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Atlas and catalogue of the mosquitoes (Diptera, Culicidae) of Luxembourg

Francis Schaffner
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Christian Ries

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1. *Culex pipiens*, female. Photo: Francis Schaffner
2. *Anopheles maculipennis* s.l., female. Photo: Francis Schaffner
3. *Aedes geniculatus*, female. Photo: Francis Schaffner

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Contents

The project MosquitoLUX – Atlas of the mosquitoes of Luxembourg

The MosquitoLUX project was implemented under the auspices of the Fondation faune-flore at the Scientific Research Centre of the National Museum of Natural History in Luxembourg. The Fondation faune-flore was the host institution and administrative manager of the project.

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- 25%: Directorate of Health

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Ministère de l'Environnement, du Climat
et du Développement durable

LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de la Santé

Direction de la santé

Atlas and catalogue of the mosquitoes (Diptera, Culicidae) of Luxembourg

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Zusammenfassung

Durch Stechmücken übertragene Krankheiten wie Chikungunya, Dengue, Usutu oder West-Nil sind in jüngster Zeit sowohl für die Gesundheit von Menschen als auch von Tieren wieder in den Vordergrund gerückt. Um Krankheitsausbrüche zu verhindern, ist eine fundierte Kenntnis über die Stechmückenfauna einer Region erforderlich. Für Luxemburg bestanden dahingehend größere Lücken.

Der hier vorliegende Atlas der Stechmücken Luxemburgs enthält eine nationale Checkliste und Verbreitungskarten aller erfassten Arten. Vorhandene Daten zum Vorkommen von Stechmücken im Land wurden identifiziert und zusammengeführt, sowie von 2019-2021 Felduntersuchungen durchgeführt, um aktuelle Informationen zu erhalten. Letztere basierten auf Stichproben von Jungtieren und Fängen erwachsener Mücken, die im Laufe von drei Jahren an ausgewählten Standorten in landwirtschaftlichen, natürlichen oder städtischen Gebieten in jedem der 137 5x5 km großen Rasterquadrate des Großherzogtums durchgeführt wurden. Individuen wurden allgemein morphologisch bestimmt, jedoch bei bekannten kryptischen Arten durch genetische Methoden unterstützt.

Aus allen Informationsquellen wurden zwei Datensätze zusammengestellt: zum einen 642 Stechmückenvorkommen aus bereits veröffentlichten oder unveröffentlichten Quellen (1997 bis 2018) und zum anderen 22 Mitteilungen von Bürger*innen und weiteren 735 neuen Stechmückenvorkommen, die von 560 über das Großherzogtum verteilten Standorten stammen (2019-2021). Alle Daten sind in einer zusammenführenden Datenbank gespeichert, die es ermöglicht, aktualisierte Karten für die 28 Stechmückenarten (31 Taxa) zu erstellen, deren Vorkommen in Luxemburg gemeldet werden kann. Die detaillierte Suche nach vorhandenen Daten ermöglichte es uns, vier Arten zu identifizieren, die in der veröffentlichten Literatur nicht vorkommen, sondern nur in der „grauen Literatur" oder in unveröffentlichten Datensätzen erwähnt wurden: *Aedes communis*, *Ae. geminus*, *Ae. vexans* und *Culiseta morsitans*.

Während unserer Feldstudie konnten wir mit Ausnahme von *Ae. cinereus* alle zuvor gemeldeten Arten erneut nachweisen (d.h. 22 / 23 Arten). Darüber hinaus wurden fünf Erstnachweise für Luxemburg getätigt: *Ae. refiki*, *Anopheles daciae*, *An. petragnani*, *Culex modestus* und *Uranotaenia unguiculata*. Aus den gesammelten

Daten geht hervor, dass *Cx. pipiens/torrentium* das am weitesten verbreitete und am häufigsten anzutreffende Taxon war und wahrscheinlich auch das häufigste Taxon in Luxemburg ist. Die anderen Taxa, die eine weite Verbreitung aufweisen, sind *An. claviger* s.s., *An. maculipennis* s.l., *Ae. geniculatus*, *Cs. annulata*, *Ae. annulipes/cantans*, *Cs. morsitans*, *Cs. longiareolata*, *An. maculipennis* s.s., *Cx. territans*, und *Ae. rusticus*. Zu den seltenen Taxa in Luxemburg gehören *Ae. cinereus/geminus*, *Ae. sticticus*, *Ae. vexans*, *Coquillettidia richiardii*, *Ae. refiki*, *An. petragnani*, *Cs. glaphyroptera*, *Cx. modestus* und *Ur. unguiculata*.

Im Vergleich zu früheren Daten deutet unsere Feldstudie nicht auf das Aussterben von Arten oder eine Verringerung ihrer Verbreitungsgebiete hin. Im Gegenteil, die Region wurde in den letzten Jahren von zwei neuen Arten besiedelt: *Ae. japonicus* und *Cs. longiareolata*. In Anbetracht ihres Vorkommens in den Nachbarländern und der Verfügbarkeit geeigneter Lebensräume in Luxemburg führen wir auch 22 Arten auf, die bei künftigen Erhebungen gefunden werden könnten. Mit der Einführung weiterer invasiver *Aedes*-Arten, die sich in artifiziellen Bruthabitaten wie z. B. Vasen entwickeln, könnten sich in der Tat weitere Veränderungen ergeben. Was Luxemburg betrifft, so gibt es keinen umweltbedingten oder klimatischen Grund, warum die Asiatische Tigermücke *Ae. albopictus* zukünftig keine Populationen im Land ausbilden sollte. Klimatische Veränderungen könnten gar die Ansiedlung und Ausbreitung weiterer wärmeangepassten Arten begünstigen (z. B. *An. petragnani*, *Ae. berlandi*), während das Verbreitungsgebiet von kälteangepassten Arten zurückgehen könnte (z. B. *Ae. communis*, *Cs. glaphyroptera*), je nachdem, wie stark die Veränderungen in Zukunft sein werden.

Eine primäre Risikobewertung der in Luxemburg vorkommenden Stechmückenarten (+ *Ae. albopictus*) zeigt, dass mindestens 15 Arten an der Übertragung von Krankheitserregern oder Parasiten beteiligt sein könnten. Insgesamt 18 Arten haben das Potenzial den Menschen zu belästigen, wobei fünf Taxa als primäre Belästigungsarten betrachtet werden können.

Abstract

Mosquito-borne diseases such as chikungunya, dengue, Usutu or West Nile, have come back to the forefront in both human and animal health. To prevent outbreaks, it is necessary to acquire a solid knowledge of the mosquito fauna in the considered area. Therefore, and since the mosquito fauna of Luxembourg was only partially known, we suggested the construction of the first comprehensive mosquito diversity and distribution database for the Grand Duchy as a base for an atlas.

This atlas of the mosquitoes of Luxembourg assembles a taxonomic list and species' distribution maps. To achieve this, we collected and synthesised existing mosquito occurrence data, and implemented field surveys to acquire original field data. The latter were based on immature sampling and adult catches performed throughout three years (2019-2021) at sites selected in agriculture, nature or urban land cover categories within each of the 137 5x5 km grid squares that cover the territory of the Grand Duchy. Specimens were identified by morphology, but in the case of known cryptic species DNA-based methods were applied in addition.

As a result, we assembled two sets of data; firstly, 642 mosquito taxa occurrences from published or unpublished sources from the period 1997 to 2018, and secondly, 22 citizen data and 735 original mosquito occurrences obtained from 560 sites distributed across the Grand Duchy and collected over the period 2019-2021. All data are stored in a unique database that allows the production of updated mosquito distribution maps for the 28 species (31 taxa) reported to occur in Luxembourg to date. The detailed search for existing data allowed us to identify four species that do not appear in published literature but are reported by grey literature or unpublished data sets: *Aedes communis*, *Ae. geminus*, *Ae. vexans*, and *Culiseta morsitans*.

During our field study, we collected all species previously reported except *Ae. cinereus* (i.e. 22 / 23 species). In addition, we recorded five species new for the country: *Ae. refiki*, *Anopheles daciae*, *An. petragnani*, *Culex modestus*, and *Uranotaenia unguiculata*. The assembled data reveal the taxon *Cx. pipiens/torrentium* to be the most widespread and frequently encountered, and probably the most abundant taxon in Luxembourg. The other taxa that show a wide-ranging distribution are *An. claviger* s.s., *An. maculipennis* s.l., *Ae. geniculatus, Cs. annulata*, *Ae. annulipes/cantans*, *Cs. morsitans*, *Cs. longiareolata*, *An. maculipennis* s.s., *Cx. territans*, and *Ae. rusticus*. Taxa that have a very limited distribution range are *Ae. cinereus/geminus*, *Ae. sticticus*, *Ae. vexans*, *Coquillettidia richiardii*, *Ae. refiki*, *An. petragnani*, *Cs. glaphyroptera*, *Cx. modestus*, and *Ur. unguiculata*.

Compared to earlier data, our field study does not indicate any species extinction or distribution range retraction. On the contrary, the territory has been colonised in recent years by two new species: *Ae. japonicus* and *Cs. longiareolata*. Considering their occurrence in neighbouring countries and the availability of suitable environments in Luxembourg, we also list 22 species which may be found in upcoming field surveys. Further possible changes could indeed occur, with the introduction of additional invasive *Aedes* container-inhabiting species. As for Luxembourg, there is no environmental or climatic reason that the Asian tiger mosquito *Ae. albopictus* will not establish populations. Climatic changes may favour the establishment and expansion of warm-climate species in Luxembourg (e.g. *An. petragnani*, *Ae. berlandi*), while cold-climate species may be disfavoured and might show a retraction of their ranges (e.g. *Ae. communis*, *Cs. glaphyroptera*), according to the degree of changes that will occur in the future.

A primary risk assessment performed for the mosquito species reported to occur in Luxembourg, plus the invasive species *Ae. albopictus,* shows that at least 15 species could be involved in pathogen or parasite transmission. Considering the nuisance aspect, 18 species may have the potential to disturb humans, with five taxa that can be considered as primary nuisance species.

Résumé

Les maladies à transmission vectorielle telles que le chikungunya, la dengue et les infections à virus Usutu ou West Nile, sont revenues sur le devant de la scène pour la santé publique comme pour la santé animale. La prévention des épidémies requiert une bonne connaissance de la faune des moustiques pour une aire considérée. Pour cette raison, et comme la faune des moustiques du Luxembourg n'était que partiellement connue, nous avons suggéré de construire la première base de données conséquente sur la distribution des moustiques du Grand-Duché, comme fondement pour un atlas.

Cet atlas des moustiques du Luxembourg réunit une liste taxonomique et des cartes de distribution des différentes espèces. Pour cette réalisation, nous avons collecté et synthétisé les données de distribution de moustiques existantes, et effectué des prospections pour acquérir des données de terrain originales. Ces dernières ont été basées sur des prélèvements de stades immatures et des captures d'adultes réalisées pendant trois années (2019-2021) sur des sites sélectionnés dans des zones de couverture du sol à caractère agricole, naturel ou urbain, dans chacune des 137 mailles de 5x5 km qui couvrent le territoire du Grand-Duché. Les spécimens ont été identifiés par la morphologie, mais dans le cas d'espèces cryptiques connues, des méthodes génétiques ont été appliquées en complément.

De ce travail résultent deux jeux de données; premièrement, 642 données de présence de moustiques issues de sources publiées ou non publiées, de la période 1997 à 2018, et deuxièmement, 22 données de sciences citoyenne et 735 données de terrain originales de présence de moustiques obtenues sur 560 sites distribués à travers le Grand-Duché et collectées pendant la période 2019-2021. Toutes les données sont stockées dans une base unique qui permet la production des cartes de distribution de moustiques à jour pour les 28 espèces (31 taxons) reportées comme présentes aux Luxembourg à ce jour. La recherche détaillée des données existantes nous a permis d'identifier quatre espèces qui n'apparaissent pas dans la littérature publiée mais sont reportées par la littérature grise ou dans des jeux de données non publiés : *Aedes communis*, *Ae. geminus*, *Ae. vexans*, et *Culiseta morsitans*.

Pendant notre étude de terrain, nous avons collecté toutes les espèces précédemment reportées sauf *Ae. cinereus* (soit 22 / 23 espèces). En plus, nous avons enregistré cinq espèces nouvelles pour le pays : *Ae. refiki*, *Anopheles daciae*, *An. petragnani*, *Culex modestus*, et *Uranotaenia unguiculata*. L'ensemble des données montre que le taxon *Cx. pipiens/torrentium* est le plus largement répandu et le plus fréquemment rencontré, et probablement le plus abondant au Luxembourg. Les autres taxons qui montrent une large distribution sont *An. claviger* s.s., *An. maculipennis* s.l., *Ae. geniculatus, Cs. annulata*, *Ae. annulipes/cantans*, *Cs. morsitans*, *Cs. longiareolata*, *An. maculipennis* s.s., *Cx. territans*, et *Ae. rusticus*. Les taxons qui ont une distribution très limitée sont *Ae. cinereus/geminus*, *Ae. sticticus*, *Ae. vexans*, *Coquillettidia richiardii*, *Ae. refiki*, *An. petragnani*, *Cs. glaphyroptera*, *Cx. modestus,* et *Ur. unguiculata*.

En comparaison aux données antérieures, notre étude de terrain ne révèle pas d'extinction d'espèce ou de rétraction de distribution. Au contraire, le territoire a été colonisé dans les récentes années par deux espèces : *Ae. japonicus* et *Cs. longiareolata*. Nous listons également 22 espèces qui pourraient être rencontrées dans des prochaines études de terrain, compte tenu de leur occurrence dans les pays voisins et de la disponibilité d'environnements favorables à ces espèces au Luxembourg. Des changements supplémentaires pourraient apparaître, avec l'introduction d'espèces invasives du genre *Aedes* qui se développent dans des contenants artificiels. Pour le Luxembourg, il n'y a pas de raison environnementale ou climatique qui empêcherait le moustique tigre asiatique *Ae. albopictus* d'établir des populations. Les changements climatiques pourraient également favoriser l'établissement et l'expansion d'espèces de climat chaud au Luxembourg (par ex. *An. petragnani*, *Ae. berlandi*), tandis que des espèces de climat froid seraient désavantagées et pourraient montrer une rétraction de leur aire de distribution (par ex. *Ae. communis*, *Cs. glaphyroptera*), en fonction du degré de changement effectif futur.

Une première évaluation du risque vectoriel réalisée pour les espèces de moustiques répertoriées au Luxembourg, plus l'espèce invasive *Ae. albopictus,* montre qu'au moins 15 espèces peuvent être impliquées dans une transmission d'agent pathogène ou de parasite. Pour ce qui est de la nuisance, 18 espèces ont le potentiel de générer une nuisance pour l'homme, avec cinq taxons qui peuvent être considérés comme des espèces nuisantes primordiales.

1 Introduction

1.1 Context

Mosquitoes (Diptera, Culicidae) have been well studied since the early XXth century, because of the severe threat they can cause to the health and well-being of humans and animals. The mosquito family is very diverse, with 3,570 valid species names recognised to date (Wilkerson et al. 2021), but some geographical areas, like Luxembourg, remain poorly studied.

During the recent decades, mosquito-borne diseases have come back to the forefront in both human and animal health. Mosquitoes are among the most important vectors, especially in tropical and semi-tropical environments, but also in temperate environments. In Western Europe, the vector context has changed considerably since the late 1990s, with the (re-)appearance of vectorial transmissions in, among others, Belgium (Usutu virus), France (chikungunya, dengue, West Nile and Zika viruses) and Germany (Sindbis, Usutu, and West Nile viruses) (Johnson et al. 2018; Giron et al. 2019; Ziegler et al. 2019; Benzarti et al. 2020). Among the causes of these changes are globalisation together with environmental and climate changes, which indirectly modify mosquito fauna and the range of pathogens present in a given region (Franklinos et al. 2019; Rocklöv & Dubrow 2020).

This context forces us to define plans for the prevention and control of mosquito-borne diseases. The effectiveness of these plans is directly related to the knowledge and understanding of the local mosquito fauna, in terms of species presence (both alien and native), abundance and seasonality (Braks et al. 2011). These data also support optimising vector control, in order to apply control measures only to threatening populations, with the best possible timing, and thus also to limit any unintended consequences of the selected measures.

At the European continental level, risk assessment for mosquito-borne diseases is based on mosquito surveillance data, and distribution maps of major vector species are available (VectorNet project: Braks et al. 2022). These maps are based on presence/absence data at NUTS3 level (i.e. departments for France, entire country for Luxembourg) and therefore only allow a very general estimate of the risk. An estimate of vectorial risk at the local level requires more precise (geolocation or, failing that, at the communal level) and up-to-date diversity and distribution data.

1.2 Project aim

The mosquito fauna of Luxembourg is only partially known, especially in terms of species' distributions. While some data is available from the literature (e.g. Beck et al. 2003), additional datasets remained unpublished at the start of our project (e.g. Zittra et al. 2021, Schaffner 2022). Only the invasive mosquito *Aedes japonicus* has been actively monitored very recently (Schaffner & Ries 2019). However, other invasive species (e.g. the tiger mosquito *Ae. albopictus*) could also be introduced into Luxembourg since some populations are established in neighbouring countries (Medlock et al. 2015; Becker et al. 2017; Ibáñez-Justicia 2020), and native species can potentially carry pathogens important to human and/or animal health (Martinet et al. 2019). Besides, while invasive species also represent a threat to the native fauna, the study of the mosquito local diversity expands our knowledge of the biodiversity and may help to highlight natural or human-induced changes.

Therefore we suggested the construction of the first comprehensive mosquito diversity and distribution database for Luxembourg, and the collection of new data with a focus on data-poor areas, with a high probability of finding new species and new populations of threatening species. We proposed the creation of an innovative mosquito surveillance network, integrating molecular data and involving international experts, as well as further scientific and technical networks and the general public. The collections of new data were based on high expertise, in order to select sampling sites according to the biology of the target species and to optimally combine the various available sampling methods. We aimed at (i) collecting qualitative, but not quantitative, data on mosquito presence/absence to assess biodiversity, vectorial risk and faunistic changes, (ii) providing an updated species list and the respective species' distribution maps, and (iii) identifying the potential for additional species to occur in the country.

2 Materials and methods

Here we propose an atlas of the mosquitoes of Luxembourg, assembling a taxonomic list and species' distribution maps. To achieve this, we (1) collected historical distribution data in a centralised database, (2) performed field surveys to collect original field data throughout the Grand Duchy, in particular to fill geographical and biological gaps (in terms of species and environments) revealed by the historical dataset, and (3) contributed to the setup of a surveillance programme of invasive *Aedes* mosquito species.

2.1 Nomenclature

Scientific names for genera, subgenera and species of mosquitoes follow the International Code of Zoological Nomenclature (ICZN) and the usage of the Systematic Catalog of Culicidae which is recognised as a reference, maintained by the Walter Reed Biosystematics Unit (http://www.mosquitocatalog.org), and recently published in the form of a book (Wilkerson et al. 2021). Genera and subgenera abbreviations used herein also follow the same catalogue (Wilkerson et al. 2021).

Informal names are provided by Wilkerson et al. (2021) in English, and are here translated/adapted to French, German and Luxembourgish based on their etymology. The latter names were determined by the third author and validated by the Centre for Luxembourgian Language (Zenter fir d'Lëtzebuerger Sprooch).

2.2 Study area

The area under investigation is the Grand Duchy of Luxembourg. The country lies between 49°26' and 50°11' North latitude and between 5°44' and 6°32' East longitude and covers an area of 2,586 km². Besides the administrative divisions, Luxembourg is divided into two biogeographical regions due to the different geological, topographical and climatic conditions.

2.2.1 The Oesling

With an area of 828 km², the Oesling in the north of the country covers about 32% (STATEC 2022b) of the country's area and belongs to the Ardennes and Eifel mountain range which is part of the Rhenish Massif. It is a plateau with an average altitude of about 450 m a.s.l. (highest elevation 560 m a.s.l.). It is only slightly indented in the northwest, but is otherwise cut by deep and steep valleys (Fig. 1).

Geologically, the entire Oesling consists of slate rocks and quartzites from the Devonian (Fig. 2). The weathering of these schists and quartzites forms shallow, stony-loamy and nutrient-poor soils in large areas. Only on the plateaus, where the sand content of the slate soils is higher, have deeper and looser soils developed which are used as arable land (Fig. 3). The wet meadows of the valleys are mainly used as cattle pastures.

The Oesling is the most densely wooded area in Luxembourg, 41.8% of the area being covered with forest. However, the formerly widespread natural beech forest community Luzulo-Fagetum can be found on only about 8% of this area. By 2011, the majority of the forest area was covered by spruce and Douglas fir plantations (approx. 44%), followed by oak coppice (approx. 30%) (ANF 2014 Fig. 4).

Natural still waters are rare in the Oesling due to the geological conditions. The standing waters in this area are almost always artificial ponds created by damming streams and springs. In addition, the region counts three large reservoirs: the Upper Sûre Lake, which at 380 ha is the largest body of water in the country (dammed valley), and the Vianden Pumped Storage Plant with its lower reservoir (97 ha) in the dammed valley of the Our, and its upper reservoir (48 ha) on the nearby Saint Nicholas Mountain (STATEC 2022b).

The Oesling has the coolest and wettest climate of the country. The mean annual temperature reaches 7.5°C on the plateaus and 9.0°C in the valleys, and the annual precipitation ranges between 800 mm and 950 mm for both the 1970-2000 and 1991-2020 climate normals (Pfister et al. 2005; Figs. 5, 6). The latest 1991-2020 normal states an annual mean of 8.7°C for Dahl situated on the plateau and a mean annual precipitation of 856 mm in Clervaux (STATEC 2022c). Despite these high levels of precipitation, no large groundwater reservoirs can form; due to the compact, water-impermeable slate and the fissures in the slate rock, which are already closed at a shallow depth, most of the precipitation runs off superficially.

2.2.2 The Gutland

With an area of 1,758 km^2, the Gutland to the south of the Oesling covers about 68% (STATEC 2022c) of the country's area and belongs to the area of the Lorraine stratified plain. It is an undulating hilly landscape (average altitude about 300 m a.s.l., highest elevation about 400 m a.s.l., lowest elevation 130 m a.s.l.) with strata and witness hills, which was formed by a succession of hard geological strata, for example sandstone and shell limestone, and soft ones such as Keuper (Fig. 1).

Geologically, the Gutland consists of the Triassic Formation (Buntsandstein, Muschelkalk, Keuper) and the Jurassic Formation (Lias with Luxembourg sandstone, loess clay, Liastone and limestone marl, Dogger, Fig. 2). These geological layers provide different soil types: light permeable sandy soils, fertile loamy soils, calcareous soils and heavy drying clay soils (Fig. 3).

The best conditions for the emergence of natural still waters and their new formation are provided by the heavy clayey soils of the Keuper and the Liassic clays and calcareous marls of the Lias. Mention should be made, for example, of the marble-rich deciduous forest areas "Bois de Biwer", "Bois de Herborn", the deciduous forests west of Rodenburg, east of Pleitringen, between Stegen and Ingeldorf, northeast of Schrondweiler, north of Folkendange and west of Colmar-Berg ("Biischtert") (all on Keuper), as well as the deciduous forest areas "Bois de Bettembourg", "Bois de Cessange", and the forests in the area of Kockelscheuer (Lias).

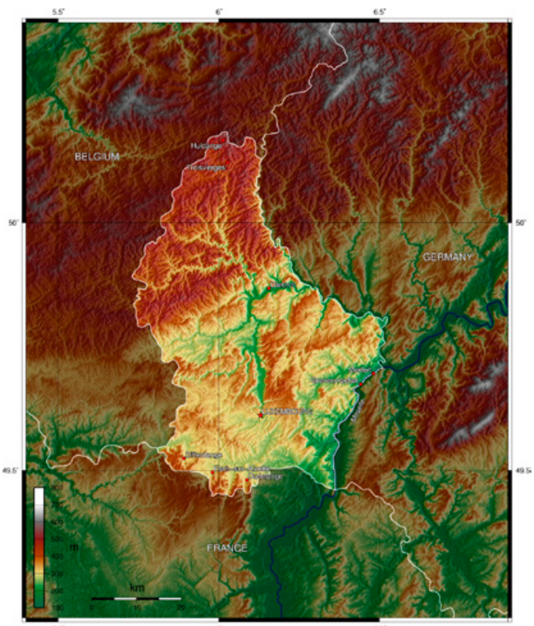


Fig. 1: Topographic map of the Grand Duchy of Luxembourg. The Oesling region in the north is easily recognisable as part of the Ardennes and Eifel mountain range in brown. © Captain Blood, Wikimedia Commons.

