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REVIEW

The Chironomidae (Diptera) diversity in the Balkan Peninsula spring systems and other small water bodies

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Abstract – Chironomidae are known to occur in small, even astatic water bodies like seepages, rheocrens, pools and wells. The Balkan Peninsula reveals a high variability of small water bodies, with springs (rheocrens, limnocrens and helocrens) being the most widely distributed. In this review, we give a brief presentation of the Chironomidae communities in valley and mountain small water bodies, and in Lake Skadar (Shkodra) spring system. River valleys offer a large variety of small freshwater habitats. Their presence strongly increases of midge (Chironomidae) diversity, providing a habitat for the cold-stenotherms and semiterrestrial taxa that do not occur in rivers and lakes. The species richness in small water bodies strongly depends on their hydrological conditions (*i.e.* perennial *vs.* astatic water bodies), size and microhabitat complexity. Mountain spring communities depend on precipitation and exhibit altitudinal zonation. The higher mountain zones (1400–1500 m a.s.l.) have the most diverse midge assemblages, due to their stable perennial hydrological conditions. Human activity may alter species composition in riparian springs, favouring taxa that are not typical to the local fauna. By studying these small aquatic habitats, the significance of their Chironomidae fauna is being recognised, thereby filling a gap in the knowledge of freshwater insects biodiversity in the Balkan region.

Keywords: River valleys / Lake Skadar / springs / species richness / small water bodies

1 Introduction

Chironomidae are an important component of freshwater macrobenthos. They are ecologically diverse and taxonomic rich aquatic insects (Oliver, 1971; Pinder, 1986). The Balkan Peninsula in Southeastern Europe is a very interesting region for Chironomidae (midge) studies. The peninsula mixes faunistic influences from Western Palearctic, Levant, and East European zoogeographical regions. Midge communities of the Balkan Peninsula's large flowing waters have been extensively investigated for a long time (*e.g.*, Janković, 1967; Milošević *et al.*, 2012, 2014, 2018, 2019). Long-term studies focused on the Danube River catchment, Croatian, Greek and Montenegrin streams (Płóciennik and Pešić, 2012; Płóciennik and Karaouzas, 2014; Čerba *et al.*, 2020), and identified community ecogeography and its faunistic distinctness. Large lakes of this region have also been a subject of extensive studies. The non-biting midges' (Chironomidae) cryptic diversity and its relation to habitat quality were recently analysed in Lake Skadar located at the border between Montenegro and Albania (Pešić *et al.*, 2018; Gadawski *et al.*, 2022). The chironomid fauna of lakes Kastoria and Volvi (localised in Greece), Ohrid (at the Albanian/Macedonia border), and Prespa (at the Greece/Albania/Macedonia border) was also investigated (*e.g.*, Smiljkov, 2001; Vallenduuk, 2013; Ntislidou *et al.*, 2019). Research on dam reservoirs has been conducted by Paunović *et al.* (2005); Marković *et al.* (2012); Smiljkov and Slavevska-Stamenković, (2006); Ilieska and Smiljkov (2020). The above studies usually include ecological or ecological/faunistic characteristics. In fact, as far as large stagnant waters are concerned, only Lake Skadar and Lake Ohrid have been extensively investigated and the list of taxa existing there is nearly complete. The fauna of other lakes and large

