Rediscovering the mosquito fauna (Diptera: Culicidae) of Estonia: an annotated checklist with distribution maps and DNA evidence

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Abstract

Female mosquitoes (Diptera: Culicidae) affect their hosts in numerous negative ways and are crucial to the spread of vector-borne pathogens. It is, therefore, important to have a detailed overview of regional mosquitoes, to be able to detect changes in species diversity and identify possible health threats. The aim of this study was to update the checklist of the mosquito fauna of Estonia for the first time since 1957. For this purpose, 24,344 adult mosquitoes (94% females) were collected in Estonia from 2008 to 2020 using various trapping methods. Specimens were primarily identified by morphological characteristics, but DNA barcoding based on the partial cytochrome c oxidase subunit 1 gene (COI) was also used. Species were included in the checklist based on historical records as well as new collections, while also considering reports from neighboring countries. Species records are supplemented with voucher specimens, distribution maps and DNA evidence. The updated checklist includes 34 species, 27 of which were confirmed with recently collected material. All in all, *Aedes communis* (de Geer, 1776) proved to be the most common mosquito in Estonia, accounting for 30.1% of the specimens collected. This is noteworthy, as this species has been implicated in the transmission of multiple disease agents present in the area. New evidence revealed the presence of *Ae. hexodontus* Dyar, 1916, *Ae. sticticus* (Meigen, 1838), *Anopheles messeae* Falleroni, 1926 and *Culiseta bergrothi* (Edwards, 1921) in Estonia.

Key words: *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, DNA barcoding, entomology, Estonia

Introduction

Mosquitoes (Diptera: Culicidae) are a notable group of insects, as they can affect the wellbeing of humans and animals alike. The haematophagous females of many mosquito species can be a serious biting nuisance, as well as transmit various pathogens. Illnesses caused by vector-borne pathogens affect more than one billion people per year, with diseases caused by mosquito-borne pathogens being responsible for the majority of the infections (WHO 2014). Furthermore, these diseases cause not only death and disability, but also notable monetary loss, further exacerbating economic inequality, as poorer populations are more vulnerable to insect bites (WHO 2017). Blood seeking mosquitoes can also be a nuisance in their own right, disrupting outdoor activities and creating considerable stress in humans and animals (Islam et al. 2017). For these reasons, mosquitoes continue to be an important subject of study, as better understanding of their biology and ecology can help predict changes and create strategies to mitigate some of the harmful effects. Mosquito species richness varies based on geographic location, with areas close to the equator supporting a greater number of species than regions at higher latitudes (Foley et al. 2007). This diversity makes mosquitoes especially significant in the tropics. However, some important species, a number of which are known to be competent vectors of pathogenic agents, can be found in colder climates as well (Martinet et al. 2019). This trend is mirrored by mosquito-borne pathogens: while the majority are confined to warm climates, a number of diseases also occur in higher latitudes and many infections are now emerging or re-emerging (Bale 2012; Liang
et al. 2015; Evans & Peterson 2019). In fact, throughout recent decades, there have been noticeable changes in the geographic distributions of both biting insects as well as vector-borne pathogens (Medlock et al. 2012; Brugueras et al. 2020). Such shifts have been driven by numerous anthropogenic and environmental factors, such as global transport routes, changes in land use, urbanization, extreme weather events and climatic fluctuations, among others (Hui 2006; Zell et al. 2008). These aspects can also cause significant changes in the relative and absolute abundance of indigenous mosquito species (Frankinos et al. 2019; Câmara et al. 2020). As a result, calls have been made for increased mosquito surveillance as well as additional empirical studies to investigate vector ecology in the changing world (Frankinos et al. 2019).

More than half of the eight Nordic-Baltic countries, as well as the neighboring Russian Federation, have published at least one update to their mosquito checklists during the last few decades. For example, a literature based list of Lithuanian Diptera was published in 2000 and included 36 mosquito species, listing five species in the genus Anopheles Meigen, 1818, 23 in genus Aedes Meigen, 1818, three in Culex Linnaeus, 1758, four in Culiseta Felt, 1904 and the species Coquillettidia richardii (Ficalbi, 1889) (Pakalniškis et al. 2000). Eleven years later, the official number of Lithuanian mosquitoes rose to 37, with the addition of Aedes geminus Peus, 1970 (Bernotienė & Lučiūnaitė 2011). Meanwhile, only 25 mosquito species had been reported from Latvia: four species of Anopheles, 17 species of Aedes, one species of Coquillettidia Dyar, 1905 and Culex, as well as two species of the genus Culiseta, as reported by Spungsis (2000). However, the author of the aforementioned study concluded that the real number of mosquitoes in Latvia was likely to be significantly higher. During this time, the mosquito checklist for European Russia was also revised, with the update featuring 64 species, including four species with doubtful presence (Gornostaeva 2000). The Swedish mosquito fauna has been relatively well researched from 2000 onwards. The most recent checklist for Sweden, based on both prior literature records and new collection efforts (Lundström et al. 2013), included 49 mosquito species: seven belonging to the genus Anopheles, 31 to Aedes, one to Coquillettidia, three to Culex and another seven to Culiseta. At the moment, 55 mosquito species are thought to be present in Sweden (Mohlmann et al., 2017; Robert et al. 2019). The mosquito fauna of Finland has been updated multiple times in the last decade. First of these was a literature review listing 38 mosquito species (Huldén & Huldén 2014), but this information was further built upon and corrected in later articles (Culverwell 2018; Culverwell et al. 2020; Culverwell et al. 2021), with the most recent list including 43 species. Similarly, a recent comprehensive overview was written about the mosquitoes of northwestern Russia, reporting a total of 46 species and comparing the results to data from neighboring countries (Khalin & Aibulatov 2020). This was followed by a publication about the northernmost records of these mosquito species (Khalin & Aibulatov 2021). In contrast, the most recent checklist of the mosquitoes of Estonia was published in the mid-Twentieth Century (Remm 1957).

Few people have studied mosquitoes in the area of present-day Estonia. Some of the earliest records concerning the Baltic mosquito fauna can be found from the first half of the Nineteenth Century onwards, attributable to the Baltic German entomologists B. A. Gimmerthal (1779–1848) and F. L. F. Sintenis (1835–1911), as well as some visiting scientists, e.g. A. Dampf Tenson (1884–1948) (Remm 1955). The first extensive research on haematophasous Diptera in Estonia was conducted during the mid-Twentieth Century. This culminated in 1955, when H. Remm (1929–1986) completed a dissertation featuring an annotated checklist of the mosquitoes of Estonia. The manuscript was based on 12,204 specimens collected from 300 study sites, as well as available museum collections, encompassing 30 mosquito species (Remm 1955). This work was published two years later in the journal Entomologicheskoe Obozrenie (Remm 1957). Afterwards, new entries relating to mosquito species present in Estonia have been few and far between. Burtin (2014) defended a master’s theses updating previous species records with currently valid synonyms and presented a study based on 691 new mosquito specimens. The manuscript included a list of 33 mosquito species likely to be present in the country, and two species suspected to occur in the country. Some of this information was later published as part of a larger study concerning urban mosquitoes, along with 1,199 additional observations (Kirik et al. 2021). Many of the mosquito species suspected to be present in Estonia were still missing reliable up to date records. Furthermore, information regarding the distribution and abundance of these mosquitoes had not been substantially updated after the contributions of Remm (1955). To remedy this, an updated checklist, supplemented with new evidence, was needed to better understand the mosquito fauna of the country. This would allow future researchers to track changes in species composition as well as better assess the risk of diseases caused by vector-borne pathogens in the region. Consequently, the aim of this study was to provide an updated checklist of the mosquitoes present in Estonia, along with voucher material, distribution maps, partial cytochrome c oxidase (MT-COI) sequences and comments concerning the abundance of each species.