In contrast, the areas of the Luxembourg Sandstone (which outcrops on about 20% of the area of the Grand Duchy), the Muschelkalk, the Buntsandstein and the Dogger are poor in natural stillwaters due to their permeable soils. Only locally, in areas with a marl layer or in the presence of subsurface loam, good conditions exist for the emergence of natural still waters (such as in the "Rockeldriesch" forest area west of Nospelt).

About 32% of the Gutland area is covered with forest, of which more than 75% are deciduous forests (beech, beech-oak and oak-hornbeam forests) (ANF 2014). The proportion of coniferous forests, at just under 24%, is significantly lower than in the Oesling (Fig. 4).

The climate of the Gutland is warmer and in many areas drier than that of the Oesling. The mean annual temperature is between 8.5° and 9.5°C for the 1970-2000 climate normals (Pfister et al. 2005), and between 9.5° and 10.7°C for the 1991-2020 normals (Fig. 5). The annual precipitation varies between 750 mm in the east and 950 mm in the west for the 1970-2000 normals (Pfister et al. 2005) and between 750 and 1000 mm for the 1991-2020 normals (Fig. 6). During that period, an annual mean of 9.9°C was observed for Luxembourg-City and 10.4°C for Grevenmacher with respective mean annual precipitation of 800 mm and 757 mm (STATEC 2022c).

2.2.3 Administrative divisions

Through an administrative approach, the territory is divided into 12 cantons (Capellen, Clervaux, Diekirch, Echternach, Esch-sur-Alzette, Grevenmacher, Luxembourg, Mersch, Redange-sur-Attert, Remich, Vianden, Wiltz) and 102 municipalities (Fig. 7). The Grand Duchy has a population of 645,397 inhabitants, as of January 2022 (STATEC 2022a). The overall density is 249.5 inhabitants/km^2, but the density is highly variable within the country, with highest values for some communes

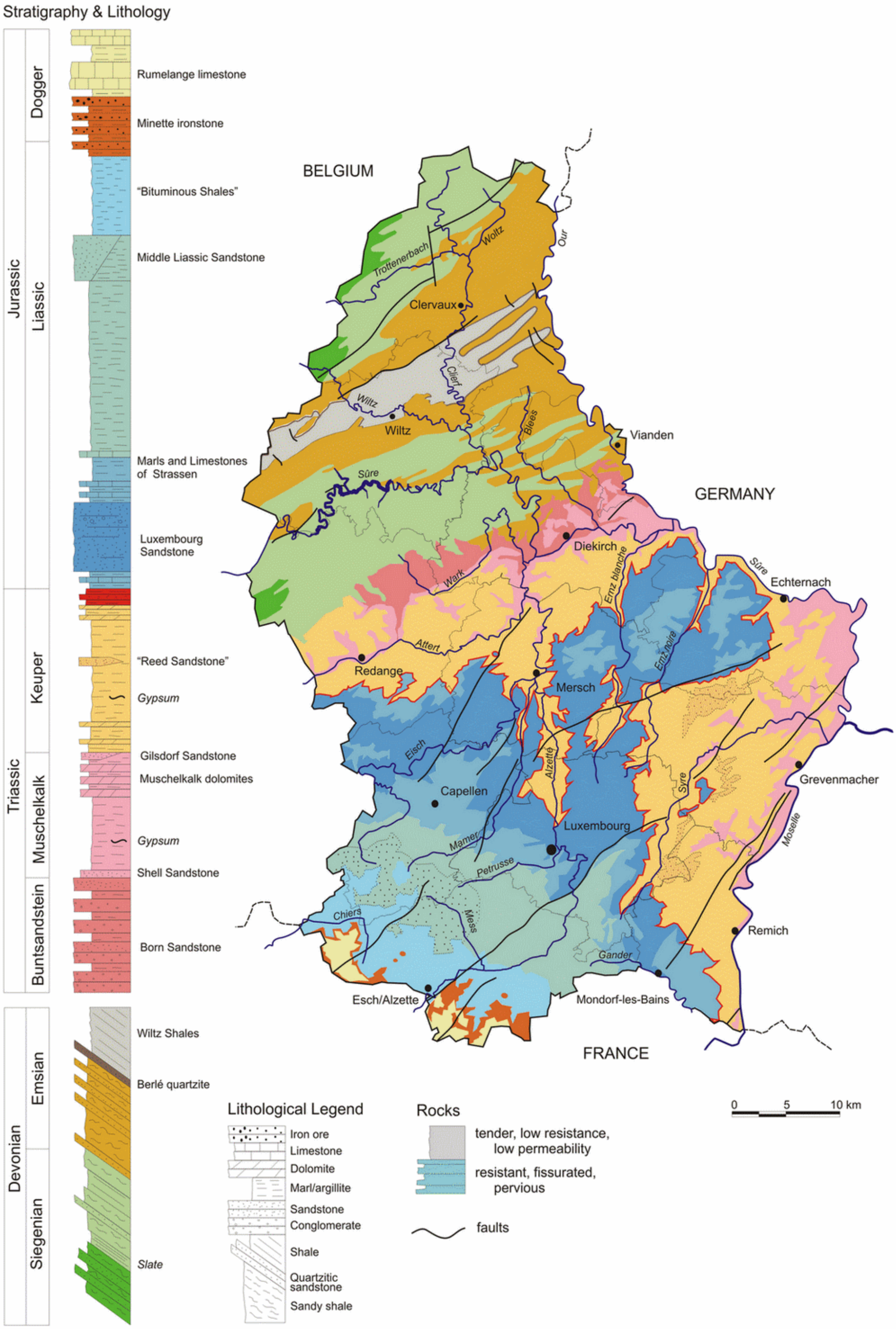


Fig. 2: Geology of the Grand Duchy of Luxembourg. Source: Ministère des travaux publics, Service géologique du Luxembourg, © SGL, 2007.

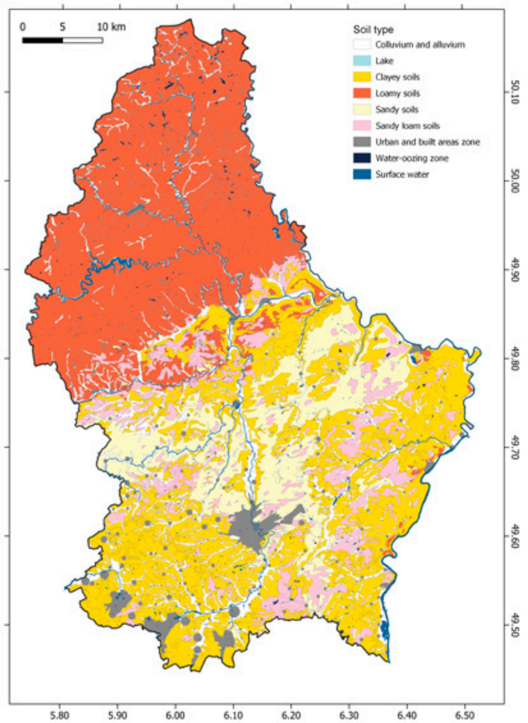


Fig. 3: Soil types of the Grand Duchy of Luxembourg. Data source: La plate-forme de données luxembourgeoise (data.public.lu).

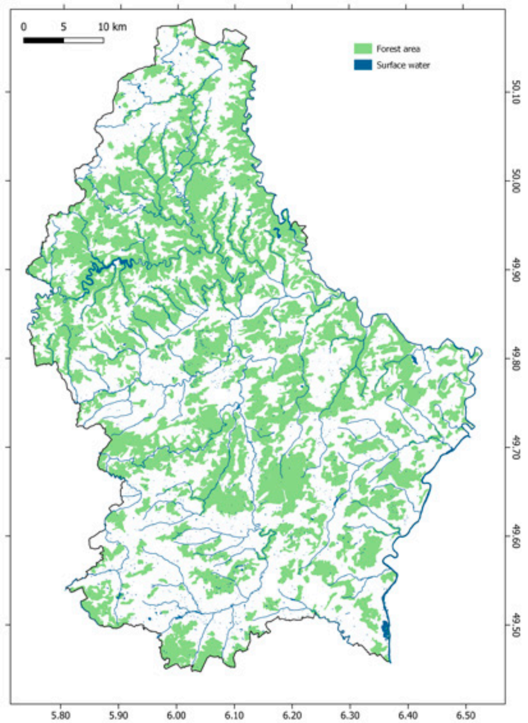


Fig. 4: Forest areas and surface waters of the Grand Duchy of Luxembourg. Data source: La plate-forme de données luxembourgeoise (data.public.lu).

of the cantons of Luxembourg (in particular the city of Luxembourg, which accounts for almost 20% of inhabitants) and Esch-sur-Alzette (south-west of the Grand Duchy; Fig. 7).

2.3 Collection and synthesis of existing data

2.3.1 Database

All current and historical data used to generate the maps in this atlas are stored in Recorder-Lux, the database on the natural heritage of the Grand Duchy of Luxembourg (MNHNL 2000). The underlying software is Recorder 6 (R6 Consortium 2022), a central component of the Museum's national data hub and a client-server database software for wildlife recording developed by the National Biodiversity Network in the UK (Walisch et al. 2019).

2.3.2 Collection and extraction of data from the literature

The collection of historical data was based on a systematic review of the literature completed by reference tracking.

- Systematic literature search

The systematic literature search was performed by questioning online databases as of 26 April 2021. The following search string was used: ((Culicidae or Mosquito$ or Aedes or Stegomyia or Ochlerotatus or Hulecoeteomyia or Georgecraigus or Anopheles or Coquillettidia or Culex or Culiseta or Orthopodomyia or Uranotaenia) and Luxembourg); all fields, without limited time frame or language restriction.

Several online databases were questioned: CAB Direct, CAB Abstracts and Global Health databases; Embase®; Google Scholar (in this case the search string was limited to (Culicidae and

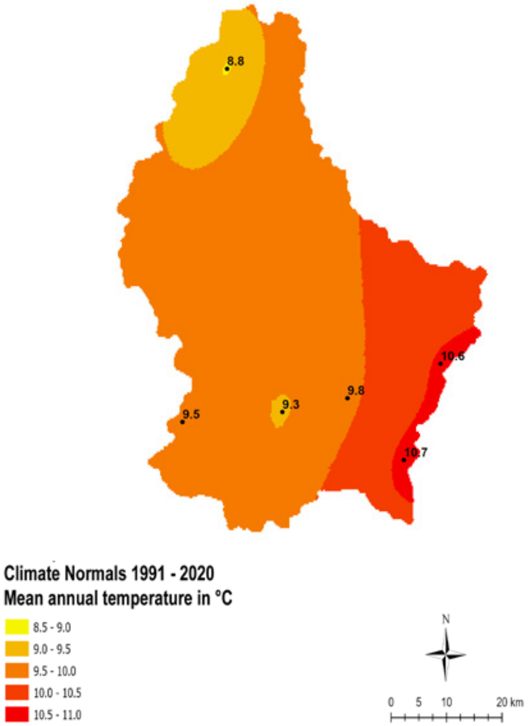


Fig. 5: Mean annual temperature in the Grand Duchy of Luxembourg (period 1991-2020). Source: Meteorological Service, Administration des services techniques de l'agriculture, Luxembourg.

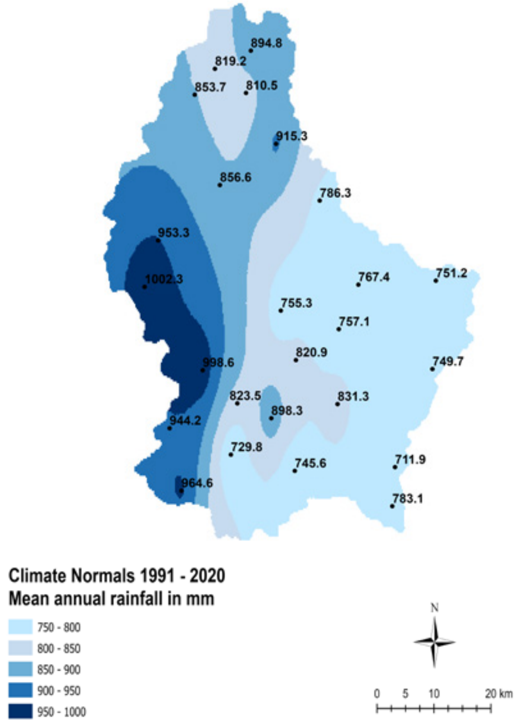


Fig. 6: Mean annual rainfall in the Grand Duchy of Luxembourg (period 1991-2020). Source: Meteorological Service, Administration des services techniques de l'agriculture, Luxembourg.

Luxembourg)); Ovid MEDLINE®, 1946 to April 26, 2021; Scopus; Web of Science, all databases (WOS, BCI, BIOSIS, CCC, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC), 1864-2021.

- Reference tracking

Additional published references (articles, grey reports, databases) were tracked in historical references and on various web sites as of December 2021.

2.3.3 Collection and extraction of unpublished datasets

An invitation to share existing databases was disseminated. The MNHNL houses some collections which contain a certain number of mosquito specimens. Those collections were revised and the data collated. Scientific collaborators of the Museum and experts were contacted and invited to incorporate (un)published datasets into the atlas. Other museums and experts were questioned about existing mosquito occurrence data in museum collections, online databases, and unpublished datasets.

2.4 Collection of new field data

The collection of new data was based on various complementary methods:

- Development of an expert-based approach to select sampling sites and methods, rather than a random approach, since the main aim of our work was to detect a maximum number of species;
- Selection of the field sampling methods and techniques according to the targeted species;
- Selection of environments to be prospected according to gaps in terms of geography, land cover, possible introduction pathways (Medlock et al. 2015; Pfeiffenschneider 2016; Ibáñez-Justicia 2020) and targeted species;

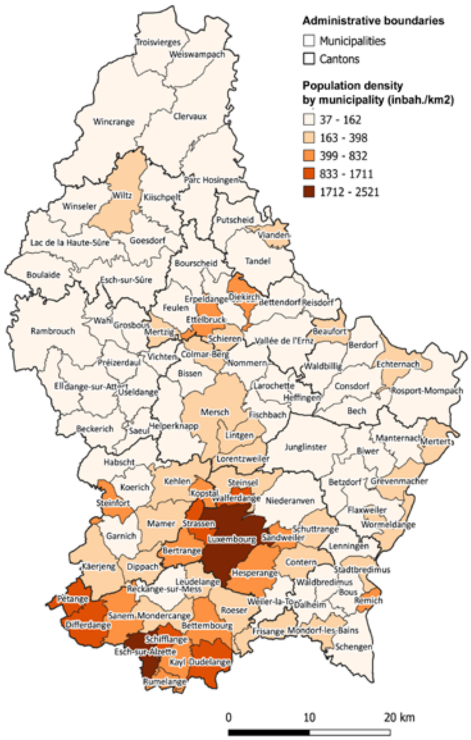


Fig. 7: Administrative map of the Grand Duchy of Luxembourg showing the population density by municipality on 1st January 2022. Data source: STATEC 2022c.

- Field data collection, data transfer and centralisation via smartphone (VECMAP® System, AVIA-GIS, Zoersel, Belgium);
- Encouragement of contributions by partners (e.g. park managers, foresters, naturalists) and by the general public (citizen scientists);
- Use of advanced morphological and molecular techniques for species identification.

2.4.1 Sampling strategy

- Study area

In this study we targeted the whole territory of the Grand Duchy of Luxembourg.

- Basic strategy

In order to cover the whole Luxembourg territory, we used the 5x5 km standard grid which identifies 137 map sections over the country (Fig. 8). For each of these sections, a sampling was scheduled in three different environments, i.e. nature, agriculture, and urban land cover categories, which resulted in a set of 411 samples. Locations were investigated according to these land cover categories and were selected from satellite images online available.

- Identification of geographical and biological gaps

Following the analysis of historical data, mosquito distribution gaps were identified and targeted samplings scheduled to fill these gaps, in terms of spatial distribution, of environments that were overlooked, and of species that may be encountered but not yet recorded. Therefore we targeted, within each map section, deciduous (beech) forests for tree-hole breeding mosquitoes, standing water bodies ('mardelles') for spring *Aedes* mosquitoes, permanent water bodies (ponds) for *Anopheles* and *Culex* species, floodlands for summer *Aedes* mosquitoes, urbanised areas for container-inhabiting mosquitoes, and points of entry (highway parking lots and international airport) for invasive *Aedes* mosquitoes. Such sites were located from available maps ('Stillgewaesser', 'SICONA-Weiher', 'WBK_mardelles', and 'Quellsuempfe' shapefiles).

- Fine tuning of the field data collection strategy and methods

Throughout the project, the strategy and methods were fine-tuned in order to investigate environments that are favourable for certain mosquito species, e.g. peat bogs or caves, in addition to the environments listed above. Sites were located using available reports (peat bogs: Felten 2012, Schneider 2013; caves and artificial subterranean cavities: Weber 2013). Additionally, some environments that looked favourable for a certain species, but which were not found at that location during a first investigation, were inspected again to confirm the presence or absence of that species. Finally, the sampling methods were adapted to raise the chances of finding a certain species. These methods include immature sampling, adult catching and trapping, and egg trapping.

- Information and activation of partner and citizen science networks

Identified partners and citizen science networks were encouraged to report mosquito occurrences. However, due to the SARS-CoV-2 pandemic,

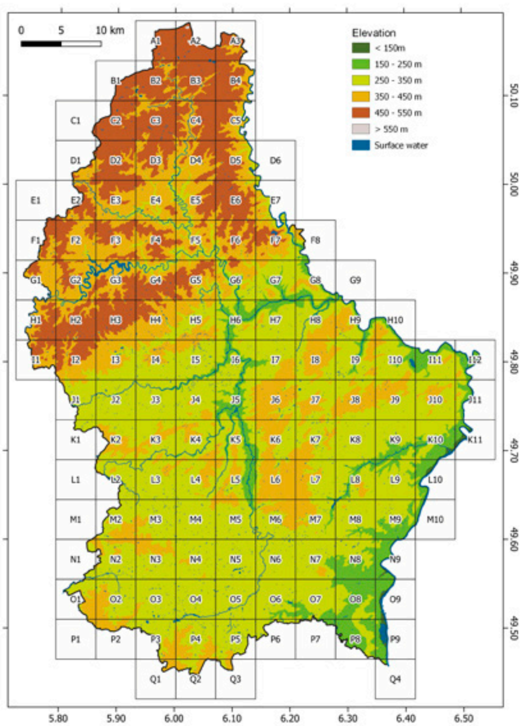


Fig. 8: Grid (5x5 km) utilised to design our sampling strategy, with elevation and surface water of Luxembourg. The territory is divided into 137 map sections. Data source: Digital Luxembourg 2022.

limited action could be performed here, since the initially planned conference and training could not be undertaken.

- Field missions

To cover the development periods of all mosquito species possibly occurring in Luxembourg, we scheduled six two-week sampling periods in April, June and September 2019, March, May and July 2020. An additional one-week period was scheduled in December 2019 to collect overwintering mosquitoes. Adjustments to this initial plan were performed in response to constraints and meteorological factors (see Field sampling results section).

2.4.2 Sampling methods

Surveillance (*Aedes* invasive species) and biodiversity assessment (native species) were implemented based on methods described in available guidelines (Schaffner et al. 2012, 2014; Medlock et al. 2018).

- Larval sampling

Larvae and pupae were collected using different techniques according to the size of the water bodies. Immatures were collected from large water bodies by using a dipper (BioQuip Products Inc, USA; Fig. 9a) or a large aquatic net (bioform, Germany; Fig. 9b) with an extendable handle. In small water bodies such as puddles or artificial containers, we used a small aquarium net (Europrix, France; Fig. 9c). In tree holes, we used a kitchen ladle (Fig. 9d), a manual rescue mucus aspirator (MedicAffaires, France; Fig. 9e) or a large cleaning pear pipette for aquariums (Fig. 9f), according to the size of the hole opening. In all cases, the sample was observed in a white 1 l plastic tray (Fig. 9f-g). Immatures were then transferred with water to a hermetic plastic vial for transport to the laboratory (Fig. 9g-h). There, 4th instar larvae were transferred into 70% ethanol solution and young larvae and pupae were kept until they grew to the 4th instar or to adults. Emerged adults were transferred into a mouth aspirator and killed in a deep freezer, and later identified. Some adults (mainly females) were dry-pinned on minutia pins (females) or stored in 70% ethanol solution (mainly males).

- Adult sampling

Mosquito adults were either collected by ways of resting catches by using a sweep net (bioform, Germany) around vegetation (Fig. 10a), or of human landing catches by sweep netting around a person. In both cases, adults were collected from the net via a mouth aspirator and brought to the laboratory. Resting catches were also performed in caves and shelters with a mechanical aspirator (handmade: Fig. 10b; InsectaZooka Field Aspirator, BioQuip, USA: Fig. 10c) or a mouth aspirator. In addition, adult trapping was performed with a Heavy Duty EVS trap (BioQuip, USA) baited with carbon dioxide released from a decommissioned fire extinguisher and equipped with a BG-CO_2 Timer® and a BG-Booster® release set (Biogents, Germany) (Fig. 10d). Traps were run overnight, at selected locations and under suitable climatic conditions (absence of heavy rain and wind). Collected adults were handled as described above.

- Egg sampling

The occurrence of *Aedes* invasive mosquitoes was also investigated using ovitraps, i.e. traps which attract container-inhabiting female mosquitoes which then lay their eggs in the trap. They consist

Fig. 9: Larval sampling with examples of tools. a. Dipper with extendable handle; b. Large aquatic net and white dish; c. Small aquatic net with white dish and sample vial; d. Kitchen ladle; e. Manual rescue aspirator; f. Cleaning pear pipette; g. Collected sample (mosquito larvae); h. Sample identification at the field laboratory. Photos: Christian Ries (b,d); Francis Schaffner (a,c,e,f,g,h).

Fig. 10: Adult sampling techniques. a. Sweep netting around vegetation; b. Resting catch with a small mechanical aspirator in a cave; c. Resting catch with a large mechanical aspirator (InsectaZooka) in a vegetation pile; d. CO_2-baited (EVS) trap. Photos: Christian Ries (a); Francis Schaffner (b,c,d).

of a black 1 l plastic bucket, filled with water by two thirds, and which has a tongue depressor added to serve as oviposition support (Fig. 11a-b). Five such ovitraps were placed at two sites identified to be possible points of entry, i.e. the two parking lots along the A6 highway (Berchem and Capellen stations, northbound sides; Fig. 11c-d). In 2019, 3 traps were run at each site, from 17/06/2019 until 13/12/2019, and checked twice in the season. In 2020, 5 traps were run at each site from 28/05/2020 until 05/11/2005, with oviposition supports checked and replaced every two weeks. These were inspected under a stereozoom microscope and eggs, if present, identified under a reflected light microscope. This surveillance was implemented following the AIMSurv2020 protocol (Miranda et al. 2022), within the *Aedes* Invasive Mosquitoes COST Action CA17108 project https://www.aedescost.eu/

2.4.3 Samples identification

- Breeding of immature specimens to adult stage (a selection of samples and species)

Pupae and some larvae were reared in the laboratory up to the emergence of adults. They were kept in a plastic container with an insect-tight lid, water and some substrate from the larval habitat, under normal temperature and light conditions. Small quantities of crushed fish food (Tetramin™) were added to provide additional food for the larvae.