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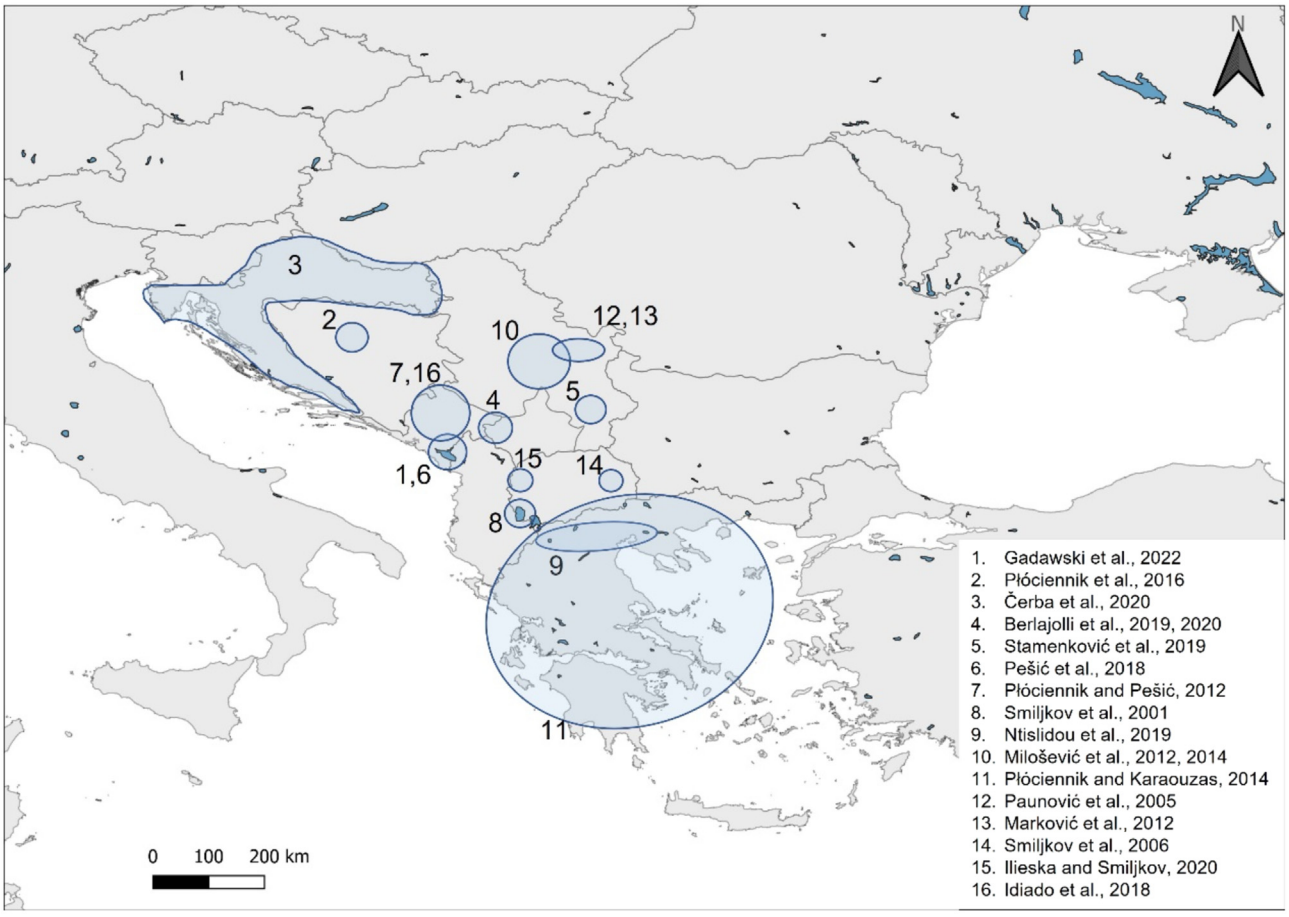

Fig. 1. The map of the Balkan Peninsula.

reservoirs has been only preliminarily studied. Although some investigations concerning large stagnant waters exist, limnological studies were rarely conducted on small and astatic (*i.e.* temporary or ephemeral) water bodies.

Stamenković *et al*. (2019) made some contributions to the ecology of freshwater ponds of south-eastern Serbia. Čerba *et al*. (2020) and Berlajolli *et al*. (2019) studied the seasonality and faunistics of mountain rheocrenes, Płóciennik *et al*. (2016) investigated the community ecology of valley springs, and Gadawski *et al*. (2022b) focused on Lake Skadar spring system's chironomid diversity.

In this study, we present a review of Chironomidae communities of small water bodies with different limnological characteristics. These include astatic and perennial springs, ditches in the basins of large river valleys, limnocrenes or natural springs and, finally, ponds. Such a variability of habitats implies a high diversity of ecological groups from stagnant to flowing waters and semiterrestrial species. The present paper describes environmental variables that drive ecological diversity in small water bodies located at the valley margins. The composition of mountain spring assemblages depends on climatic conditions which includes the temperature and precipitation change with altitude. The presented review discusses how mountain midge fauna zonation is linked to the rheocrence perennial type. Finally, we give the first insight into the Chironomidae diversity of the Lake Skadar spring system in Montenegro.

2 Study area – topography, hydrology, and biogeography of the Balkan Peninsula

The Balkan Peninsula forms an irregular, inverted triangle of land which extends from Central Europe in the north to the Eastern Mediterranean in the south. The Balkans lies in the south of the rivers Save, Drava and Danube and are surrounded by the Adriatic and Ionian Seas in the west, the Mediterranean Sea in the south and the Aegean, Marmara and Black Seas in the east (Fig. 1). It is situated in the biogeographical region where continental Europe, western Asia, the Mediterranean and Black Seas meet (Skoulikidis *et al*., 2022). The Balkan mainland is hilly and mountainous, while its northern and north-eastern parts transfer into plains. It covers nearly 16% of the European area. The region is characterised by a high diversity of aquatic fauna and flora, with a high proportion of endemic species. Such a high level of biodiversity is a result of the region's geologic and palaeoclimatic history, as well as the geophysical variety of inland water bodies (Griffiths *et al*., 2004; Skoulikidis *et al*., 2022). The Balkan rivers and streams flowing through abrupt and narrow mountain valleys host

Fig. 2. Photos of the small water bodies inhabited by Chironomidae: A – pond, B – rheocrene) Zeta Valley (country: Montenegro); C, D) a typical springs in the Accursed Mountains (country: Republic of Kosovo); E, F) a typical springs of Lake Skadar (country: Montenegro).

highly diverse freshwater communities (Skoulikidis *et al*., 2022).

The most remarkable feature of the Balkans is definitely their high degree of endemism, compared to the rest of Europe (Pešić and Glöer, 2013). The Dinarides–Hellenides mountain chain creates a biogeographical barrier which separates the eastern and western species assemblages. The west–east division of the Balkan aquatic and terrestrial biota is evidenced by phylogenetic studies. Species richness increases from south to north, while the proportion of endemic species increases from north to south (Skoulikidis *et al*., 2022).