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Material and methods

Study area. Estonia is the northernmost country of the three Baltic nations: it is located on the eastern shore of the Baltic Sea and shares a land border with Latvia and Russia. Estonia is situated on the East-European Plain and is therefore relatively flat, with a mean altitude of about 50 m above sea level (Raukas 1995). The country has a population density of 30.6 inhabitants per km², which is relatively low compared to other European nations (Eurostat 2021; Statistics Estonia 2020). Furthermore, 51.4% (relative error (RE) ±1.1%) of Estonia is consists of forests, 27.0% (RE ±1.9%) of the land is in agricultural use and bogs and inland waters make up 4.9% (RE ±5.1%) and 1.7% (RE ±8.8%) of the country, respectively (Environment Agency 2020). Estonia is considered to be part of the Boreal Region according to the European Commission (Sundseth et al. 2009), but belongs to the temperate continental climate zone with warm summers based on the updated Köppen-Geiger classification system (Kottek et al. 2006; Beck et al. 2018).

Mosquito collection. Adult mosquitoes were collected from 2008 to 2020 from various locations in Estonia, both from the mainland and the three largest islands: Saaremaa, Hiiumaa and Muhu (Fig. 1). Fieldwork took place from the start of May until mid-October, and included collection sites in the countryside, suburbs and urban greenpaces. In rural areas, mosquitoes were collected in farmyards, pastures, lakesides, wetlands and forest. Collection points were chosen to cover as many biomes as possible, while allowing insect traps to be emptied regularly and be supervised by volunteers. Collection sites were sampled for different periods of time due to both limited personnel and the variety of different collection methods. Most specimens were caught with the battery powered Mosquito Magnet Independence traps (Woodstream Corp., Lancaster, USA) baited with Octenol (C₅H₁₀O), but mosquitoes were also collected with sweep nets, EVS light traps (Bioquip Products, Rancho Dominguez, USA) baited with dry ice, Malaise traps (cf. Tomasson et al. 2014) and window traps (cf. Sammet et al. 2016). Mosquito Magnet and EVS traps were emptied every two to four days, but Malaise and window traps were emptied a few times over the summer. Information concerning the use of sweep nets can be found in a previous publication (Kirik et al. 2021). It is important to note that Mosquito Magnet and EVS traps use bait to attract host seeking arthropods and therefore predominantly capture female mosquitoes. As a result, the newly acquired data for the checklist primarily consists of information obtained from the collection of females.

The majority of mosquitoes were stored in tubes at -20°C as dry material, but some older samples were kept in 76% ethanol at 4°C or at room temperature. Mosquitoes were identified to species or species-group level by the first author based on morphological markers, using keys of Becker et al. (2020). The resulting identifications were used to make general inferences concerning the prevalence of each taxonomic group. Based on the number of individuals collected from 2008 to 2020, species were designated as abundant (>1,001 individuals), common (501–1,000 individuals), infrequent (101–500 individuals) or rare (<100 individuals) for ease of discussion. Maps showing the new and historic collection sites of each species were constructed using Adobe Photoshop CS5 Extended (Adobe, San Jose, USA) and arranged into figures. Up to three mosquitoes from every species collected were selected as voucher specimens, pinned and stored at room temperature in the Entomological Collection [IZBE] of the Estonian University of Life Sciences. The remainder of the material is also stored in the university. At least one voucher specimen of each species was subjected to DNA barcoding to further validate species identification and to help distinguish morphologically similar or isomorphic species.

DNA analysis. DNA extraction was carried out using one to three legs from each mosquito. The material was homogenized with the handheld Kontes Pellet Pestle (DWK Life Sciences GmbH, Mainz, Germany) and DNA extraction was completed using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Species identification was carried out based on the 710 bp partial sequence of the cytochrome c oxidase subunit 1 gene (COI), using the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Additionally, 16 Culex pipiens mosquitoes were analyzed for the presence of the intercellular bacteria, Wolbachia, based on the symbionts wsp gene. This was done using primers wsp-81F (5'-TGGTCAATAAGTGATGAAAGAC-3') and wsp-691R (5'-AAAATTAAACGCCTACTCCA-3') (Braig et al. 1998). All polymerase chain reaction (PCR) mixtures consisted of 1 µl template DNA, 12.5 µl DreamTaq PCR Master Mix (Thermo Fisher Scientific, Waltham, USA), 0.5 µl of each 20 pmol/l primer and 10.5 µl ddH₂O. For degraded material, 1.0 µl MgCl₂ (25 mM) (Thermo Fisher Scientific, Waltham, USA) and 0.5 µl dimethyl sulfoxide (DMSO) (ITW Reagents Division, Glenview, USA) were added as needed at the expense of ddH₂O. The PCR program for COI included a 15 min first denaturation stage at
94°C, followed by 60 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 44°C and 30 sec of synthesis at 72°C, capped by a 10 min final synthesis stage at 72°C. The PCR program for amplifying the \textit{Wolbachia} wsp gene was set up according to Shaikevich \textit{et al.} (2019b).

PCR products were checked for positive signals by electrophoresis on a 1.6% agarose gel infused with 3.8 µl of ethidium bromide, run for 1 h at 120 V and 70 mA. Six µl of each sample were mixed with 1 µl of DNA Gel Loading Dye (6X) (Thermo Fisher Scientific, Waltham, USA) before it was added to the gel. GeneRuler 100 bp DNA Ladder, ready-to-use (Thermo Fisher Scientific, Waltham, USA) was used as a reference. Successfully amplified PCR products were sequenced at the Institute of Genomics Core Facility using Sanger sequencing (University of Tartu, Tartu, Estonia). Forward and reverse nucleotide strands were combined into consensus sequences and cleaned in BioEdit version 7.2.6.1 (Hall 1999). Resulting barcodes were checked against the information stored at GenBank (National Institutes of Health, Bethesda, USA) using both the US National Library of Medicine nucleotide BLAST tool (National Institutes of Health, Bethesda, USA) and the Barcode of Life Data (BOLD) Systems workbench developed by Ratnasingham & Hebert (2007). The partial \textit{COI} sequences of 49 voucher specimens are deposited in GenBank. The GenBank accession numbers for the species are provided for below.

\textbf{Data availability.} The mosquito count data generated during this research can be found online at FigShare (DOI: 10.6084/m9.figshare.16817395) or obtained from the corresponding author.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{map.png}
\end{figure}

\section*{Results}

\textbf{General results.} The updated checklist includes 34 species based on material collected in Estonia from 2008 to 2020, information provided previously by Kirik \textit{et al.} (2020, 2021) and historical records and studies from neighbor-
ing countries. More specifically, the newly collected material included in this study consists of 24,344 adult mos-
quitos (94.2% female), which by themselves helped confirm the presence of 27 species. Most of these mosquitoes
were identified based on their morphological characteristics, but some were also submitted for genetic identification
based on mitochondrial COI sequences. All mosquitoes that were collected belong to one or other of five genera:
Aedes, Anopheles, Coquillettidia, Culex and Culiseta. In the following checklist, information on mosquito species in
Estonia is summarized and annotated with brief comments. Regrettably, the historical material underlying the first
checklist compiled by Remm (1957) no longer exists; thus, seven species not encountered during the recent collec-
tions, are included based on only literature sources.

