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Taxonomy & Inventories

Chironomidae (Diptera) of the Šventoji and Žeimena sub-basins in Lithuania

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Abstract

Background

Chironomidae, commonly known as non-biting midges, are key indicators of the health and biodiversity of freshwater ecosystems. They are also one of the most abundant and diverse groups of aquatic invertebrates. Although Chironomidae are ecologically important, abundant and diverse, there has been limited focused research on this group in Lithuania. Our study addresses this gap by analysing the diversity of Chironomidae in six Lithuanian streams.

New information

In this study, we present a comprehensive list of Chironomidae collected from six streams with similar hydrological characteristics: three dammed and three undammed. Lithuania is home to 158 species of known species of Chironomidae, comprised of 22 species in the subfamily Tanypodinae, 87 in the Chironominae, 42 in the Orthocladiinae, four in the Diamesinae and three in the Prodiamesinae. Throughout our research, we collected

11,296 chironomid specimens using a D-shaped aquatic net. Amongst these specimens, we identified 89 species representing 65 genera and five subfamilies, including 37 species and 12 genera, were recorded for the first time in Lithuania. The subfamily Chironominae, with 28 genera and 6,816 specimens, has exhibited the highest abundance of non-biting midges both in terms of genera and individuals. Amongst the streams investigated, the Luknelė was the richest in diversity, harbouring 37 genera and 2,657 individuals, accounting for about 55% of the Chironomidae genera found during the research. Our findings significantly enhance the understanding of the Lithuanian Chironomidae fauna, marking the first comprehensive study of such a kind, as previous knowledge of this fauna has been derived only from related studies.

Keywords

Chironominae, Diamesinae, Orthocladiinae, Prodiamesinae, Tanypodinae, checklist, streams

Introduction

Rivers and streams occupy only 0.51% of Lithuania's territory (Gailiušis et al. 2001). The country boasts approximately 29,000 streams longer than 0.25 km, with a cumulative length of about 64,000 km (Kilkus and Stonevičius 2011). Around 80% of these streams (representing about 51% of the total stream length) are very small, measuring up to 3 km in length (Jablonskis et al. 2007). There are 3,646 small streams ranging from 3 to 10 km, collectively accounting for 24.5% of the total stream length. Medium-sized rivers, ranging from 10 to 100 km, constitute 20.4% of the total stream length, with a total of 75 such streams present in the country. Notably, only 17 rivers in Lithuania exceed 100 km in length, comprising just 0.1% of the total stream length. The Nemunas Basin is the largest river basin in Lithuania, covering an area of 46,695.4 km² within the country, with a total basin area of 97,863.5 km². The Nemunas River, flowing through this Basin, is the longest river in Lithuania, stretching for 475 km within Lithuania out of its total length of 937.4 km. The investigated streams are part of two sub-basins. The first sub-basin is the Šventoji River, the longest river flowing entirely within Lithuania, with a length of 246 km and a basin area of 6,889 km^2. The second sub-basin is the Žeimena, with Žeimena River measuring 79.6 km in length and encompassing a basin area of 2,793 km^2 (Jablonskis et al. 2007).

Diptera is the predominant group of macroinvertebrates in freshwater ecosystems, typically comprising the largest biomass and it is also one of the most extensively researched groups in freshwater environments (Ivković et al. 2020). Amongst aquatic macroinvertebrates, the Chironomidae family is generally the most abundant, both in individual numbers and species diversity (Farias et al. 2012, Rocha et al. 2012, Dantas et al. 2024). With over 6,000 species inhabiting various biotopes and occupying diverse niches, non-biting midges exhibit a wide array of trophic specialisations and lifestyles (Stur and Ekrem 2020). Given their tendency to dominate freshwater fauna,

Chironomidae are considered important bioindicators for assessing freshwater (Lencioni et al. 2012). Consequently, they play a crucial role in monitoring, protecting and conserving freshwater environments (Cortelezzi et al. 2020).

In Lithuania, more than 150 species of Chironomidae have been recorded (Pakalniškis et al. 2006, Ruginis 2007, Móra and Kovács 2009). However, only a few isolated identifications have been made in previous studies (Móra and Kovács 2009) and comprehensive research on this family is yet to be conducted in Lithuania. Based on studies of Chironomidae diversity in the neighbouring countries, it is likely that several times more species exist in the Lithuania (Schartau et al. 2010, Paasivirta 2014). The aim of this study was to expand knowledge about the diversity and distribution of Chironomidae in streams of Lithuania.

Materials and methods

Hydrography and geography of the sampling area

The study was conducted in Lithuania, specifically within the Nemunas Basin, focusing on the sub-basins of the Šventoji and Žeimena Rivers. To ensure accurate data for the comparison, over 200 rivers and streams in Lithuania were screened and evaluated, based on data from the Rivers, Lakes and Ponds Cadastre of the Republic of Lithuania (UETK) data (Lietuvos Respublikos aplinkos ministerija 2024). Chironomidae specimens were collected from two types of streams: dammed (Skerdyksna, Šešuola, Dubinga) and undammed (Plaštaka, Kiauna, Luknelė). The streams were grouped into three pairs and then selected, based on their similarities in terms of geographic location, morphometric and hydrographic characteristics. The sample collection sites are situated in three protected areas: Anyksčiai and Asveja Regional Parks (Plaštaka, Šešuola, Dubinga and Skerdyksna) and Aukštaitija National Park and Labanoras Regional Park (Luknelė and Kiauna) (Fig. 1). All the streams are in the central-eastern part of Lithuania, with altitude ranges from 80 m to 150 m above sea level and traverse various landscapes, including natural or semi-natural forests and meadows, as well as agricultural and urbanised areas. The flow rate varied between the study sites, with a steady increase downstream in the undammed streams. However, the dammed streams varied considerably. For example, the Skerdyksna stream in the upper reaches passes through agricultural fields and is reclaimed, resulting in very low flow velocities. In the middle of the stream, at study sites 6 and 7, the stream is dammed. Upstream of the dam, a pond is formed, leading to stagnant water flow. Downstream of the dam, the flow velocity increases and the stream follows a natural course, characterised by a habitat-rich environment. The Šešuola stream is dammed twice, so all study sites were chosen at the dams. Study sites 23 and 21 are located in the ponds, resulting in stagnant water flow. Study site 24 is situated below the dam and in agricultural fields, where the stream section is reclaimed, with consistently low water levels averaging about 15 cm, leading to very low water velocity. Below the second dam, at study site 22, the river section is natural, which increases the flow rate. However, due to the dam and the varying rainfall, the stream depth fluctuates significantly during the season, ranging from 5 cm to 30 cm. The Dubinga stream, the

