

Project report.

A survey of mosquito species (Diptera, Culicidae) in Luxembourg, 2016.

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Abstract. From July to October 2016 mosquito collections were conducted at ten different sites throughout Luxembourg using CO₂-baited traps. In total, seven mosquito species were recorded with *Culex pipiens/torrentium* exhibiting the widest distribution and highest frequency (175 specimens, including 46 *Culex pipiens* and 26 *Cx. torrentium*). The other taxa were seldom recorded: *Anopheles plumbeus* (3 specimens), *Coquillettidia richiardii* (1), *Culiseta annulata* (1), *Aedes cinereus/geminus* (1), and *Aedes vexans* (1), the latter being new to the fauna of the Grand Duchy.

Keywords. Mosquitoes, Culicidae, invertebrates, Luxembourg

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1. Introduction

While being on holidays in August 2015 in Vienna, Austria, the first author of the present report has been infected by the West Nile virus through a mosquito bite. After this experience, questions rose about the situation in his home country Luxembourg: Which mosquito species do occur in the Grand Duchy? Are there invasive alien species and vectors of pathogens amongst them? Are there invasive alien mosquito species on their way to Luxembourg?

This was the motivation to start a pilot project to gather data on Culicidae in Luxembourg and gain first experience in their trapping and identification. It was intended as a first inventory of mosquito species of the Grand Duchy with a focus on exotic invasive species like the Asian tiger mosquito (*Aedes albopictus*) or the East Asian bush mosquito (*Aedes japonicus*).

Earlier, a study about the mosquito fauna of Luxembourg was conducted in 2001 (Beck et al. 2003). Before that study, Luxembourg was the only European country without published records of Culicidae (Snow & Ramsdale, 1999). Later in 2010 and 2011, two Luxembourg health institutions (CRP Santé, Laboratoire national de santé) conducted a research project on the prevalence and diversity of mosquitoes and mosquito-borne pathogens in Luxembourg (Muller & Reye 2012). Unfortunately the project was terminated before finalisation, by the end of 2011.

2. Methods

Mosquito collections were conducted from July to October 2016 at ten different sites throughout the country, in cooperation with local and regional partner organisations and private persons (Fig. 1 & Tab. 2).

We operated Biogents BG-Sentinel™ 2 mosquito traps once a week on each site during 24 hours. The traps were run either on batteries or connected to the electric mains supply. We used a combination of CO₂ (bottles) and Biogents BG-Lure™ to attract mosquitoes.

The catches were conserved in deep freezers until their transfer to the Department for Ecology of the Luxembourg National Museum of Natural History at the end of the catching period.

On June 14th and 15th, 2017, the last author identified the catches at the Museum (Fig. 2). The by-catch was not identified.

During these same two days, the last author did train two museum staff members in the identification of mosquitoes using the CD-ROM entitled “The Mosquitoes of Europe” (Schaffner et al. 2001).

