psectrotanypus spp.	0.38	36.25	0	0	0	0
Tanypus kraatzi Kieffer, 1912	0.38	0.38	0	0	0	0
Xenopelopia sp.	0.13	0	0	0	0	0
Orthocladiinae						
Cricotopus/Orthocladius	0	0	0	0	0.33	0
Cricotopus gr. *sylvestris*	0	0.38	4	0	0	0.75
Psectrocladius gr. *limbatellus/ sordidellus*	2.13	0.75	2.67	0	0	0
Pseudosmittia sp.	0	0.5	0	0	0	0
Thienemannia sp.	0.13	0	0	0	0	0
Chironominae						
Chironomini						

table continued

Chironomus annularis agg.	7	60.75	0	0	0	0
Chironomus dorsalis agg.	0	0	4.33	0	0	0
Chironomus plumosus agg.	0	0	0	1	0	0
Chironomus riparius agg.	110.38	52	16.67	0	0	0
Chironomus spp.	0	0.5	0.33	0	0	0
Dicrotendipes lobiger (Kieffer, 1921)	0	1.5	0	0	0	0
Endochironomus tendens (Fabricius, 1775)	0	0.13	0	0	0	0
Glyptotendipes barbipes (Staeger, 1839)	0	0.25	0	0	0	0
Kiefferulus tendipediformis (Goetghebuer, 1921)	0.5	0.38	0	0	0	0
Microchironomus cf. *deribae*	0	16	0	267	0.67	1.25
Polypedilum nubeculosum (Meigen, 1804)	0	0.13	0	0	1.33	0
Polypedilum nubifer (Skuse, 1889)	0	0	0	222	0.67	0
Tanytarsini						
Micropsectra spp.	1.13	0	0.33	0	0	0
Tanytarsus gr. *pallidicornis*	0	0.13	0	0	0	0

Seasonal dynamics of chironomid communities across the study sites

The highest taxa richness (S) and Shannon diversity (H) across all study sites were recorded in spring and summer months (Fig. 3), with exception of Oblačinska salt marsh where the highest taxa richness was recorded in winter (Fig. 3a). At Oblačinska salt marsh *Chironomus riparius* agg. dominated in abundance throughout the whole year (Figs. 4a, c and d) except during summer when *Chironomus annularis* agg. was the most abundant (Fig. 4b). *C. annularis* agg. was the most abundant at Bresničić salt marsh in summer and winter (Figs. 5b, d), *C. riparius* agg. dominated in abundance in spring (Fig. 5a), while *Microchironomus* cf. *deribae* dominated in autumn (Fig. 5c). *Procladius* spp. and *C. riparius* agg. were the most abundant taxa at Lalinačka salt marsh during spring (Fig. 6a). *C. dorsalis* agg. and *C. riparius* agg. dominated in abundance at Lalinačka salt marsh in autumn months (Fig. 6b), while *Natarsia* sp. was the only recorded taxon at this locality during winter months (Fig. 6c). As mentioned above, Lalinačka salt marsh was dry during whole summer in 2017. At both Okanj bara and Slano Kopovo chironomids were recorded only in summer. *M.* cf. *deribae* dominated in abundance at Okanj bara (Fig. 7a), while *Polypedium nubeculosum* was the most abundant taxon at Slano Kopovo (Fig. 7b). Finally, chironomids were recorded only in summer and autumn months at Rusanda site. *Procladius* spp. was the most abundant taxon during summer months (Fig. 7c), and it was the only recorded taxon in autumn (Fig. 7d).

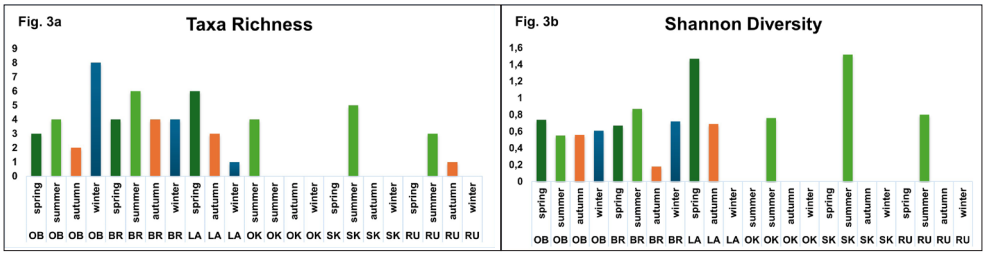

Figure 3. Variations of taxa richness (a) and Shannon diversity (b) across the study sites and through different seasons. Each season is marked by different colour. Study sites: Oblačinska salt marsh, OB; Bresničićka salt marsh, BR; Lalinačka salt marsh, LA; Okanj bara, OK; Slano Kopovo, SK; Rusanda, RU.