third dammed stream, is dammed only in its downstream, while the upstream is natural or semi-natural, flowing through several small villages without intensive agriculture. The stream is quite deep in the upper and middle reaches, with study sites 1 and 2 maintaining an average depth of around 40 cm throughout the season. The last two survey sites in this stream were at the dam, with survey site 3 located in a pond, resulting in a stagnant water flow. Behind the dam, the stream's velocity and depth varied due to dam operations, with the depth ranging from 0 cm to 15 cm, causing highly variable flow velocity. The substrate of the studied streams varied not only between different streams, but also within the same stream at different study sites. Sand was the predominant substrate overall, but the composition and distribution of substrates varied between habitats. The substrate composition at the study sites included: silt, clay, mud, sand (the predominant substrate), granules, pebbles, cobbles and boulders. Additionally, some of the pristine sites were rich in detritus, with layers up to 0.5 m thick and contained dead wood trunks, some banks also being covered with abundant aquatic vegetation (Table 1).

Table 1.

List of the six researched streams along with pertinent information.

Sub-basin	Stream	Length (km)	Discharge (m^3/s)	Catchment area (km^2)	Dammed	Coordinates of sampling sites			
Žeimena	Dubinga	18.1	4.01	405.9	Yes	1 55°00'51.1"N, 25°38'16.4"E	2 55°00'56.4"N, 25°42'14.4"E	3 54°59'32.9"N, 25°45'16.6"E	4 54°59'27.2"N, 25°45'32.8"E
Žeimena	Skerdyksna	13.9	0.50	42.2	Yes	5 54°56'44.7"N, 25°50'37.2"E	6 54°56'50.9"N, 25°45'35.6"E	7 54°56'47.5"N, 25°45'29.4"E	8 54°57'08.5"N, 25°43'18.7"E
Žeimena	Luknelė	13.6	0.54	45.0	No	9 55°12'49.1"N, 25°56'32.1"E	10 55°13'22.0"N, 25°52'04.6"E	11 55°14'20.9"N, 25°51'14.6"E	12 55°15'00.0"N, 25°50'22.5"E
Žeimena	Kiauna	17.9	2.95	308.7	No	13 55°14'48.5"N, 25°58'14.5"E	14 55°15'50.5"N, 25°56'28.6"E	15 55°17'44.4"N, 25°53'38.0"E	16 55°18'29.6"N, 25°53'12.9"E
Šventoji	Plaštaka	18.1	0.82	88.3	No	17 55°18'29.12″N, 25°1'51.20″E	18 55°18'50.94″N, 25°3'41.15″E	19 55°20'9.35″N, 25°2'11.69″E	20 55°16'40.62″N, 24°59'10.82″E
Šventoji	Šešuola	15.6	0.65	91.7	Yes	21 55°15'10.2"N, 24°58'40.9"E	22 55°15'14.9"N, 24°58'44.0"E	23 55°10'56.5"N, 24°56'28.0"E	24 55°11'03.7"N, 24°56'33.3"E

Sampling and identification

The research was conducted in 2021 and 2022, spanning from May to September. Sampling was conducted across six streams with four research sites in each, resulting in sampling from 24 sites in total. Samples were gathered every two weeks using a D-shaped aquatic net mesh size of 1 mm (Fig. 2). At each site, a 1 m² area was randomly selected for sampling, which was conducted using the Kick Sampling method (Letovsky et al. 2012). Samples collected were transferred into 2 litre zip-lock bags filled with 99% propylene glycol. Upon collection, all samples were stored in a refrigerator at 4°C in the Life Sciences Centre of Vilnius University. Subsequently specimens which were identified as belonging to the Chironomidae family underwent meticulous separation and were preserved in containers filled with 97% ethanol.

Chironomidae larvae were identified by using taxonomic keys, based on morphology (morpho) available from Epler (2001), Orendt et al. (2011) and Andersen et al. 2013. The systematics and nomenclature of taxa follow Andersen et al. (2013). For molecular analysis, larvae from 200 specimens were selected. Total genomic DNA was extracted from each larva using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's protocol. Partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified using primers LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR amplification was performed in a thermal cycler (Eppendorf) in 30 µl reaction volumes containing 3 µl genomic DNA, 1.5 µl of each primer (0.5 µM), 15 µl of DreamTaq PCR Master Mix (Thermo Scientific) and 9 µl of nuclease free water (Thermo Scientific). The cycling parameters were as follows: initial denaturation at 95°C for 3 minutes (1 cycle), denaturising at 95°C for 30 seconds, annealing at 49°C for 30 seconds, extension at 72°C for 60 seconds (35 cycles in total) and final extension at 72°C for 10 minutes (1 cycle). PCR products were purified using the GeneJet PCR purification kit (Thermo Scientific) and sequenced at Macrogen Europe BV (Amsterdam, the Netherlands). The amplification primers were also used as sequencing primers. DNA sequences for each specimen were aligned in the BioEdit Sequence Alignment Editor (Hall 1999) and compared by BLAST (National Library of Medicine 2024). GenBank accession numbers for each individual are given in the Check List.

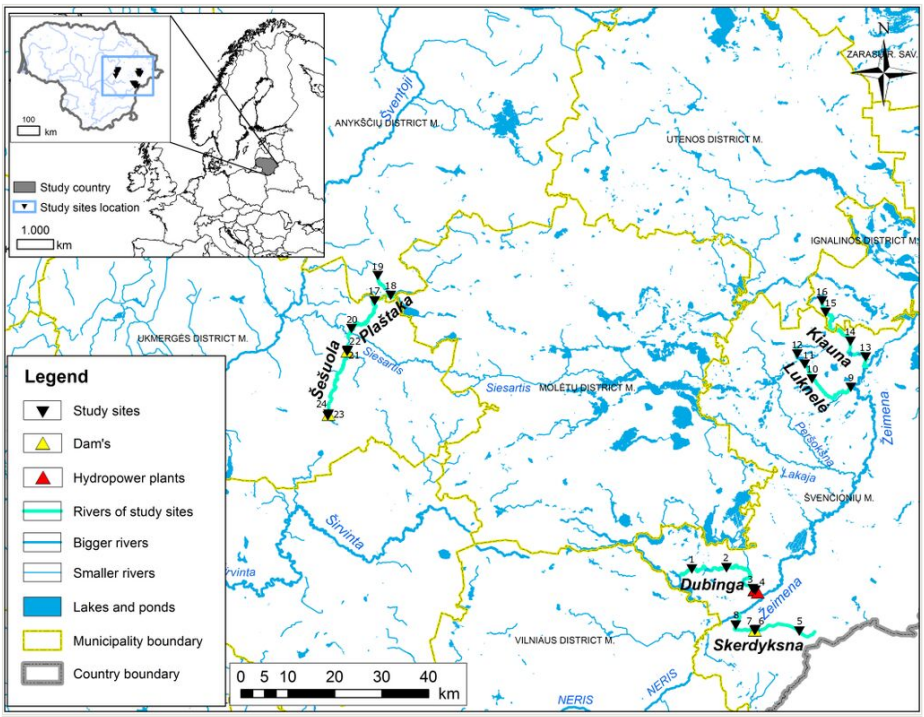

Figure 1. doi

Map of Lithuania highlighting the studied streams and their locations.