Annotated checklist

Genus Aedes Meigen, 1818

1. Aedes (Aedes) cinereus Meigen, 1818
(Fig. 2A)


Voucher material: 1♀, Tartu (58° 21′ 26″ N, 26° 42′ 60″ E), 14.VI.2017, T. Kesküla leg., H. Kirik det., sweep
net, IZBE0210180; 1♂, Tartu (58° 21′ 23″ N, 26° 44′ 31″ E), 24.IX.2017, T. Kesküla leg., H. Kirik det., sweep
net, IZBE0210247; 1♀, Tartu (58° 23′ 24″ N, 26° 42′ 55″ E), 14.IX.2016, H. Kirik leg., H. Kirik det., sweep net,
IZBE0210181, GenBank: OK465139.

Comment: 1,436 mosquitoes (5.9% of all specimens collected) were identified as Ae. cinereus. This species is
abundant in Estonia and can be found almost everywhere from June to September. Also, Ae. cinereus can be numer-
ous at times, especially towards the end of the summer, based on the data of the present study. It should be noted that
adult females are difficult to differentiate from the closely related Ae. geminus based on morphology alone; thus,
specimens of Ae. geminus could also be among the specimens of Ae. cinereus collected during the study.

2. Aedes (Aedimorphus) vexans (Meigen, 1830)
(Fig. 2B)


Voucher material: 1♀, Tartu (58° 21′ 23″ N, 26° 44′ 31″ E), 13.VIII.2017, T. Kesküla leg., H. Kirik det., sweep
net, IZBE0210182, GenBank: OK465140; 1♀, Tartu (58° 22′ 17″ N, 26° 41′ 58″ E), 21.VIII.2017, H. Kirik leg., H.
Kirik det., sweep net, IZBE0210183, GenBank: OK465141; 1♂, Tartu (58° 21′ 16″ N, 26° 40′ 53″ E), 06.VI.2015,
T. Kesküla leg., O. Kurina det., sweep net, IZBE0210184.

Comment: 366 mosquitoes (1.5% of all specimens collected) were identified as Ae. vexans. This species was
infrequently collected, but specimens could be found throughout the warm season, with a peak of activity in August.
However, Ae. vexans has been found to emerge in large numbers after floods (Schäfer & Lundström 2009), so their
low numbers in this study could be due to sampling bias.

3. Aedes (Ochlerotatus) annulipes (Meigen, 1830)
(Fig. 2C)

Published sources: Remm (1957: 154), Burtin (2014: 43), Kirik et al. (2021: 11, as part of the Aedes (Ochlerotatus
annulipes) group).

Voucher material: 1♀, Mändjala (58° 12′ 56″ N, 22° 19′ 56″ E), 16.VI.2015, L. Tummeleht leg., H. Kirik
det., Mosquito Magnet trap, IZBE0210241, GenBank: OK465167; 1♂, Omedu (58° 45′ 09″ N, 27° 02′ 23″ E),
06.VI.2015, O. Kurina leg., O. Kurina det., sweep net, IZBE0210185.

Comment: Aedes annulipes belongs to the Ae. annulipes group, along with Ae. cantans (Meigen, 1818), Ae.
Aedes (Ochlerotatus) cyprius Ludlow, 1920, *Ae. euedes* Howard, Dyar & Knab, 1913, *Ae. excrucians* (Walker, 1956), *Ae. flavescens* (Müller, 1764), *Ae. riparius* Dyar & Knab, 1907, etc. (Becker et al. 2020). *Aedes annulipes* can be morphologically distinguished from others species of the group, but this can be difficult when it comes to adult females. This is because of the variability in their morphological traits as well as inconclusive DNA evidence, making species identification time and resource extensive. For the purposes of this study, mosquitoes with morphology similar to *Ae. annulipes* were designated as specimens of the *Ae. annulipes* group. In total, 2,091 individuals (8.6% of all specimens collected) were identified as simply belonging to the group. These mosquitoes were active from May to October, but were most numerous in June. Two specimens, which corresponded well to both the morphological description of *Ae. annulipes* and the partial COI sequences found in online databases, were chosen as the local voucher specimens.

4. *Aedes (Ochlerotatus) cantans* (Meigen, 1818)  
(Fig. 2C)  

**Published sources:** Remm (1957: 152, as *Aëdes maculatus* Meigen, 1804), Burtin (2014: 43), Kirik et al. (2021: 11, under the *Aedes (Ochlerotatus) annulipes* group).  

**Voucher material:** 1 ♀, Tartu (58° 22′ 17″ N, 26° 41′ 58″ E), 06.VI.2017, H. Kirik leg., H. Kirik det., sweep net, IZBE0210121, GenBank: OK465165; 1 ♂, Tartu (58° 23′ 54″ N, 26° 44′ 40″ E), 17.V.2015, O. Kurina leg., O. Kurina det., sweep net, IZBE0210121; 1 ♀, Tartu (58° 21′ 1″ N, 26° 41′ 30″ E), 24.VI.2017, T. Kesküla leg., H. Kirik det., sweep net, IZBE0210121, GenBank: OK465166.  

**Comment:** *Aedes cantans* belongs to the *Ae. annulipes* group. The species of this group can be distinguished based on morphological characteristics, but due to the variability of some traits, as well as inconclusive results of DNA barcoding, mosquitoes similar to *Ae. cantans* were designated as specimens of the *Ae. annulipes* group. In all, 2,091 mosquitoes (8.6% of all specimens collected) were as belonging to the *Ae. annulipes* group. These individuals were found throughout the warm months, but were most numerous in June. Voucher material was chosen from among specimens that best corresponded to the morphological traits of *Ae. cantans* and matched well with reliable DNA sequences in online databases.

5. *Aedes (Ochlerotatus) caspius* (Pallas, 1771)  
(Fig. 2D)  

**Published source:** Remm (1957: 152).  


**Comment:** 206 mosquitoes (0.9% of all specimens collected) were identified as *Ae. caspius*. *Aedes caspius* is a halophilic species mainly found in Estonia near the brackish water of the Baltic Sea. These mosquitoes are common and at times numerous near the coastline, but are rarely found further inland. Thus, their relatively low numbers collected in this study is due to collection bias. *Aedes caspius* appears to be active throughout summer.

6. *Aedes (Ochlerotatus) cataphylla* Dyar, 1916  
(Fig. 2E)  

**Published sources:** Remm (1957: 154), Khalin et al. (2020: 66), Kirik et al. (2021: 11).  

**Voucher material:** 1 ♀, Tartu (58° 23′ 44″ N, 26° 43′ 44″ E), 06.VI.2017, H. Kirik leg., H. Kirik det., sweep net, IZBE0210188, GenBank: OK465143; 1 ♀, Tartu (58° 20′ 52″ N, 26° 41′ 37″ E), 14.VI.2017, T. Kesküla leg., H. Kirik det., sweep net, IZBE0210189, GenBank: OK465144; 1 ♂, Tartu (58° 23′ 40″ N, 26° 44′ 05″ E), 14.VI.2017, H. Kirik leg., H. Kirik det., sweep net, IZBE0210244.  

**Comment:** 3,951 mosquitoes (16.2% of all specimens collected) were identified as *Ae. cataphylla*, making it
the third most common mosquito species in Estonia. This species is abundant almost everywhere in the country and can be numerous at times. As is typical for a spring-time species, *Ae. cataphylla* are most active in May, but some specimens can be found until September.