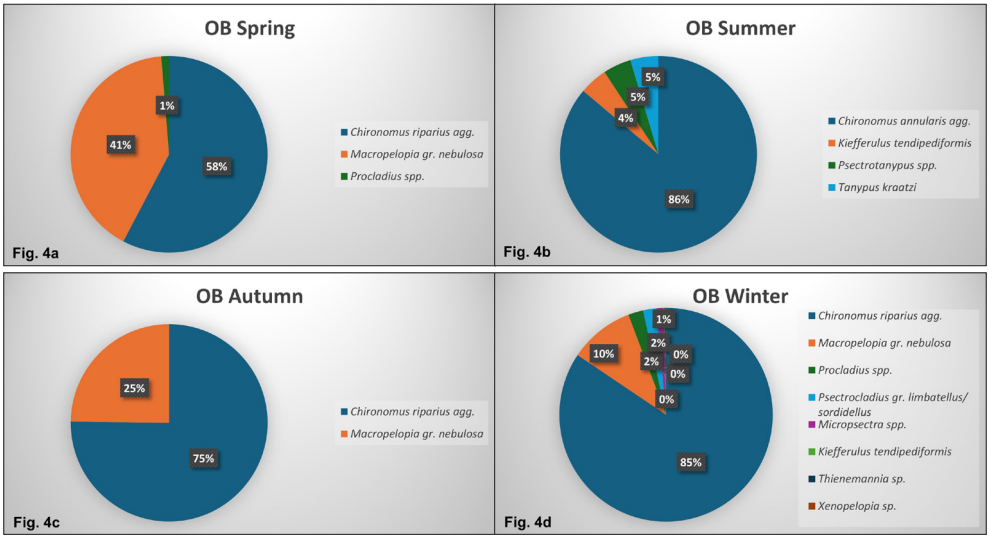

Figure 4. Community structure of chironomids at Oblačinska salt marsh (OB). a) spring, b) summer, c) autumn, d) winter.

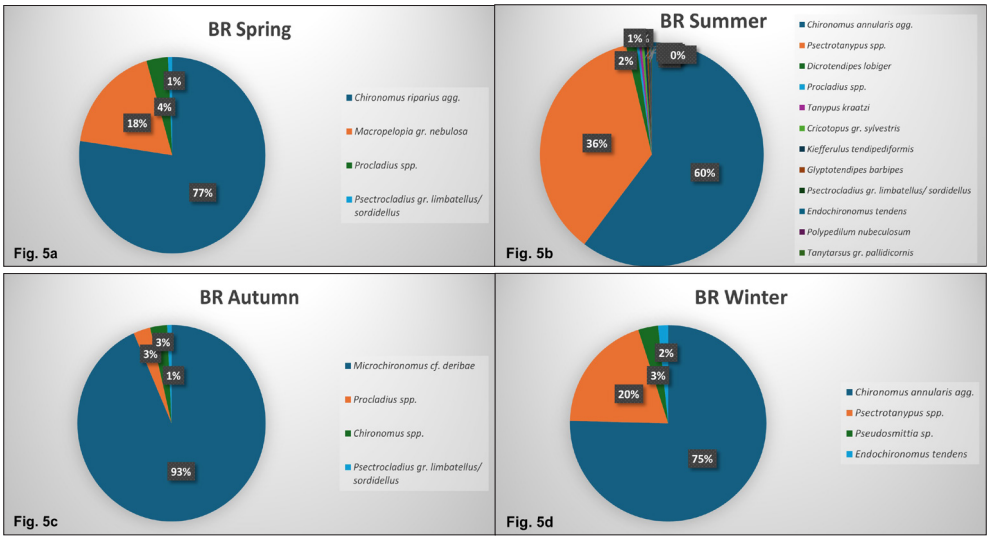

Figure 5. Community structure of chironomids at Bresničićka salt marsh (BR). a) spring, b) summer, c) autumn, d) winter.