Sample-based rarefaction curves were produced to visually compare the genera richness of non-biting midges amongst the researched streams and to assess the sampling effort (Gotelli and Colwell 2001). The similarity of chironomid assemblages collected over two study years from dammed and undammed streams was visualised using non-metric multidimensional scaling (NMDS) ordination, based on the Bray–Curtis similarity index. The analyses were conducted using PAST 4.07b software (Hammer et al. 2001).

Figure 2. doi

Sample collection using the Kick Sampling method by using D-shaped aquatic net.

Check list of Chironomidae collected across six streams: Skerdyksna, Šešuola, Dubinga, Plaštaka, Kiauna and Luknelė

Order Diptera Linnaeus, 1758

Suborder Nematocera Dumeril, 1805

Infraorder Culicomorpha Hennig, 1948

Family CHIRONOMIDAE Newman, 1834

Subfamily Tanypodinae Skuse, 1889

Tribe Anatopyniini Fittkau, 1962

Genus *Anatopynia* Johannsen, 1905

Anatopynia sp.

Notes: Morpho identification. First record for Lithuania (Lapinskaitė 1968).

Tribe Coelotanypodini Coffman, 1978

Genus *Clinotanypus* Kieffer, 1913

Clinotanypus nervosus (Meigen, 1818)

Notes: Morpho-molecular identification (GenBank ID: PQ458089; PQ458090; PQ458091). First record for Lithuania (Lapinskaitė 1968).

Tribe Macropelopiini Zavřel, 1929

Genus *Apsectrotanypus* Fittkau, 1962

Apsectrotanypus trifascipennis (Zetterstedt, 1838)

Notes: Morpho-molecular identification (GenBank ID: PQ458073; PQ458074). First record for Lithuania (Móra and Kovács 2009).

Genus *Macropelopia* Thienemann, 1916

Macropelopia nebulosa (Meigen, 1804)

Notes: Morpho identification. New record for Lithuania.

Macropelopia notata (Meigen, 1818)

Notes: Morpho identification. First record for Lithuania (Sæther and Spies 2005).

Genus *Psectrotanypus* Kieffer, 1909

Psectrotanypus varius (Fabricius, 1787)

Notes: Morpho-molecular identification (GenBank ID: PQ458163). First record for Lithuania (Grigelis 1999).

Tribe Pentaneurini Hennig, 1950

Genus *Ablabesmyia* Johannsen, 1905

Ablabesmyia (*Ablabesmyia*) *longistyla* Fittkau, 1962

Notes: Morpho-molecular identification (GenBank ID: PQ458066; PQ458067; PQ458068; PQ458069; PQ458070; PQ458071). First record for Lithuania (Sæther and Spies 2005).

Ablabesmyia (*Ablabesmyia*) *monilis* (Linnaeus, 1758)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Ablabesmyia (*Ablabesmyia*) *phatta* (Egger, 1864)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Conchapelopia* Fittkau, 1957

Conchapelopia melanops (Meigen, 1818)

Notes: Morpho-molecular identification (GenBank ID: PQ458092; PQ458093; PQ458094; PQ458095; PQ458096; PQ458097). New record for Lithuania.

Genus *Krenopelopia* Fittkau, 1962

Krenopelopia binotata (Wiedemann, 1817)

Notes: Morpho identification. First record for Lithuania (Sæther and Spies 2005).

Genus *Larsia* Fittkau, 1962

Larsia atrocincta (Goetghebuer, 1942)

Notes: Morpho-molecular identification (GenBank ID: PQ458114; PQ458115). New record for Lithuania.

Genus *Zavrelimyia* Fittkau, 1962

Zavrelimyia melanura (Meigen, 1804)

Notes: Morpho identification. First record for Lithuania (Pliūraitė and Kesminas 2004).

Tribe Procladiini Roback, 1971

Genus *Procladius* Skuse, 1889

Procladius (*Holotanypus*) *crassinervis* (Zetterstedt, 1838)

Notes: Morpho-molecular identification (GenBank ID: PQ458151). New record for Lithuania.

Procladius (*Holotanypus*) *culiciformis* (Linnaeus, 1767)

Notes: Morpho-molecular identification (GenBank ID: PQ458152; PQ458153; PQ458154). New record for Lithuania.

Procladius (*Holotanypus*) *denticulatus* Sublette, 1964

Notes: Morpho-molecular identification (GenBank ID: PQ458155; PQ458156; PQ458157). New record for Lithuania.

Procladius (*Holotanypus*) *fuscus* Brundin, 1956

Notes: Morpho identification. New record for Lithuania.

Procladius (*Holotanypus*) *pectinatus* (Kieffer, 1909)

Notes: Morpho-molecular identification (GenBank ID: PQ458158). New record for Lithuania.

Procladius sp. Skuse, 1889

Notes: Morpho-molecular identification (GenBank ID: PQ458159). First record for Lithuania (Grigelis 1999).

Tribe Tanypodini Skuse, 1889

Genus *Tanypus* Meigen, 1803

Tanypus (*Tanypus*) *kraatzi* (Kieffer, 1912)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Tanypus (*Tanypus*) *vilipennis* (Kieffer, 1918)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Subfamily Diamesinae Kieffer, 1922

Tribe Diamesini Kieffer, 1922

Genus *Potthastia* Kieffer, 1922

Potthastia sp.

Notes: Morpho identification. First record for Lithuania (Gasiūnas 1959).

Genus *Pseudodiamesa* Goetghebuer, 1939

Pseudodiamesa (*Pachydiamesa*) *arctica* (Malloch, 1919)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Subfamily Prodiamesinae Sæther, 1976

Genus *Monodiamesa* Kieffer, 1922

Monodiamesa bathyphila (Kieffer, 1918)

Notes: Morpho-molecular identification (GenBank ID: PQ458130; PQ458131). First record for Lithuania (Grigelis 1999).