7. *Aedes (Ochlerotatus) communis* (de Geer, 1776)  
(Fig. 2F)

**Published sources:** Remm (1957: 155), Burtin (2014: 44), Kirik *et al.* (2021: 11).

**Voucher material:** 1♀, Törve (58° 37′ 40″ N, 26° 23′ 48″ E), 27.VI–01.VII.2018, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210190, GenBank: OK465145; 1♀, Tartu (58° 23′ 40″ N, 26° 44′ 05″ E), 06.VI.2017, H. Kirik leg., H. Kirik det., sweep net, IZBE0210191, GenBank: OK465146; 1♂, Tartu (58° 23′ 24″ N, 26° 44′ 40″ E), 17.V.2015, O. Kurina leg., O. Kurina det., sweep net, IZBE0210192.

**Comment:** 7,316 mosquitoes (30.1% of all specimens collected) were identified as *Ae. communis*, making it the dominant species in Estonia. *Aedes communis* can be found everywhere in the country. It is especially numerous during May and June, but individuals can be found until October. Importantly, there appears to be two distinct mitochondrial lineages in the area, which can make DNA barcoding difficult, as some COI sequences appear to be very similar to the North American *Ae. tahoensis* (Dyar, 1916) (Kirik *et al.* 2020).

8. *Aedes (Ochlerotatus) cyprius* Ludlow, 1920  
(Fig. 2G)

**Published source:** Remm (1957: 153).

**Voucher material:** 1♀, Lasila (59° 16′ 47″ N, 26° 13′ 24″ E), 31.V.2016, M. Kruus leg., H. Kirik det., Malaise trap, IZBE0210193, GenBank: OK465147; 1♀, Lasila (59° 16′ 47″ N, 26° 13′ 24″ E), 31.V.2016, M. Kruus leg., H. Kirik det., Malaise trap, IZBE0210194, GenBank: OK465148.

**Comment:** Four mosquitoes were identified as *Ae. cyprius*. It was very rare among the mosquitoes collected during this study, but it can be found at the beginning of the warm season in May and June. The only individuals collected in the country from 2008 to 2020 were caught in northern Estonia. The low numbers captured could also be due to collection bias.

9. *Aedes (Ochlerotatus) diantaeus* Howard, Dyar & Knab, 1913  
(Fig. 2H)

**Published sources:** Remm (1957: 154), Burtin (2014: 45), Kirik *et al.* (2021: 11).

**Voucher material:** 1♂, Tartu (58° 23′ 44″ N, 26° 43′ 44″ E), 07.VI.2013, V. Burtin leg., V. Burtin det., sweep net, IZBE0210010.

**Comment:** In this study, only one mosquito was identified as *Ae. diantaeus*. This single specimen was collected in southeastern Estonia at the beginning of June. More research is needed to better understand the abundance of this species in the country.

10. *Aedes (Ochlerotatus) dorsalis* (Meigen, 1830)  
(Fig. 3A)

**Published source:** Remm (1957: 152).

**Voucher material:** None.

**Comment:** One male and four females of *Ae. dorsalis* have been reported from Estonia (Remm 1957). However, the specimens have not been preserved and cannot be verified. No specimens were found during the present study. *Aedes dorsalis* has been reported from Lithuania (Pakalniškis *et al.* 2000), Latvia (Spungis 2000), provinces
adjacent to Estonia in northwestern Russia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell et al. 2021) and Sweden (Lundström et al. 2013). Therefore, this species is likely to be present in Estonia as well. The absence of specimens in collections made during this study could be due to insufficient trapping, as well as collection bias, as *Ae. dorsalis* is halophilic and has been known to be associated with floods (Becker et al. 2020).

11. *Aedes* (*Ochlerotatus*) *excrucians* (Walker, 1856)  
(Fig. 3B)

**Published sources:** Remm (1957: 154), Burtin (2014: 46), Kirik et al. (2021: 11).


**Comment:** 193 mosquitoes (0.8% of all collected specimens collected) were identified as *Ae. excrucians*. The species appears to be uncommon in the country based on this study, but individuals can be found from May to October, although the peak of their activity seems to be in June.

12. *Aedes* (*Ochlerotatus*) *flavescens* (Müller, 1764)  
(Fig. 3C)

**Published sources:** Remm (1957: 153), Burtin (2014: 46), Kirik et al. (2021: 11).


**Comment:** 77 mosquitoes (0.3% of all specimens collected) were identified as *Ae. flavescens*. This species seems to be rare in Estonia, but specimens can be found throughout the warm season, from May to October. It is slightly more numerous in May.

(Fig. 3D)

**Published sources:** None.

**Voucher material:** 1♀, Tõrve (58° 35′ 56″ N, 26° 22′ 20″ E), 27.VI–01.VII.2018, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210201, GenBank: OK465154; 1♀, Undi (58° 29′ 44″ N, 26° 54′ 00″ E), 15–16.VII.2016, L. Tummeleht leg., H. Kirik et al. (2021: 11).

**Comment:** 35 mosquitoes (0.1% of all specimens collected) were identified as *Ae. hexodontus*. This species appears to be relatively rare in Estonia and from 2008 to 2020 it was only collected in May and June.

14. *Aedes* (*Ochlerotatus*) *intrudens* Dyar, 1919  
(Fig. 3E)

**Published sources:** Remm (1957: 156), Burtin (2014: 47), Kirik et al. (2021: 11).

**Voucher material:** 1♀, Tartu (58° 21′ 26″ N, 26° 42′ 60″ E), 04.VI.2017, T. Kesküla leg., H. Kirik det., sweep net, IZBE0210204, GenBank: OK465157; 1♀, Viivre (58° 04′ 52″ N, 25° 31′ 26″ E), 18–19.VI.2016, H. Kirik leg.,

Comment: 189 mosquitoes (0.8% of all specimens collected) were identified as *Ae. intrudens*. This species was uncommon in Estonia during the fieldwork of this study. *Aedes intrudens* appears to be active from May to July, but it is more numerous in May.

15. *Aedes (Ochlerotatus) leucomelas* (Meigen, 1804)
(Fig. 3F)

**Published sources:** Remm (1957: 154), Kirik *et al.* (2021: 11).


Comment: 211 mosquitoes (0.9% of all specimens collected) were identified as *Ae. leucomelas*. This species seems to be uncommon in Estonia, much less common than the closely related *Ae. cataphylla*. Mosquitoes identified as *Ae. leucomelas* were most numerous in May, but some individuals were found until October during this study.

16. *Aedes (Ochlerotatus) nigrinus* (Eckstein, 1918)
(Fig. 3G)

**Published sources:** Remm (1957: 155), Burtin (2014: 48).

**Voucher material:** None.

Comment: Three males and 23 females of *Ae. nigrinus* were previously reported from Estonia (Remm 1957), but the specimens have not been preserved. No specimens were collected during this study. However, *Ae. nigrinus* has also been recorded in Lithuania (Pakalniškis *et al.* 2000), provinces in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Harbach *et al.* 2017; Culverwell *et al.*, 2021) and Sweden (Lundström *et al.* 2013). Therefore, this species is likely to be present in Estonia as well.

17. *Aedes (Ochlerotatus) punctor* (Kirby, 1837)
(Fig. 3H)

**Published sources:** Remm (1597: 155), Burtin (2014: 48), Kirik *et al.* (2021: 11).

**Voucher material:** 1♀, Tartu (58° 22’ 52” N, 26° 42’ 49” E), 06.VI.2017. H. Kirik leg., H. Kirik det., sweep net, IZBE0210210, GenBank: OK465161; 1♀, Tartu (58° 22’ 52” N, 26° 42’ 49” E), 19.VI.2017, H. Kirik leg., H. Kirik det., sweep net, IZBE0210211, GenBank: OK465162; 1♂, Tartu (58° 23’ 54” N, 26° 44’ 40” E), 17.V.2015, O. Kurina leg., O. Kurina det., sweep net, IZBE0210212.