Since the Miocene, the isolation of the western Balkan river basins has favored a rich endemic aquatic fauna (Bianco, 1986; Gasc, 1997; Skoulikidis *et al*., 2022; Pešić and Glöer, 2013). Strong biogeographic differences are caused by the climatic factors, such as continental and dry climate with harsh and cold winters or high proportion of karstic subterranean rivers, especially in the western part. They contain remarkable subterranean communities (Skoulikidis *et al*., 2022). Many aquatic invertebrates endemic to the Balkan Peninsula were described in the previous decades and new taxa are described each year, such as leeches (Hirudinea) *i.e. Dina crnogorensis* Grosser and Pešić 2022; crustaceans (Crustacea) *i.e. Gammarus bosniacus* Schäferna, 1923, *Gammarus sketi* Karaman, 1989; mites (Hydrachnida) *i.e. Sperchon milisai* Pešić 2022; insects *i.e. Micropsectra uva*, Giłka, Wojciech, Zakrzewska, Marta, Baranov, Viktor A. and Dominiak, Patrycja, 2013, *Rhyacophila macedonica* Karaouzas, Valladolid and Ibrahimi 2022; snails *i.e. Bythinella marici* Glöer and Pešić, 2014, *Lanzaia pesici* Glöer, Grego, Erőss and Fehér, 2015. Many cryptic species, endemic even for particular rheocrenes are recorded (Mamos *et al*., 2014; 2016).

UNESCO and the Hellenic Public Power Corporation reported the total river discharge in the European Mediterranean region of 330 km^3 per year (UNEP/MAP 2003, www.rivdis.sr.unh.edu). As much as 85 km^3/year is generated by all Balkan rivers. The eastern Balkan basins exhibit a semi-arid climate causing low discharge from 4 to 14.9 L/s/km^2. On the other hand, the western Balkan basins are characterised by high precipitation and specific discharge ranges between 18.3 and 34.6 L/s/km^2. The hydrological and thermal regime depends on the seasonal distribution and type of precipitation caused by rain or snow, as well as on hydrogeological features (*e.g.* karstic or alluvial aquifers, degree of surface/subsurface flow interactions). Balkan rivers reveal a strong seasonal hydrology, mostly flashy and with low summer flow (Skoulikidis *et al*., 2022).

The hydrological character and geological background of springs and various small water bodies in the Balkan Peninsula are described by Pešić *et al*. (2022a). The small water bodies

are diverse and differ in origins. In the Dinaric Mountains, there are numerous karst springs formed in tectonic depressions. They have complicated hydrogeological relations, as drainage of surface water is achieved through many surface and underground flows. Depending on the locality, morphology and karstification properties, the average infiltration rate can depend on the intensity of precipitation (Stevanović *et al*., 2022). In the uplands and mountains, there are also numerous riparian springs that feed dense stream networks. The riparian springs remain natural in many regions, such as Bosnia and Herzegovina or the Accursed Mountains in Republic of Kosovo. However, they are often neglected in the conservation efforts, as larger rheocrenes and limnocrenes are used by local people as a source of drinking water (Pešić *et al*., 2022b). In valleys, gravel peats and fluvial lakes of diverse size form dynamic floodplain systems. Small water bodies, such as ditches, ponds, wells and fountains often serve as refuge for aquatic fauna during dry season and remain important for local invertebrate diversity (Dražina *et al*., 2022).