Genus *Odontomesa* Pagast, 1947

Odontomesa fulva (Kieffer, 1919)

Notes: Morpho-molecular identification (GenBank ID: PQ458134). First record for Lithuania (Pliūraitė 2001b).

Genus *Prodiamesa* Kieffer, 1906

Prodiamesa olivacea (Meigen, 1818)

Notes: Morpho-molecular identification (GenBank ID: PQ458160; PQ458161; PQ458162). First record for Lithuania (Bubinas and Jagminienė 2001).

Subfamily Orthocladiinae Kieffer, 1911

Genus *Acricotopus* Kieffer, 1921

Acricotopus lucens (Zetterstedt, 1850)

Notes: Morpho-molecular identification (GenBank ID: PQ458072). New record for Lithuania.

Genus *Brillia* Kieffer, 1913

Brillia sp.

Notes: Morpho identification. First record for Lithuania (Pliūraitė 2001a).

Genus *Chaetocladius* Kieffer, 1911

Chaetocladius (*Chaetocladius*) *piger* (Goetghebuer, 1913)

Notes: Morpho identification. New record for Lithuania.

Genus *Corynoneura* Winnertz, 1846

Corynoneura sp.

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Cricotopus* van der Wulp, 1874

Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)

Notes: Morpho-molecular identification (GenBank ID: PQ458098). First record for Lithuania (Grigelis 1999).

Cricotopus (*Cricotopus*) *cylindraceus* (Kieffer, 1908)

Notes: Morpho identification. New record for Lithuania.

Cricotopus (*Cricotopus*) *festivellus* (Kieffer, 1906)

Notes: Morpho identification. New record for Lithuania.

Cricotopus (*Isocladius*) *perniger* (Zetterstedt, 1850)

Notes: Morpho identification. New record for Lithuania.

Cricotopus sp.

Notes: Morpho-molecular identification (GenBank ID: PQ458100). First record for Lithuania (Grigelis 1999).

Cricotopus (*Isocladius*) *sylvestris* Fabricius, 1794

Notes: Morpho-molecular identification (GenBank ID: PQ458099). First record for Lithuania (Grigelis 1999).

Genus *Epoicocladius* Sulc and Zavřel, 1924

Epoicocladius ephemerae (Kieffer, 1924)

Notes: Morpho identification. First record for Lithuania (Virbickas and Pliūraitė 2002).

Genus *Eukiefferiella* Thienemann, 1926

Eukiefferiella sp.

Notes: Morpho identification. First record for Lithuania (Pliūraitė 1999).

Genus *Halocladius* Hirvenoja, 1973

Genus *Heterotrissocladius* Spärck, 1923

Heterotrissocladius marcidus (Walker, 1856)

Notes: Morpho identification. New record for Lithuania.

Genus *Limnophyes* Eaton, 1875

Limnophyes minimus (Meigen, 1818)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Metriocnemus* van der Wulp, 1874

Metriocnemus (*Metriocnemus*) *eurynotus* (Holmgren, 1883)

Notes: Morpho identification. First record for Lithuania (Sæther and Spies 2005).

Genus *Nanocladius* Kieffer, 1913

Nanocladius (*Nanocladius*) *dichromus* (Kieffer, 1906)

Notes: Morpho-molecular identification (GenBank ID: PQ458132; PQ458133). First record for Lithuania (Grigelis 1999).

Genus *Orthocladius* van der Wulp, 1874

Orthocladius (*Orthocladius*) *decoratus* (Holmgren, 1869)

Notes: Morpho identification. New record for Lithuania.

Orthocladius (*Orthocladius*) *oblidens* (Walker, 1856)

Notes: Morpho-molecular identification (GenBank ID: PQ458135; PQ458136; PQ458137). New record for Lithuania.

Orthocladius (*Orthocladius*) *rubicundus* (Meigen, 1818)

Notes: Morpho-molecular identification (GenBank ID: PQ458138). First record for Lithuania (Grigelis 1999).

Genus *Paracladius* Hirvenoja, 1973

Paracladius conversus (Walker, 1856)

Notes: Morpho identification. First record for Lithuania (Sæther and Spies 2005).

Genus *Parakiefferiella* Thienemann, 1936

Parakiefferiella sp.

Notes: Morpho identification. First record for Lithuania (Ashe and Cranston 1990).

Genus *Parametriocnemus* Goetghebuer, 1932

Parametriocnemus sp.

Notes: Morpho identification. New record for Lithuania.

Genus *Paraphaenocladius* Thienemann, 1924

Paraphaenocladius sp.

Notes: Morpho-molecular identification (GenBank ID: PQ458064; PQ458065). New record for Lithuania.

Genus *Psectrocladius* Kieffer, 1906

Psectrocladius (*Psectrocladius*) *limbatellus* (Holmgren, 1869)

Notes: Morpho identification. New record for Lithuania.

Psectrocladius (*Psectrocladius*) *psilopterus* Thienemann, 1906)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Psectrocladius (*Psectrocladius*) *sordidellus* (Zetterstedt, 1838)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Rheocricotopus* Brundin, 1956

Rheocricotopus (*Rheocricotopus*) *fuscipes* (Kieffer, 1909)

Notes: Morpho identification. New record for Lithuania.

Genus *Synorthocladius* Thienemann, 1935

Synorthocladius semivirens (Kieffer, 1909)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Thienemanniella* Kieffer, 1911

Thienemanniella sp.

Notes: Morpho identification. New record for Lithuania.

Genus *Zalutschia* Lipina, 1939

Zalutschia sp.

Notes: Morpho identification. First record for Lithuania (Pliūraitė 1999).

Subfamily Chironominae Newman, 1834

Tribe Chironomini Newman, 1834

Genus *Chironomus* Meigen, 1803

Chironomus (*Chironomus*) *acidophilus* Keyl, 1960

Notes: Morpho-molecular identification (GenBank ID: PQ458075). New record for Lithuania.

Chironomus (*Chironomus*) *cingulatus* Meigen, 1830

Notes: Morpho-molecular identification (GenBank ID: PQ458076; PQ458077; PQ458078; PQ458079). New record for Lithuania.

Chironomus (*Chironomus*) *curabilis* Belyanina, Sigareva and Loginova, 1990

Notes: Morpho-molecular identification (GenBank ID: PQ458080; PQ458081). New record for Lithuania.

Chironomus (*Chironomus*) *melanescens* Keyl, 1961

Notes: Morpho-molecular identification (GenBank ID: PQ458082). New record for Lithuania.

Chironomus (*Chironomus*) *melanotus* Keyl, 1961

Notes: Morpho-molecular identification (GenBank ID: PQ458083). New record for Lithuania.