Comment: 4,594 mosquitoes (18.9% of all specimens collected) were grouped as *Ae. punctor/punctodes* Dyar, 1922, although males were identified as *Ae. punctor*. *Aedes punctor* is known to be more common than *Ae. punctodes* (Culverwell *et al.*, 2021), but further DNA analysis or larval collections are required to make definitive conclusions about the presence of *Ae. punctodes* in Estonia. The two females of *Ae. punctor/punctodes* chosen as voucher specimens were identified as *Ae. punctor* by DNA barcoding. These mosquitoes were especially numerous in May during this study, but some individuals were found until October.

18. *Aedes (Ochlerotatus) riparius* Dyar & Knab, 1907
(Fig. 4B)

**Published source:** Remm (1957: 153).
Voucher material: None.

Comment: *Aedes riparius* belongs to the *Ae. annulipes* group and can be difficult to identify. A specimen corresponding to morphologically to *Ae. riparius* was found during this study, but DNA barcoding identified it as *Ae. annulipes/cantans*. One male, four females and one larva of *Ae. riparius* have previously been reported from Estonia (Remm 1957), but these individuals have not been preserved and their identification cannot be verified. *Aedes riparius* has been found in Lithuania (Pakalniškis et al. 2000), Latvia (Spungis 2000), provinces in Northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell et al. 2021) and Sweden (Lundström et al. 2013); consequently, *Ae. riparius* is likely present in Estonia as well.

19. *Aedes (Ochlerotatus) sticticus* (Meigen, 1838)

(Fig. 4A)

Published source: Kirik et al. (2021: 11).

Voucher material: 1♀, Külitse (58° 20′ 5″ N, 26° 35′ 57″ E), 01–03.VIII.2020, V. Oborina leg., H. Kirik det., Mosquito Magnet trap, IZBE0210213, GenBank: OK465163; 1♂, Tartu (58° 20′ 52″ N, 26° 41′ 37″ E), 17.07.2017, T. Keskiä leg., H. Kirik det., sweep net, IZBE0210249; 1♀, Tartu (58° 23′ 40″ N, 26° 44′ 05″ E), 05.VII.2017, H. Kirik leg., H. Kirik det., sweep net, IZBE0210214, GenBank: OK465164.

Comment: 231 mosquitoes (1.0% of all specimens collected) were identified as *Ae. sticticus*. This species appears to be uncommon in the country based on the current collections. However, the abundance of *Ae. sticticus* has been found to depend on floods, and they can be numerous at certain times (Schäfer & Lundström 2009). Their relatively low numbers in this study could be due to collection bias. Interestingly, *Ae. sticticus* was encountered more often in August, which is unusual compared to most *Aedes* species in the area. Overall, some *Ae. sticticus* can be found in Estonia throughout the summer, from June to September.

Genus *Anopheles* Meigen, 1818

20. *Anopheles (Anopheles) algeriensis* Theobald, 1903

(Fig. 4C)

Published source: Remm (1957: 150).

Voucher material: None.

Comment: Nine *An. algeriensis* females have been previously reported from Estonia, but these specimens have not been preserved and cannot be verified. No new specimens were found during this study. Also, Sweden is the closest country to Estonia where *An. algeriensis* has been collected (Lundström et al. 2013). In fact, this species appears to be most common in Mediterranean and Balkan countries (Scholte et al. 2011).

21. *Anopheles (Anopheles) claviger* (Meigen, 1804)

(Fig. 4D)

Published sources: Remm (1957: 150, as *Anopheles bifurcatus* Linnaeus, 1758), Burtin (2014: 35), Kirik et al. (2021: 11).

Voucher material: 1♀, Ülenurme (58° 19′ 3″ N, 26° 43′ 23″ E), 14–17.VIII.2020, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210219, GenBank: OK465168; 1♀, Undi (58° 29′ 44″ N, 26° 54′ 00″ E), 15–16.VIII.2016, L. Tummeleht leg., H. Kirik det., Mosquito Magnet trap, IZBE0210220, GenBank: OK465169; 1♂, Tartu (58° 23′ 24″ N, 26° 42′ 55″ E), 03.IX.2013, V. Burtin leg., V. Burtin det., sweep net, IZBE0210007.

Comment: 1,038 mosquitoes (4.3% of all specimens collected) were identified as *An. claviger*. This species is abundant in Estonia, especially during August, but some individuals can be found from May to October. *Anopheles claviger* was the most common anopheline mosquito in this study.
22. *Anopheles (Anopheles) maculipennis* Meigen, 1818 s.s.  
(Fig. 4E)

**Published sources:** Remm (1957: 151), Burtin (2014: 36, as *An. maculipennis* s.l.), Kirik *et al.* (2021: 11, part of the *An. maculipennis* complex).

**Voucher material:** 1♂, Ülenurme (58° 23′ 40″ N, 26° 44′ 05″ E), 19.VIII.2016, H. Kirik leg., H. Kirik det., sweep net, IZBE0210243, GenBank: OK465173.

**Comment:** *Anopheles maculipennis* is the nominotypical species of the *An. maculipennis* complex. Three mosquitoes were identified as *An. maculipennis* s.s among of 20 specimens of the *An. maculipennis* complex that were subjected to DNA barcoding. These results indicate that *An. maculipennis* is likely to be quite uncommon in the country, as only 215 mosquitoes (0.9% of all collected specimens collected) were identified as belonging to the *An. maculipennis* complex, and presumably *An. maculipennis* makes up a small portion of these individuals. The true abundance of the *An. maculipennis* complex is likely to have been underestimated in this study due to collection bias.

23. *Anopheles (Anopheles) messeae* Falleroni, 1926  
(Fig. 4E)

**Published source:** Kirik *et al.* (2020: 5).

**Voucher material:** 1♀, Punaküla (58° 20′ 09″ N, 25° 20′ 04″ E), 31.V.–03.VI.2018, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210221, GenBank: OK465170; 1♀, Tartu (58° 21′ 26″ N, 26° 42′ 00″ E), 04.IX.2017, T. Kesküla leg., H. Kirik det., sweep net, IZBE0210222, GenBank: OK465171; 1♀, Tartu (58° 21′ 13″ N, 26° 40′ 45″ E), 17.IX.2017, T. Kesküla leg., H. Kirik det., sweep net, IZBE0210223, GenBank: OK465172.

**Comment:** *Anopheles messeae* belongs to the *An. maculipennis* complex. Based on COI sequences, 17 mosquitoes among 20 specimens of the complex subjected to genetic analyses were identified as *An. messeae* or *An. daciae* Linton, Nicolescu & Harbach, 2004 (in Nicolescu *et al.* 2004). This indicates that *An. messeae//daciae* most likely make up the majority of the 215 (0.9% of all specimens collected) specimens of the complex collected in this study. *Anopheles messeae* and *An. daciae* are difficult to distinguish based on COI sequences at this time, but the ribosomal internal transcribed spacer 2 (ITS2) region of one Estonian *An. messeae* specimen was sequenced in a previous study (Kirik *et al.* 2020). All in all, individuals belonging to the *An. maculipennis* complex could be found from May to October, but were more numerous in August. The abundance of mosquitoes of the complex may be underestimated in this study due to collection bias.