- Morphological identification and preparation of reference specimens

Mosquito larvae and adults (females and males) were classified to species level or, if not possible, to a group of morphologically closely-related species based on standard identification keys (Schaffner et al. 2001; Becker et al. 2020) using a

Fig. 11: Egg sampling. a. Ovitrap with a tongue depressor as oviposition support (2020); b. Ovitrap fixed on a tree, with a block of polystyrene as oviposition support (2019); c. Trapping site at Berchem station (ovitrap placed in the bush, left side); d. Trapping site at Capellen station (ovitrap placed in the tree). Photos: Christian Ries (c,d); Francis Schaffner (a,b).

stereo microscope (Fig. 9h). Several subsamples of mosquito larvae and adults were preserved in ethanol (larvae and immature exuviae, male genitalia) or dry pinned in an insect box (adults) to serve as reference specimens (Fig. 12). Some of these specimens have been integrated into the reference collection of Luxembourg Culicidae deposited at the MNHNL.

- Molecular identification of sibling species and species complexes

Different DNA-based identification tools were selected to best provide reliable group-specific taxonomic identification when morphological determination was not possible down to species level. DNA sequencing of the ribosomal internal transcribed spacer 2 (ITS2) was applied to diagnose *Anopheles* spp. (Collins & Paskewitz 1996; Linton et al. 2001), whereas polymerase chain reaction (PCR) fingerprinting via the acetylcholinesterase-2 (ACE-2) assay of Smith & Fonseca (2004) was conducted to genetically distinguish *Culex torrentium* from *Culex pipiens* s.s. by their specific PCR banding patterns. In addition, a rapid PCR assay that builds on the ACE-2 assay and also uses polymorphisms in the flanking region of the CQ11 microsatellite locus was run for the identification of specimens of the *Cx. pipiens* complex (i.e. *Cx. pipiens* form Pipiens and form Molestus) and possible hybrids (Bahnck & Fonseca 2006). New sequences were deposited in the BOLD System (Tab. 1). Examples of *de novo* sequences used to genetically determine further *Anopheles* spp. materials are given in Figure 13. All laboratory steps and bioinformatic analyses were conducted in the facilities of the MNHNL.

Fig. 12: Examples of mounted adult mosquitoes deposited at the MNHNL collection. Photo: Francis Schaffner.

2.4.4 Data analysis and production of maps

A number of field and laboratory data were gathered throughout the study. Field data were collected through the VECMAP smartphone app, including the location's name and geo-coordinates, start and end dates and times, sampling method, land cover, adult/larval habitat type, and some larval habitat characteristics. Every sampling had a unique site ID+date association. This data set was then downloaded into an Excel™ file and further filled in the laboratory with complementary admin data (e.g. commune, grid square) and sampling results (e.g. species, stages, numbers).

Hereafter, for each species we synthesise our findings, describing in particular the frequency of positive samplings, the spatial distribution of observed occurrences (according to the 5x5 km grid squares, communes, cantons, and geographical regions), the temporal distribution of our observations (season months), and the larval habitat types that proved positive for the considered species.

Maps were produced by Paul Braun at MNHNL using the open source geographic information system QGIS (QGIS Development Team 2022).

3 Introduction to the mosquitoes of Luxembourg

The family Culicidae belongs to the Diptera, suborder Nematocera, infraorder Culicomorpha. Mosquitoes are small two-winged insects characterised by long and fragile-looking legs, and elongated, piercing mouthparts (called proboscis). In the western Palaearctic region, 147 mosquito

Tab. 1: List of *Anopheles* specimens with ITS2 sequences deposited to the Barcode Of Life Data (BOLD) System and results of reverse molecular taxonomic assignment, collection site and date. F: Adult females; L: Larva; IDs starting with 'ATL' are our site identification number (numbers: site; lowercase letter: station).

Molecular assignment	Collection site	Stage	Collection date	BOLD ID
Anopheles claviger s.s.	ATL039a; Haff Réimech, Schengen	L	20/06/2019	MNHNL164-22
Anopheles daciae	ATL027; Canach, Lenningen	F	21/06/2019	MNHNL165-22
Anopheles daciae	ATL039a; Haff Réimech, Schengen	L	20/06/2019	MNHNL166-22
Anopheles daciae	ATL086a; Schwunnendal, Lintgen	L	27/05/2020	MNHNL167-22
Anopheles daciae	ATL315; Haff Réimech, Schengen	F	11/03/2021	MNHNL168-22
Anopheles maculipennis s.s.	ATL029b; Mensdorf, Betzdorf	L	21/06/2019	MNHNL169-22
Anopheles maculipennis s.s.	ATL086a; Schwunnendal, Lintgen	L	27/05/2020	MNHNL170-22
Anopheles maculipennis s.s.	ATL101a; Bettembourg	L	25/05/2021	MNHNL171-22
Anopheles petragnani	ATL064; Hoscheid, Bourscheid	L	16/03/2021	MNHNL172-22

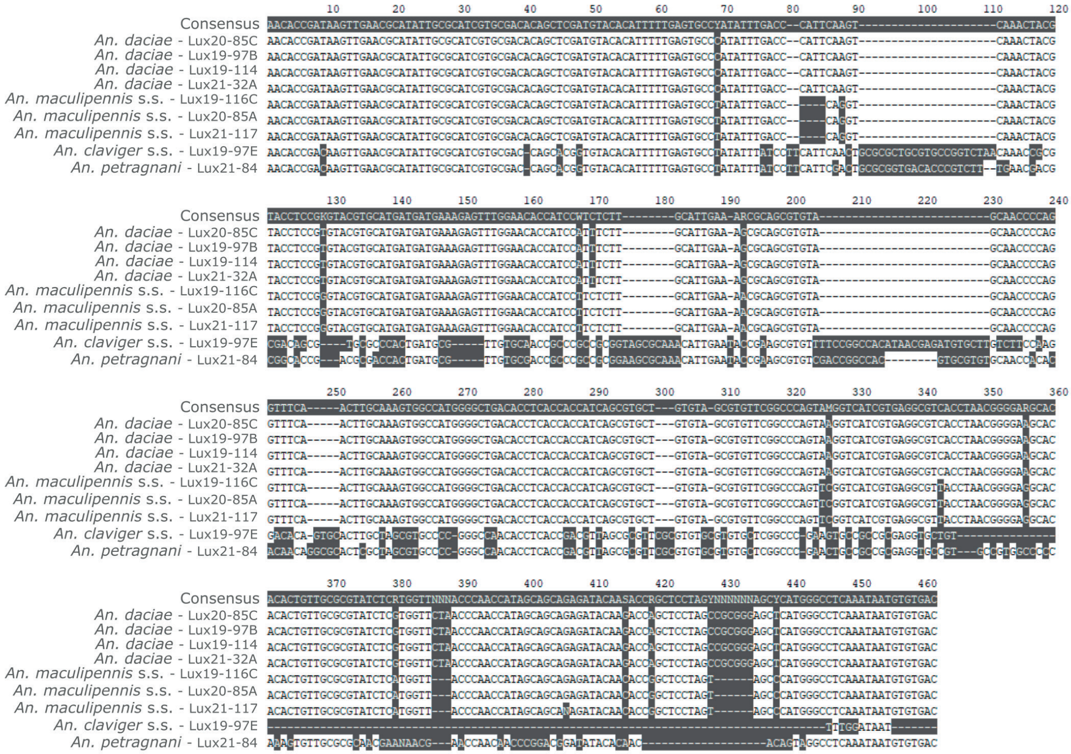


Fig. 13: Examples of *de novo* ITS2 sequences used to genetically determine *Anopheles* spp. specimens from the fieldwork campaigns, Luxembourg, 2019-2021.

species are currently reported to occur, including four species that are exotic and invasive (Robert et al. 2019). All these species belong to seven different genera, and their biology varies significantly in terms of e.g. number of generations per year, overwintering stages, feeding behaviour, host preferences, and larval habitat. In addition, the species distribution highly differs, from species endemic to a small area (e.g. Macaronesia) to species that became cosmopolitan thanks to human transportation.

3.1 General biology of mosquitoes

Mosquitoes live in almost every conceivable environment where water occurs, a necessity for their larval development. Only seas and oceans have not been colonised, but brackish marshes or salted rock pools are. They undergo complete metamorphosis within the water, and the adults are winged and capable of extensive flight and diverse behaviours on land (Fig. 14). Only the females require a blood meal, to develop their eggs, but both sexes feed on plant sugar. Females of most species feed primarily on the blood of wild or domestic animals, and only a few are dedicated human-feeders. For some, blood-feeding is either optional or altogether lacking, with eggs being developed autogenously. Female mosquitoes search for their blood meal by detecting organic substances released from the host, foremost carbon dioxide. Eggs are laid either on the water surface, on the emerged inner sides of water containers, or on the ground of submersible areas. In the first case they rapidly develop into the first larval instar (within 48 hours); in the other two cases they can stand for weeks, months or even years until submersion, which then provokes embryonic development. Larvae develop in the water through four instars within about a week, or for several weeks or months in winter time, feed on microparticles and breathe the air at the water surface. The fourth instar transforms into a pupa which swims and breathes like larvae, but does not feed anymore, and transforms into an adult which emerges after 2 to 3 days. Emerged adults will then swarm and mate, seek sugar and blood meals. After a few days and following blood digestion, females will produce eggs and search for a suitable oviposition site. Freshly laid eggs will then start to develop within a new cycle, or enter into diapause until the next favourable season.

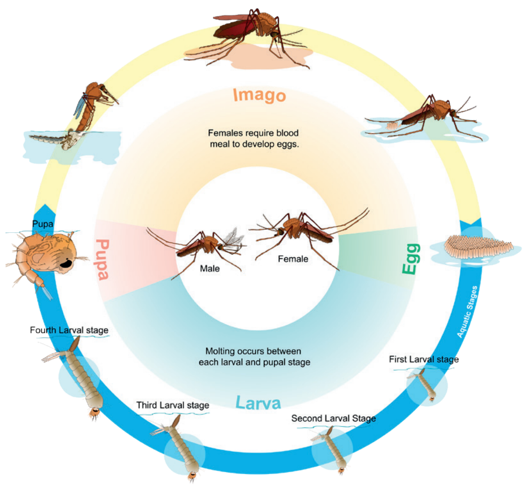


Fig. 14: Life cycle of a *Culex* mosquito. Source: Ladyof-Hats, Public domain via Wikimedia Commons.

3.2 About the significance of mosquitoes in Luxembourg

In the early 20th century, an alarmingly rapid increase and a worrying mass occurrence of the northern house mosquito (*Culex pipiens* s.l.) was recorded (Weinachter 1911; Massard 2019). A few years later, following two years of investigations, Sunnen published a 24 page booklet containing an introduction to the biology of the house mosquito (Fig. 15), instructions and measures for its control, and a municipal ordinance of the city of Luxembourg for the control of the house mosquito (Sunnen 1918; Fig. 16).

The mosquito plague was caused less by breeding in stagnant water or open vessels than by breeding in underground septic tanks (cesspits). Through a newspaper notice, Sunnen gained the cooperation of the public in searching for breeding sites, an early form of citizen science so popular today. Through this action, 50-60 more breeding sites were identified in Limpertsberg. It turned out that cesspits with water flushing were extremely favourable reproduction sites, where all reproductive stages from eggs to imagos could be observed simultaneously almost all year round. Street catch basins and old, underground rainwater collection systems were also identified as excellent breeding sites.

Sunnen derived three measures from his investigations: 1- Open containers with standing water must either be hermetically sealed or emptied weekly and left empty for at least an entire day; 2- Water cisterns must be hermetically closed; and 3- All cesspits must be inspected for mosquitoes and treated if necessary.

On 16th March 1918, the City of Luxembourg regulated mosquito control to the effect that all house owners were obliged to destroy mosquitoes hibernating in cellars, sheds and stables with an insecticide or by other effective means during the periods 1st to 15th December and 1st to 15th February (Fig. 16). Furthermore, in the period between 1st April and 1st September, all purposeless accumulations of water must be avoided, rain barrels must be covered and cesspits must be tightly sealed. Finally, standing waters must be stocked with fish. The municipal ordinance provided for appropriate penalties for violators. In order to most safely achieve the purpose of winter control of the hibernating female mosquitoes, in the following years the work was carried out from house to house by a separate staff of the municipal hygiene service. Based on a municipal ordinance of 8th June 1921, the residents had to grant access to the extermination columns, each consisting of 2 men, from 8 am to 6 pm (City of Luxembourg 1934).

Malaria was historically occurring in Luxembourg, when the disease was frequent all over Europe up to Scandinavia, and until the 20th century (Boualam et al. 2021). During the 19th century in particular, many cases of malaria infections were reported (as “fièvres intermittentes” or “fièvres paludéennes”) from several localities with e.g. in 1857-1859, 72 and 65 cases out of 169 and 101 inhabitants in Eisborn and Imbringen, respectively (Kleyr 1862). Mosquitoes were not known to be responsible for these fevers, but the wetlands’ standing water was supposed to be

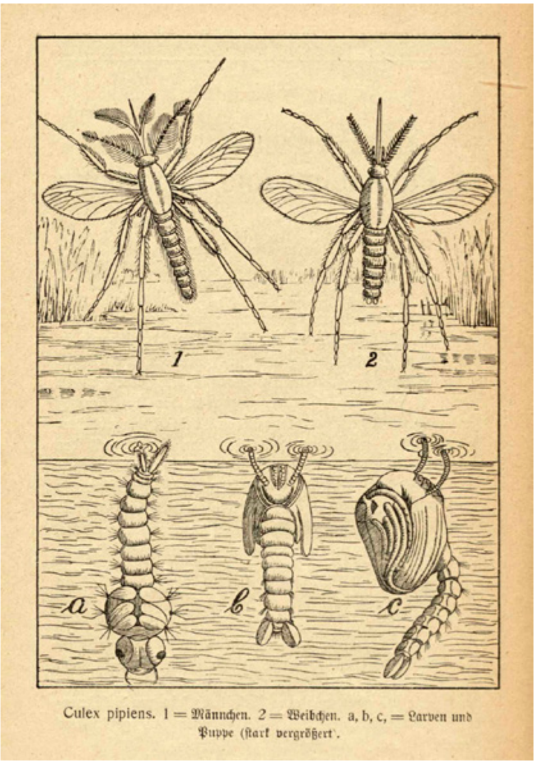


Fig. 15: The northern house mosquito *Culex pipiens*, as illustrated in Sunnen (1918).

unhealthy. Therefore, significant planning and constructions were implemented in the Weiss and Ernz valleys to reduce standing water. More recently, Luxembourg has faced five so-called 'airport malaria cases' in 1997 (2 cases) and 1999 (3 cases) (Hemmer 1999). People diagnosed positive for *Plasmodium falciparum* did not travel to any malaria endemic country but lived about 2 to 4 km from the airport, where they must have been bitten by infected *Anopheles* specimens introduced by aeroplanes.

Within Europe, mosquito-borne diseases were not considered a threat anymore after the elimination of dengue in the 1930s, and malaria in the 1970s. The concern was raised again in the late 1990s, with the (re-)emergence of malaria in Greece, and West Nile and Usutu infections in several countries (Schaffner et al. 2014; Martinet et al. 2019). Also, the intensification of travel and trade generated an increase of imported cases of dengue, chikungunya or Zika, and the introduction and expansion of invasive mosquito vector species such as *Aedes albopictus*. As a result, local transmission of these viruses occurs nowadays almost yearly in north-western Europe (Martinet et al. 2019).

As with its neighbouring countries, Luxembourg is at risk for the introduction of pathogens and invasive vector species, and native vector species may also occur. Therefore our study was aimed at assessing the mosquito biodiversity, which is associated with the local environment and climate, to support the assessment of the threat and the risks to public health, to animal health and to the environment.

3.3 Data about the local mosquito fauna data preceding our field study

The first part of our study consisted of gathering all accessible data on mosquito occurrences in Luxembourg. We particularly focused on and extracted distribution data.

3.3.1 Historical and recent entomological studies

During our study, we inventoried all data collected outside of this work, gathered through a literature search and data sharing. Published literature was collected with a search string run on CAB Direct (n=76), Embase (n=21), Ovid MEDLINE® (n=16), Scopus (n=277), and Web of Science (n=16) databases by 27/04/2021 (as last query), resulting in a total of 330 references. Deletion of double entries and non-relevant references reduced the list to 30 articles. An additional search was performed on Google Scholar on 29/04/2021, generating a list of 510 references of which only four references not collected by the previous search mentioned distribution of mosquito species in Luxembourg. Finally, six articles or grey literature documents were also identified by reference tracking, and unpublished data sets were identified (n=1) by actively searching various websites and communicating with museum curators and mosquito project leaders. This resulted in a final list of 24 references that cite mosquito occurrences in Luxembourg, but among these, only six provide usable distribution data (i.e. species occurrence at a precise geographical location; Tab. 2).

Die Stechmückenplage in Luxemburg und Umgebung

von

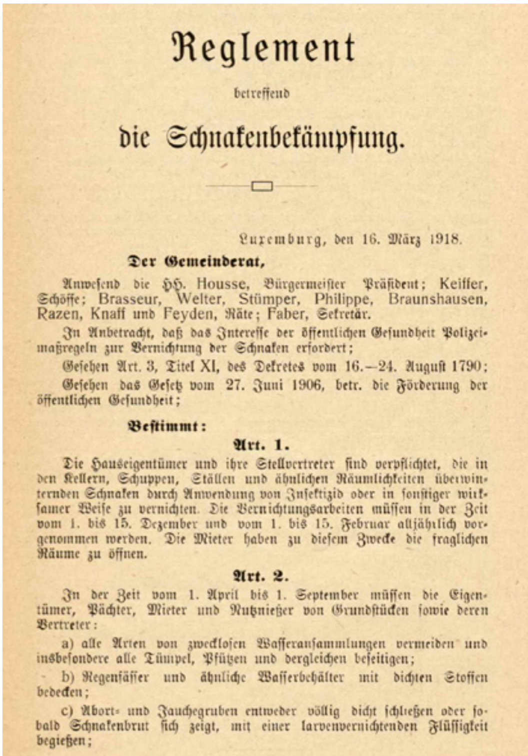
M. SUNNEN, Lehrer.

Preis: 1 Franken.

1918

Luxemburg. — Druck und Verlag von P. Worré-Mertens.

J. P. Worré, Nachfolger.



Reglement

betreffend

die Schnakenbekämpfung.

Luxemburg, den 16. März 1918.

Der Gemeinderat,

Anwesend die HH. Housse, Bürgermeister Präsident; Keiffer, Schöffe; Brasseur, Welter, Stümper, Philippe, Braunshausen, Razen, Knaff und Feyden, Räte; Faber, Sekretär.

In Anbetracht, daß das Interesse der öffentlichen Gesundheit Polizeimaßregeln zur Vernichtung der Schnaken erfordert;

Gesehen Art. 3, Titel XI, des Dekretes vom 16.—24. August 1790;

Gesehen das Gesetz vom 27. Juni 1906, betr. die Förderung der öffentlichen Gesundheit;

Bestimmt:

Art. 1.

Die Hauseigentümer und ihre Stellvertreter sind verpflichtet, die in den Kellern, Schuppen, Ställen und ähnlichen Räumlichkeiten überwinternden Schnaken durch Anwendung von Insektizid oder in sonstiger wirksamer Weise zu vernichten. Die Vernichtungsarbeiten müssen in der Zeit vom 1. bis 15. Dezember und vom 1. bis 15. Februar alljährlich vorgenommen werden. Die Mieter haben zu diesem Zwecke die fraglichen Räume zu öffnen.

Art. 2.

In der Zeit vom 1. April bis 1. September müssen die Eigentümer, Pächter, Mieter und Nutznießer von Grundstücken sowie deren Vertreter:

a) alle Arten von zwecklosen Wasseransammlungen vermeiden und insbesondere alle Tümpel, Pfützen und dergleichen beseitigen;

b) Regenfässer und ähnliche Wasserbehälter mit dichten Stoffen bedecken;

c) Abort- und Jauchegruben entweder völlig dicht schließen oder sobald Schnakenbrut sich zeigt, mit einer larvenvernichtenden Flüssigkeit begießen;

Fig. 16: The first historical document that refers to mosquito nuisance, with the regulation adopted by the City of Luxembourg on 16th March 1918. Source: Sunnen 1918.

- Published data

The first report of the occurrence of mosquitoes in Luxembourg dates back to 1910, 1916 and 1917 when nuisance due to *Culex pipiens* and *Culiseta annulata* (the latter known as *Culex annulatus* at that time) was reported and control actions were planned in the city of Luxembourg (Sunnen 1918; Massard 2019). In 1997 and 1999, a few airport malaria cases were reported but the mosquito vector species was not identified (most probably introduced exotic *Anopheles* spp.) (Hemmer 1999). The next mosquito record is related to one of our observations (Schaffner F., unpublished data in Proft et al. 1999; see also below), in the frame of the development of a polymerase chain reaction (PCR) assay to identify members of the *Anopheles maculipennis* complex. The same year, Snow and Ramsdale (1999) published a distribution chart for European mosquitoes, in which the column for Luxembourg remained empty of any record. This absence of data triggered German colleagues to organise a field study in 2001, performing larval sampling and adult trapping at 12 locations throughout the Grand Duchy (Beck et al. 2001, 2003). As a result, they listed the occurrence of 15 species (Tab. 3). The same list of species was given in another work (Schaffner et al. 2001), based on conference communication (Beck et al. 2001) and on personal (FS) unpublished data. Among the other identified documents that report mosquito occurrences in Luxembourg, five refer to the Beck et al. (2003) data, for *Anopheles claviger* (Schaffner 2002) or *Cx. pipiens* and *Cx. torrentium* (Hesson et al. 2011; Weitzel et al. 2011; Becker et al. 2012; Brugman et al. 2018). In 2013, an extensive work on the subterranean fauna of Luxembourg, including flies and midges, was published (Weber 2013), but Culicidae were not identified to species or genus levels. However, these samples together with more recent ones, collected over the period 2007-2015, were identified later and published in the frame of a pan-European analysis of mosquito fauna in caves and artificial underground cavities (Zittra et al. 2021). In this article, five species are

Tab. 2: Reports of mosquito occurrences in Luxembourg, in the literature identified through several search methods. LS: Literature online databases search; GS: Google Scholar search; RT: Reference tracking.