Chironomus (*Chironomus*) *pallidivittatus* Malloch, 1915

Notes: Morpho-molecular identification (GenBank ID: PQ458085). New record for Lithuania.

Chironomus (*Chironomus*) *piger* Strenzke, 1959

Notes: Morpho-molecular identification (GenBank ID: PQ458086; PQ458087). New record for Lithuania.

Chironomus (*Chironomus*) *plumosus* (Linnaeus, 1758)

Notes: Morpho identification. First record for Lithuania (Grigelis et al. 1981).

Chironomus (*Chironomus*) *pseudothummi* Strenzke, 1959

Notes: Morpho-molecular identification (GenBank ID: PQ458084; PQ458088). New record for Lithuania.

Chironomus (*Chironomus*) *riparius* Meigen, 1804

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Chironomus (*Chironomus*) *salinarius* Kieffer, 1915

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Cladopelma* Kieffer, 1921

Cladopelma sp.

Notes: Morpho identification. First record for Lithuania (Ashe and Cranston 1990).

Genus *Cryptochironomus* Kieffer, 1918

Cryptochironomus albofasciatus (Staeger, 1839)

Notes: Morpho-molecular identification (GenBank ID: PQ458101; PQ458102). New record for Lithuania.

Cryptochironomus obreptans (Walker, 1856)

Notes: Morpho-molecular identification (GenBank ID: PQ458103). New record for Lithuania.

Cryptochironomus rostratus Kieffer, 1921

Notes: Morpho-molecular identification (GenBank ID: PQ458104). New record for Lithuania.

Genus *Cryptotendipes* Beck and Beck, 1969

Cryptotendipes sp.

Notes: Morpho identification. First record for Lithuania (Sæther and Spies 2005).

Genus *Demicryptochironomus* Lenz, 1941

Demicryptochironomus (*Demicryptochironomus*) *vulneratus* (Zetterstedt, 1838)

Notes: Morpho-molecular identification (GenBank ID: PQ458105; PQ458106). First record for Lithuania (Ashe and Cranston 1990).

Genus *Dicrotendipes* Kieffer, 1913

Dicrotendipes nervosus (Staeger, 1839)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Dicrotendipes tritomus Thienemann & Kieffer, 1916

Notes: Morpho-molecular identification (GenBank ID: PQ458107). First record for Lithuania (Grigelis 1999).

Genus *Einfeldia* Kieffer, 1924

Einfeldia pagana (Meigen, 1838)

Notes: Morpho-molecular identification (GenBank ID: PQ458108; PQ458109). First record for Lithuania (Grigelis 1999).

Genus *Endochironomus* Kieffer, 1918

Endochironomus albipennis (Meigen, 1830)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Endochironomus tendens (Fabricius, 1775)

Notes: Morpho-molecular identification (GenBank ID: PQ458110; PQ458111). First record for Lithuania (Grigelis 1999).

Genus *Glyptotendipes* Kieffer, 1913

Glyptotendipes (*Phytotendipes*) *cauliginellus* (Kieffer, 1913)

Notes: Morpho-molecular identification (GenBank ID: PQ458112). First record for Lithuania (Grigelis 1999).

Glyptotendipes (*Phytotendipes*) *pallens* (Meigen, 1804)

Notes: Morpho identification. First record for Lithuania (Pliūraitė and Kesminas 2004).

Glyptotendipes (*Trichotendipes*) *signatus* (Kieffer, 1909)

Notes: Morpho identification. New record for Lithuania.

Genus *Harnischia* Kieffer, 1921

Harnischia fuscimanus Kieffer, 1921

Notes: Morpho-molecular identification (GenBank ID: PQ458113). First record for Lithuania (Pliūraitė 2001a).

Genus *Microtendipes* Kieffer, 1915

Microtendipes chloris (Meigen, 1818)

Notes: Morpho-molecular identification (GenBank ID: PQ458117; PQ458118; PQ458119; PQ458120; PQ458121). First record for Lithuania (Grigelis 1999).

Microtendipes pedellus (De Geer, 1776)

Notes: Morpho-molecular identification (GenBank ID: PQ458122; PQ458123; PQ458124; PQ458125; PQ458126; PQ458127). First record for Lithuania (Grigelis 1999).

Microtendipes rydalensis (Edwards, 1929)

Notes: Morpho-molecular identification (GenBank ID: PQ458128). New record for Lithuania.

Microtendipes sp.

Notes: Morpho-molecular identification (GenBank ID: PQ458129). First record for Lithuania (Grigelis 1999).

Microtendipes tarsalis (Walker, 1856)

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Parachironomus* Lenz, 1921

Parachironomus vitiosus (Goetghebuer, 1921)

Notes: Morpho-molecular identification (GenBank ID: PQ458139; PQ458140; PQ458141). First record for Lithuania (Ashe and Cranston 1990).

Genus *Paracladopelma* Harnisch, 1923

Paracladopelma camptolabis (Kieffer, 1913)

Notes: Morpho identification. First record for Lithuania (Pliūraitė and Kesminas 2004).

Genus *Paratendipes* Kieffer, 1911

Paratendipes albimanus (Meigen, 1804)

Notes: Morpho-molecular identification (GenBank ID: PQ458142; PQ458143; PQ458144; PQ458145). First record for Lithuania (Grigelis 1999).

Genus *Polypedilum* Kieffer, 1912

Polypedilum (*Uresipedilum*) *convictum* (Walker, 1856)

Notes: Morpho-molecular identification (GenBank ID: PQ458146). First record for Lithuania (Grigelis 1999).

Polypedilum (*Uresipedilum*) *cultellatum* Goetghebuer, 1931

Notes: Morpho-molecular identification (GenBank ID: PQ458147). New record for Lithuania.

Polypedilum (*Polypedilum*) *nubeculosum* (Meigen, 1804)

Notes: Morpho identification. First record for Lithuania (Ashe and Cranston 1990).

Polypedilum (*Tripodura*) *pullum* (Zetterstedt, 1838)

Notes: Morpho identification. New record for Lithuania.

Polypedilum (*Tripodura*) *scalaenum* Schrank, 1803

Notes: Morpho-molecular identification (GenBank ID: PQ458148; PQ458149). First record for Lithuania (Grigelis 1999).

Polypedilum (*Pentapedilum*) *sordens* (Wulp, 1875)

Notes: Morpho identification. First record for Lithuania (Ashe and Cranston 1990).

Polypedilum sp.

Notes: Morpho-molecular identification (GenBank ID: PQ458150). First record for Lithuania (Lapinskaitė 1968).