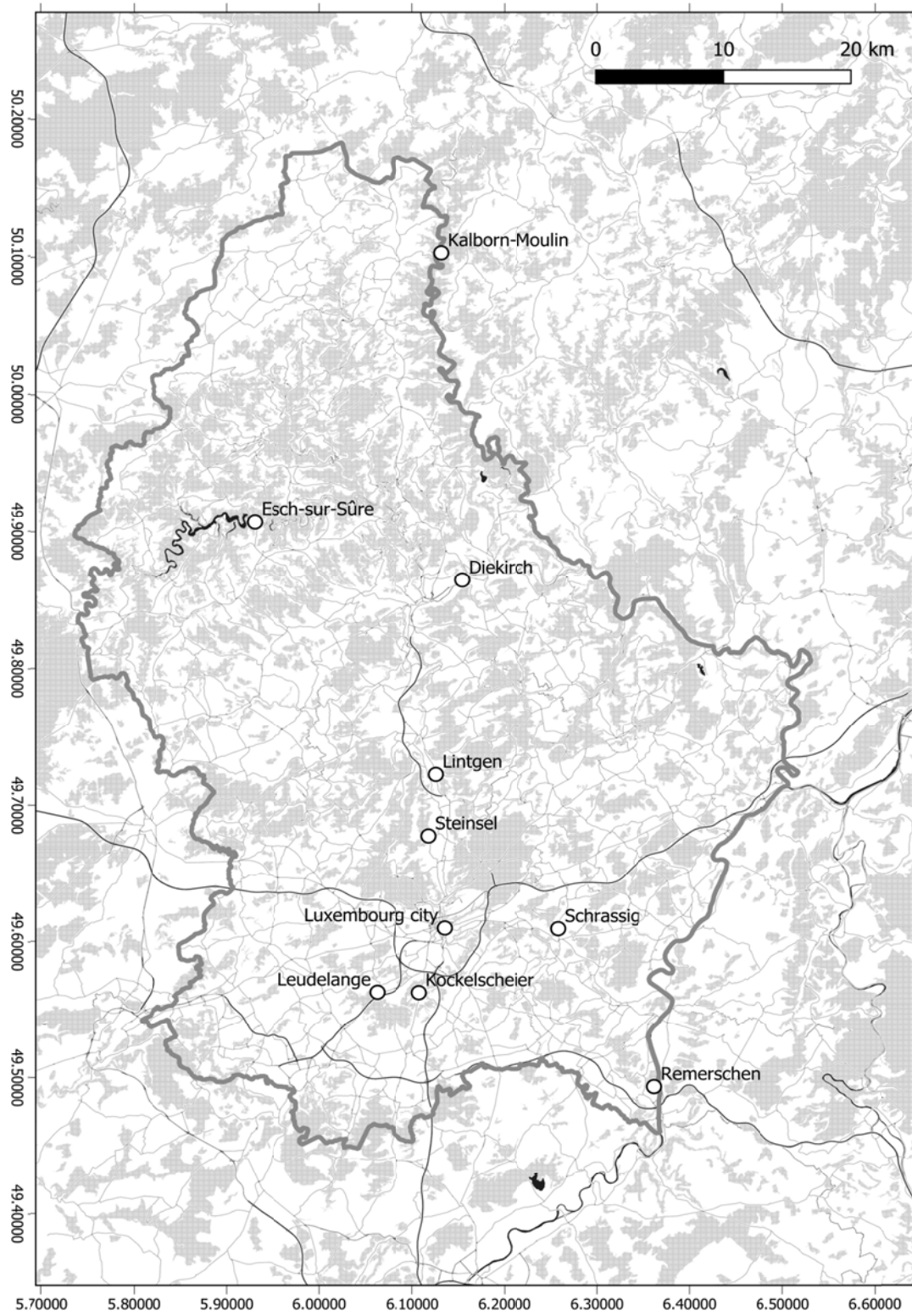


Fig. 1. Map of the trapping sites, July-October 2016.



Fig. 2. F.I.t.r.: Svenja Christian (Department of Invertebrate Zoology MNHNL) and Dr Francis Schaffner (University of Zurich) in the Lab of the Department of Ecology. Photo: Dr Christian Ries, MNHNL, 15 June 2017.

As for the differentiation of the sister species *Culex pipiens* and *Cx. torrentium*, 72 samples were analysed by the multiplex PCR developed by Smith and Fonseca (2004). Briefly, the KAPA Express Extract Kit (KAPA-BIOSYSTEMS, USA) was used for DNA extraction from each specimen following the manufacturer's instructions. The multiplex PCR targeting the acetylcholinesterase-2 (*ace-2*) locus was carried out in 10 μ l reactions, each one containing 5 μ l Platinum Green PCR 2X mix (Invitrogen, USA), 0.3 pmol of each primer ACEquin, ACEtorr, ACEpip and B1246s (Smith and Fonseca, 2004), approximately 50 ng extracted DNA and ultra pure water up to the volume of 10 μ l. PCR conditions were as described by Smith and Fonseca (2004).

3. Results

A total of 182 specimens from seven mosquito species were caught in 90 catches performed at the 10 sites (Tables 1 & 2), the most frequent being *Culex pipiens/torrentium* (175 specimens in 9 out of 10 sites). The analysis of 72 specimens by the multiplex PCR assay resulted in 46 individuals of *Cx. pipiens* complex and 26 individuals of *Cx. torrentium*. In this study *Aedes vexans* was detected for the first time in Luxembourg.

No mosquitoes were caught in the trap situated in Diekirch and as there were 16 by-catch specimens in 11 catches we assume that the trap was not used in a place suitable to be visited by mosquitoes. All other sites revealed the presence of mosquitoes, *Cx. pipiens/torrentium* showing the widest distribution (nine sites among ten), while the other species were found at two or one site only. The site of Kockelscheuer showed the highest diversity with five species among the seven detected in our study.