Source	Publication date	Reference	Taxa target	Country target	No. of taxa for LUX	No. of data	No. of extracted data1	Comment
PL	1918	Sunnen (1918)	Pest species	Luxembourg	2	2	0	No precise distribution data; Reports nuisance due to Culex pipiens and Culiseta annulata
PL	1999	Hemmer (1999)	*Anopheles* sp.	Luxembourg	0	0	0	Absence of distribution data; Airport malaria cases in Luxembourg, 1997 (2 cases), 1999 (3 cases)
PL	1999	Proft et al. (1999)	*Anopheles maculipennis* complex	Austria, France, Germany, Italy, Luxembourg, Netherlands, Portugal	1	1	0	Original distribution data: Anopheles maculipennis s.s. at Bettembourg but no geo coordinates; Same data reported (precisely) in Schaffner (2022); First molecular ID of Anopheles maculipennis group
PL	1999	Snow & Ramsdale (1999)	Culicidae	Europe	0	0	0	Absence of distribution data for Luxembourg; Stimulated the study of Beck et al. (2003)
PL	2001	Schaffner et al. (2001)	Culicidae	Europe 3rd	15	0	0	Occurrences at country level; Refer to Beck et al. (2001) (data published in Beck et al. (2003), Hemmer (1999), Proft et al. (1999), Schaffner F. pers. obs. (1998-2000)
PL	2002	Schaffner (2002)	*Anopheles claviger* complex	Palaearctic region	1	0	0	Occurrence at country level for Anopheles claviger; Refers to Beck et al. (2001); One precise data for the Belgian Luxembourg province
PL	2003 (2001)	Beck et al. (2001, 2003)	Culicidae	Luxembourg	15	32	32	Original distribution data; Reference publication is Beck et al. (2003); Specimen numbers of Culex pipiens/torrentium completed by Weitzel et al. (2011)
PL	2011	Hesson et al. (2011)	*Culex pipiens/ torrentium*	Sweden	2	0	0	Refer to Beck et al. (2003)
PL	2011	Weitzel et al. (2011)	*Culex pipiens/ torrentium*	Europe	2	5	0	Same data as Beck et al. (2003), but dates missing
PL	2012	Becker et al. (2012)	*Culex pipiens/ torrentium*	Europe	2	5	0	Same data as Weitzel et al. (2011) and Beck et al. (2003)
GL/DS	2012	Muller & Reye (2012)	Culicidae	Luxembourg	14	197	165	Longitudinal study; Work not finalised; Data partially available only; First report of Aedes communis and Aedes vexans

Source	Publication date	Reference	Taxa target	Country target	No. of taxa for LUX	No. of data	No. of extracted data1	Comment
PL	2013	Weber (2013)	Culicidae	Luxembourg	0	0	0	Identification to genus/species level not done; Sample identification results are given in the table on unpublished data sets
PL	2018	Brugman et al. (2018)	*Culex pipiens*	Europe	2	0	0	Refer to Beck et al. (2003) and Weitzel et al. (2011)
PL	2019	Koban et al. (2019)	*Aedes japonicus*	Europe	1	0	0	Occurrence at country level (Aedes japonicus); Refer to our finding (website neobiota.lu)
PL	2019	Martinet et al. (2019)	Culicidae	North-western Europe	15	0	0	No precise species occurrences
PL	2019	Massard (2019)	Pest species	Luxembourg	2	0	0	Occurrences at country level (Culex pipiens and Culiseta annulata); Refers to Sunnen (1918), and other historical documents
GL/DS	2019	Ries et al. (2019)	Culicidae	Luxembourg	8	100	23	Longitudinal study; Confirmation of Aedes vexans
PL	2019	Robert et al. (2019)	Culicidae	Western Palaearctic region	16	0	0	Occurrences at country level; Native: 15 spp; Invasive: 1 sp.; Refer to Beck et al. (2001) and our finding (website neobiota.lu)
PL/DS	2019	Schaffner & Ries (2019)	*Aedes japonicus*	Luxembourg	10	160	160	Targeted field study; Original distribution data; First report of Aedes japonicus and Culiseta longiareolata; Shared raw dataset
PL	2020	Cebrián-Camisón et al. (2020)	*Aedes japonicus*	Europe	1	0	0	Occurrence at country level (Aedes japonicus); Refers to Schaffner & Ries (2019)
PL	2021	De Wolf et al. (2021)	*Culex modestus*	Belgium	1	0	0	Occurrence at country level (Culex modestus); Refer to Ries (mosquitoes-lu webpage)
PL/DS	2021	Zittra et al. (2021)	Culicidae	Luxembourg	6	79	79	Mosquitoes collected in caves; First molecular evidence of presence of Culex pipiens both forms Pipiens and Molestus; First report of Culiseta glaphyroptera; Include data from Weber (2013) and more recent ones
DS	2019	Schaffner (2022)	Culicidae	Luxembourg	19	71	71	Intuitive field samplings; First findings of Aedes geminus, Culex hortensis, Culex torrentium, and Culiseta morsitans

1 Data to be used for mapping, i.e. a species occurrence at a precise geographical location.

listed, including the first report of *Cs. glaphyroptera*, to which *Ae. sticticus* had to be added (observed once and listed in the dataset but not mentioned in the article), yielding 79 distribution data. A second field study was performed in 2018, to investigate the presence and distribution of the invasive mosquito species *Ae. japonicus* (Schaffner & Ries 2019). That study provided 160 occurrence data, for 10 taxa, and reported the presence of *Cs. longiareolata*. The same year, Martinet et al. (2019) and Robert et al. (2019) published countries' species lists, for north-western Europe in the first case, for the western Palaearctic region in the second case. They listed 15 and 16 species, respectively, referring to Beck et al. (2003) and our unpublished findings (including *Ae. japonicus*, at website neobiota.lu). The *Ae. japonicus* record was subsequently also mentioned in Koban et al. (2019) and Cebrián-Camisón et al. (2020). Finally, one of our findings of the present work, i.e. the presence of *Cx. modestus* in Luxembourg, was mentioned by De Wolf et al. (2021), referring to our webpage (mosquitoes.lu). In summary, while 6 published documents report occurrences of mosquitoes at municipality or geocoordinate levels, only three of them provide original and accurate enough distribution data to be used for our mapping (Beck et al. 2003; Schaffner & Ries 2019; Zittra et al. 2021) (Tab. 2).

- Grey literature

Between 2010 and 2016, two field studies targeting mosquitoes were implemented in Luxembourg. A first study conducted in 2010-2011 by health institutions (Centre de Recherche Publique - Santé, Laboratoire national de santé) was investigating tick and mosquito occurrence and the prevalence of pathogen infection in these vectors (Muller & Reye 2012). Unfortunately the project was terminated before finalisation, by the end of 2011, and data are only partially available. The authors analysed 530 mosquito adults collected in 2010, listed 6 mosquito taxa, and did not detect any prevalence of West Nile virus (from 90 specimens; unpublished data). Further investigation at MNHNL of samples collected in 2011 (2,553 specimens collected at 90 sites) revealed the occurrence of 14 taxa, of which *Ae. communis* and *Ae. vexans* are reported for the first time (unpublished data). A total of 165 distribution data (i.e. a species occurrence at a geolocation, whatever the number of specimens observed) could be extracted from the dataset (Tab. 2). In 2016, a second study was implemented to assess the presence of native and invasive mosquito vector species in the territory (Ries et al. 2019). Ten different sites throughout the country were studied by the MNHNL in cooperation with local and regional partner organisations and private persons. As a result, eight taxa were observed and 23 distribution data could be extracted from the dataset (Tab. 2).

- Museum collections

A number of museum collections have been searched for mosquito specimens from Luxembourg. Only the collection of the MNHNL provides some observations, which are all integrated in the online GBIF database (see next section). The searched collections are:

- Institut de recherches pour le développement, Montpellier: no Culicidae from Luxembourg (28/04/21).
- Institut Pasteur de Paris: Printed catalogue of the entomological collection: no Culicidae from Luxembourg (30/03/22).
- Institut royal des sciences naturelles de Belgique, Bruxelles: no Culicidae from Luxembourg (21/03/22).
- Musée zoologique de Strasbourg: no Culicidae from Luxembourg (18/03/22).
- Muséum national d'histoire naturelle, Paris: no Culicidae from Luxembourg (30/10/00).
- National Museum of Natural History Luxembourg: collections and observation data, see GBIF online data below.
- Natural History Museum, London: no Culicidae from Luxembourg listed in the online catalogue (27/04/21) (Natural History Museum, 2021).
- Naturalis Biodiversity Center, Leiden: no Culicidae from Luxembourg listed in the online catalogue (27/04/21).
- Staatliches Museum für Naturkunde Stuttgart: no Culicidae from Luxembourg listed in the GBIF online catalogue (27/04/21).

- Global Biodiversity Information Facility (GBIF) catalogue

The GBIF online catalogue comprises 130 data (27/04/21) for Luxembourg, which include:

- Six data at family or genus levels only, not considered here;
- 120 occurrences from collections and observation data of the MNHNL, referring to F. Schaffner (Turpel & Walisch 2021); all data included in the dataset of Schaffner & Ries (2019);
- Three occurrences from International Barcode of Life project dataset (The International Barcode of Life Consortium 2016); all data included in the data set of Schaffner & Ries (2019).

● Other datasets and unpublished data

Unpublished data were sought from ongoing national (neighbouring countries) or international projects:

- Our (FS) personal observations on distribution and biology of mosquitoes in Europe revealed 71 distribution data for Luxembourg in the period 1997-2018; these address 19 taxa, providing first observations in Luxembourg of *Ae. geminus*, *Cx. hortensis*, *Cx. torrentium*, and *Cs. morsitans* (Schaffner 2022; Tab. 2).
- Mückenatlas project, Germany (mueckenatlas.com): no data for Luxembourg (16/03/2022).
- MEMO project, Belgium (Monitoring of Exotic Mosquitoes in Belgium): no data for Luxembourg (21/03/2022).
- Signalement-moustiques, France (signalement-moustique.anses.fr): not possible to report data for Luxembourg (21/03/2022).
- TIGER – Plateforme d'information sur le moustique tigre asiatique dans la région du Rhin supérieur (tiger-platform.eu): no data for Luxembourg (21/03/2022).
- International Citizen Science project Mosquito-Alert (Spain, expanded by AIM-COST): no usable mosquito data for Luxembourg (24/03/2022).

3.3.2 Species list from data collected prior to our field study

A total of 642 mosquito taxa occurrences could be retrieved from the published literature, museum collections, grey literature and data sets, and unpublished data, from 1997 to 2018 (Fig. 18), of which 530 included species/site data. Imprecise data are not considered here (e.g. Sunnen 1918). These data are distributed over 106 squares (77.4%) of our 5x5 km grid (Fig. 17). A total of 23 mosquito species (27 taxa) were reported to occur in Luxembourg prior to our study (Tab. 3). Among these, four species that do not appear in published literature are reported by grey literature or unpublished data sets: *Ae. communis*, *Ae. geminus*, *Ae. vexans*, and *Cs. morsitans*.

3.4 Original mosquito distribution data

3.4.1 Citizen science data 2019-2021

In this section we collate all data reported by citizens to the MNHNL directly or via the iNaturalist application to GBIF, and that could be validated (on sample or photo) as a mosquito species occurrence in Luxembourg. For the period 2019-2021, a total of 22 citizen data could be gathered. Most of these data were sent to the MNHNL, while one was reported to GBIF (*Aedes rusticus*; Ueda 2021). A total of 28 mosquito specimens could be identified, originating from 11 different locations (and 11 different communes) (Fig. 20). These specimens were caught as resting or biting adults (26 females, 2 males), and belong to 5 different taxa (Fig. 19). While many samples were sent by citizens as suspected to be Tiger mosquitoes (*Ae. albopictus*), this species was not recorded; instead there were five occurrences of the invasive species *Ae. japonicus* and 17 occurrences of native mosquito species.

3.4.2 Field sampling results 2019-2021

● Sampling effort

A total of 608 sites were investigated. Among them, 540 (88.8%) were inspected at a single station (larval habitat or adult catching stations), while 68 (11.2%) were inspected at 2 up to 7 stations. At a defined site and for a defined mosquito habitat, we sorted the samples according to stations when (i) the spatial distance from each other was more than approx. 20 m, (ii) the mosquito habitats showed some slight differences rendering it possible to have a slightly different mosquito fauna (iii) the sampling method differed. All communes of Luxembourg (n=102) had at least one sampling site on its territory (5 communes), up to a maximum of 25 (Schengen), the median value being 5 sampling sites (Fig. 21).

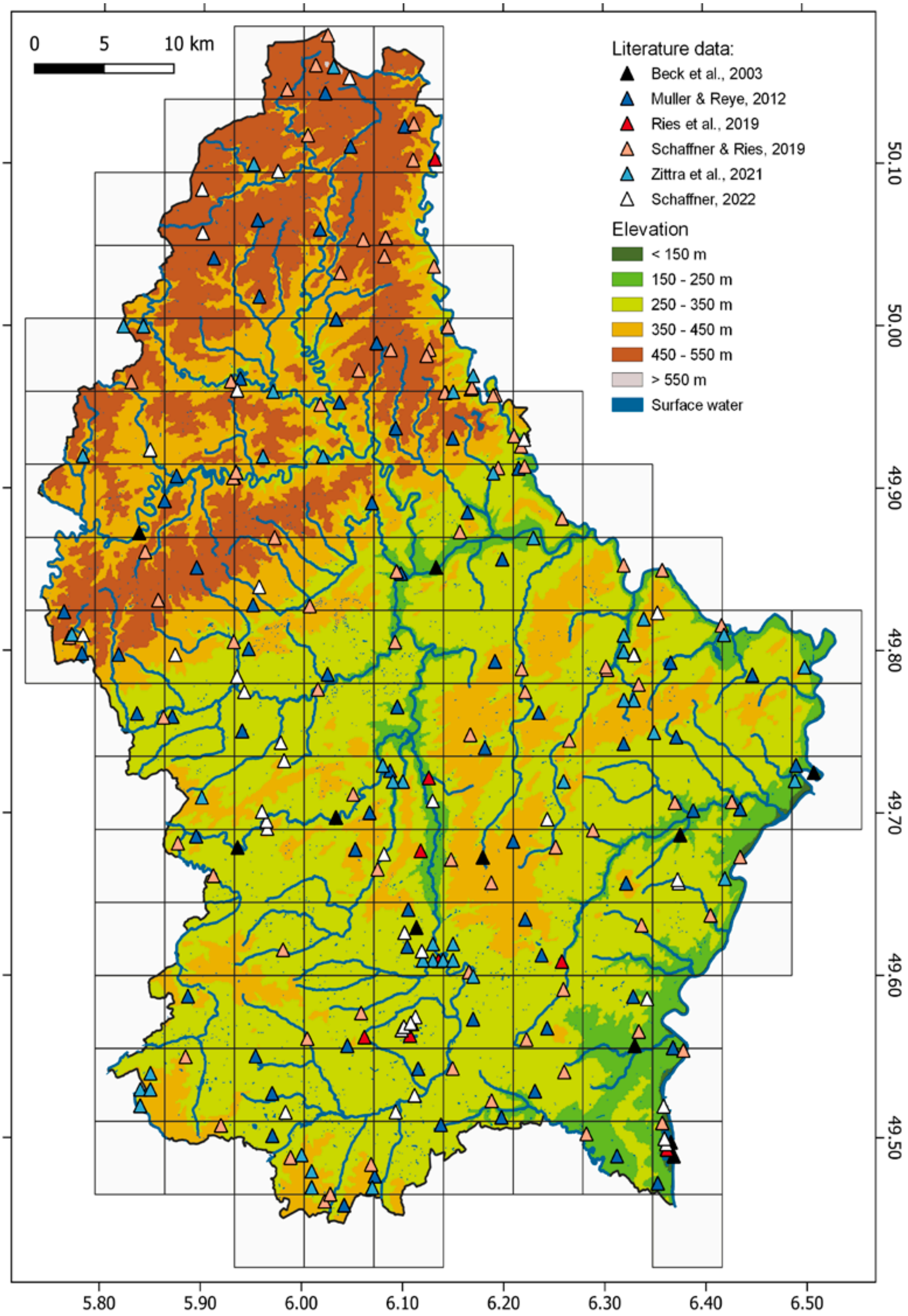


Fig. 17: Spatial distribution of mosquito distribution data collected prior to our study throughout Luxembourg, 1997-2021.

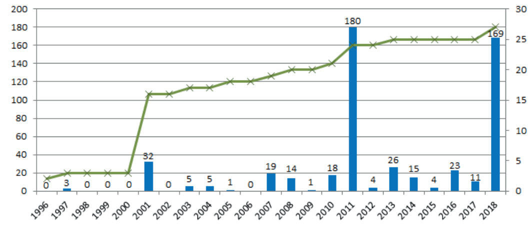


Fig. 18: Temporal distribution of mosquito distribution data collected prior to our study (n=530). Numbers of distribution data per year of observation (blue, left axis) and cumulated number of mosquito species observed (green, right axis). Imprecise data are not considered in data number but in species list (e.g. Sunnen 1918).

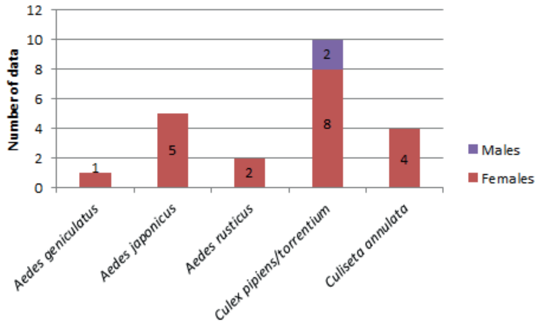


Fig. 19: Number of citizen data per mosquito taxon, Luxembourg, 2019-2021.

Among the 137 5x5 km grid squares, 136 (93.3%) had at least one and up to 17 sampling sites, and only one (0.7%) square could not be sampled since no suitable place was found at that very small part of the territory (square C1, incomplete, located at the north-west border, encompassing Agriculture land cover only) (Fig. 22). The median value is 4 sampling sites per square, and the highest value (17) is for the square P9, incomplete, located at the south-eastern territory border, but covering Schengen and the large wetlands and nature reserve of Haff Réimech. The second highest value (14) is for the square I9, full, which includes the Mullerthal nature park, where we intensified our investigations to confirm the occurrence of *Culiseta glaphyroptera*, previously reported by Zittra et al. (2021).

All sampling sites were classified into one of the three land cover categories, Agriculture, Nature, and Urban, used for designing the sampling strategy. Overall, we identified 163, 257 and 188 sampling sites for the respective categories, with median numbers of 1 (range 0-5), 2 (0-13), and 1 (0-7) sampling sites of the respective category per 5x5 km grid square. The aim of having at least one sampling site in each of the three categories could be achieved for 65 out of 137 grid squares (47.4%). 17 grid squares (12.4%) had only one category represented and 54 (39.4%) had 2 categories represented (Fig. 23). Most of the grid squares had at least one sampling site in Urban land cover (n=123, 89.8%) and/or in Nature land cover (n=109, 79.6%), while sampling sites in Agriculture land cover could be identified in only 88 grid squares (64.2%) (Fig. 24). Since the effort to spot sampling sites was similar over the grid squares, the deviation from the designed plan is a consequence of the difficulty to identify, if not the absence, of suitable mosquito development sites in certain grid squares, i.e. incomplete squares located at the territory borders (e.g. the very incomplete square C1 which has only Agriculture land cover). Also, while the occurrence of mosquito larval habitats in urban areas is easy to spot, this is more difficult in Agriculture and in Nature, in particular in the Oesling region where the landscape is very hilly and deciduous forests are rare (Fig. 25).

The samples were collected within ten periods, between 3rd April 2019 and 19th August 2021 (Fig. 26). All initially scheduled 7 field mission periods were performed, but due to the SARS-CoV-2 pandemic, the two-week period planned in March 2020 was interrupted and postponed to March 2021. In addition, to fill some remaining geographical gaps and to benefit from favourable weather conditions (heavy summer rainfalls), one sampling week was performed in May 2021 and a few complementary samples were collected in August 2021.

Subsequently, the highest number of samplings was performed in spring months (n=448; 49.9%) when more mosquito species are known to develop, than in summer (n=269; 30.0%) and autumn (n=152; 16.9%) months (Fig. 27). The lowest number of samplings was performed in winter months (n=28; 3.1%) when only a few mosquito species can be collected as larvae or adults, since the majority of the species overwinter at the egg stage. During that period, resting catches represented half of the samplings (n=14; 50.0%) while during all other seasons larval samplings were the vast majority (88.4 to 98.7% per season).

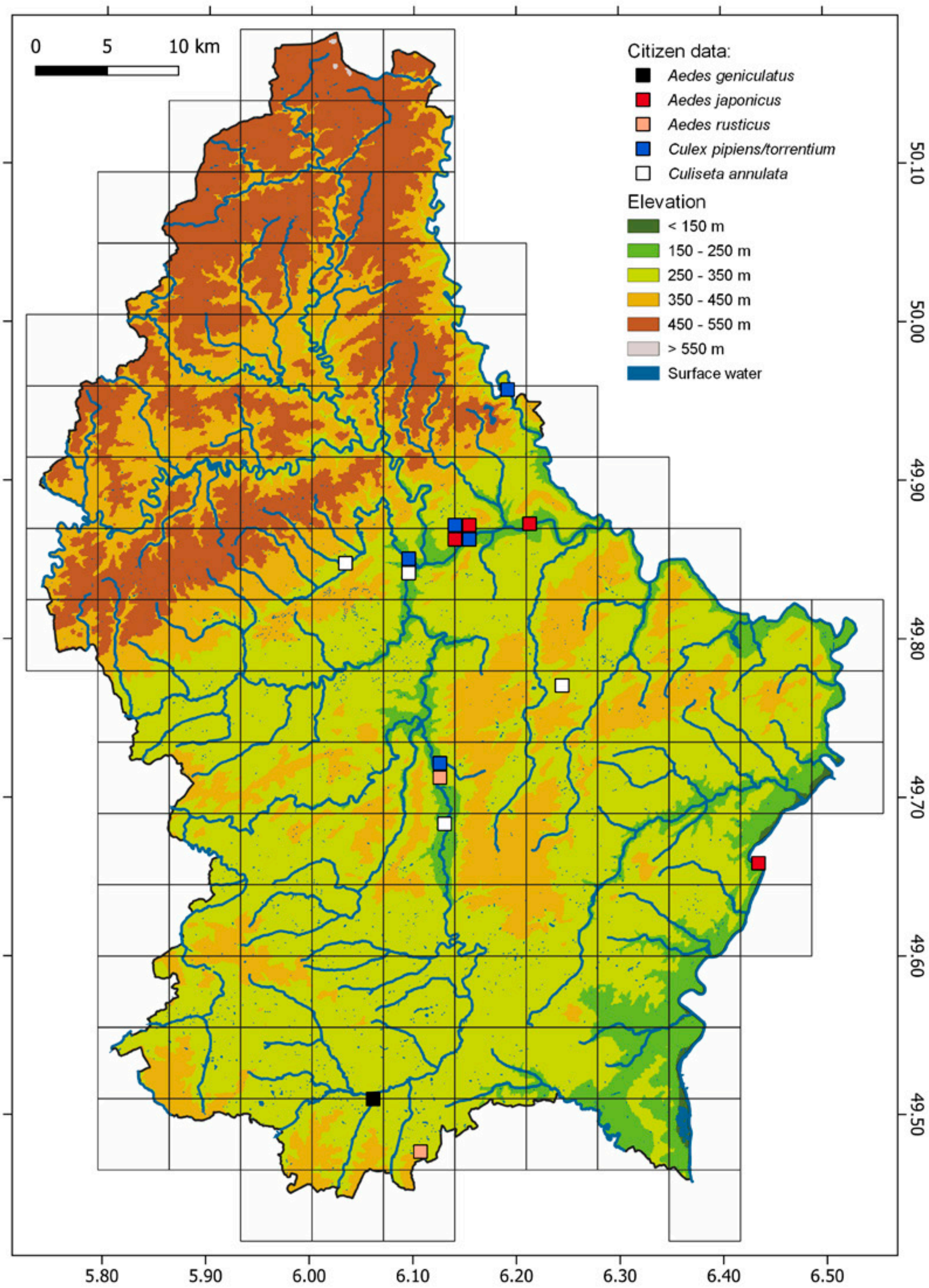


Fig. 20: Distribution of validated citizen data across Luxembourg, 2019-2021 (n=21). When several species are reported at a location, the squares are shown side by side.

Tab. 3: List of mosquito species reported to occur in Luxembourg prior to our study, with year of first observation and the mention of first report. NC: Not confirmed; NS: Not specified; X: Listed species.

Mosquito taxa	Reference for first observation		1- Sunnen (1918)	2- Proft et al. (1999)	3- Beck et al. (2001, 2003)	4- Schaffner et al. (2001)	5- Muller & Reye (2012)	6- Ries et al. (2019)	7- Martinet et al. (2019)	8- Massard (2019)	9- Robert et al. (2019)	10- Schaffner & Ries (2019)	12- Zittra et al. (2021)	11- Schaffner (2022)
	First field observation	Sampling period	1910-1917	NS	2001	NS	2010-2011	2016	NS	1910-1920	NS	2018	2007-2015	1997-2018
Aedes (Aedes) cinereus/geminus	-							X	X					X
Aedes (Aed.) cinereus	2001	3			First	X					X			
Aedes (Aed.) geminus	2010	11												First
Aedes (Aedimorphus) vexans	2016	6					First	X						
Aedes (Dahliana) geniculatus	2001	3			First	X	X		X		X	X		X
Aedes (Hulecoeteomyia) japonicus	2018	10									X	First		
Aedes (Ochlerotatus) annulipes	2001	3			First	X			X		X			X
Aedes (Och.) annulipes/cantans	-													X
Aedes (Och.) cantans	2001	3			First	X	X		X		X			X
Aedes (Och.) communis	2011	5					First							
Aedes (Och.) punctor	2001	3			First	X	X		X		X			X
Aedes (Och.) sticticus	2001	3			First	X			X		X		X	
Aedes (Rusticoidus) rusticus	2001	3			First	X			X		X			X
Anopheles (Anopheles) claviger s.s.	2001	3			First	X	X		X		X	X		X

Tab. 3: (cont.)