3 Diversity in river valleys

Up until now, there has been primarily research on chironomids of river valleys in the Balkan Peninsula. They were investigated in Serbia (*e.g.* Janković, 1975; Zivić *et al*., 2001; Milošević *et al*., 2011), Montenegro (Płóciennik and Pešić, 2012), Greece (Karaouzas and Płóciennik, 2016) and Albania (Bitušík and Trnková, 2019). These studies focused particularly on streams, even though large river valleys in southern Europe contain smaller freshwater tributaries and sources (Fig. 2). These include springs, small brooks situated in the valley margins and foothills, as well as additional seepages, small ponds, wells, and ditches. Each of these habitats has a characteristic type of Chironomidae community. Idiado *et al*. (2018) and Gadawski *et al*. (unpublished data) have investigated a variety of small water bodies in the Zeta Valley basin in Montenegro (Figs. 1, 2, Tab. 1). The distinctive aquatic fauna diversity of this basin results in high species richness. As many as 57 Chironomidae taxa from 46 sites were recorded. The fauna of small rheocrenes is clearly distinct from other types of investigated habitats. It contains mainly species from subfamilies Orthocladiinae and Chironominae which is a typical of fast-flowing waters on mineral bottoms. These include but not limited to *Orthocladius* type S sensu Brooks *et al*. (2007), *Cricotopus bicinctus*-type sensu Brooks *et al*. (2007), *Paratanytarsus intricatus* (Goetghebuer, 1921), *Paratendipes albimanus* (Meigen, 1804) and *Chironomus* spp. (Tab. 1). Small rheocrenes located in forested and agricultural areas are inhabited by ubiquitous species from genera *Chironomus* and *Procladius*, as well as semiterrestrial ones like *Smittia* species. Slow-flowing rheocrenes with semiaquatic habitats have a diverse fauna, including species typical of cold and oligotrophic habitats and semiaquatic ones, such as *Micropsectra* spp., *C. bicinctus*-type, *Limnophyes* spp., *Zavrelimyia* spp., *Bryophaenocladius* cf. *flexidens* (Brundin, 1947), *Diamesa* spp., *Rheocricotopus fuscipes* (Kieffer, 1909) or *Synorthocladius semivirens* (Kieffer, 1909) (Tab. 1). Seepages and ditches are similar in terms of their fauna and include many rheophile species: *Rheocricotopus effusus* (Walker, 1856), *O.* type S, *Zavrelimyia*, *C. bicinctus*-type, *Corynoneura* cf. *antennalis* Kieffer, 1921, *R. fuscipes*. Astatic waters in marginal zones of mainstream valleys have poor diversity of fauna dominated by *Limnophyes* spp. and *Thienemannimyia*-type genera agg. sensu Brooks *et al*. (2007) (Tab. 1). In smaller river basins of the northern Balkans, like the Cvrcka River basin, human impact weighs on the environmental character of marginal valley springs, but Chironomidae communities defy simple classification, often depending more on bottom substrate complexity than oxygen concentration and conductivity reflecting chemical water pollution (Płóciennik *et al*., 2016). Hard bottom substrates may play the most important role in species composition. Even in the case of upland valleys where the altitude ranges is within less than 500 m a.s.l., elevation influences spring midge assemblages. There are clear differences in diversity and abundance in valley springs. Communities which reveal the highest diversity are characteristic of natural springs without organic waste and algae vegetation, manifesting anthropogenic disturbance. The high abundance of *Chironomus* species can be seen in rheocrenes, that are outliers of the springs' general environmental pattern. Different zones of rheocrenes, particularly eucrenon and hypocrenon, are characterised by distinct benthic communities and indicator species. For instance, *Prodiamesa olivacea* (Meigen, 1818) prefers the spring source, while members of the genus *Paraphaenocladius* prefer spring brooks (Pešić *et al*., 2016; Płóciennik *et al*., 2016). These species preferences conclude that the small water bodies of valleys have variant hydrological charterer and can also be transformed by local anthropogenic variables. These variations in the hydrological regimes are the main reasons for river valleys to host patchy diverse communities. These diverse communities can be found in hydrological systems consisting of spring, ditches, wetlands, ponds and other small water bodies hosting Chironomidae fauna that can overrich in diversity the main river channel. Instead of protecting individual species, protecting the natural habitats can protect the biodiversity within it (Lauge-Madsen, 1995). If human activity becomes sustainable, it may increase the richness of habitats suitable for chironomids as an ecologically diverse and resistant group of insects.

4 Altitudinal zonation in mountain springs

The Balkan Peninsula is a mountainous region. In the higher altitudes, small water bodies are mostly springs (rheocrenes and brooks) that are sources for intermittent and perennial streams. The mountain springs are specific habitats, where hydrological conditions strongly depend on weather conditions, which are substantially different in the Mediterranean mountains than in lowlands (Čerba *et al*., 2020). Seasonal variations in temperature, such as much lower temperatures in high altitudes than in valleys, and high precipitation have an important influence on invertebrate communities. The structure of habitat conditions in streams also variate with altitudinal plant communities zonation (Rahbek, 1995, Słowińska and Jaskuła, 2021). The Chironomidae communities were recently investigated in the Accursed Mountains by Berlajolli *et al*. (2019). While investigating the seasonality of two springs near the city of Peja, they found that Chironomidae and Amphipoda constitute the main component of the

Table 1. Drivers of Chironomidae diversity obtained from literature data.