Table 1. Number of trappings and mosquito specimens per site, July-October 2016.

<i>Sites</i>	<i>Partner organisations and private partners</i>	<i>Trappings</i>	<i>Specimens</i>
Diekirch	Administration de la nature et des forêts	11	0
Esch-sur-Sûre	Naturpark Öwersauer (PNHS)	12	9
Kalborn-Moulin	natur&ëmwelt – Fondation Hëllef fir d’Natur	10	6
Kockelscheuer	natur&ëmwelt – Haus vun der Natur	11	48
Leudelange	Susanne Folschette	10	27
Lintgen	Sandra Cellina	9	23
Luxembourg city	MNHNL	11	19
Remerschen	Biodiversum	5	16
Schrassig	Pierre Kalmes	6	24
Steinsel	Manou Pfeiffenschneider	5	10

Table 2. Mosquito species and number of specimens caught, July-October 2016.

<i>Species</i>	<i>Sites</i>	<i>Specimens</i>
<i>Aedes cinereus/geminus*</i>	Kockelscheuer	1
<i>Aedes vexans</i>	Kockelscheuer	1
<i>Anopheles plumbeus</i>	Kockelscheuer, Steinsel	3
<i>Coquillettidia richiardii</i>	Remerschen	1
<i>Culex pipiens/torrentium</i>	All sites but Diekirch	175
<i>Culex pipiens</i>	All sites but Diekirch and Steinsel	46
<i>Culex torrentium</i>	All sites but Diekirch	26
<i>Culiseta annulata</i>	Remerschen	1

* Sister species not identified here.

4. Discussion

Due to administrative delays, the present project could not be implemented throughout the whole season suitable to catch mosquitoes which extends basically from March to October. We collected samples from July to October only and missed the earlier months March to June.

With the exclusive use of CO₂- and lure-baited BG Sentinel 2 mosquito traps, we caught seven mosquito species. Beck et al. (2003) collected fifteen species back in 2001 combining three sampling methods: CO₂-baited trapping, human landing catches and larval dipping in adjacent breeding sites. From over 2,500 mosquitoes collected in 2011, Muller & Reye (2012) sequenced 204 specimens and found six species. A provisional checklist combining the findings of the three studies can be found in table 3.

Table 3. List of culicidae found by Beck et al. (2003) in 2001, Muller & Reye (2012) in 2011 and the present study in 2016. First records (field data) from 1997 are from Francis Schaffner (*Anopheles maculipennis* s.s. published in Proft et al. 1999; *Cx. pipiens*, *Cx. torrentium*: unpublished data).

Species	2001	2011	2016	First record
<i>Aedes annulipes</i> (Meigen, 1830)	x			2001
<i>Aedes cantans</i> (Meigen, 1818)	x			2001
<i>Aedes cinereus</i> Meigen, 1818 / <i>geminus</i> Peus, 1970	x		x	2001
<i>Aedes geniculatus</i> (Olivier, 1791)	x			2001
<i>Aedes punctor</i> (Kirby, 1837)	x	x		2001
<i>Aedes rusticus</i> (Rossi, 1790)	x			2001
<i>Aedes sticticus</i> (Meigen, 1838)	x			2001
<i>Aedes vexans</i> (Meigen, 1830)			x	2016
<i>Anopheles claviger</i> s.s. (Meigen, 1804)	x	x		2001
<i>Anopheles maculipennis</i> s.s. Meigen, 1818	x	x		1997
<i>Anopheles plumbeus</i> Stephens, 1828	x	x	x	2001
<i>Coquillettidia richiardii</i> (Ficalbi, 1889)	x		x	2001
<i>Culex pipiens</i> Linnaeus, 1758	x	x	x	1997
<i>Culex territans</i> Walker, 1856	x			2001
<i>Culex torrentium</i> Martini, 1925	x	x	x	1997
<i>Culiseta annulata</i> (Schrank, 1776)	x	x	x	2001

In summary, one mosquito species, *Aedes vexans*, is added to the known fauna from Luxembourg, which reaches now the number of 16 species.

5. Conclusions

There is still quite a lack of knowledge about Culicidae in Luxembourg, several areas of the country and environment types have not yet been investigated. A more global survey should be considered to produce a complete checklist and an atlas of the mosquitoes of Luxembourg. Further data sources should be considered and the remaining samples from the Muller & Reye study should be analysed with molecular methods.

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