Mosquito taxa	Reference for first observation: First field observation	Sampling period	1- Sunnen (1918) 1910-1917	2- Proft et al. (1999) NS	3- Beck et al. (2001, 2003) 2001	4- Schaffner et al. (2001) NS	5- Muller & Reye (2012) 2010-2011	6- Ries et al. (2019) 2016	7- Martinet et al. (2019) NS	8- Massard (2019) 1910-1920	9- Robert et al. (2019) NS	10- Schaffner & Ries (2019) 2018	12- Zittra et al. (2021) 2007-2015	11- Schaffner (2022) 1997-2018
Anopheles (Ano.) maculipennis s.l.	2001	3			First		X				X			X
Anopheles (Ano.) maculipennis s.s.	1997	2		First		X	X							X2
Anopheles (Ano.) plumbeus	2001	3			First	X	X	X	X		X	X		
Coquillettidia (Coquillettidia) richiardii	2001	3			First	X	X	X	X		X			
Culex (Culex) pipiens	1910	1+8	First		X1	X	X	X	X	X	X	X	X3	X3
Culex (Cux.) pipiens/torrentium	-							X				X		X
Culex (Cux.) torrentium	1997	11			X1	X	X	X	X		X	X	X	First
Culex (Maillotia) hortensis	2005	11										X		First4
Culex (Neoculex) europaeus/territans	2001	3			First	X			X		X			X
Culiseta (Allotheobaldia) longiareolata	2018	10										First		
Culiseta (Culicella) morsitans	2003	11												First
Culiseta (Culiseta) annulata	1916	1+8	First		X	X	X	X	X	X	X	X	X	X
Culiseta (Cus.) glaphyroptera	2007	12											First	
No. of taxa	27		2	1	15	15	13	8	14	2	16	10	5	18

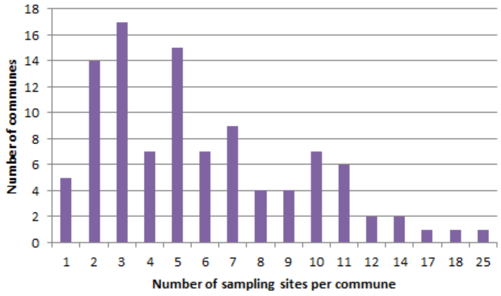


Fig. 21: Distribution of sampling sites per commune of Luxembourg, 2019-2021.

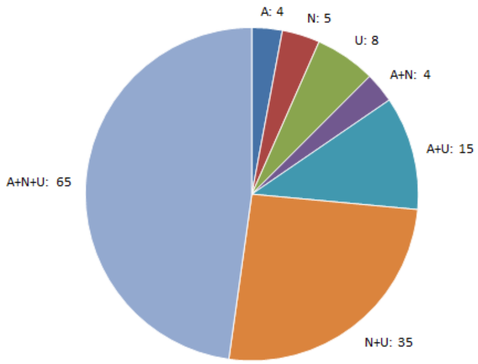


Fig. 23: Proportions of 5x5 km grid squares of Luxembourg with sampling sites (n=136) distributed in 1, 2 or 3 land cover categories, 2019-2021. A: Agriculture; N: Nature; U: Urban.

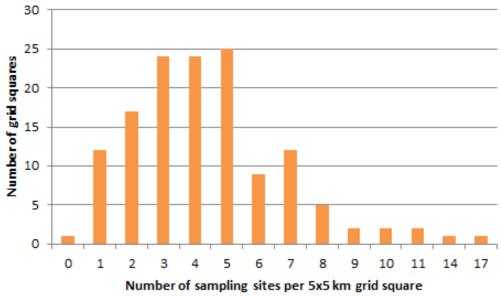


Fig. 22: Distribution of sampling sites per 5x5 km grid squares (n=137) over Luxembourg, 2019-2021.

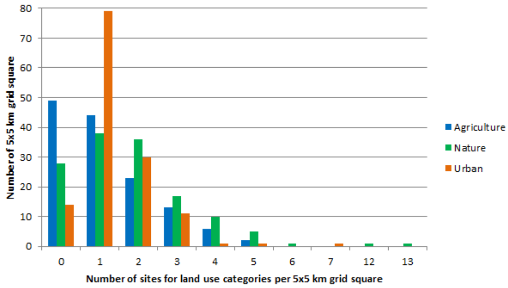


Fig. 24: Distribution of sampling sites for land cover categories per 5x5 km grid squares (n=137) over Luxembourg, 2019-2021.

Overall, samples proved positive (=presence detected) for mosquitoes at 560 (92.1%) out of these 608 sites, while 48 sites (7.9%) remained negative. Almost half of the sites yielded a single mosquito species (n=277, 49.5%), while 241 (43.0%) sites yielded 2 or 3 species (177, 31.6% and 64, 11.4%, respectively) and only 42 (7.5%) sites yielded from 4 up to 8 species, the latter for a single site (Fig. 28).

A total of 4,489 samples were gathered during our field study in Luxembourg, 2019-2021 (Fig. 29). Most of them, i.e. 3,592 (80%), are ovitrap/days implemented at two sites, to survey the possible introduction of invasive *Aedes* mosquito species via vehicles at highway parking places, in 2019 and 2020 (726 and 2,866 ovitrap/days, respectively). All samples remained negative for mosquito eggs.

Among the other samples (n=897), larval dippings are the most abundant (n=809, 90.2%), since our sampling strategy was basically designed on that method. A large part of these dippings proved positive (n=674, 80.3%), while 135 (16.7%) remained negative. Resting catches were performed on 68 occasions, mainly during winter time, to seek resting/hibernating adult mosquitoes indoors in caves and shelters or outdoors in vegetation. A good proportion proved positive (n=45, 66.2%), mainly in caves, cellars and wood shelters, while others, mainly in vegetation and stone piles, remained negative (n=23, 33.8%). Larval dippings and indoor resting catches could be performed under any climatic conditions, while CO_2-baited trappings and human landing catches could not be performed

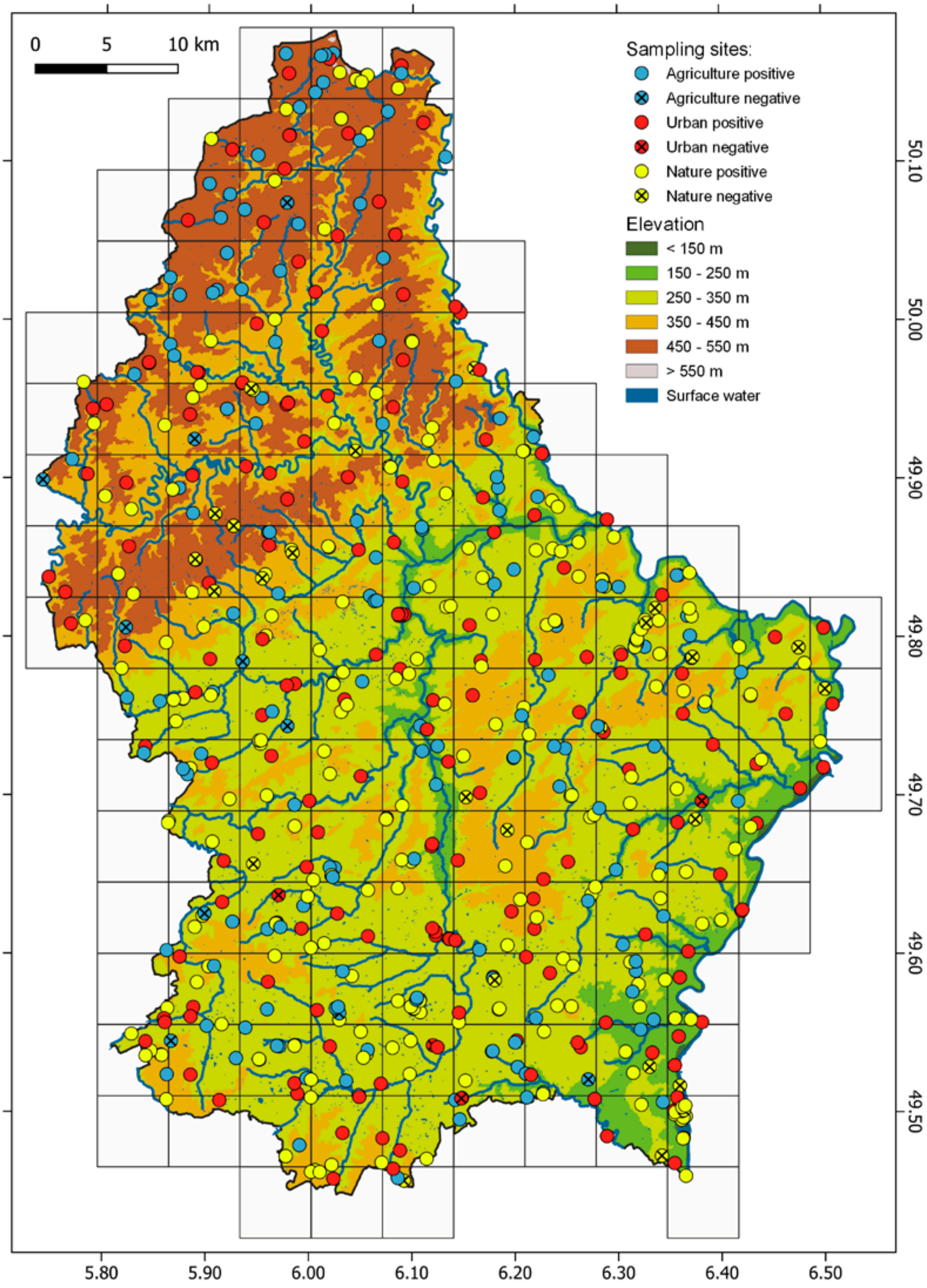


Fig. 25: Distribution of investigated sampling sites throughout Luxembourg, 2019-2021. Crossed circle: site without sample; Full circles: site providing at least one sample; Land cover categories: Agriculture - blue, Nature - yellow, Urban - red.

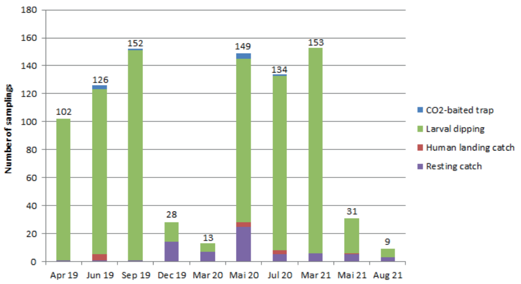


Fig. 26: Numbers of sampling performed during our atlas field study in Luxembourg, 2019-2021 (n=897), according to the sampling methods and the periods. Ovitraps are not shown (n=3,592; performed in periods June-September 2019 and June-October 2020).

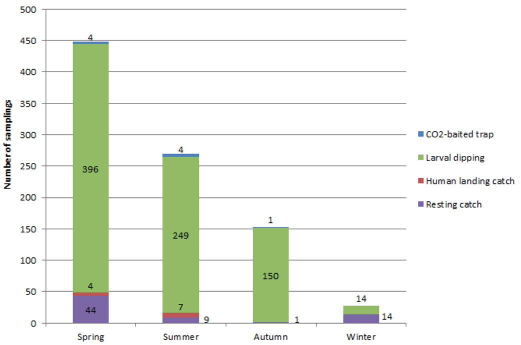


Fig. 27: Numbers of sampling performed during our atlas field study in Luxembourg, 2019-2021 (n=897), according to the sampling methods and the season months. Spring: March-May; Summer: June-August; Autumn: September-November; Winter: December-February. Ovitraps are not shown (n=3,592; performed in periods June-September 2019 and June-October 2020).

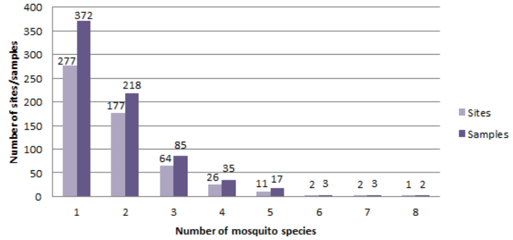


Fig. 28: Mosquito species diversity at sites and within samples, all methods and all sites in Luxembourg, 2019-2021.

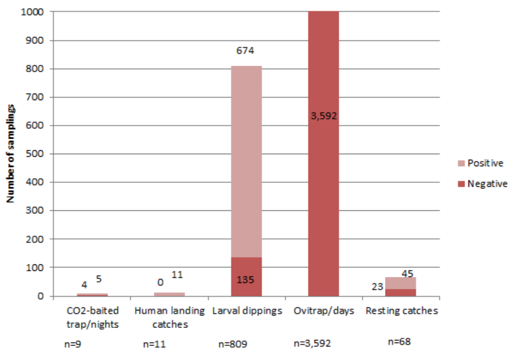


Fig. 29: Sampling effort performed during our field study in Luxembourg, 2019-2021, according to the sampling method. Negative (dark pink): no mosquito species collected; Positive (light pink): at least one mosquito species observed in the sample.

during windy and rainy periods. Therefore only a limited number of such samplings has been performed. Carbon dioxide-baited trappings were performed at locations suspected to harbour some mosquito diversity, i.e. wetlands or large ponds, to confirm or complete the larval dippings. More than half of them (n=5, 55.6%) proved positive, but four (44.4%) remained negative. Finally, human landing catches all (n=11) yielded positive results. Such catches were performed and reported only in the presence of biting mosquitoes, usually near a larval breeding site. We did not report the absence of biting mosquitoes, which was *de facto* observed during our larval search at breeding sites.

Similarly across sites, half of the samples included a single mosquito species (n=372, 50.6%), while 303 (41.2%) sites yielded 2 or 3 species (218, 29.7% and 85, 11.6%, respectively) and only 60 (8.2%) sites yielded from 4 up to 8 species, the latter for two samples only (Fig. 28).

- Sampling results

As a result, 27 species (31 taxa) were observed during our field study (Tab. 4). All 23 species that were reported in the literature and observed prior to our study (Tab. 3) were also collected here except *Ae. cinereus*, of which the occurrence could not be confirmed here, while only its sibling species *Ae. geminus* was found. In addition, four

Tab. 4: Mosquito species collected during our field study over Luxembourg, 2019-2021. BT: CO_2-baited trapping; HC: Human landing catch; LD: Larval dipping; RC: Resting catch; X: Listed species; First: First observation for Luxembourg during our study; In brackets: Not identified to species level.

Mosquito taxa	Species reported in literature prior to our study	Species sampled during our field study	Number of occurrences per sampling method			
			BT	HC	LD	RC
Aedes (Aedes) cinereus/geminus	(X)	(X)			7	2
Aedes (Aed.) cinereus	X	-				
Aedes (Aed.) geminus	X	X			4	
Aedes (Aedimorphus) vexans	X	X			1	3
Aedes (Dahliana) geniculatus	X	X		2	90	
Aedes (Hulecoeteomyia) japonicus	X	X			16	
Aedes (Ochlerotatus) annulipes	X	X			15	7
Aedes (Och.) annulipes/cantans	(X)	(X)	3	6	72	8
Aedes (Och.) cantans	X	X			34	2
Aedes (Och.) communis	X	X		1	15	3
Aedes (Och.) punctor	X	X		1	11	1
Aedes (Och.) sticticus	X	X		2	4	2
Aedes (Rusticoidus) refiki	-	First			3	
Aedes (Rusticoidus) rusticus	X	X		3	77	6
Anopheles (Anopheles) claviger s.s.	X	X			133	1
Anopheles (Ano.) daciae	-	First	1	1	26	3
Anopheles (Ano.) maculipennis s.l.	(X)	(X)	2		22	2
Anopheles (Ano.) maculipennis s.s.	X	X			64	
Anopheles (Ano.) petragnani	-	First			1	
Anopheles (Ano.) plumbeus	X	X			22	
Coquillettidia (Coquillettidia) richiardii	X	X	2	2	2	5
Culex (Barraudius) modestus	-	First	2		12	
Culex (Culex) pipiens	X	X	1	1	219	20
Culex (Cux.) pipiens/torrentium	(X)	(X)			99	4
Culex (Cux.) torrentium	X	X			136	5
Culex (Maillotia) hortensis	X	X			30	6
Culex (Neoculex) europaeus/territans	X	X			86	
Culiseta (Allotheobaldia) longiareolata	X	X			48	
Culiseta (Culicella) morsitans	X	X			81	2
Culiseta (Culiseta) annulata	X	X			75	9
Culiseta (Cus.) glaphyroptera	X	X			2	5
Uranotaenia (Pseudoficalbia) unguiculata	-	First	1		5	1
No. of species (taxa)	23 (27)	27 (31)				

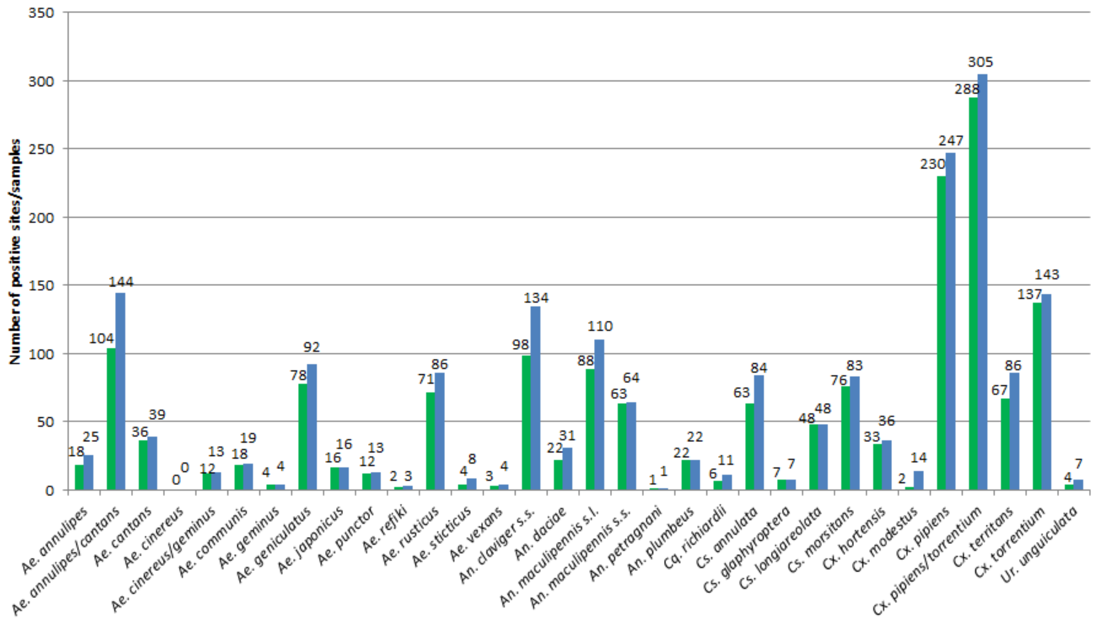


Fig. 30: Numbers of sites (green) and samples (blue) positive for the different mosquito taxa listed to occur in Luxembourg, in our field study, 2019-2021, all methods (32 taxa; Positive sites: n=560, range per taxon=0-388, median=22; Positive samples: n=735, range per taxon=0-414, median=29).

species are reported for the first time to occur in Luxembourg: *Ae. refiki*, *An. daciae*, *Cx. modestus*, and *Uranotaenia unguiculata*. Only one invasive mosquito species, *Ae. japonicus*, was observed. All 27 species were observed as larvae, pupae, adult females and adult males, except *An. petragnani* which was observed only once, as sampled larvae. All species but *An. daciae* and *An. maculipennis* s.s. were identified by morphology, while molecular identification was performed to allow the identification of the previously mentioned two species (60 and 127 specimens, respectively) or to confirm the identification of *An. petragnani*, *Cx. pipiens*, and *Cx. torrentium* (1; 1,211; and 531 specimens, respectively).

A total of 22,114 specimens were observed and counted, including 180 eggs, 15,760 larvae, 2,621 pupae, 1,707 adult males and 1,828 adult females. Because of the necessity to rear them to obtain other stages for morphological identification, eggs were collected only in case of the absence of larvae. Some of the adults (n=2,779) were obtained from reared pupae or larvae, in particular when adults were necessary to morphologically identify the species with certainty. However, 756 adult specimens were directly collected in the field. Positive larval dippings (n=674, Fig. 29) yielded from 1 to 200 immatures, with a median number of six immatures per sample.

Overall, 12 taxa (10 species) were frequently found in Luxembourg, i.e. at more than 10% of the positive sites. Among them, the *Cx. pipiens/torrentium* populations were the most frequently observed at 288 sites (i.e. 51.4% of all positive sites) and in 305 samples. In these samples, the *Cx. pipiens* complex (i.e. *Cx. pipiens* form Pipiens and form Molestus) was more common than the *Cx. torrentium* species (230 sites, 41.1%, and 247 samples; and 137 sites, 24.5%, and 143 samples; respectively). The 9 other taxa (7 species) were recorded at 63 to 104 sites (11.3 to 18.6% of the positive sites, in 84 to 137 samples). Ten other taxa (9 species) were commonly found, i.e. at between 12 to 48 sites (2.1 to 8.6% of the sites, in 13 to 48 samples). Finally, 9 species were rarely collected, at less than 10 sites (1 to 7 sites, i.e. 0.2 to 1.3% of the positive sites, and 1 to 14 samples). Among them, *An. petragnani* was detected at a single site, and both *Ae. refiki* and *Cx. modestus* at two sites only (Fig. 30).