Ecosystem	Zone	Dominant substrate	Common taxa	Drivers of diversity	References
Adult males					
Spring	Rheocrene		*M. uva*		Giłka *et al.* 2013
River	Potamal	Sand, stone	*O. rivicola*		Gadawski *et al.* (2022b)
Spring	Rhithral	Sand, stone	*O. rivicola*, *Tanytarsus* sp., *P. nubeculosum*	Fast water current, low water temperature	Gadawski *et al.* (2022b)
Lake	Open-lake	Silt	*Synendotendipes lepidus* (Meigen, 1830)	Accumulation of detritus	Gadawski *et al.*, (2022b)
Lake	Lake-littoral	Stone	*P. nubeculosum*, *Polypedilum scalaenum* (Schrank, 1803), *Cladotanytarsus mancus* (Walker, 1856) agg.	Accumulation of detritus, high water temperature	Gadawski *et al.* (2022b)
Lake	Open-lake	Macrophytes	*Procladius choreus* (Meigen, 1804)	Accumulation of detritus	Gadawski *et al.* (2022b)
Spring	Krenal	Sand, stone	*P. nubeculosum*, *C. alpestris*	Low water temperature, high oxygen concentration	Gadawski *et al.* (2022b)
River	Potamal	Silt	*C. bicinctus*, *C. edwardsi*, *P. nubeculosum*	Accumulation of detritus, slow water current	Gadawski *et al.* (2022b)
Lake	Open-lake	Silt	*S. lepidus*, *P. nubeculosum*	High water temperature	Gadawski *et al.* (2022b)
Lake	Lake-littoral	Macrophytes	*Paratanytarsus dissimilis* (Johannsen, 1905), *Glyptotendipes pallens* (Meigen, 1804), *Stictochironomus* sp., *P. laetipes*, *Cladopelma virescens* (Meigen, 1818), *C. sylvestris*	High water temperature, presence of macrophytes	Gadawski *et al.* (2022b)
Pupal exuviae					
Lakes	Lake-littoral	FPOM, macrophytes	*C. plumosus*, *C.* Pe 1, *C.* Pe 2, C. Pe 3, *Paratanytarsus bituberculatus* (Edwards, 1929), *C. alpestris*, *Tanytarsus miriforceps* (Kieffer, 1921), *G. pallens*, *Glyptotendipes foliicola* Kieffer, 1918, *Kiefferulus tendipediformis* (Goetghebuer, 1921), *Tanypus punctipennis* Meigen, 1818, *Paratanytarsus laetipes* (Zetterstedt, 1850), *Paratanytarsus inopertus* (Walker, 1856), *Sergentia* sp., *Cryptotendipes*	Accumulation of detritus, low oxygen concentration, high water temperature	Gadawski *et al.* (2022b)

Table 1. (continued).

Ecosystem	Zone	Dominant substrate	Common taxa	Drivers of diversity	References
			pseudotener (Goetghebuer, 1922)		
Lakes	Open-lake	Stone	*Tanytarsus chinyensis* (Goetghebuer, 1922)	Hard substrate covered by algae	Gadawski *et al*. (2022b)
Lakes	Lake-littoral	Macrophytes	*P. bituberculatus*, *Tanytarsus usmaensis* Pagast, 1931, *C. plumosus, C. sylvestris*, *Endochironomus albipennis* (Meigen, 1830)	Presence of macrophytes, water temperature	Gadawski *et al*. (2022b)
Larvae					
Springs	Rheocrene (particularly eucrenon and hypocrenon)	Organic soil	*P. olivacea*, *Paraphaenocladius* spp., *Chironomus* spp.,	Rheocrenes	Płóciennik *et al*. (2016)
Springs	Krenal	Sand, stones of mineral origin	*O.* type S, *C. bicinctus*-type, *P. intricatus*, *P. albimanus* and *Chironomus* spp.,	Fast-flowing water	Idiado *et al*. (2018)
Springs	Rheocrene	Organic (mostly acidic) accumulations	*Micropsectra* spp., *C. bicinctus*-type, *Limnophyes* spp., *Zavrelimyia*, *B.* cf. *flexidens*, *Diamesa* spp., *R. fuscipes*, *S. semivirens*, *Smittia* spp.,	Slow flowing water, cold and oligotrophic habitats, semiterrestrial	Idiado *et al*. (2018)
Seepages and ditches	Rheocrene	Hard substratum	*R. effusus*, *O.* type S, *Zavrelimyia*, *C. bicinctus*-type, *C.* cf. *antennalis*, *R. fuscipes*	Fast-flowing water, low temperature	Idiado *et al*. (2018)
Marginal zones of mainstream valleys	Astatic waters	Fine sediment, organic soil	*Limnophyes* spp., *Thienemannimyia*-type genera agg.	Fast-flowing water, low temperature	Idiado *et al*. (2018)
Mountain springs	Rheocrene	Variant microhabitats	*R. effusus*, *C. dentiforceps* agg., *Diamesa* spp., *Eudactylocladius* spp., *Euorthocladius* spp., *M.* type A, *M. contracta*-type, *P. penerasus*, *R. effusus*, *T. bavarica*	Flowing water, low temperature	Berlajolli *et al*. (2020)
Springs	Krenal and rhithral springs	Sand, stones	*P. nubeculosum*, *C. alpestris*, *Tanytarsus* spp.	Fast-flowing water, low temperature	Gadawski *et al*. (2022b)
Lakes, suclacustrine springs	Lake-littoral	Boulders, stones and coarse gravel	genera: *Acricotopus* spp., *Corynoneura* (*e.g.*, *C. gratias* and *C.*	Algae covering the surface of rocks, wood and other sunken objects	Gadawski *et al*. (2022b)

Table 1. (continued).