Genus *Synendotendipes* Grodhaus, 1987

Synendotendipes impar (Walker, 1856)

Notes: Morpho-molecular identification (GenBank ID: PQ458166). First record for Lithuania (Pliūraitė 1999).

Genus *Stenochironomus* Kieffer, 1919

Stenochironomus (*Stenochironomus*) *gibbus* (Fabricius, 1794)

Notes: Morpho-molecular identification (GenBank ID: PQ458164; PQ458165). New record for Lithuania.

Genus *Stictochironomus* Kieffer, 1919

Stictochironomus sp.

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Tribelos* Townes, 1945

Tribelos intextus (Walker, 1856)

Notes: Morpho-molecular identification (GenBank ID: PQ458171; PQ458172). First record for Lithuania (Ashe and Cranston 1990).

Genus *Xenochironomus* Kieffer, 1921

Xenochironomus xenolabis Kieffer, 1916

Notes: Morpho identification. First record for Lithuania (Pliūraitė 1999).

Tribe Tanytarsini Zavřel, 1917

Genus *Cladotanytarsus* Kieffer, 1921

Cladotanytarsus mancus (Walker, 1856)

Notes: Morpho identification. First record for Lithuania (Ashe and Cranston 1990).

Genus *Micropsectra* Kieffer, 1908

Micropsectra apposita (Walker, 1856)

Notes: Morpho identification. New record for Lithuania.

Micropsectra contracta Reiss, 1965

Notes: Morpho-molecular identification (GenBank ID: PQ458116). First record for Lithuania (Grigelis 1999).

Genus *Neozavrelia* Goetghebuer, 1941

Neozavrelia sp.

Notes: Morpho identification. New record for Lithuania.

Genus *Paratanytarsus* Thienemann and Bause, 1913

Paratanytarsus sp.

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Stempellina* Thienemann and Bause, 1913

Stempellina sp.

Notes: Morpho identification. First record for Lithuania (Grigelis 1999).

Genus *Tanytarsus* van der Wulp, 1874

Tanytarsus multipunctatus Brundin, 1947

Notes: Morpho-molecular identification (GenBank ID: PQ458168; PQ458169; PQ458170). New record for Lithuania.

Genus *Virgatanytarsus* Pinder, 1982

Virgatanytarsus sp.

Notes: Morpho-molecular identification (GenBank ID: PQ458167). New record for Lithuania.

Analysis

A grand total of 11,296 non-biting midge specimens, comprising 89 species representing 65 genera and five subfamilies, were gathered from the sampling sites from six streams. Most specimens were identified to the subfamily and genus level, based on both morphological characteristics and analysis of partial COI sequences.

The highest richness of chironomids was observed in the subfamily Chironominae, which included 28 genera and 45 species. In contrast, the lowest richness was found in Diamesinae, with two genera and two species (Fig. 3). The Orthocladiinae exhibited a richness of 21 genera and 21 species, while Prodiamesinae showed a richness of 13 genera and 19 species.

In terms of abundance, the subfamilies Chironominae (6816 specimens) and Tanypodinae (2476 specimens) were the most prevalent, together accounting for 82.46% of collected specimens. Conversely, the subfamilies Prodiamesinae and Diamesinae

subfamilies had the fewest individuals, with specimens 176 and six specimens, respectively.

Amongst the genera, the most abundant were *Ablabesmyia* Johannsen, 1905 (944 specimens), *Microtendipes* Kieffer, 1915 (661 specimens), *Procladius* Skuse, 1889 (474 specimens), *Tanytarsus* van der Wulp, 1874 (349 specimens), *Polypedilum* Kieffer, 1912 (290 specimens), *Chironomus* Meigen, 1803 (254 specimens), *Conchapelopia* Fittkau, 1957 (168 specimens), *Prodiamesa* Kieffer, 1906 (156 specimens), *Micropsectra* Kieffer, 1908 (151 specimens), *Paratendipes* Kieffer, 1911 (104 specimens) and *Psectrocladius* Kieffer, 1906 (103 specimens) (Fig. 4).

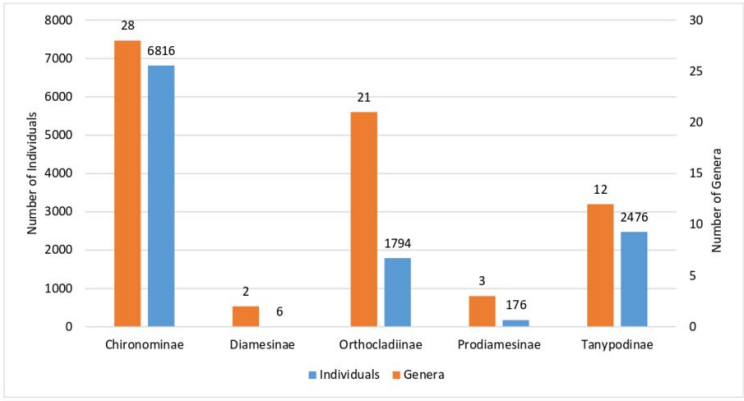

Figure 3. doi

The amount of individuals (blue bars) and the distribution across genera (orange bars) of non-biting midges' (Chironomidae) subfamilies collected during the study.

The Table below lists the subfamilies, genera and the abundance of specimens in each of the studied streams: Dubinga, Kiauna, Luknelė, Plaštaka, Skerdyksna and Šešuola in 2021 and 2022, spanning from May to September (Table 2).

The genera richness of non-biting midges reached an asymptote in all the streams studied, except for the Kiauna stream, suggesting that additional genera may still be discovered (Fig. 5).

Amongst the undammed streams, the Luknelė had the highest abundance of individuals and the greatest number of genera identified. It also emerged as a stream with the highest abundance and diversity of chironomids in terms of genera across all the streams studied. In contrast, the Šešuola stream had the lowest number of individuals, the least

diversity of genera across all the studied streams and was the only stream that was dammed twice (Fig. 6). Since three of the streams studied were dammed and three were undammed, NMDS analysis was conducted to compare them based on chironomid assemblages. The visual analysis showed that the genera composition of chironomids in dammed and undammed streams partially overlapped (Fig. 7).