3.5 Taxonomic list of the mosquitoes of Luxembourg

Subfamily *ANOPHELINAE* Grassi 1900

Genus *Anopheles* Meigen 1818

Subgenus *Anopheles* Meigen 1818

1 *Anopheles claviger* (Meigen 1804)

2 *Anopheles petragnani* Del Vecchio 1939

3 *Anopheles daciae* Linton, Nicolescu, & Harbach 2004

4 *Anopheles maculipennis* s.s. Meigen 1818

5 *Anopheles plumbeus* Stephens 1828

Subfamily *CULICINAE* Meigen 1818

Tribe *Aedini* Neveu-Lemaire 1902

Genus *Aedes* Meigen 1818

Subgenus *Aedes* Meigen 1818

6 *Aedes cinereus* Meigen 1818

7 *Aedes geminus* Peus 1970

Subgenus *Aedimorphus* Theobald 1903

8 *Aedes vexans vexans* (Meigen 1830)

Subgenus *Dahliana* Reinert, Harbach, & Kitching 2006

9 *Aedes geniculatus* (Olivier 1791)

Subgenus *Hulecoeteomyia* Reinert, Harbach, & Kitching 2006

10 **Aedes japonicus* (Theobald 1901)

Subgenus *Ochlerotatus* Lynch Arribálzaga 1891

11 *Aedes annulipes* (Meigen 1830)

12 *Aedes cantans* (Meigen 1818)

13 *Aedes communis* (De Geer 1776)

14 *Aedes punctor* (Kirby 1837)

15 *Aedes sticticus* (Meigen, 1838)

Subgenus *Rusticoidus* Shevchenko & Prudkina 1973

16 *Aedes refiki* Medschid 1928

17 *Aedes rusticus rusticus* (Rossi 1790)

Tribe *Culicini* Meigen 1818

Genus *Culex* Linnaeus 1758

Subgenus *Barraudius* Edwards 1921

18 *Culex modestus* Ficalbi 1890

Subgenus *Culex* Linnaeus 1758

19 *Culex pipiens pipiens* Linnaeus 1758

Form Pipiens Linnaeus 1758

Form Molestus (Forskål 1775)

20 *Culex torrentium* Martini 1925

Subgenus *Maillotia* Theobald 1907

21 *Culex hortensis hortensis* Ficalbi 1889

Subgenus *Neoculex* Dyar 1905

22 *Culex territans* Walker 1856

Tribe *Culisetini* Belkin 1962

Genus *Culiseta* Felt 1904

Subgenus *Allotheobaldia* Broelemann 1919

23 *Culiseta longiareolata* (Macquart 1838)

Subgenus *Culicella* Felt 1904

24 *Culiseta morsitans* (Theobald 1901)

Subgenus *Culiseta* Felt 1904

25 *Culiseta annulata* (Schrank 1776)

26 *Culiseta glaphyroptera* (Schiner 1864)

Tribe *Mansoniini* Belkin 1962

Genus *Coquillettidia* Dyar 1905

Subgenus *Coquillettidia* Dyar 1905

27 *Coquillettidia richiardii* (Ficalbi 1889)

Tribe *Uranotaeniini* Lahille 1904

Genus *Uranotaenia* Lynch Arribálzaga 1891

Subgenus *Pseudoficalbia* Theobald 1912

28 *Uranotaenia unguiculata unguiculata* Edwards 1913

* Alien introduced species

4 Catalogue of the mosquitoes of Luxembourg

4.1 Genus *Anopheles* Meigen 1818

Members of the genus *Anopheles* are usually medium-sized to small mosquitoes with long and slender legs and relatively narrow wings (Becker et al. 2020). The proboscis is straight, long and slender. The palps are as long as the proboscis in

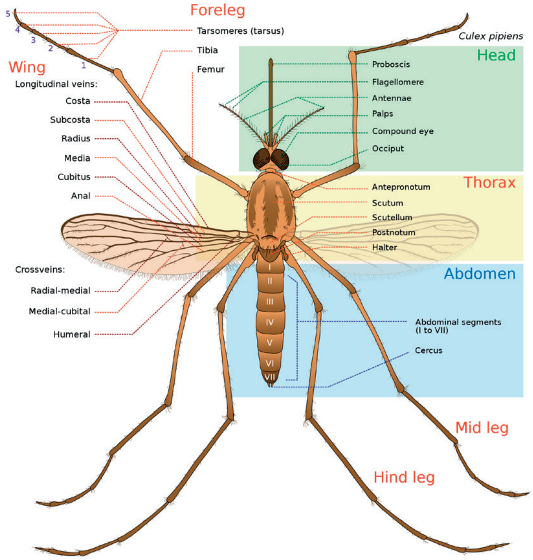


Fig. 31: General morphology of a female Culicinae mosquito (*Culex pipiens*), dorsal view. Thoracic pleura (sides of the thorax) are visible only in lateral view; Abdominal segments are composed of terga (dorsal plates) and sterna (ventral plates). Source: LadyofHats, Public domain via Wikimedia Commons.

both sexes, while they are much shorter than the proboscis in females of all other mosquito genera. The scutellum is evenly rounded with a more or less regular row of long setae. The wings of species occurring in temperate regions of Europe are dark-scaled, with or without dark spots; species with dark and pale patches occur in more warm areas, where the genus is more diversified. Legs are usually unicolour. The abdomen is usually unicolour, without scales, and with a blunt apex and short, round cerci. Larvae breed mainly in (semi-)permanent water bodies, rarely in artificial or natural containers. Because of the absence of a respiratory tube (siphon), they hold themselves horizontally at the water surface (Fig. 38), while larvae of all other genera hold themselves more or less perpendicularly to the water surface when breathing. Eggs are laid singly, usually directly on the water surface, supported by lateral floats; most of them do not survive desiccation. It is generally the adult females that overwinter, rarely the larvae. Females mainly bite mammals or birds, at sunset and during the night, also indoors. The *Anopheles* species are the vectors of human malaria parasites. They also contribute to the transmission of arboviruses (e.g. Tahyna virus), canine filariasis helminths, myxomatosis virus and tularaemia bacteria (Schaffner et al. 2001). *Anopheles* specimens are occasionally introduced by aeroplanes, and some species have been shown to be invasive in tropical areas.

While species from the two subgenera *Anopheles* and *Cellia* occur in the Palaearctic region, only species of the subgenus *Anopheles* are present in temperate Europe.

4.1.1 *Anopheles* (*Anopheles*) *claviger* complex

The *An. claviger* species complex comprises two so-called "sibling species":

Anopheles (*Ano.*) *claviger* sensu stricto (Meigen 1804)

Informal names: en: Club-lipped German nail mosquito; de: Keulenlippige Nadelstechmücke; lb: Keellëpsen-Nolmustik; fr: Moustique-aiguille aux palpes en massue.

Anopheles (*Ano.*) *petragnani* Del Vecchio 1939

Informal names: en: Petragnani Italian nail mosquito; de: Petragnani-Nadelstechmücke; lb: Petragnani-Nolmustik; fr: Moustique-aiguille de Petragnani.

Adults of *An. claviger* s.l. are large and uniformly brownish (Fig. 32). The palps and the proboscis are entirely dark-scaled. The dorsal part of the head (vertex) bears a distinct tuft of broad white scales. The scutum (Fig. 31) shows a patch of pale scales on its anterior margin. The legs are entirely dark, as are the wings, on which the scales are evenly distributed, without concentrations of dark scales. Females of *An. petragnani* are described to be darker in colouration than *An. claviger* s.s., but this character is of little value to distinguish both species (Becker et al. 2020). These may be distinguished based on subtle larval characters and most reliably identified using molecular techniques (Kampen et al. 2003; Mathieu & Schaffner 2005). *Anopheles claviger* s.s. adults could be mixed up with those of *An. plumbeus*, but the latter are smaller with a darker colouration.

Immature habitats for *An. claviger* s.l. are diverse but the species shows a preference for cool, clean waters, such as shaded woodland pools, springs, ditches or pond edges with aquatic vegetation (Schaffner 2002). They are commonly collected in wells, cisterns and small streams in the Mediter-

Fig. 32: Adult female of *Anopheles claviger* s.s. Photo: Anders Lindström.

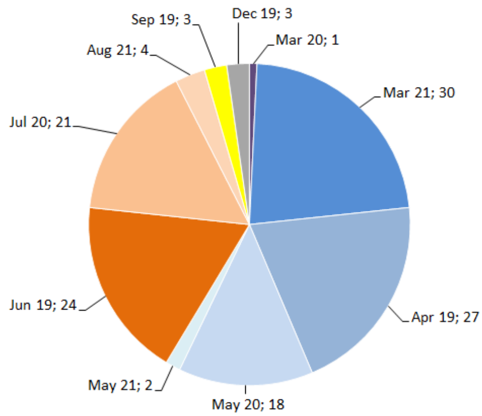


Fig. 33: Temporal distribution of *Anopheles claviger* s.s. immature samples, Luxembourg, 2019-2021 (133 samples). Blue: spring months; Orange: summer months; Yellow: autumn months; Grey: winter months.

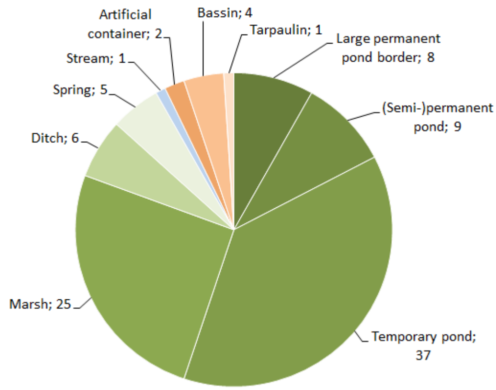


Fig. 34: Categories of larval habitats in which *Anopheles claviger* s.s. was observed, Luxembourg, 2019-2021 (98 sites).

ranean. Immatures of *An. petragnani* tolerate warmer larval habitat waters (Becker et al. 2020). Unlike most *Anopheles* that oviposit directly on the water surface, *An. claviger* s.s. lays its eggs in the damp substrate slightly above the waterline (Wilkerson et al. 2021). *Anopheles claviger* s.l. is multivoltine, producing 2 or 3 generations per year, with northern populations peaking in May and September. There, *An. claviger* s.s. overwinters as larvae and is able to develop and pupate in winter. In warmer southern parts of its distribution, the species is active year-round.

Female *An. claviger* s.s. are primarily exophilic (i.e. biting outdoors), feeding on both humans and large mammals after dusk (Wilkerson et al. 2021). *Anopheles claviger* s.s. is a competent malaria vector and the species played an important role in malaria parasite (*Plasmodium* sp.) transmission in the Near East and Central Asia. It is suspected to participate in the transmission of other pathogens such as Bunyavividae viruses (Tahyna and Batai viruses), myxomatosis virus, *Dirofilaria* sp. and *Setaria labiatopapillosa* nematodes, anaplasmosis, borreliosis and tularaemia bacteria (Schaffner 2002). By contrast, *An. petragnani* appears solely zoophilic, and has never been implicated in pathogen transmission.

Anopheles claviger s.s. was first collected in Luxembourg in 2001 (Beck et al. 2001, 2003; Tab. 2), and 46 precise locations are known from the literature. In our study, we identified 98 additional sites. In total (155 sites), the species was observed in 88 5x5 km grid squares (out of 137; 64.2%), 78 communes (out of 102; 76.5%) distributed over all 12 cantons (Fig. 36). Thus the species can be considered as common in the Grand Duchy.

Our field study yielded 133 larval samplings and one adult resting catch positive for *An. claviger* s.s. (two males; Fig. 30). Immatures were found all year round (Fig. 33), but mainly in spring (n=78 samples; 58.6%) and summer (n=49; 36.8%) months. A total of 459 larvae and 63 pupae were sampled. Larval habitats mainly belonged to stagnant temporary water bodies (n=73; 74.5%) (Fig. 34; 35a). Additionally, (semi-)permanent

Fig. 35: Larval habitats of *Anopheles claviger* s.s. (a) and *Anopheles petragnani* (b) in Luxembourg: a. Temporary pond with vegetation (site ATL461, Bockholtz, Parc Hosingen, 07/07/20, found in association with *Anopheles maculipennis* s.s.); b. Marsh puddle (site ATL064, Hoscheid, Bourscheid, 16/03/21). Photos: Francis Schaffner.

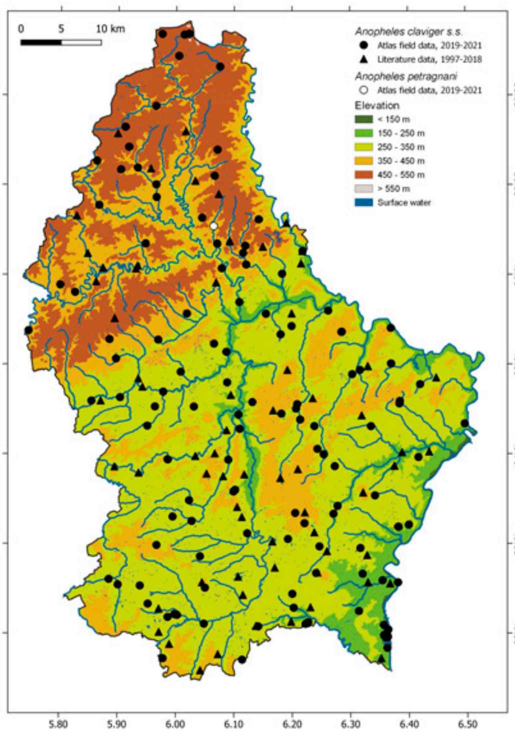


Fig. 36: Distribution of *Anopheles claviger* (black) and *Anopheles petragnani* (white) in Luxembourg.

water bodies with vegetation (n=17, 17.3%) were well represented and a few man-made containers were found positive for the species (n=7, 6%), as well as a single running water site (a small stream without current). More than half of the larval habitats were ponds (permanent, semi-permanent or temporary; n=54; 55.1%), followed by water bodies in marshes (n=25; 25.5%). Other natural habitats such as ditches, springs, and streams were much less frequent (6; 5; and 1, respectively). The positive man-made habitats consisted of basins (4), artificial containers (2) and tarpaulin (1).

The sibling species *An. petragnani* was found for the first time in Luxembourg during our study, in a single positive sample collected at Bourscheid on 16/03/2021 (4 larvae). The habitat consisted of swampy puddles in an enclosed valley (Fig. 35b). The species was identified by morphology (larvae) and confirmed by DNA sequencing (one specimen).

Anopheles claviger s.l. occurs throughout the western Palearctic, from the United Kingdom across Europe up to China and Central Siberia, and south to North Africa (Schaffner 2002). While the nominotypic species is found all over the above-mentioned geographic range, *An. petragnani* is largely restricted to the western Mediterranean Basin and is sometimes found in sympatry with *An. claviger* s.s. (Schaffner et al. 2001; Wilkerson et al. 2021). Our finding of *An. petragnani* in Luxembourg, together with the recent record in Germany (Baden-Württemberg: Black Forest; Becker et al. 2016), suggest a northwards spread of the species. Its occurrence in Luxembourg is the species' current northernmost record.

4.1.2 *Anopheles* (*Anopheles*) *maculipennis* complex

The Maculipennis complex comprises several members that can hardly be distinguished by morphology. They have been discovered based on differences in biology and behaviour, particularly when comparing their population abundance with the intensity of malaria transmission in different regions. Some members of the Maculipennis complex are indeed efficient malaria vectors, e.g. *An. atroparvus* Van Thiel 1927 in western Europe, *An. labranchiae* Falleroni 1926 in the Mediterranean regions, *An. sacharovi* Favre 1903 in the Near East, while others do not play any significant role. In the Palaearctic region, twelve complex members are currently recognised, of which two have been observed to date in Luxembourg, based on molecular identification. Hereafter for our study, we consider all observations of members of the Maculipennis complex under the name *An. maculipennis* sensu lato, and for the nominal taxon *An. maculipennis* sensu stricto, and for *An. daciae* only specific observations where those species could be identified (by analysing ITS2 sequences).

Fig. 37: Adult female of *Anopheles maculipennis* s.l. Photo: Francis Schaffner.

Fig. 38: Larvae of *Anopheles maculipennis* s.l. Photo: Francis Schaffner.

Anopheles (*Ano.*) *maculipennis* sensu stricto Meigen 1818

Informal names: en: Spot-winged German nail mosquito; de: Geflecktflügige Nadelstechmücke; lb: Nolmustik mat gefleckte Flilleken; fr: Moustique-aiguille aux ailes tachetées.

Anopheles (*Ano.*) *daciae* Linton, Nicolescu, & Harbach 2004

Informal names: en: Dacia Romanian nail mosquito; de: Rumänische Nadelstechmücke; lb: Rumänesch Nolmustik; fr: Moustique-aiguille roumain.

Adults of *An. maculipennis* s.l. are, in temperate regions, large and medium brown in colour (Fig. 37). The characteristic features, which differ from all other European *Anopheles* species, are the wings with an aggregation of dark scales forming several distinct spots. The proboscis and the palps are dark brown; the palps are nearly as long as the proboscis. The vertex has a tuft of long, whitish, anteriorly directed narrow scales and setae. The scutum has a broad greyish median stripe and the lateral parts are brown anteriorly and blackish brown posteriorly (Becker et al. 2020). Legs are uniformly brown.

Immatures of *An. maculipennis* s.l. develop mainly in (semi-)permanent water bodies with vegetation with clean, calm and cool water, such as lake borders, ponds and ditches. Eggs are laid directly on the water surface, singly but often close to each other. Larvae feed at the water surface on floating debris, often close to vegetation which provides protection from predators. They often show a greenish colour as a result of feeding on phytoplankton (Fig. 38). Members of the Maculipennis complex are multivoltine. Females have

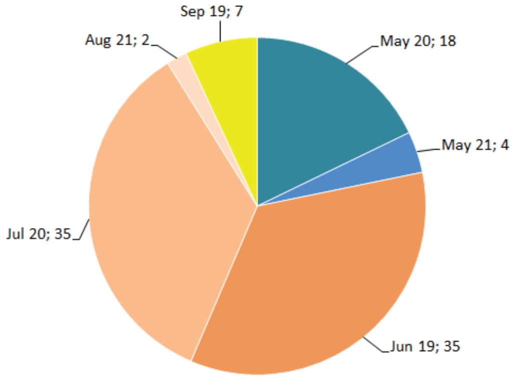


Fig. 39: Temporal distribution of *Anopheles maculipennis* s.l. immature samples, Luxembourg, 2019-2021 (112 samples). Blue: spring months; Orange: summer months; Yellow: autumn months.

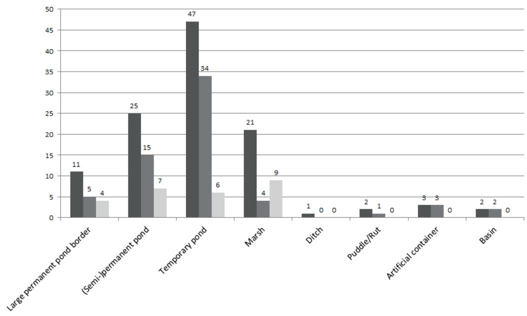


Fig. 40: Categories of larval habitats in which *Anopheles maculipennis* s.l. (black), *An. maculipennis* s.s. (deep grey) and *An. daciae* (light grey) were observed, Luxembourg, 2019-2021 (88, 63, and 22 sites, respectively).

Fig. 41: Larval habitats of *Anopheles daciae* (a,d) and *Anopheles maculipennis* s.s. (b,c,d) in Luxembourg: a. Semi-permanent pond with vegetation (site ATL157, Surré, Boulaide, 26/06/19, in association with *Culex pipiens*); b. Pond with dead leaves (site ATL164, Rossmillen, Weiswampach, 26/06/19); c. Basin (site ATL414, Bildgeshaff, Bettendorf, 05/07/20, in association with *Culex hortensis* and *Culex pipiens/torrentium*); d. Temporary pond with vegetation (site ATL336, Bergem, Mondercange, 03/07/20). Photos: Francis Schaffner.

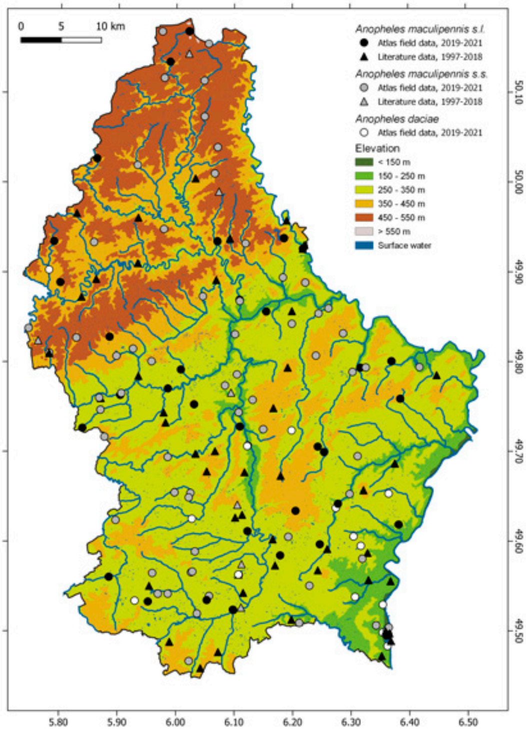


Fig. 42: Distribution of *Anopheles maculipennis* s.l. (black), *Anopheles maculipennis* s.s. (grey) and *Anopheles daciae* (white) in Luxembourg.

a complete winter diapause and overwinter in natural and artificial shelters (e.g. hollowed trees, caves, cellars, bunkers).

Females of *An. maculipennis* s.s. are zoophilic, but they can bite humans. They are usually endophilic (i.e. biting indoors) and nocturnal, and the daytime resting sites are stables and dwellings. The species has probably only played a very minor role in malaria transmission in Europe; it was reported as a vector in the Balkans. It could be involved in the transmission of Batai, Sindbis and West Nile viruses, canine filariasis helminths, myxomatosis virus and tularaemia bacteria (Schaffner et al. 2001). The taxon *An. daciae* has been identified only recently (2004) based on molecular differentiation, and the species behaviour is not yet known, but females have been found infected in nature by Batai virus (Scheuch et al. 2018).

Anopheles maculipennis s.l. is the third mosquito taxon reported from Luxembourg (Tab. 2), based on our personal (FS) field data and identified by molecular methods as belonging to the species *An. maculipennis s.s.* (Proft et al. 1999). To date, 22 precise locations are known from the literature (15 for *An. maculipennis* s.l. and 7 for *An. maculipennis* s.s.) and our atlas field study resulted in the identification of 88 additional sites (Fig. 42). In total (110 sites), *An. maculipennis* s.l. was observed in 72 5x5 km grid squares (out of 137; 52.6%), 54 communes (out of 102; 52.2%) distributed over all 12 cantons. Thus the taxon can be considered as common in the Grand Duchy.

Immatures of *An. maculipennis* s.l. were frequently found during our study (Fig. 30), with 110 positive samples, including 101 larval samplings and 9 adult catches. We identified the complex member in samples from 78 sites among 88. In these 78 sites, *An. maculipennis* s.s. was found more frequently than *An. daciae*, i.e. in 63 (80.8%) and 22 (28.2%) sites, respectively (Fig. 42).

Immatures of *An. maculipennis* s.l. were collected (534 larvae and 45 pupae) mainly during summer months (n=72 samples; 71.3%); they were also observed in spring (May; n=22; 21.8%) and in autumn (September; n=7; 6.9%) (Fig. 39). Adults were caught (24 females and one male) by both CO_2-baited traps and human landing, i.e. one female per trap night on 22/09/2019, 20/05 and 28/05/2020 and one female in a 30 minute catch on 04/07/2020. The latter human bait was performed in a camper van, door open, in the early night (22:40); the specimen was found to be *An. daciae*, as was one of the specimens caught by trapping. Overwintering females were caught by resting catches performed in wooden shelters (three catches) at the Haff Réimech (Wintrange) nature reserve and in a cellar and an adjacent barn (one catch) at Bech-Kleinmacher, in December 2019, March 2020 and March 2021 (18 females in total). Specimens from three out of these four samples belonged to *An. daciae*.

Both *An. daciae* and *An. maculipennis* s.s. were found mainly in stagnant temporary water bodies (n=15; 57.7% and n=38; 59.4%, respectively) as well as in (semi-)permanent water bodies with vegetation (n=11; 42.3% and n=20; 31.3%, respectively). A few samples collected in man-made containers or in semi-natural water bodies without vegetation revealed the presence of *An. maculipennis* s.s. (n=6; 9.4%) (Figs 40; 41).

Anopheles maculipennis s.s. is widely distributed in Europe and its range extends eastwards to Southwest Asia and the Persian Gulf (Becker et al. 2020). *Anopheles daciae* was first identified in Romania (Nicolescu et al. 2004), and later detected throughout Europe, from England to the Near East (Turkey and Iran) (Becker et al. 2020).

4.1.3 *Anopheles (Anopheles) plumbeus*

Anopheles (*Ano.*) *plumbeus* Stephens 1828

Informal names: en: Leaden Irish nail mosquito; de: Bleifarbige Nadelstechmücke; lb: Bläifaarweg Nolmustik; fr: Moustique-aiguille gris plomb.

Adults of *An. plumbeus* are medium-sized and uniformly blackish (Fig. 43). The palps and the proboscis are entirely black-scaled. The vertex has a tuft of narrow, pure white scales, which is directed anteriorly, and yellowish longer setae (Becker et al. 2020). The scutum shows a patch of pure white, narrow scales on its anterior margin. Lateral parts of the scutum are blackish brown, with a median longitudinal grey stripe which covers at least 1/3 of the width of the scutum. The legs are entirely black, and the wings have veins that are densely covered with dark-brown, lanceolate scales, without concentrations of scales forming patches.

Immatures of *An. plumbeus* develop almost exclusively in tree holes, mainly of deciduous hard-wood trees (Fig. 47a). The accumulated rain water is usually dark brown due to the dissolved tannins and pigments derived from the wood. Occasionally, the species breeds in containers, preferentially in dark containers such as used

Fig. 43: Adult female of *Anopheles plumbeus*. Photo: Francis Schaffner.

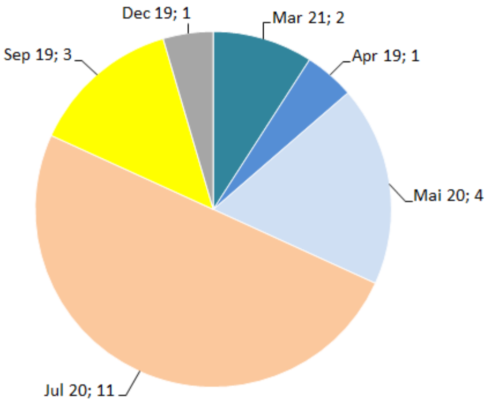


Fig. 44: Temporal distribution of *Anopheles plumbeus* immature samples, Luxembourg, 2019-2021 (22 samples). Blue: spring months; Orange: summer months; Yellow: autumn months; Grey: winter months.