Ecosystem	Zone	Dominant substrate	Common taxa	Drivers of diversity	References
			edwardsi), *Cricotopus* (*e.g.*, *C. sylvestris* and *C. bicinctus*), *Dicrotendipes* (*e.g.*, *D. lobiger*, *D. nervosus*, *D. notatus*, *D. pulsus*), *Limnophyes* (*e.g.*, *L. minimus* and *L. natalensis*), *Orthocladius* (*e.g.*, *O. abiskoensis*, *O. excavatus*, *O. luteipes, O. oblidens, O. pedestris, O. rivicola, O. rubicundus*), *Parachaetocladius* (*e.g.*, *P. abnobaeus*), *Paratrichocladius* (*e.g.*, *P. rufiventris*) and *Pseudosmittia* (*e.g.*, *P. trilobata*)		
Springs	Permanent suclacustrine spring (below 30m depth)	Sand, stones of mineral origin	*P. convictum*, *C. plumosus*	Fast-flowing water, low temperature	Gadawski *et al.* (2022b)
Springs, pools	Springs forming pools, also temporary	Sand, stones, silt	*O. rivicola*, *C. bicinctus*, *C. edwardsi* and *P. nubeculosum*	Slow-flowing water	Gadawski *et al.*, (2022b)
Springs	Permanent and temporary rheocrene	Sand, stones	*B. bifida*, *C. pallidula*, *E. brevicalcar*, *E. clypeata*, *T. ejuncidus*, *T. laeta*	Fast-flowing water	Gadawski *et al.*, (2022b)

assemblages. The investigated springs were very small water bodies where Chironomdiae abundance and species composition strongly varied seasonally, depending on their life cycles. Because midges have a terrestrial dispersal stage, they easily colonise small water bodies that are distant from each other, but they are also absent in small aquatic habitats during some part of their life cycle. Two first larval stages are also very small and difficult to detect. This all affects their detectable seasonal biodiversity pattern (Berlajolli *et al.*, 2019). This problem, along with difficult logistical access to the mountain regions are the reasons for the relatively low faunistic recognition of mountain midge fauna in the southern Balkans. In comparison, less elevated regions of Croatia and Serbia are better studied. The mountainous midge fauna of Balkans has not been investigated extensively especially in the south-west (Płóciennik and Pešić, 2012; Idiado *et al.*, 2018; Čerba *et al.*, 2020). Recent studies on nearly 40 springs distributed over a wide altitude range in the Accursed Mountains indicate high Chironomidae species richness in mountain springs (Berlajolli *et al.*, 2020). In this area, a set of 42 taxa (from species to genus level) were collected. Orthocladiinae were the most species-rich subfamily among Chironomidae. The midge community composition of mountain springs depends on local physical and chemical environmental variables, including the morphological microhabitat composition, like in other freshwater ecosystems. However, the randomness of these factors, related to the distance between springs, changeable weather, and other incidental factors, influence species dispersion. This would make these communities much more variant than the midge communities of the large lowland water bodies. The altitudinal gradient of Chironomidae communities follows mainly the precipitation-related hydrological conditions. Conductivity is

the secondary factor influencing the composition of midge communities, revealing higher values at lower elevations. Finally, the local physical factors such as the presence of submerged branches and water temperature, varying by altitude, influence the midge assemblages. In the Accursed Mountains, four altitudinal zones can be recognized: (1) the upland zone (470-700 m a.s.l.) dominated by *R. effusus*, *Chaetocladius dentiforceps* (Edwards, 1929) agg., *Diamesa* spp., and *Micropsectra* type A sensu Brooks *et al.* (2007); (2) the lower mountain zone (700-1400 m a.s.l.) dominated by *Diamesa* spp., *M.* type A and *Orthocladius* (*Euorthocladius*) species; (3) the higher mountain zone (1400–1500 m a.s.l.) with dominant *R. effusus*, *Euorthocladius* spp., *Tvetenia bavarica* (Goetghebuer, 1934), and *Micropsectra contracta*-type sensu Brooks *et al.* (2007); and (4) the topmost alpine zone (1500–1700 m a.s.l.) settled mainly by *Orthocladius* (*Eudactylocladius*) species, *Paraphaenocladius penerasus* (Edwards, 1929), and Dixidae. The higher mountain zone (1400–1500 m a.s.l.) has the most diverse midge assemblages due to its stable perennial hydrological conditions.

Research on mountain rheocrenes and brooks prove that the local fauna variability coming from the randomness of colonisation pattern has in fact an ecogeographical background. Bedrock geology, climate zonation and riparian vegetation patterns shape Chironomidae zoogeography on a large spatial scale. This was also proven for larger rheocrenes communities in mainland Greece (Płóciennik and Karaouzas, 2014).

5 Diversity in Lake Skadar suclacustrine springs (in the Montenegro/Albania border region)

A unique combination of geological and climatic factors, as well as the complex geological history, makes the Mediterranean region one of the 25 most important biodiversity and endemism hot-spots worldwide (Myers *et al.*, 2000; Woodward, 2009; Blondel *et al.*, 2010). Skadar Lake is one of the main components of this region, well-known for its high freshwater biodiversity and endemicity. Many invertebrate taxa are reported to be endemic for the lake or its surroundings, *i.e. Spirosperma scodraensis* (Hrabě 1958), *Tubificidarum hrabei* Karaman, 1973, *Trichodrilus montenegrinus* Karaman, 1973, Dina nesemanni Grosser, Rewicz, Jovanović, Zawal & Pešić 2023 *Nitocrella longa* Karanovic, 2000, *Pseudocandona regisnikolai* Karanovic and Petkovski, 1999, *Laurogammarus scutarensis* (Schäferna, 1923); (Pešić *et al.*, 2018; Grosser *et al.*, 2023). It is the biggest lake in the Balkan Peninsula, situated in the Zeta-Skadar valley, supplied by a system of springs, predominantly associated with the local network of karstic connections (Glöer and Pešić, 2009; Pešić and Glöer, 2013). The large diversity and richness of the lake basin fauna could be explained by its location in the climatic zone, habitat diversity, spring system connection, environmental protection programmes and relatively low human impact.