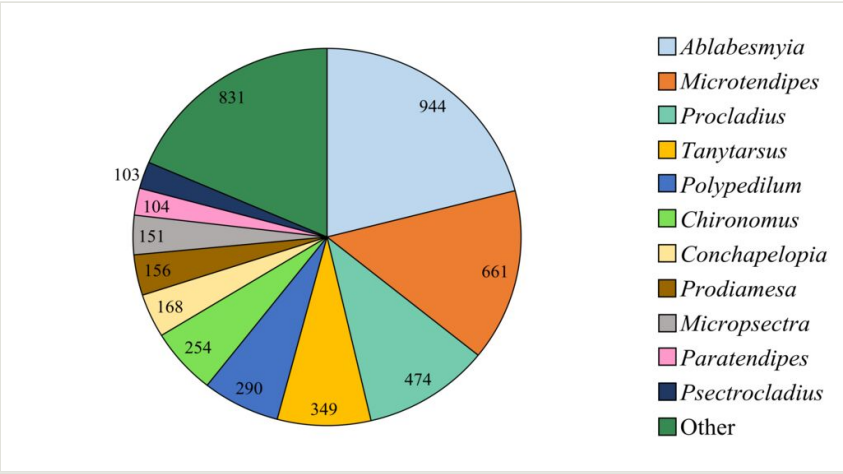

Figure 4. doi

The number of specimens of non-biting midges (Chironomidae) representing each genus after excluding those with less than 100 collected specimens.

Table 2.

List of non-biting midges (Chironomidae) sub-families and genera and their abundance collected of six streams in Lithuania, 2021 to 2022: Dubinga, Kiauna, Luknelė, Plaštaka, Skerdyksna and Šešuola.

Sub-family	Genera	Dubinga	Kiauna	Luknelė	Plaštaka	Skerdyksna	Šešuola
Tanypodinae	*Anatopynia*		1	1			
	Clinotanypus	5	1	4	13	47	10
	Apsectrotanypus			10			
	Macropelopia		1	6	2		
	Psectrotanypus				4		
	Ablabesmyia	119	206	412	75	38	94
	Conchapelopia	4	39	73	10	16	26
	Krenopelopia			2			
	Larsia		4	2			
	Zavrelimyia			5		1	

Sub-family	Genera	Dubinga	Kiauna	Luknelė	Plaštaka	Skerdyksna	Šešuola
	Procladius	84	68	54	81	153	34
	Tanypus		2		2		4
Diamesinae	*Potthastia*	4					
	Pseudodiamesa			1		1	
Prodiamesinae	*Monodiamesa*	10		1	2		
	Odontomesa				7		
	Prodiamesa	69	41	13		3	30
Orthocladiinae	*Acricotopus*				23		
	Brillia	1					
	Chaetocladius				1	1	
	Corynoneura	23	9	15	14	5	17
	Cricotopus	16		2		4	15
	Epoicocladius	3		1			
	Eukiefferiella		1		2		
	Heterotrissocladius			4			
	Limnophyes			1			
	Metriocnemus				1		
	Nanocladius					2	
	Orthocladius		4	5			64
	Paracladius		3		1		1
	Parakiefferiella	15	9	7			3
	Parametriocnemus				3		
	Paraphaenocladius			58			
	Psectrocladius	91	3	5	2	2	
	Rheocricotopus			19			
	Synorthocladius	5			2		1
	Thienemanniella			1	1		
	Zalutschia	2		7			
Chironominae	*Chironomus*	38	29		85	59	12
	Cladopelma		2				

Sub-family	Genera	Dubinga	Kiauna	Luknelė	Plaštaka	Skerdyksna	Šešuola
	Cryptochironomus	8	4	4	6	2	
	Cryptotendipes				4	1	
	Demicryptochironomus	1		15			
	Dicrotendipes					3	3
	Einfeldia				1	1	17
	Endochironomus	2	5		9	2	8
	Glyptotendipes	14					2
	Harnischia			2	10		
	Microtendipes	238	152	39	158	63	10
	Parachironomus					19	15
	Paracladopelma	1			1	5	
	Paratendipes	3	8		60	28	5
	Polypedilum	167	10	17	58	4	21
	Synendotendipes	9	1	1		3	5
	Stenochironomus	8	1				
	Stictochironomus		1	2		2	
	Tribelos	14					
	Xenochironomus			2			
	Cladotanytarsus	5	11	27	13		
	Micropsectra	69		13	32	3	34
	Neozavrelia	7					
	Paratanytarsus	1	1	1		3	3
	Stempellina		1				
	Tanytarsus	97	82	37	101	22	21
	Virgatanytarsus	16					

Discussion

Our study represents the first comprehensive investigation into the Chironomidae family in Lithuania. Despite studying only six streams with similar characteristics within one region of Lithuania, the gathered chironomids material encompasses over 50% of all known non-biting midges species in the country. However, given the limited scope of our

research and its outcomes, it is apparent that the findings may not entirely reflect the actual state of Chironomidae in Lithuania.

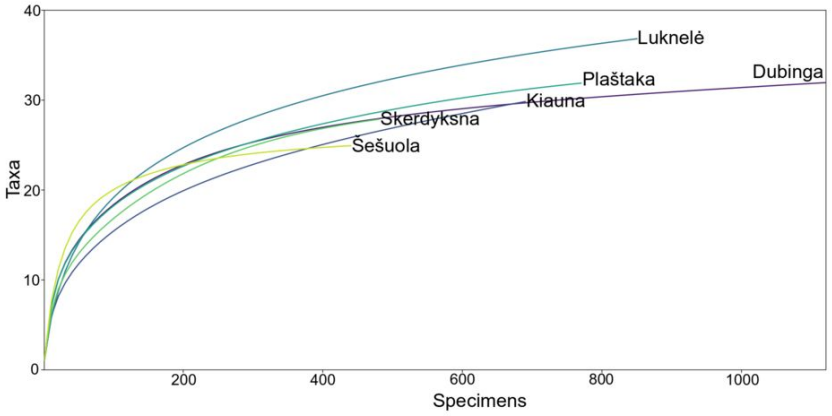

Figure 5. doi

Sample-based genera accumulation (rarefaction) curves, with 95% confidence intervals, of the Chironomidae genera' richness collected in six streams.

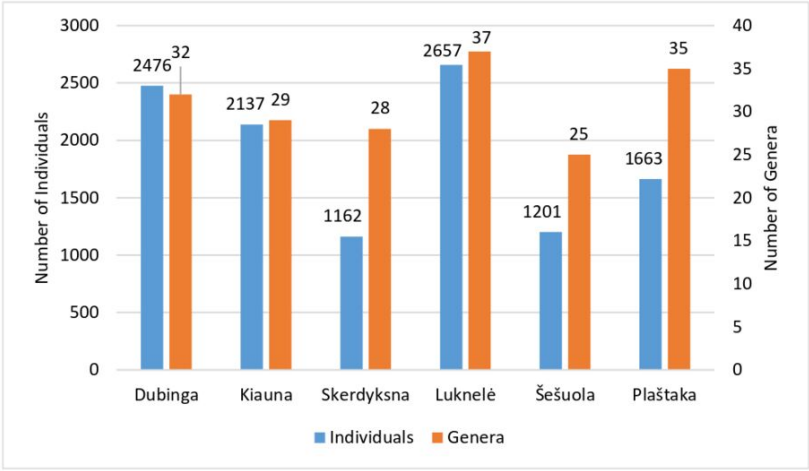

Figure 6. doi

Number of genera (orange bars) and specimens (blue bars) of non-biting midges (Chironomidae) in all streams researched.