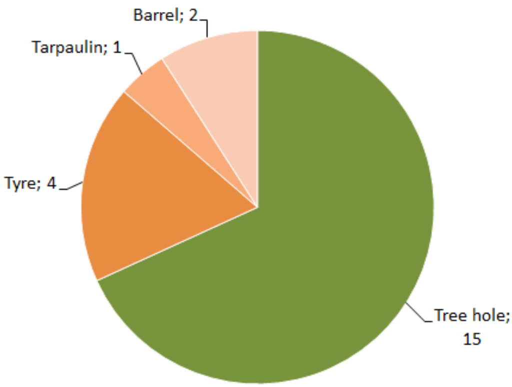


Fig. 45: Categories of larval habitats in which *Anopheles plumbeus* was observed, Luxembourg, 2019-2021 (22 sites).

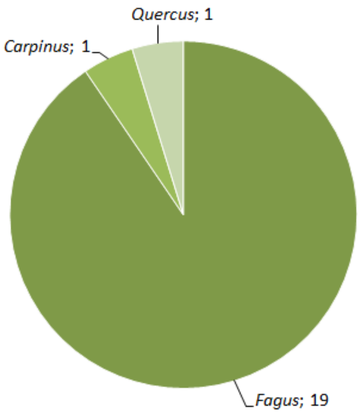


Fig. 46: Tree species in which *Anopheles plumbeus* was observed, Luxembourg, 2019-2021 (15 sites, 22 tree holes).

Fig. 47: Larval habitats of *Anopheles plumbeus* in Luxembourg: a. Tree hole (site ATL310, Mullerthal, Berdorf, 06/07/20, in association with *Aedes geniculatus*); b. Tyres and tarpaulins (site ATL321, Gosseldange, Lintgen, 06/07/20, in association with *Culex torrentium*). Photos: Francis Schaffner.

tyres, and in the presence of large amounts of organic matter. Females lay their eggs on the side of the tree hole, and hatching occurs when the hole is flooded. Hibernation takes place in the egg or larval stage and females occur from the end of spring to the autumn. The species has 2 or 3 generations per year.

Females bite humans, mammals, birds and reptiles. They can be responsible locally for severe nuisance, depending on the size and number of artificial breeding sites (e.g. manure pits of unexploited farms; Dekoninck et al. 2011; Schaffner et al. 2012; Ibañez-Justicia & Cianci 2015). The species has been shown experimentally to transmit human malaria plasmodiums (Schaffner et al. 2012) and West Nile virus (Schaffner et al. 2001), but due to its ecology, it is considered to be of minor epidemiological

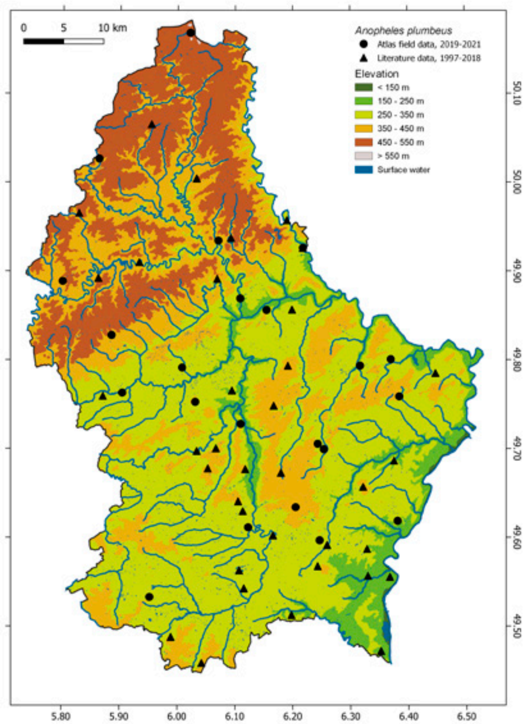


Fig. 48: Distribution of *Anopheles plumbeus* (black) in Luxembourg.

importance (Becker et al. 2020). However, where favourable larval habitats occur, e.g. city parks or unexploited farms, a transmission risk has to be considered (Schaffner et al. 2012).

Anopheles plumbeus was among the species collected during the first country-wide study performed in Luxembourg in 2001 (Beck et al. 2001, 2003; Tab. 2), and 36 precise locations are known from the literature. In our study, we identified 22 additional sites (Fig. 48). In total (58 sites), the species was collected in 45 5x5 km grid squares (out of 137; 32.8%) and 43 communes (out of 102; 42.2%) distributed over all 12 cantons. Thus the species can be considered as common in the Grand Duchy.

In our study, we found *An. plumbeus* in 22 larval samplings of which half of them were collected in summer months (n=11 samples; 50.0%), but immatures were also found in spring (samples: n=7; 31.8%), autumn (n=3; 13.6%) and even in winter (n=1; 4.5%) (Fig. 44). A total of 84 larvae and nine pupae were sampled.

Most of the samples were collected in natural containers, i.e. tree holes (n=15; 68.2%), while the species was also observed to breed in man-made containers (n=7; 31.8%), i.e. tyres (n=4), barrels (n=2) and tarpaulin (n=1) (Figs 45; 47a-b). Positive tree holes were mainly in beech trees (*Fagus sylvatica*, n=19) but also in oak and common hornbeam (*Quercus* sp. and *Carpinus betulus*; N=1 each) (Fig. 46).

This Palaearctic species is widely distributed throughout Europe, up to the Middle East and North Africa (Robert et al. 2019).

4.2 Genus *Aedes* Meigen 1818

Species of the genus *Aedes* are of variable size, with extensive and variable scaling patterns. The proboscis is long, straight and entirely scaled. The palps (Fig. 31) of the female are usually very short, while males have palps more or less as long as the proboscis. The scutum has a species-specific pattern of coloured scales. The scutellum has three lobes that are scarcely scaled but have groups of setae. The wing veins are covered with numerous dark scales, and sometimes scattered pale scales are present. Legs are scaled, entirely dark or with a pattern (rings) of pale and dark scales. The abdomen is covered with flat and coloured broad scales. The apex of the abdomen differs from other Culicinae by its tapered last segments and usually distinct cerci, which are elongated and rarely rounded. Larvae have a more or less long respiratory tube (siphon). They develop mainly in stagnant temporary water bodies, more rarely in artificial and natural containers. Eggs are laid singly on the ground of submersible areas, or on the inner side of containers; they are resistant to desiccation, for months or years. Most of the species overwinter as diapausing eggs, rarely as larvae. Females mainly bite mammals, outdoors, during daytime in shadowed places, but particularly at sunset and sunrise. The *Aedes* genus includes the main vectors of arboviruses such as dengue, chikungunya or yellow fever viruses; they may also be involved in the transmission of canine filariasis helminths, myxomatosis virus and tularaemia bacteria (Schaffner et al. 2001). A number of species are responsible for severe nuisance and thus targeted by mosquito control campaigns. Some container-inhabiting *Aedes* species are recognised as major invasive organisms.

The *Aedes* genus is the more diversified one in the Palaearctic region. It comprises more than 40 subgenera, of which the ten subgenera *Acartomyia*, *Aedes*, *Aedimorphus*, *Dahliana*, *Fredwardsius*, *Georgecraigus*, *Hulecoeteomyia*, *Ochlerotatus*, *Rusticoidus* and *Stegomyia* occur in the European region. Most of these subgenera were recently created, in relation to morphology-based phylogenetic studies of the tribe Aedini, which comprises approximately one-quarter of the known species of mosquitoes. Whilst initial studies proposed the creation of 74 new genera from the single *Aedes* genus within a natural classification based on monophyletic groups of species (Reinert et al. 2009 and earlier), generating a debate about the classification system, a successive study suggested restoring the usefulness of the classification for the operational community, showing the monophyletic groups at subgenera or informal species groups ranks to preserve stability (Wilkerson et al. 2015). This classification, including posterior changes, is reflected in the Systematic Catalog of Culicidae (http://www.mosquitocatalog.org; Wilkerson et al. 2021) and followed herein.

4.2.1 *Aedes (Aedes) cinereus/geminus*

While most of Culicinae males have palps as long the proboscis, those from the subgenus *Aedes* Meigen 1818 (12 species) have palps that are more or less as short as the females'. Members of this subgenus are also characterised by the proboscis which is about as long as the fore femur (distinctly longer in all other *Aedes* subgenera). The patches of scales on the thoracic pleura (lateral sides of the thorax) are weakly developed and the wings of the adults are dark scaled. The apex of the abdomen is pointed, with cerci of moderate length. The most characteristic features for the members of the *Aedes* subgenus are found in the male genitalia (Becker et al. 2020).

Aedes (*Aed.*) *cinereus* Meigen 1818

= *Aedes cinereus* Meigen 1818, sensu Reinert et al. (2009)

Informal names: en: Ashy European pointy mosquito; de: Aschgraue Spitzstechmücke; lb: Äschegro Iergermustik; fr: Moustique pointu cendré.

Aedes (Aed.) geminus Peus 1970

= *Aedes geminus* Peus 1970, sensu Reinert et al. (2009)

Informal names: en: Twin German pointy mosquito; de: Zwillings-Spitzstechmücke; lb: Zweelaf-Iergermustik; fr: Moustique pointu jumeau.

These two sibling species have been described based on morphological differences in the male genitalia. No other diagnostic characters, at any stage, are known to differentiate them accurately. Even the examination of mtDNA and rDNA genes or protein profiles (MALDI-TOF mass spectrometry) did not reveal any conclusive differentiation, raising the question of species validity (FS and A. Lindström, unpublished data).

Our comprehensive country-wide sampling gathered 13 observations of *Ae. cinereus/geminus* and among these, solely *Ae. geminus* could be identified on 4 occasions, by examining male genitalia. Among the five historical records gathered from the literature, three are attributed to *Ae. cinereus/geminus* (Schaffner & Ries 2019; Schaffner 2022) and a single one to both *Ae. cinereus* (Beck et al. 2003) and *Ae. geminus* (Schaffner 2022). Though the latter was based on solid male genitalia identification, the former relies on female identification only and may therefore be attributed to the *Ae. cinereus/geminus* taxon. However, it is not excluded that the undetected *Ae. cinereus* morphological form might occur in Luxembourg. Overall, the taxon *Ae. cinereus/geminus* is recorded to date from 18 sites distributed over 11 5x5 km grid squares (out of 137; 8.0%) and 13 communes (out of 102; 12.7%) from 7 cantons, but in the Gutland only (Fig. 53). The species can be considered as common in the Grand Duchy.

Adults of *Ae. cinereus/geminus* are medium-sized to rather small mosquitoes (Fig. 49). The proboscis is dark brown with lighter scales on its ventral surface and the palps are entirely dark brown. The scutum has a fawn-brown colouration and the thoracic pleura are light brown with patches of broad creamy scales. The legs are almost entirely dark-scaled. The abdominal terga (dorsal plates of the abdominal segments; Fig. 31) have dark brown scales on the dorsal surface, without pale transverse bands. Each tergum has also pale scales which form lateral patches that are usually joined, forming longitudinal stripes at the sides of the abdomen, which are not easily visible in dorsal view (Becker et al. 2020). Larvae have a rather long and slender siphon, with pecten teeth unevenly spaced.

Fig. 49: Adult female of *Aedes cinereus/geminus*. Photo: Anders Lindström.

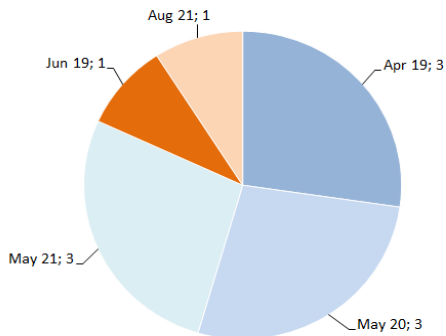


Fig. 50: Temporal distribution of *Aedes cinereus/geminus* immature samples, Luxembourg, 2019-2021 (22 samples). Blue: spring months; Orange: summer months.

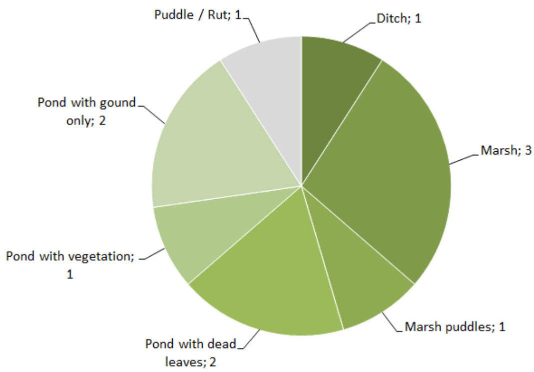


Fig. 51: Categories of larval habitats in which *Ae. cinereus/geminus* was observed, Luxembourg, 2019-2021 (22 sites).

Fig. 52: Larval habitats of *Aedes cinereus/geminus* (a) or *Aedes geminus* (b) in Luxembourg: a. Pond with dead leaves (site ATL364, Herborn, Rosport-Mompach, 22/05/20, in spring the site was inhabited with *Aedes annulipes/cantans*, *Aedes rusticus*, and *Culiseta morsitans*); b. Pond with ground only (site ATL360, Olingen, Betzdorf, 22/05/20). Photos: Francis Schaffner.

Both *Ae. cinereus* and *Ae. geminus* have at least two generations per year. Larvae hatch in spring, later than the typical snowmelt mosquitoes (e.g. *Ae. communis*) and adults occur from late spring until early autumn. Larvae develop after flooding of their larval habitats by rainfall, often together with *Ae. sticticus* and *Ae. vexans*. They develop in various habitats, most often at the edges of semi-permanent, partly shaded pools of floodplains, but also in marshes and *Sphagnum* sp. bogs, at the edges of lakes covered by emerged vegetation, or in woodland ponds and ditches (Schaffner et al. 2001; Becker et al. 2020). The species overwinters at the egg stage.

Females of both *Ae. cinereus* and *Ae. geminus* are anthropophilic and mammophilic. They bite

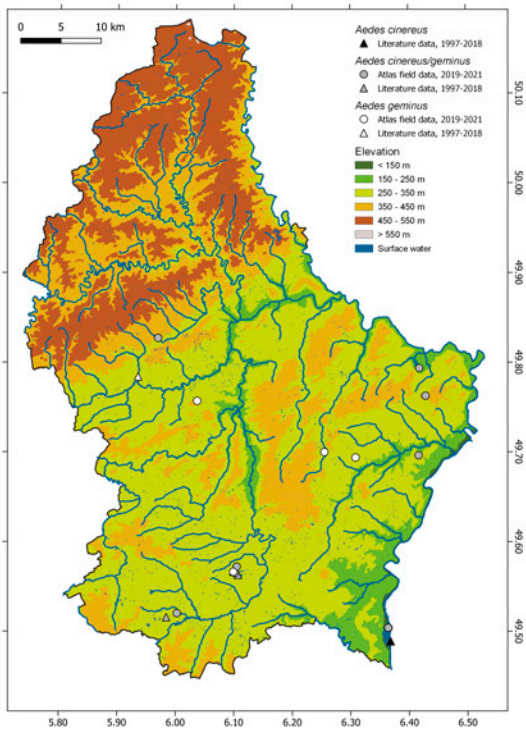


Fig. 53: Distribution of *Aedes cinereus* (black), *Aedes cinereus/geminus* (grey) and *Aedes geminus* (white) in Luxembourg. The presence of *Aedes cinereus* has still to be confirmed.

outdoors, under tree cover or in open areas, at dusk but also during the day; they can fly quite far from their breeding sites and can cause great annoyance, when present in large numbers. The taxon is a vector of Sindbis virus and is infected in nature by Tahyna and West Nile viruses; it may also play an important role as vector (mechanical transmission) in tularaemia epidemics (Schaffner et al. 2001; Martinet et al. 2019).

The taxon *Ae. cinereus/geminus* was commonly found in our study (Fig. 30), in 11 larval samplings (yielding 129 larvae and two pupae) and 2 resting catches (2 females; Fig. 53). Most of the larval samples were collected in spring months (n=9 samples; 81.8%), and immatures were also found in summer (n=2; 18.2%) following the heavy rainfall period of August 2021 (Fig. 50). At that same period, adults could be caught in two resting catches.

Almost all immature samples were collected in stagnant temporary water bodies (n=10 samples; 90.9%), while a single sample originated from a semi-natural water body, i.e. a group of puddles and ruts with little vegetation (Fig. 51). Among the natural breeding sites, marshes and ponds were the most frequently inhabited (n=4 and 5, 36.4 and 45,5%, respectively). The remaining site was a ditch. Sites from where *Ae. geminus* could be identified were all relatively small temporary water bodies, e.g. ponds from fallen trees, ground pool, puddles and ruts (Figs 52; 55).

While *Ae. cinereus* has a Holarctic distribution, *Ae. geminus* is only recorded in the western Palaearctic region, mainly in middle and western Europe (Becker et al. 2020).

Fig. 54: Adult female of *Aedes vexans*. Photo: Anders Lindström.

4.2.2 *Aedes (Aedimorphus) vexans*

The subgenus *Aedimorphus* Theobald 1903 comprises approximately 110 species and subspecies. The majority of them can be found in the Ethiopian and Oriental regions. The taxon *Ae. vexans* is one of the most widely distributed species and it shows some cryptic composition across its range. Currently, four subspecies are recognised (Wilkerson et al. 2021), of which only the nominal form, i.e. *Ae. vexans vexans* (hereafter *Ae. vexans*), occurs in the western Palaearctic region.

Aedes (*Adm.*) *vexans vexans* (Meigen 1830)

= *Aedimorphus vexans* (Meigen 1830), sensu Reinert et al. (2009)

Informal names: en: Vexing German pointy mosquito, Vexans mosquito; de: Lästige Spitzstechmücke, Wiesenmücke, Rheinschnake; lb: Stëppeleg Iergermustik; fr: Moustique pointu fâcheux.

Adults of *Ae. vexans* are medium-sized brownish mosquitoes (Fig. 54). The proboscis is dark-scaled but with white scales ventrally, and palps are dark-scaled but with some white scales apically. The scutum is dark brown with curved, narrow dark and pale scales forming indistinct patches. The thoracic pleura have some patches of broad pale scales. The wing veins (Fig. 31) are covered with moderately broad dark scales and isolated pale scales at the bases of the costa and subcostal. The abdominal terga have white basal bands with the distal parts dark-scaled. These basal bands on terga III–VI are distinctly narrowed in the middle, forming a bilobed pattern. The extremity of the abdomen is pointed, and the cerci are long and narrow. All the tarsomeres of the hind legs have narrow basal pale rings which usually do not exceed more than 1/4 of the length of the tarsomeres, thus much shorter than for the ringed *Ae. annulipes/cantans* (Becker et al. 2020). Larvae have a rather long and slender siphon, with pectin teeth unevenly spaced.

Aedes vexans is a multivoltine species. In Europe, larvae occur from middle spring to the end of summer, with an abundance peak between May and July (Schaffner et al. 2001). Immatures develop predominantly in inundation areas such as floodplains of rivers or lakes with fluctuating water levels. Their preferred breeding sites are temporary water bodies which are present only a few days to weeks after a flood, such as flooded meadows, poplar cultures, willow and reed areas (Becker et al. 2020). They often breed in the company of *Ae. sticticus* and *Ae. cinereus/geminus*. The egg is the overwintering stage. Larvae often hatch in huge numbers after a flood, and their development to adults is rapid, from 4 to 25 days, depending on temperature and food abundance. Adults can disperse over long distances, e.g. 15 km or more.

Females of *Ae. vexans* feed mainly on mammals. In relation to their abundance, they can generate severe nuisance to humans, outdoors and mainly at sunset, at locations surrounding floodplain wetlands. Therefore mosquito control campaigns do target that species, e.g. in the Upper Rhine Valley (in both France and Germany). The species is considered a vector of Tahyna virus, is infected in nature by Batai, Usutu and Zika viruses, and is a laboratory vector of chikungunya, West Nile and Zika viruses; it also probably plays a role in the transmission of tularaemia bacteria and *Diro-*

Fig. 55: Larval habitat and catching place of *Aedes vexans* in Luxembourg: marsh border (site ATL593a, Haff Réimech, Schengen, 25/05/21; *Aedes vexans* larvae were found on 18/08/21 in association with *Aedes cinereus/geminus*, *Anopheles claviger* s.s., and *Culex pipiens*. Photo: Francis Schaffner.

filaria and *Setaria* nematodes (Schaffner et al. 2001; Elizondo-Quiroga et al. 2018; Scheuch et al. 2018; Martinet et al. 2019).

Only two records of *Ae. vexans* exist in the literature, with the first record dating back to 2012 (Muller & Reye 2012; Ries et al. 2016; Tab. 2); both records are based on longitudinal adult trappings. The species was also rarely collected in our study, with a unique larval sampling, but three resting catches, collected at three different sites. Thus in total, the species is reported from five sites, located in four 5x5 km grid squares (out of 137; 2.9%), five communes (out of 102; 4.9%; i.e. Dudelange, Bettembourg, Luxembourg/Kockelscheuer, Roeser and Schengen/Schwebsingen) and three cantons of the Gutland only (Fig. 56). The species can be considered as rare in the Grand Duchy.

In our study, all four samples were collected in summer, following the heavy rainfall period of August 2021. Immatures (one sample, 23 larvae)

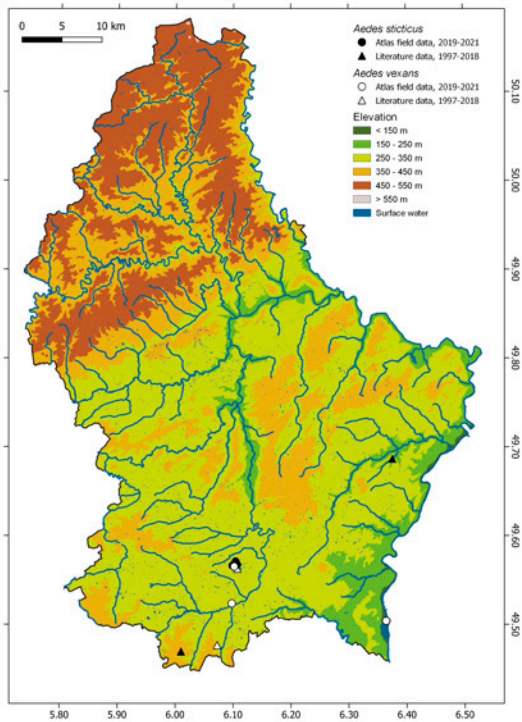


Fig. 56: Distribution of *Aedes sticticus* (black) and *Aedes vexans* (white) in Luxembourg.

were collected in a reed marsh, north of the Haff Réimech nature reserve, together with larvae of *Aedes cinereus/geminus*, *An. claviger* s.s. and *Cx. pipiens* (Fig. 55). Adults were caught (three resting catches, six females, one male) together with *Ae. sticticus* and *Ae. cinereus/geminus*, among others.

Aedes vexans vexans has a Holarctic distribution.

4.2.3 *Aedes (Dahliana) geniculatus*

The subgenus *Dahliana* Reinert, Harbach, & Kitching 2006 comprises only 3 species which all occur in the western Palaearctic region. *Aedes geniculatus* shows the broadest distribution, while *Ae. echinus* (Edwards 1920) is restricted to the Mediterranean Basin, and *Ae. gilcolladoi* Sánchez-Covisa, Rodríguez Rodríguez, & Guillén Llera 1985 is only known from Spain (Wilkerson et al. 2020).

Aedes (*Dah.*) *geniculatus* (Olivier 1791)

= *Dahliana geniculata* (Olivier 1791), sensu Reinert et al. (2009)

Fig. 57: Adult female of *Aedes geniculatus*. Photo: Francis Schaffner.