As mentioned above, one characteristic feature of the Lake Skadar water balance is its high inflow from many temporary and permanent karstic springs. The coast of the lake includes numerous bays with a variety of such springs (Pešić *et al.*, 2018, 2019). Some of them are sublacustrine and situated in cryptodepressions. Water entering the lake is substantially harder than river water, probably owing to its direct limestone source (Lasca *et al.*, 1981; Grabowski *et al.*, 2018). There are about thirty such springs. The best-known and investigated are Karuč and Raduš, with the latter being the deepest at about 60 m. The Chironomidae diversity at such depths is very low, with only two species reported up to now from the depth of 55 m: *Polypedilum convictum* (Walker, 1856) and *Chironomus plumosus* (Linnaeus, 1758). Communities of different habitat types in Skadar Lake are given in Table 1. (Gadawski *et al.*, 2022b).

The rich species diversity of Chironomidae is supported by various habitats present in the Lake Skadar catchment. The southern and south-western shoreline of the lake is rocky, with boulders, stones and coarse gravel. This part of the Lake Skadar is settled mostly by *Acricotopus* sp., *Corynoneura* (*e.g.*, *Corynoneura gratias* Schlee, 1968 and *C. edwardsi* Brundin, 1949), *Cricotopus* (*e.g.*, *Cricotopus sylvestris* (Fabricius, 1794) and *Cricotopus bicinctus* (Meigen, 1818)), *Dicrotendipes* (*e.g.*, *Dicrotendipes lobiger* (Kieffer, 1921); *Dicrotendipes nervosus* (Staeger, 1839); *Dicrotendipes notatus* (Meigen, 1818); *Dicrotendipes pulsus* (Walker, 1856)); *Limnophyes* (*e.g.*, *Limnophyes minimus* (Meigen, 1818) and *Limnophyes natalensis* (Kieffer, 1914)), *Orthocladius* (*e.g.*, *Orthocladius abiskoensis* Thienemann, 1937, *Orthocladius excavatus* Brundin, 1947, *Orthocladius luteipes* Goetghebuer, 1938, *Orthocladius oblidens* (Walker, 1856), *Orthocladius pedestris* Kieffer, 1909, *Orthocladius rivicola* Kieffer, 1911, *Orthocladius rubicundus* (Meigen, 1818)), *Parachaetocladius* (*e.g.*, *Parachaetocladius abnobaeus* (Wülker, 1959)), *Paratrichocladius* (*e.g.*, *Paratrichocladius rufiventris* (Meigen, 1830)) and *Pseudosmittia* (*e.g.*, *Pseudosmittia trilobata* (Edwards, 1929)) (Tab. 1) (Gadawski *et al.*, 2022b).

The most recent study (Gadawski *et al.*, 2022b) reports that krenal and rhithral springs in Skodra spring system, with sandy and stone bottoms, are also inhabited by such species as *Polypedilum nubeculosum* (Meigen, 1804), *Chironomus alpestris* Goetghebuer, 1934 and species belonging to the genus *Tanytarsus*. Some of the springs are grouped in small areas, forming pools with sandy and stony bottoms but covered with silt where slower water current can be observed. Such places are inhabited mostly by species such as *O. rivicola*, *C. bicinctus*, *C. edwardsi* and *P. nubeculosum* (Tab. 1). Some of the species reported in the spring system were characteristic exclusively for running waters *e.g.*, *Brillia bifida* (Kieffer, 1909), *Conchapelopia pallidula* (Meigen, 1818), *Eukiefferiella brevicalcar* (Kieffer, 1911), *Eukiefferiella clypeata* (Kieffer, 1923), *Tanytarsus ejuncidus* (Walker, 1856), *Thienemannimyia laeta* (Meigen, 1818) (Tab. 1). Generally, species belonging to the *Cricotopus* genus are more abundant in polluted ecosystems of Skadar Lake *e.g.*, species belonging to groups: *Cricotopus pilosellus* Brundin, 1956, *Cricotopus tibialis* (Meigen, 1804), *Cricotopus fuscus* (Kieffer, 1909), *Cricotopus magus* Hirvenoja, 1973, *Cricotopus tremulus* (Linnaeus, 1758), *Cricotopus pulchripes* Verrall, 1912, *Cricotopus septentrionalis* Hirvenoja, 1973, *Cricotopus claripes* Hirvenoja, 1973, *Cricotopus tristis* (Hirvenoja, 1973). In the Skadar Lake basin, larvae of *C. sylvestris* are very frequent in habitats associated with

emergent and submerged vegetation with growing aquatic macrophytes and algae (Gresens *et al.*, 2012; Moller Pilot, 2013).