24. *Anopheles (Anopheles) plumbeus* Stephens, 1828  
(Fig. 4F)

**Published source:** Remm (1957: 150).

**Voucher material:** None.

**Comment:** There are historical records of four *An. plumbeus* females collected from Estonia (Remm 1957). No specimens were identified during this study, but this may be because of collection bias. *Anopheles plumbeus* has also been reported from Lithuania (Pakalniškis *et al.* 2000) and Sweden (Lundström *et al.* 2013), but not from other countries neighboring Estonia.

**Genus Coquillettidia Dyar, 1904**

25. *Coquillettidia (Coquillettidia) richiardii* (Ficalbi, 1889)  
(Fig. 4G)

**Published sources:** Remm (1957: 152, as *Mansonia richiardii*), Burtin (2014: 38), Kirik *et al.* (2021: 11).

Comment: 787 mosquitoes (3.2% of all specimens collected) were identified as *Cq. richiardii*. This species is common in the country and specimens have been found from June to October, with a peak of activity in July.

Genus *Culex* Linnaeus, 1758

26. *Culex* (*Culex*) *pipiens* Linnaeus, 1758
(Fig. 4H)

Published sources: Remm (1957: 157), Burtin (2014: 39), Kirik *et al*. (2021: 11, as *Cx. (Cux.) pipiens/torrentium*).


Comment: Adults of *Cx. pipiens* are difficult to distinguish from *Cx. torrentium* by morphological characteristics alone. However, when 12 *Cx. pipiens/torrentium* females were subjected to DNA barcoding, five (41.7%) were identified as *Cx. pipiens*. In 2013, 64 (48.5%) male mosquitoes were identified as *Cx. pipiens* compared to 68 (51.5%) determined to be *Cx. torrentium*. In 2017, however, 84 (60.9%) males were identified as *Cx. pipiens* and only 54 (39.1%) were identified as *Cx. torrentium*. Based on this information, *Cx. pipiens* and *Cx. torrentium* could be present in relatively similar numbers in Estonia. It is possible that the true relative abundance of *Cx. pipiens* is underestimated in this study due to collection bias. Mosquitoes identified as *Cx. pipiens/torrentium* were most numerous in September. Also, *Cx. pipiens* specimens in Estonia were found to be infected with the intercellular symbiont *Wolbachia pipientis*, which agrees with the published literature (Bergman & Hesson 2021; Inácio da Silva *et al*. 2021). No attempts were made to identify the “molestus” biotype of *Cx. pipiens* among the specimens collected during the study.

27. *Culex* (*Culex*) *torrentium* Martini, 1925
(Fig. 4H)

Published sources: Remm (1057: 157, as *Culex exilis* Dyar, 1924), Burtin (2014: 40), Kirik *et al*. (2021: 11, as *Cx. (Cux.) pipiens/torrentium*).

Voucher material: 1♀, Tartu (58° 22′ 17″ N, 26° 41′ 58″ E), 27.IX.2016, H. Kirik leg., H. Kirik det., sweep net, IZBE0210228, GenBank: OK465178; 1♀, Tartu (58° 23′ 40″ N, 26° 44′ 05″ E), 08.IX.2016, H. Kirik leg., H. Kirik det., sweep net, IZBE0210229, GenBank: OK465179; 1♂, Tartu (58° 23′ 44″ N, 26° 43′ 44″ E), 27.IX.2016, H. Kirik leg., H. Kirik det., sweep net, IZBE0210245.

Comment: The adult females of *Cx. torrentium* are difficult to distinguish from *Cx. pipiens* based on only morphological characteristics. Of 12 females subjected to DNA barcoding, seven (58.3%) were identified as *Cx. torrentium*. Adult males of the two species can be distinguished based on structures of their genitalia. In 2013, 68 (51.5%) among 132 males were identified as *Cx. torrentium*. In 2017, only 54 (39.1%) males were determined to be *Cx. torrentium* compared to 84 (60.9%) of individuals identified as *Cx. pipiens*. All things considered, it is reasonable to assume that *Cx. torrentium* makes up about half of the 1,236 (5.1% of all collected mosquitoes collected) identified as *Cx. pipiens/torrentium* during in this study. However, the true relative abundance of these mosquitoes may have been underestimated in this study due to collection bias. *Culex torrentium* and *Cx. pipiens* are most active at the end of summer, when they become dominant. Seven *Cx. torrentium* caught in Estonia were analyzed for *Wolbachia pipientis* using the wsp gene for detection. The results were negative, which is in line with the findings of Bergman & Hesson (2021).
FIGURE 5. Maps showing the historic and current collection points of individual mosquito species in Estonia. A, Culex (Neoculex) territans; B, Culiseta (Culicella) fumipennis; C, Culiseta (Culicella) morstians; D, Culiseta (Culicella) ochroptera; E, Culiseta (Culiseta) alaskaensis; F, Culiseta (Culiseta) annulata; G, Culiseta (Culiseta) bergrothi. For details, see Fig. 2, except for data for Cs. bergrothi, which is provided according to Khalin & Aibulatov (2020).
28. *Culex* (*Neoculex*) *territans* Walker, 1856
(Fig. 5A)

**Published sources:** Remm (1957: 157, as *Culex apicalis* Adams, 1903), Burtin (2014: 41), Kirik et al. (2021: 11).

**Voucher material:** 1♀, Tartu (58° 22′ 52″ N, 26° 42′ 49″ E), 08.IX.2016, H. Kirik leg., H. Kirik det., sweep net, IZBE0210230, GenBank: OK465180; 1♂, Tartu (58° 22′ 17″ N, 26° 41′ 58″ E), 20.IX.2016, H. Kirik leg., H. Kirik det., sweep net, IZBE0210250.

**Comment:** 50 mosquitoes (0.2% of all specimens collected) were identified as *Cx. territans*, making it the least common *Culex* species in Estonia. *Culex territans* was collected from July to October. This species is likely more common than the results of this fieldwork indicate, and their very low numbers are expected to be because of collection bias.

Genus *Culiseta* Felt, 1904

29. *Culiseta* (*Culicella*) *fumipennis* (Stephens, 1825) (Fig. 5B)

**Published source:** Remm (1957: 152, as *Theobaldia fumipennis*).

**Voucher material:** None.

**Comment:** Five females of *Cs. fumipennis* were previously reported from Estonia (Remm 1957). No individuals were found during this study. Of countries neighboring Estonia, *Cs. fumipennis* has been collected in Sweden (Lundström et al. 2013) and a province in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020).

30. *Culiseta* (*Culicella*) *morsitans* (Theobald, 1901)
(Fig. 5C)

**Published source:** Remm (1957: 152, as *Theobaldia morsitans*).


**Comment:** 41 mosquitoes (0.2% of all specimens collected) were identified as *Cs. morsitans*, making it the most commonly collected *Culiseta* species during this study. *Culiseta morsitans* was found in insect traps from June to September. The low number of individuals collected is likely due to collection bias.

31. *Culiseta* (*Culicella*) *ochroptera* (Peus, 1935)
(Fig. 5D)

**Published sources:** Remm (1957: 152, as *Theobaldia ochroptera*), Kirik et al. (2021: 11).


**Comment:** 11 mosquitoes were identified as *Cs. ochroptera*. These specimens were collected from August to October. The low number of individuals collected is likely to be due to sampling bias.
32. *Culiseta (Culiseta) alaskaensis* (Ludlow, 1906) (Fig. 5E)

**Published source:** Remm (1957: 151, as *Theobaldia alaskaensis*).

**Voucher material:** 1 ♀, Undi (58° 29′ 44″ N, 26° 54′ 00″ E), 21–22.IV.2015, L. Tummeleht leg., H. Kirik det., Mosquito Magnet trap, IZBE0210237, GenBank: OK465184; 1 ♀, Ülenurme (58° 19′ 3″ N, 26° 43′ 23″ E), 11–14.VIII.2020, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210238, GenBank: OK465185; 1 ♂, Kibuvitsa (58° 46′ 3″ N, 26° 30′ 46″ E), May to October 2020, L. Laaser leg., H. Kirik det., light trap, IZBE0210242.