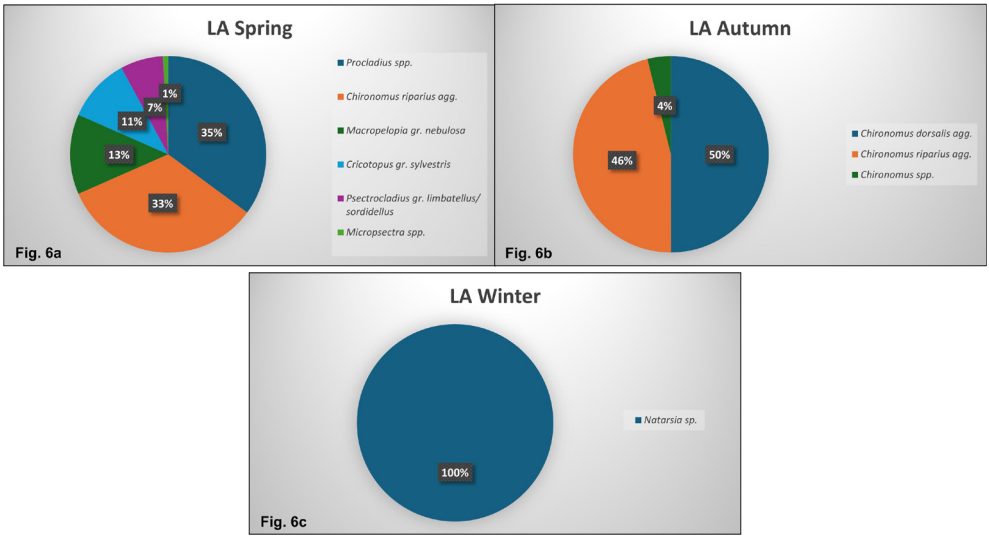

Figure 6. Community structure of chironomids at Lalinačka salt marsh (LA). a) spring, b) autumn, c) winter.

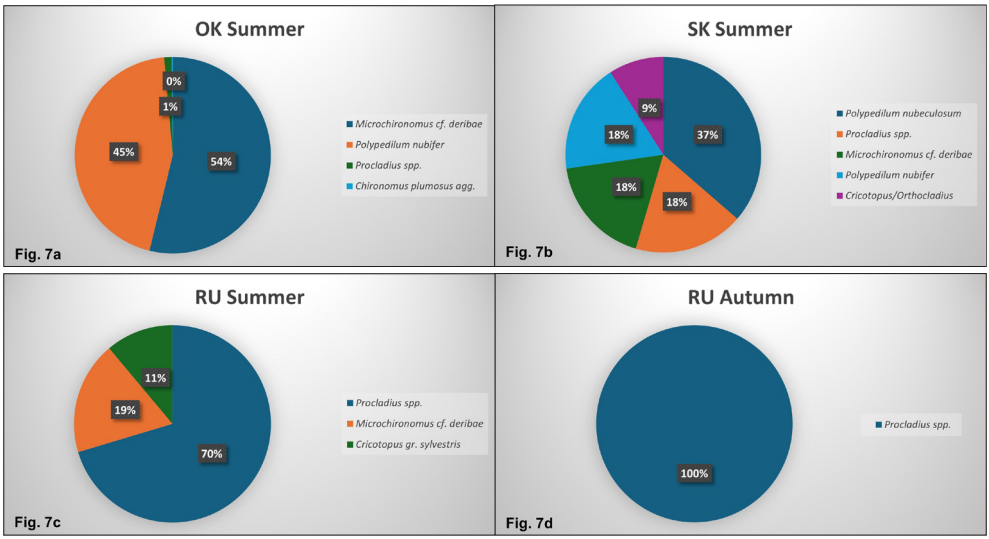

Figure 7. Community structure of chironomids. a) Okanj bara (OK) in summer, b) Slano Kopovo (SK) in summer, c) Rusanda (RU) in summer, d) Rusanda (RU) in autumn.

DISCUSSION

Our results indicate low taxa richness of chironomids in the Pannonian salt marshes in Serbia (from 3 to 5 taxa per study site) but higher taxa richness of chironomids in the salt marshes in southern Serbia (from 9 to 17 taxa per study site). Lower diversity of chironomids in salt marshes from the northern part of the country comparing to the localities in the southern part of the country in our study was most probably the

consequence of more extreme environmental conditions in Pannonian salt marshes. For instance, Lake Rusanda has the highest measured salinity of all saline lakes in the Carpathian Basin (13 g L^{-1}; Boros et al., 2014; Boros, V.-Balogh, Vörös, & Horváth, 2017). Previous studies pointed out that taxa richness of aquatic macroinvertebrates tends to decrease with increased salinity in salt lakes due to the direct negative effects of high salinity and/or due to the decline in macrophyte diversity (Boda et al., 2019). In addition, the small surface of salt marshes in the southern part of the country in our study allows them to be completely overgrown by vegetation (Figs. 2d-f), which provides suitable conditions for higher number of chironomid species by increasing habitat complexity (Mormul, Thomaz, Takeda, & Behrend, 2011).