The Skadar Lake ecosystem is complex. The lake itself is relatively young, and its central basin is a unified soft-bottom habitat, however it hosts diverse midge fauna associated with macrophytes. The littoral zone has rich collector, shredder and scraper assemblages living on a hard substratum. River mouths are dwelled by a taxa common for stagnant and flowing waters. Nevertheless, all that taxa are mostly widespread in Europe. High Chironomidae cryptodiversity and potential endemism in Lake Skadar is dependent mainly on old spring system that hosted the local biodiversity and speciation (Grabowski et al., 2018).

6 Future prospects

Wider sampling along with acquiring diverse sampling technique (*i.e.*, aquatic and terrestrial) is very important for better understanding on small water bodies midge communities. The extensive sampling will allow the molecular tools to delimit cryptic species and improve the understanding of species diversity. Moreover, molecular methods can establish association between life stages and so that unknown or morpho-species, described based on larval stages, can be known. Investment in molecular barcoding may complement the ongoing faunistic studies, revealing endemics, cryptic species and genetically isolated populations (Gadawski *et al.*, 2022a). The progress of knowledge on chironomids suggests that many species have large distribution ranges, but the presence of endemic cryptic species in the Skadar lake area and Balkan Peninsula generally cannot be excluded (Gadawski *et al.*, 2022a) such as the recently discovered endemic chironomid species *M. uva* from Croatia (Giłka *et al.*, 2013). In-depth faunistic studies of the Skadar Lake using molecular techniques have quite recently revealed high molecular, partly cryptic diversity (Gadawski *et al.*, 2022a). This discovery suggests that many cryptic species may exist in understudied habitats even in such well-studied freshwater bodies as Skadar Lake's watershed. It can be concluded that a wider sampling effort should be applied to the Skadar Lake area Chironomidae investigation. The effort must be put in to coordinate the knowledge of immature stages sampled from the region to those based on adults. This can be accomplished using molecular methods, so that both the faunistic and biomonitoring studies may benefit. This purpose could be achieved through the implementation of sampling in different habitats in diffrent seasons of the year, and by implementing molecular techniques such as DNA barcoding (Brodin *et al.*, 2013; Montagna *et al.*, 2016; Baloğlu *et al.*, 2018; Gadawski *et al.*, 2022a). The method could be very useful for developing species checklists and for further improvement of the reference library of DNA barcodes for the region. Moreover, recognising the immature stage of European Chironomidae at the species level with good efficiency is a goal for the taxonomy of these insects, but also a very important prerequisite to make feasible the monitoring of European freshwater quality through DNA metabarcoding using Chironomidae as bioindicators (Brodin *et al.*, 2013; Cranston *et al.*, 2013; Carew *et al.*, 2013; Carew and Hoffmann, 2015). However, identifying immature stages to species level often can not be achieved without the implementation of DNA-based methods.

7 Discussion and conclusions

Due to their size, isolation and seasonality, springs shelter diverse midge communities. Their conditions imply high spatial (natural) variability of community composition. In the river valleys, there is a large variety of springs and pools located from the peripheral valley slopes to the floodplain. Non-biting midges depend on microhabitat and water body permanence. Seasonal pools and seepages can host mainly semiterrestrial chironomid taxa, while perennial springs and ponds usually have species-rich fauna in valleys. There is also a clear gradient in community composition from flowing to more stagnant waters. Habitat complexity plays a crucial role for midges and the bottom substratum determines functional feeding groups. Human activity may favour taxa that are not a typical component of assemblages in the local fauna.

The mountains have a significant impact on the climate of the peninsula. The northern and central parts of the Balkans have a central European climate, characterised by cold winters, warm summers, and well-distributed rainfall. The southern and coastal areas, however, have a Mediterranean type of climate, with hot, dry summers and mild, relatively rainy winters (Allcock *et al.*, 2020). This causes the dominance of cold stenotherm taxa mainly in springs at a higher elevation. Species composition in the mountain springs follows altitudinal zonation. Air temperature and precipitation influence the perennial hydrology of springs. For that reason, the higher mountain zone (1400-1500 m a.s.l.) has the most diverse midge communities. The bedrock plays a secondary role in influencing physical and chemical water conditions.

Precipitation and evaporation in the Lake Skadar catchment (including springs), as well as its bathymetry, causes large water level fluctuations from 4.5 to 10.4 m above the sea level. The highest precipitation in the region occurs during the spring while the lowest during late summer (Pešić *et al.*, 2018, 2019). It causes the seasonal flooding of the springs and provides connection routes between them. It allows the exchange of fauna elements. Chironomid fauna inhabiting the spring system is less diverse compared to more varied lake habitats with macrophytes or submerged wood with higher water temperatures. On the other hand, some rare and less abundant species can be found in springs. In the Mediterranean lowlands, high temperature is a limiting factor for many benthic invertebrates. Many species occur only along the coast of Lake Skadar, in the vicinity of sublacustrine springs or at the mouths of rivers where water parameters vary from those observed within the lake.

Non-biting midge communities of small water bodies are an important and underestimated component of freshwater biodiversity. It is especially important in the Mediterranean Region – a biodiversity hot-spot in dry and warm climates.

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Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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