**Comment:** 37 mosquitoes (0.2% of all specimens collected) were identified as *Cs. alaskaensis*, making it the second most common *Culiseta* species in Estonia based on this study. *Culiseta alaskaensis* appears to be active from April to August. The low number of individuals collected is likely to be due to sampling bias.

33. *Culiseta (Culiseta) annulata* (Schrank, 1776) (Fig. 5F)

**Published sources:** Remm (1957: 151, as *Theobaldia annulata*), Burtin (2014: 42), Kirik et al. (2021: 11).

**Voucher material:** 1 ♀, Ülenurme (58° 19′ 3″ N, 26° 43′ 23″ E), 11–14.VIII.2020, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210239, GenBank: OK465186; 1 ♀, Ülenurme (58° 19′ 3″ N, 26° 43′ 23″ E), 14–17.VIII.2020, H. Kirik leg., H. Kirik det., Mosquito Magnet trap, IZBE0210240, GenBank: OK465187; 1 ♂, Tartu (58° 21′ 23″ N, 26° 44′ 31″ E), 17.IX.2017, T. Kesküla leg., H. Kirik det., sweep net, IZBE0210246.

**Comment:** 28 mosquitoes (0.1% of all specimens collected) were identified as *Cs. annulata*. These mosquitoes were found throughout the warm season, from May to October, and seem to have the longest period of activity of all of the *Culiseta* species in Estonia. The low number of individuals collected is likely to be due to collection bias.

34. *Culiseta (Culiseta) bergrothi* (Edwards, 1921) (Fig. 5G)

**Published source:** Khalin et al. (2020: 74).

**Voucher material:** None.

**Comment:** One female of *Cs. bergrothi* collected in southeastern Estonia is preserved in the Zoological Institute of the Russian Academy of Sciences (Khalin & Aibulatov 2020). This species was previously noted to be present in Estonia according to Fauna Europaea (Snow & Ramsdale 2014), but the origin of that record is unknown as no other records have been found by the authors. *Culiseta bergrothi* has also been reported from provinces in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell et al. 2021) and Sweden (Lundström et al. 2013).

**Notes on species not included in the list above**

**Genus Aedes** Meigen, 1818

*Aedes (Aedes) geminus* Peus, 1970

**Published sources:** None.

**Voucher material:** None.

**Comment:** *Aedes geminus* has been reported from Lithuania (Pakalniškis et al. 2000; Bernotienė & Lučiūnaitė 2011), a province in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell et al. 2021) and Sweden (Lundström et al. 2013). Therefore, it may also be present in Estonia, but more work is needed to verify this, as the adult females of this species are difficult to distinguish from those of *Ae. cinereus*. 

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**Aedes (Dahliana) geniculatus** (Olivier, 1791)

**Published sources:** None.

**Voucher material:** None.

**Comment:** *Aedes geniculatus* has been reported from Lithuania (Pakalniškis *et al.* 2000), provinces in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell *et al.* 2021) and Sweden (Lundström *et al.* 2013), and could be present in Estonia. This species probably has not been collected during fieldwork due to collection bias.

**Aedes (Ochlerotatus) euedes** Howard, Dyar & Knab, 1913

**Published sources:** None.

**Voucher material:** None.

**Comment:** *Aedes euedes* is a member of the *Ae. annulipes* group. It reported from Lithuania (Pakalniškis *et al.* 2000), Latvia (Spungis 2000), provinces in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell *et al.* 2021) and Sweden (Lundström *et al.* 2013). While no specimens have been reported from Estonia thus far, the species is likely to be present but overlooked, especially as this species appears to be much less common in the Nordic-Baltic region than *Ae. annulipes* and *Ae. cantans*, which also belong to the *Ae. annulipes* group (Khalin & Aibulatov 2020; Lundström *et al.* 2013).

**Aedes (Ochlerotatus) pullatus** (Coquillett, 1904)

**Published source:** Kirik *et al.* (2021: 11).

**Voucher material:** None.

**Comment:** Mosquitoes with morphological characteristics most similar to *Ae. pullatus* were found during the study, but DNA barcoding identified them as *Ae. communis*. These specimens had scales on the hypostigmal area of the thorax, which is unusual for *Ae. communis*. *Aedes pullatus* has been previously reported from Lithuania (Pakalniškis *et al.* 2000), provinces in northwestern Russia adjacent to Estonia (Khalin & Aibulatov 2020), Finland (Culverwell 2018; Culverwell *et al.* 2021) and Sweden (Lundström *et al.* 2013). However, the occurrence of this species in Estonia remains uncertain.

**Aedes (Ochlerotatus) punctodes** Dyar, 1922

**Published sources:** None.

**Voucher material:** None.

**Comment:** Females of *Ae. punctodes* are difficult to distinguish from the females of *Ae. punctor*. This species has been reported from Finland (Culverwell 2021) and Sweden (Lundström *et al.* 2013), and could also be present in Estonia, but this requires further research.

**Aedes (Rusticoidus) rusticus** (Rossi, 1790)

**Published sources:** None.

**Voucher material:** None.

**Comment:** *Aedes rusticus* has been recognized as a species in Estonia in several mosquito checklist (Khalin & Aibulatov 2020; Snow & Ramsdale 1999; Robert *et al.* 2019). However, as was pointed out by Huldén & Huldén (2014) *Ae. rusticus* has been referred to historically by the synonym *Ae. maculatus*, but that name has also been mistakenly applied to *Ae. cantans*. In fact, the first checklist of mosquitoes in Estonia by H. Remm also included *Ae. maculatus*, but the number of specimens collected, as well as the description of their bionomics indicates that the species is *Ae. cantans*, not *Ae. rusticus*. This misunderstanding may be the reason why *Ae. rusticus* has been
reported to be present in Estonia, although no verified specimens have been collected in the country. However, *Ae. rusticus* has also been thought to be present in Lithuania (Pakalniškis et al. 2000) and Latvia (Spungs 2000), and has been reported from Sweden (Lundström et al. 2013). Therefore, this species could also be present in Estonia.

**Genus Anopheles Meigen, 1818**


Published sources: None.

Voucher material: None.

Comment: It can be difficult to distinguish *An. daciae* from *An. messeae* based on morphology. Nucleotide polymorphisms in ITS2 sequences are currently the best way to distinguish these two species, but this was not done in this study. *Anopheles daciae* has been reported from Finland (Culverwell et al. 2020; Culverwell et al. 2021), and could also be present in Estonia.

Discussion

This is the first comprehensive update to the mosquito fauna of Estonia since the publication of the original checklist by Remm (1957). The new checklist was compiled based on 24,344 adult mosquitoes (94.2% females) collected from 2008 to 2020, while also considering historic records and information from neighboring countries. Regrettably, mosquitoes collected by Remm, and used to compile the first checklist, have not been preserved and could not be verified. In total, the contemporary list includes 34 species, 27 of which were confirmed with voucher specimens; however, no specimens were collected to confirm the presence of seven other species in the country. All in all, additional collection efforts are required for a more thorough and detailed overview of the local mosquito fauna.