Diversity (S and H) and community structure of chironomid communities in the studied salt marshes showed fluctuations related to the season. Seasonal variations in the structure of chironomid communities have often been observed (Milošević et al., 2013; Tóth, Árva, Nagy, & Specziár, 2013; Głowacki et al., 2023), which is not surprising given that many environmental factors show cyclic variation during a year. The lowest diversity at the majority of study sites was recorded in winter. The exception was Oblačinska salt marsh where the highest taxa richness was recorded in winter. This locality dried up completely in late summer-early autumn, so the community started forming in the late autumn when the salt marsh was filled with water again. In the winter months, this locality reached the maximum water level. Earlier study of aquatic macroinvertebrates in temporary ponds showed that the diversity of macroinvertebrates is the highest during maximum water level and the lowest when ponds start filling with water (Bazzanti, Baldoni, & Seminara, 1996).

Most of the recorded chironomids in the studied salt marshes were common in freshwaters and were typical for eutrophic ecosystems. Similar species composition of chironomids was also recorded in soda pans in Hungary (Boda et al., 2019). Analysis of environmental parameters of soda pans in Carpathian basin, including Pannonian saline habitats in Serbia, indicated hypertrophic and polyhumic conditions in these saline waters (Boros et al., 2017). Meso- or eutrophic conditions in study sites in southern Serbia could be expected based on the recorded chironomid communities. Interesting taxon recorded at Bresničićka salt marsh, *Pseudosmittia* sp. is semi-terrestrial or terrestrial (Moller Pillot, 2013). Several species from this genus inhabit marshes (Moller Pillot, 2013) and typically are found in waterlogged grounds (e.g., peatlands; Cao et al., 2019; Cao, Langdon, Shen, Li, & Pan, 2024). The depth of Bresničićka salt marsh was very shallow throughout the whole study year and in winter months it resembled on a waterlogged meadow (Fig. 2e), which created suitable conditions for development of *Pseudosmittia* larvae. Some of the recorded taxa, such as *Chironomus annularis*, *C. plumosus*, *M.* cf. *deribae*, *P. nubifer*, *Procladius* spp. and *Tanypus kraatzi* have been found to tolerate higher concentrations of chlorides in water (Moller Pillot & Vallenduuk, 2007; Moller Pillot, 2009). Taxa with high salinity resistance*, P. nubifer* and *M.* cf. *deribae*, were highly abundant in study sites in the northern part of the country (Table 1) which, along with low taxa richness, indicate high salinity of these habitats. *M.* cf. *deribae* was the most tolerant to high salinity among all the recorded species (Moller Pillot, 2009) and, to our knowledge, this was the first

record of this species for the chironomid fauna in Serbia, according to the published data (Janković, 1978, 1985; Milošević et al., 2011, 2018; Popović et al., 2016, 2022). This species has been recorded from many European countries along the seacoast (Moller Pillot, 2009), also in Rusia, Belarus and Ukraine (Antczak, Płóciennik, Rewicz, Baranov, & Bilecka, 2016; Golovatyuk, Zinchenko, & Nazarova, 2020) while in south-eastern Europe, so far it has been recorded in Hungary, Romania and Moldova (Antczak et al., 2016; Boda et al., 2019). *M.* cf. *deribae* prefers shallow standing waters and since it is well adapted to high salinity it often occurs in brackish waters (Moller Pillot, 2009). In Hungary, the larvae of this species have been recorded in a shallow saline lake with conductivity of 6778 μS cm^{-1} (Boda et al., 2019). The first record for chironomid fauna in Serbia here was also given for *Tanytarsus* gr. *pallidicornis*. Other species from this genus have been recorded in Serbia, but in faunistic studies for chironomids in Serbia the *Tanytarsus* larvae were identified only to the genus level (Milošević et al., 2011). *T. pallidicornis* is a eurytopic species, widely distributed in Europe. In Balkan Peninsula so far it has been recorded in Montenegro, Albania and Croatia (Bitušík & Trnkova, 2019; Čerba et al., 2020; GBIF Secretariat, 2024).

CONCLUSIONS

The salt marshes in Serbia are poorly investigated, especially in terms of faunal composition. Our study contributes to the knowledge of their biota and to the knowledge of the chironomid fauna in Serbia, which are important components of aquatic habitats, including salt marshes. Overall, we recorded a total of 25 taxa two of which are new to the Serbian chironomid fauna. Salt marshes studied in the southern part of the country had higher taxa richness comparing to Pannonian salt marshes. Besides low taxa richness, chironomid communities in the Pannonian region were characterised by the dominance by halophilic and halotolerant taxa, which indicate higher salinity levels in these habitats than in salt marshes in southern Serbia. Diversity and community structure of chironomids in the study sites showed seasonal fluctuations. The lowest diversity at the majority of study sites was recorded in winter. The present findings set the basis for future research and conservation measures in these unique and fragile habitats.

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