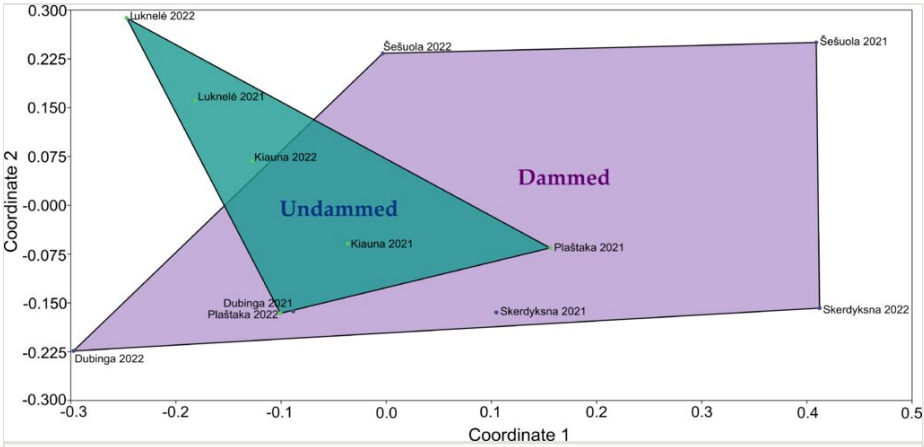

Figure 7. doi

Non-metric multidimensional scaling (NMDS) ordination (stress = 0.167) representing Chironomidae assemblages in dammed (purple) and undammed (blue) streams.

The prevalence of subfamilies in terms of genera and number of individuals reveals notable trends, aligning closely with observations made in Croatia by Čerba et al. (2020), who investigated chironomid fauna across diverse freshwater habitats. Their study, like ours, underscores the dominance of the Chironominae subfamily, while also noting a relatively lower richness within the Prodiamesinae subfamily.

According to the latest data on Lithuanian non-biting midge species (Pakalniškis et al. 2006, Ruginis 2007, Móra and Kovács 2009), our research contributes significantly to the understanding of the biodiversity within the Chironomidae family in Lithuania. The discovery of several species and genera new to Lithuania underscores the richness of the region's chironomid fauna and highlights the potential for further entomological exploration. By identifying a substantial proportion of the known Orthocladiinae genera and species, our study sheds light on the ecological complexity and diversity of this subfamily. The identification of new species and genera within the Chironominae and Tanypodinae subfamilies further enhances the existing taxonomic knowledge and provides a valuable foundation for future ecological and environmental studies. These findings not only expand the taxonomic records, but also offer insights into the distribution and ecological roles of these subfamilies in Lithuanian freshwater ecosystems.

Considering the research on Chironomidae conducted in neighbouring countries, it becomes evident that the faunistic knowledge of non-biting midges in Lithuania is relatively limited. In Europe, there are over 190 genera and more than 1260 species of Chironomidae (Paasivirta 2014, Serra et al. 2016, Serra et al. 2017). Germany, in particular, is known for extensive Chironomidae research, with significant efforts concentrated in the Land of Brandenburg (Orendt 2018). While major studies have been conducted in Brandenburg, research has also been carried out in other regions of Germany (Orendt 2000, Brunke 2004, Orendt et al. 2014). Currently, over 165 genera

and 780 species of Chironomidae are known in Germany, although there is still a need for further investigation (Chimeno et al. 2022, Chimeno et al. 2023).

Poland has also made significant contributions to Chironomidae research, with studies covering diversity, ecology, biology and other related areas (Płóciennik 2009, Płóciennik et al. 2015, Płóciennik et al. 2018, Leszczyńska et al. 2019, Pleskot et al. 2019, Głowacki et al. 2023). More than 420 species of Chironomidae are known in Poland (Płóciennik 2009). Ukraine, another country in the region where research on Chironomidae has been conducted since the early 20th century, still faces a significant lack of comprehensive study (Baranov 2011). Despite numerous surveys and the documentation of over 300 Chironomidae species, recent investigations have identified 40 additional species and one new genus, suggesting that the research remains incomplete (Baranov 2011, Bitušík et al. 2024). In the Baltic States of Latvia and Estonia, the level of faunistic knowledge of Chironomidae is similar to Lithuania, with a relatively low number of known species (Spuņģis and Kalniņš 2003). This indicates a general trend of limited research in the region regarding non-biting midges.

According to data from Finland, their national Chironomidae assemblage is extensively researched, with over 780 species of non-biting midges currently documented. However, the dynamic nature of changing climate is impacting the diversity, leading to fluctuations in species composition, with several chironomids already listed on the Red List of Finnish Species (Paasivirta 2014, Engels et al. 2019, Hyvärinen et al. 2019). In Sweden, approximately 900 species of Chironomidae are known, which accounts for more than 70% of the total number of Chironomidae species documented in Europe and the country is one of the regional leaders in the research of this group (SLU Swedish Species Information Centre 2024). Norway is another country in the region actively involved in Chironomidae research, with ongoing studies conducted even in challenging environments such as Svalbard and Jan Mayen. More than 70 species of chironomids are currently known in these areas, contributing to a total of over 650 species of Chironomidae documented in Norway (Elven and Søli 2016, Stur and Ekrem 2020).

Based on the rarefaction results analysis, we can conclude that the detection of non-biting midges in the six streams was effective. Although the NMDS analysis revealed significant overlap in genus composition between dammed and non-dammed streams, this does not imply that dams have no impact on Chironomidae diversity and ecology. In conclusion, the understanding of Chironomidae diversity in Lithuania is still evolving. By leveraging the insights gleaned from neighbouring countries, there is the urgent need for Lithuania to continue its research efforts. This should extend beyond the borders of our country, encompassing regional and global initiatives aimed at conserving biodiversity and grappling with the challenges posed by environmental shifts on non-biting midge communities and populations. It would not only enrich the scientific landscape of Lithuania, but also contribute meaningfully to the collective endeavour of safeguarding our natural heritage for future generations.

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