The updated checklist includes numerous changes compared to the historic list, which featured 30 species based on 12,204 mosquitoes (Remm 1957). Most importantly, four species were added to the list: *An. messeae*, *Ae. hexodontus*, *Ae. sticticus* and *Cs. bergrothi*. While the inclusion of the first three species is backed by numerous recently collected specimens, *Cs. bergrothi* is included based on a single specimen from Estonia stored at the Zoological Institute of the Russian Academy of Sciences (Khalin & Aibulatov 2020). The possible occurrence of seven additional species, *An. daciae*, *Ae. geminus*, *Ae. guniculatus*, *Ae. euedes*, *Ae. pullatus*, *Ae. punctodes* and *Ae. rusticus*, was discussed. Those species are present in neighboring countries, but could not be included in the updated list without evidence to verify their presence in Estonia. Interestingly, when comparing the original checklist with the present one, the same four species have remained the most numerous, making up the majority of the specimens collected in both cases. *Aedes communis* remains the most common mosquito in Estonia, as it made up 29.7% of all specimens collected in 1957 and 30.1% of all mosquitoes collected between 2008 and 2020 in the present study. *Aedes communis* is followed by *Ae. puncctor*, *Ae. cataphylia* and mosquitoes of the *Ae. annulipes* group. It is important to note that all four species are most active during late spring or early summer: the first three are especially numerous during May, while members of the *Ae. annulipes* group tend peak in June. Naturally, there were also numerous differences in the abundance of various species between the two checklists, but it is unknown whether these were due to genuine change or merely because of differences in collection methods and study sites.

Several mosquito species are likely underrepresented in this study due to the chosen collection sites and methods of collection. In fact, the relatively low numbers of many *Aedes*, *Anopheles*, *Culex* and *Culiseta* species collected in this study are likely due to collection bias and further work is needed to understand their true abundance in Estonia. Also, study sites were mostly concentrated in southeastern Estonia, covering the areas of the eastern Lowlands and Drumlins, as well as the southern Uplands (Villoslada et al. 2017). Islands and the coast of the mainland were also covered, but require more long-term collecting effort to better understand how brackish water affects the local mosquito fauna. For example, it is clear that *Ae. caspius* is common in these areas, but other salinity tolerant species, for example *Ae. dorsalis*, require further research. It is likely that the makeup and bionomics of the costal mosquito fauna are markedly different from areas on the mainland. Collection sites of the current study generally mimicked the locations reported by Remm (1957). However, the central area of the mainland, including parts of the Central Estonian Plain, as well as the Pandivere Uplands and the Northern Plain (Villoslada et al. 2017), received little attention in both cases. It is also important to note that this research was based solely on active adult mosquitoes, the
vast majority of which were females due to the chosen collection methods. In future, more work should be done to collect overwintering adults, which would allow for a more efficient collection of mosquito species that may not be attracted to baited traps. Moreover, collections larvae would not only improve the checklist, but would also provide additional information about the ecology of the species. It would likewise be beneficial to collect more male mosquitoes. While males do not require blood meals and are thus far less studied, they provide additional verification of the occurrence of some species. For example, the presence of *Ae. diantaeus*, which is morphologically similar to both *Ae. intrudens* and *Ae. communis*, was finally verified in Estonia based on a male specimen. Also, many female mosquitoes of the *Ae. annulipes* group can be difficult to identify due to overlapping morphological characteristics, as well as inconclusive results of DNA barcoding. However, it is relatively easy to distinguish the males of these species based on structures of their genitalia.

An updated checklist allows for a better understanding of the mosquito-borne pathogens circulating among local dipterans. For example, tularemia is a disease caused by the bacterium *Francisella tularensis* (McCoy & Chapin), which occurs throughout the northern hemisphere. It manifests in humans with influenza-like symptoms and numerous other ailments, based on the route of infection (Maurin & Gyuranecz 2016). *Francisella tularensis* is normally confined to animals on a few islands of Estonia, but one or two human infections occur in the country almost every year (Health Board 2012, 2016, 2020). Although mosquitoes are one of several arthropods capable of transmitting the bacterium, natural infections have been detected in *Ae. cinereus*, *Ae. communis*, *Ae. puctor, Ae. sticticus*, *Ae. vexans* and *Cx. pipiens* (Lundström et al. 2011; Dryselius et al. 2019). Furthermore, the filarial nematode *Dirofilaria repens* Railliet & Henry appears to also have become established in Estonia, since it has been found in local dogs several times since 2008 (Jokelainen et al. 2016). The mosquito-borne *Dirofilaria repens* normally parasitizes subcutaneous tissues of carnivores and is often asymptomatic in dogs, but can also infect humans, resulting in skin nodules, ocular dirofilariosis or other complications (Capelli et al. 2018; Ciucu et al. 2020; Pupić-Bakrač et al. 2021). In fact, autochthonous human cases have already been reported in countries neighboring Estonia (Melbarde-Gorkusa et al. 2011; Pietikäinen et al. 2017). Thus far, numerous species belonging to the genera *Aedes*, *Anopheles*, *Coquillettidia* and *Culex* have been indicated in carrying *Dirofilaria repens*, as reported by Kronefeld et al. (2014), Kemenesi et al. (2015), Şuleșco et al. (2016) and Shaikевич et al. (2019a). Importantly, Shaikевич et al. (2019a) found that *Ae. communis* could be one of the species effective in spreading *Dirofilaria* species in Russia. Additionally, there are also some mosquito-borne viruses circulating in northern Europe (Francy et al. 1989; Barzon 2018). For example, Sindbis virus, which is carried long distances by migrating birds and transmitted to humans by mosquitoes, is especially noteworthy in the Nordic countries (Kurkela et al. 2005; Bergqvist et al. 2015), but the virus has also been isolated from birds in Estonia (Uryvayev et al. 1992). Generally, ornithophilic species like *Cx. pipiens*, *Cx. torrentium* and *Cs. morsitans* are thought to be important carriers of the Sindbis virus (Francy et al. 1989). Based on this information, *Ae. communis*, which is overall the most numerous mosquito in Estonia, and *Cx. pipiens/torrentium*, which are especially active at the end of summer, are the most likely species to become important vectors in the country.

There are still notable gaps in our knowledge of mosquito diversity in Estonia, as biting dipterans were largely ignored during the latter half of the last century and the country still lacks a continuous mosquito monitoring program. Furthermore, scenarios of climate change predict that the annual mean temperature is likely to increase by 2.3–4.5°C in Estonia by the year 2100, and during the same time the average yearly precipitation could increase anywhere between 4–46% (Kont et al. 2003). This will likely influence the length of time suitable for mosquito development, as well as the availability of larval habitats in the country. Also, it is well known that alterations in land use, international trade and travel have led to changes in the diversity and distribution of various arthropods, including many mosquitoes (Brugueras et al. 2020; Medlock et al. 2012; Rochlin et al. 2016; Brugueras et al. 2020). Hence, there is a clear need for further studies on both blood-sucking dipterans and insect-borne pathogens in Estonia. Extra attention should be paid to the international airport and large harbors, which can act as entry points for non-native mosquitoes (Sukehito et al. 2013; Ibáñez-Justicia et al. 2020). Furthermore, mosquito collection activities should be more evenly spread out in Estonia to sample as many biotypes as possible. Finally, insect-borne pathogens require more attention. For example, how important mosquitoes are in transmitting *Francisella tularensis* in the region and which species carry *Dirofilaria repens* in Estonia remains to be investigated.
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THE MOSQUITO FAUNA (DIPTERA: CULICIDAE) OF ESTONIA


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