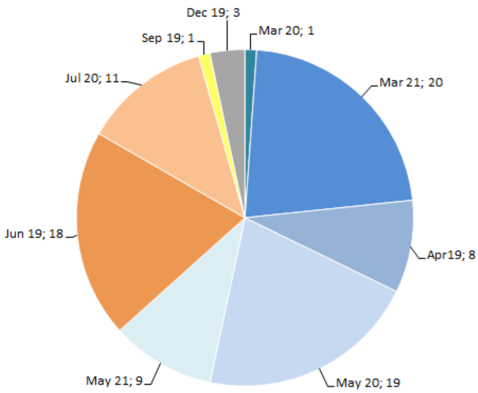


Fig. 58: Temporal distribution of *Aedes geniculatus* immature samples, Luxembourg, 2019-2021 (90 samples). Blue: spring months; Orange: summer months; Yellow: autumn months; Grey: winter months.

Informal names: en: White-kneed French pointy mosquito; de: Weißknie-Spitzstechmücke; lb: Wäissknéi-Iergermustik; fr: Moustique pointu à genoux blancs.

Adults of *Ae. geniculatus* are large mosquitoes (Fig. 57). The proboscis and palps are dark scaled, and the proboscis is longer than the fore femur. The scutum has prominent white and dark scale patterns. The thoracic pleura have patches of broad white scales. The wing veins are covered with dark scales only. The legs have obvious white knee spots, and all tarsomeres are uniformly dark. The abdominal terga have distinct lateral white patches, sometimes with white basal bands. The abdomen extremity is less pointed than for other *Aedes*, since the cerci are short and blunt. This, together with the obvious black and white pattern and white knee spot, immediately separates the females from all other females of the genus *Aedes* (Becker et al. 2020). Larvae have a relatively short siphon that bears a short pecten, in a dense row of relatively long teeth. They are also covered with many rigid star-shaped setae, of which the number and length of the branches allows the differentiation of the three species of the *Dahliana* subgenus.

Aedes geniculatus produces one or two generations per year. Adults appear at the end of spring and are numerous in summer, if the rains are abundant enough. Immatures typically inhabit tree holes, but they can also occur in small water collections such as rock holes and abandoned tyres or artificial basins full of tree leaves and branches, and very rarely in undergrowth ditches. The water from these sites is always rich in organic plant matter and in tannins. The colonised tree holes can be found at various heights, between roots or in tree stumps of different deciduous hard-wood trees such as ash, beech, hornbeam, oak, or plane. They often occur with other phytotelm-inhabiting species such as *An. plumbeus*. The larval development takes at least 2 weeks but, when the temperature is low, the duration of the development can last more than one year. Females lay 70 to 130 eggs individually on the container's inner sides, near to the water level. These eggs are resistant to frost and to desiccation. The hatching occurs asynchronously during the 2 or 3 months following an important flood. The postponed egg hatching and the long larval diapauses cause a permanent presence of larvae within the sites. The species overwinters mainly at the egg stage and occasionally at the larval stage if the winter is not too harsh. In forests where deciduous old trees are abundant, the adults can become an important nuisance. However, their heavy and unsustained flight does not allow the females to go far away to search for blood meals (Schaffner et al. 2001).

Females are day and crepuscular biters, mainly outdoors, and readily feed on humans, but also on other mammals, birds and reptiles. The species does not seem to be involved in the transmission of parasitic diseases to humans. However, females are capable of transmitting chikungunya, West Nile and yellow fever viruses in the laboratory, and have been found naturally infected by tularaemia (Schaffner et al. 2001; Martinet et al. 2019).

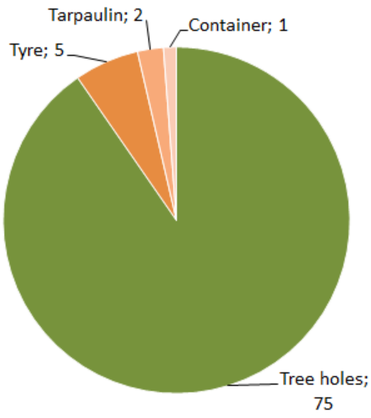


Fig. 59: Categories of larval habitats in which *Aedes geniculatus* was observed, Luxembourg, 2019-2021 (83 sites).

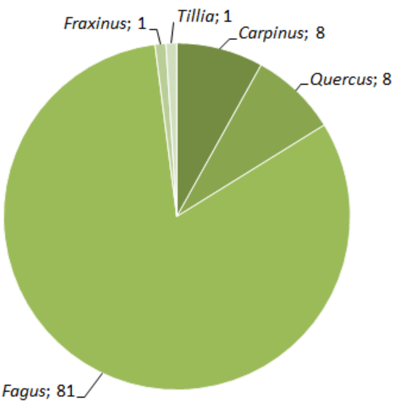


Fig. 60: Tree species in which *Aedes geniculatus* was observed, Luxembourg, 2019-2021 (75 sites, 99 tree holes).

Fig. 61: Larval habitats of *Aedes geniculatus* in Luxembourg: a. Tree hole in a beech stump (site ATL046, Geyershaff, Bech, 07/04/19, in association with *Aedes japonicus* and *Anopheles plumbeus*); b. Tyre under trees (site ATL067, Sonlez, Winseler, 09/04/19). Photo: Francis Schaffner.

Aedes geniculatus was among the species collected during the two countrywide studies performed in Luxembourg in 2001 and 2010 (Beck et al. 2001, 2003; Muller & Reye 2012; Tab. 2), in a survey targeting the container-inhabiting species *Ae. japonicus* (Schaffner & Ries 2019), and in an intuitive sampling set (Schaffner 2022). Thus, 32 precise locations are known from the literature. In addition, one location is known from citizen reports (Fig. 20) and 78 sites were identified during our field study (Fig. 62). In total (111 sites), the species was collected in 71 5x5 km grid squares (out of 137; 51.8%) and 67 communes (out of 102; 65.7%) distributed over all 12 cantons. Thus the species can be considered as common in the Grand Duchy.

During our study, *Ae. geniculatus* was found in 90 larval samplings and 2 adult catches (Fig. 30). Immature samples were collected in all seasons but mostly during spring (n=57 samples; 63.3%) and summer (n=29; 32.2%) months, but also in winter (n=3; 3.3%) and autumn (n=1; 1.1%) months (Fig. 58). A total of 1,096 larvae and 79 pupae were sampled, and 4 adult females were caught. The latter were collected by human landing catches in June and July.

Most of the samples were collected in natural containers, i.e. tree holes (n=75; 90.4%), while the species was also observed to breed in man-made containers (n=8; 9.6%), i.e. tyres (n=5), tarpaulin (n=2) and other containers (n=1) (Figs 59; 61).

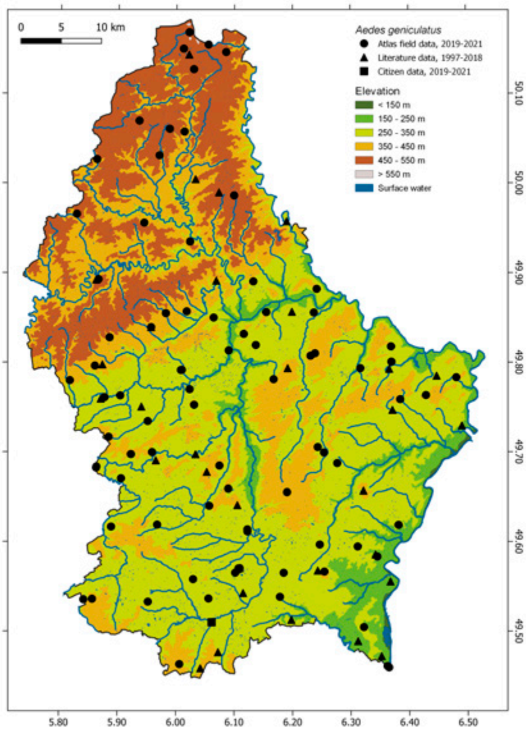


Fig. 62: Distribution of *Aedes geniculatus* (black) in Luxembourg.

Positive tree holes were mainly found in beech trees (*Fagus sylvatica*, n=81) but also in oak and common hornbeam (*Quercus* sp. and *Carpinus betulus*; N=8 each), more rarely in ash and lime (*Fraxinus excelsior* and *Tilia* sp.; n=1 each) (Fig. 60).

This Palaearctic species is common in most of Europe, where deciduous forests occur; it is also present in parts of North Africa and the Middle East (Robert et al. 2019).

4.2.4 *Aedes (Hulecoeteomyia) japonicus*

The subgenus *Hulecoeteomyia* Theobald 1904 comprises 18 species that are distributed mainly across the Oriental region (Wilkerson et al. 2021). However, two species have been shown to be invasive and subsequently occur in Europe since the beginning of the current century: *Ae. japonicus* and *Ae. koreicus* (Edwards 1917). Adults of this subgenus have pale bands on hind tarsomeres I–III (Becker et al. 2020).

Aedes (*Hul.*) *japonicus* (Theobald 1901)

= *Hulecoeteomyia japonica* (Theobald 1901), sensu Reinert et al. (2009)

Informal names: en: Tokyo Japanese pointy mosquito, Asian bush mosquito, Asian rock pool mosquito; de: Asiatische Buschmücke, Japanischer Buschmoskito; lb: Japanesch Iergermustik; fr: Moustique pointu japonais.

The Japonicus Group was recently revised (Wilkerson et al. 2022) and is now considered to comprise, besides *Ae. japonicus*, *Ae. (Hul.) amamiensis* Tanaka, Mizusawa, & Saugstad 1979, *Ae. (Hul.) shintienensis* Tsai & Lien 1950 and *Ae. (Hul.) yaeyamensis* Tanaka, Mizusawa, & Saugstad 1979 (the latter three taxa recently elevated from subspecies of *Ae. japonicus* to the species rank), *Ae. (Hul.) koreicus* (Edwards, 1917), and *Ae. (Hul.) bhutanensis* Somboon & Harbach, 2020. All taxa are native to Asia, and only *Ae. japonicus* is found outside its native range.

Adults of *Ae. japonicus* are medium- to large-sized mosquitoes of blackish appearance with white scales on the body and legs (Figs 63; 148). Proboscis and palps are entirely dark-scaled. The scutum is dark, with a scaling pattern forming five distinct golden-yellowish stripes of scales. Thoracic pleura have patches of white broad scales. Wing veins are dark-scaled, with some pale scales at the base of the costa. The hind tarsomeres I-III (Fig. 31) have broad pale basal rings, while hind tarsomeres IV and V are usually entirely dark-scaled, sometimes with a few pale scales or an incomplete ring on hind tarsomere IV. Abdominal

Fig. 63: Adult female of *Aedes japonicus*. Photo: Francis Schaffner.

terga II–VII are dark-scaled with lateral patches of white scales at the base of each segment and often with a basomedian patch of pale scales (Becker et al. 2020). Larvae are usually large and have a relatively long siphon with a pecten covering an extensive part of the siphon and with large and spaced distal teeth.

Aedes japonicus is a multivoltine species. It displays a winter diapause, at the egg stage in the northern parts of its distribution area, and at the larval stage in the southern part. The larvae are present from the beginning of spring to the end of autumn. They develop in a large variety of small natural or artificial breeding sites. They prefer shady waters, rich in organic matter, and rock holes seem to be their preferred sites in their native range. In Europe, they have been observed mainly in used tyres, rain water casks, vases, catch basins, small plastic containers, tarpaulins, and tree holes (Medlock et al. 2015). Its successful colonisation of Europe and North America has been attributed to its larval habitat plasticity and to its adult cold tolerance which allows a prolonged seasonal activity range in temperate climates (Wilkerson et al. 2021).

An environmental risk assessment according to the ISEIA protocol (Invasive Species Environmental Impact Assessment; Branquart 2009) highlighted that the species shows a high dispersal potential, a medium risk of colonisation of high conservation value habitats, a medium risk of adverse impacts on native species and a low risk of alteration of ecosystem functions (Ries et al. 2017). A detailed risk assessment according to the Harmonia+ protocol (D'Hondt et al. 2015) confirms the relatively low environmental impact of *Ae. japonicus*, similar to the one of *Ae. albopictus*, and a medium animal and human impact score, but lower than the one of *Ae. albopictus* (Schaffner & Ries 2019).

Females of *Ae. japonicus* prefer forested areas and usually bite during the daytime, outdoors. They feed on a great variety of mammals and birds such as dogs, pigs, deers, rodents or chickens. They are more reluctant to bite humans and not as aggressive biters as floodwater mosquitoes like *Ae. vexans* or *Ae. sticticus* (Becker et al. 2020). In the laboratory, the species is a vector of Japanese encephalitis and West Nile viruses, and to some degree also of dengue, chikungunya and Zika viruses. Individuals naturally infected with Usutu

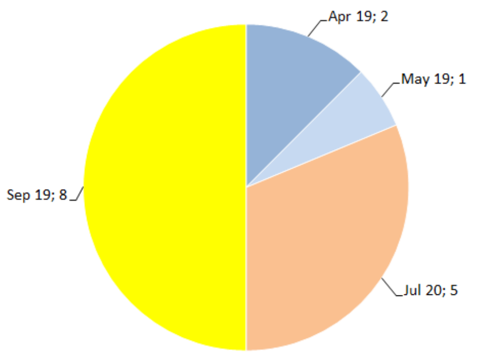


Fig. 64: Temporal distribution of *Aedes japonicus* immature samples, Luxembourg, 2019-2021 (16 samples). Blue: spring months; Orange: summer months; Yellow: autumn months.

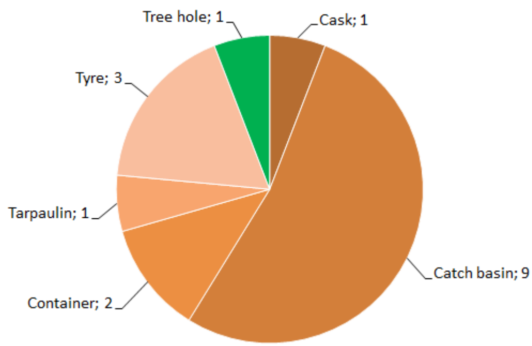


Fig. 65: Categories of larval habitats in which *Aedes japonicus* was observed, Luxembourg, 2019-2021 (16 sites; 17 container type units).

and West Nile virus were collected in Austria and the USA, respectively (Schaffner et al. 2013b; Martinet et al. 2019; Vilibic-Cavlek et al. 2020).

The taxon *Ae. japonicus* is known to occur in Luxembourg since 2018 and the first targeted study revealed its presence at 18 sites (Schaffner & Ries 2019). We here report three additional sites from citizen data (Fig. 20) and 16 sites from our field study (Fig. 67). In total (37 sites), the species is known from 26 5x5 km grid squares (out of 137; 19.0%) and 25 communes (out of 102; 24.5%) distributed over 9 cantons out of 12. Thus the species can be considered as common in the Grand Duchy.

Fig. 66: Larval habitats of *Aedes japonicus* in Luxembourg: a. Garden plastic container (site ATL044, Mertert, 07/04/19); b. Tyre under vegetation (site ATL500, Erpeldange-sur-Sûre, 10/07/20, in association with *Anopheles plumbeus* and *Culex pipiens*). Photos: Francis Schaffner.

In our field study, the species was found in 16 larval samplings (yielding 57 larvae and 36 pupae) that were collected mainly during autumn months (n=8 samples; 50,0%) but also during summer (n=5; 31.3%) and spring (n=3; 18.8%) months (Fig. 64).

Most of the samples were collected in artificial containers (n=16; 93.8%), while the species was also observed to breed in one tree hole. Positive man-made containers were mainly catch basins (n=9 out of 16; 56.3%) (Figs 61a; 65; 66).

The native range of *Ae. japonicus* includes southern China, Japan, Korea, southern Russia, and Taiwan (Tanaka et al. 1979). The species was first established in North America in the late 1990s, and a few years later in Europe (Belgium, 2002) where it is now reported from 16 countries, mainly in central Europe, plus Spain (ECDC 2022). The

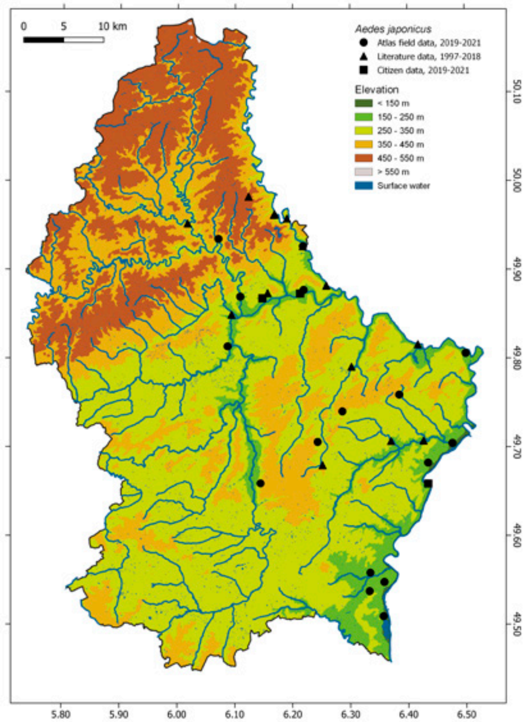


Fig. 67: Distribution of *Aedes japonicus* (black) in Luxembourg.

species was first detected in Luxembourg in July 2018, thanks to a mosquito sample collected by a private individual and sent to the MNHNL. Subsequently, two field surveys were performed to assess the distribution of the species in the Grand Duchy, revealing its presence over at least 550 km^2 (21% of the country land), in the eastern part of the country (Schaffner & Ries 2019). Our present study shows a clear spread of the species towards the south and west, with a known distribution range of around 1,000 km^2 by August 2021 (Fig. 67). Since the local climatic and environmental conditions are suitable to the species (Wint et al. 2020) and preferred larval habitats are abundant, the whole Grand Duchy may be colonised within a few years.

4.2.5 *Aedes (Ochlerotatus) annulipes*

The subgenus *Ochlerotatus* Lynch Arribálzaga comprises around 190 species and subspecies (Wilkerson et al. 2021). Morphological heterogeneity in the subgenus is high, and several species groups show distinct characters (Becker et al. 2020):

- the Annulipes Group, with adults showing tarsi with broad basal rings, represented in Europe by the ten species *Ae. annulipes*, *Ae. behningi* (Martini 1926), *Ae. cantans*, *Ae. cyprius* Ludlow 1919, *Ae. eudes* Howard, Dyar & Knab 1912, *Ae. excrucians* (Walker 1856), *Ae. flavescens* (Müller 1764), *Ae. mercurator* Dyar 1920, *Ae. riparius* Dyar & Knab 1907, and *Ae. surcoufi* (Theobald 1912);
- the Caspius Group, with adults having tarsi with pale rings comprising two tarsomeres (the apex of one and the base of the following), represented by the six species *Ae. berlandi* Séguy 1921, *Ae. caspius* (Pallas 1771), *Ae. dorsalis* (Meigen 1830), and *Ae. pulcritarsis* (Rondani 1872);
- the Communis Group, with adults having entirely dark-scaled tarsi, represented by the ten species *Ae. cataphylla* Dyar 1916, *Ae. coluzzii* Rioux, Guilvard & Pasteur 1998, *Ae. communis*, *Ae. detritus* Haliday 1833, *Ae. hungaricus* Mihályi 1955, *Ae. impiger* Walker 1848, *Ae. leucomelas* (Meigen 1804), *Ae. nigrinus* (Eckstein 1918), *Ae. pionips* Dyar 1919 and *Ae. sticticus*;
- the Intrudens Group, with adults also having entirely dark-scaled tarsi but particular differences can be found in the male hypopygium, which are unique for this group represented by the three species *Ae. diantaeus* Howard, Dyar & Knab 1912, *Ae. intrudens* Dyar 1919 and *Ae. pullatus* (Coquillett 1904);
- and the Punctor Group with adults also having entirely dark-scaled tarsi but members of this group differ from the others by the structure of the abdominal segment X of the larvae (the saddle surrounds completely or almost completely the anal segment), represented by the four species *Ae. hexodontus* Dyar 1916, *Ae. nigripes* (Zetterstedt 1838), *Ae. punctodes* Dyar 1922 and *Ae. punctor*.

From the Annulipes Group, only *Ae. annulipes* and *Ae. cantans* have been observed in Luxembourg to date. Adults of these two taxa closely resemble each other and, despite a slightly different scaling pattern on the scutum, can hardly, if not at all, be separated. The same accounts for the larval stage, and only the examination of the male genitalia allows an accurate sorting of these two species. Thus, many of our findings are attributed to *Ae. annulipes/cantans*, while samples for which male genitalia could be examined are attributed to one of these two species. Moreover, *Ae. annulipes* and *Ae. cantans* often share the same larval habitats and other biological traits. Therefore, and similarly to *Ae. cinereus/geminus*, we hereafter address *Ae. annulipes/cantans* and mention the specificities of each of them.

Fig. 68: Adult female of *Aedes annulipes*. Photo: Anders Lindström.

Fig. 69: Adult female of *Aedes cantans*. Photo: Anders Lindström.

Aedes (*Och.*) *annulipes* (Meigen 1830)

= *Ochlerotatus (subgenus uncertain) annulipes* (Meigen 1830), sensu Reinert et al. (2009)

Informal names: en: Ring-footed European pointy mosquito; de: Ringelfüßige Spitzstechmücke; lb: Réngelfouss-Iergermustik; fr: Moustique pointu annelé.

Aedes (*Och.*) *cantans* (Meigen 1818)

= *Ochlerotatus (subgenus uncertain) cantans* (Meigen 1818), sensu Reinert et al. (2009)

Informal names: en: Singing European pointy mosquito; de: Singende Spitzstechmücke; lb: Sangereg Iergermustik: fr: Moustique pointu chantant.

Adults of *Ae. annulipes/cantans* are large- to medium-sized mosquitoes (Figs 68; 69). The proboscis and the palps have mixed pale and dark scales, the latter more numerous in *Ae. cantans*. The scutum has a defined median stripe of brown scales, and the lateral parts are covered with creamish or greyish scales; the median brown stripe is more obvious in *Ae. annulipes*. Thoracic pleura have large patches of broad white scales. The wing veins are covered with intermixed dark and pale scales; dark scales are more numerous in *Ae. cantans*. The femora and tibia have mixed dark and pale scales, with pale scales more numerous in *Ae. annulipes*, and all legs have a white knee spot. Tarsomere I has mixed dark and pale scales, and tarsomeres II–V have broad white basal rings, usually wider in *Ae. annulipes*. The abdominal terga I-VIII have white basal bands, sometimes narrow and indistinct in *Ae. cantans*; the apical parts of the terga have some pale scales mixed among the darker ones, and pale scales are more numerous in *Ae. cantans*. The general colouration of the integument in *Ae. annulipes* is more brownish and the scaling more yellowish than in *Ae. cantans* (Becker et al. 2020). Larvae have a medium-sized siphon, with a regular pecten (teeth evenly spaced).

Both *Ae. annulipes* and *Ae. cantans* have only one generation per year. In some cases, and in particular when the larval sites are belatedly flooded, the emergence can be postponed, but always with poor densities (Schaffner et al. 2001). The larvae appear at the end of the winter, and they develop in open meadow pools, at forest edges and in deciduous forests, preferentially in temporary, or at the edge of, semi-permanent pools with dead leaves, where both species are often found together (Becker et al. 2020). Adults are present over several weeks to months during late spring and summer; they do not fly far away from the larval habitat. The eggs are laid on the ground of dried-out breeding sites. They go into diapause until the next season.

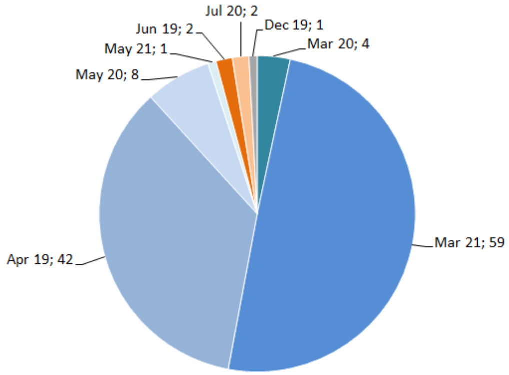


Fig. 70: Temporal distribution of *Aedes annulipes/cantans* immature samples, Luxembourg, 2019-2021 (119 samples). Blue: spring months; Orange: summer months; Green: autumn months; Grey: winter months.

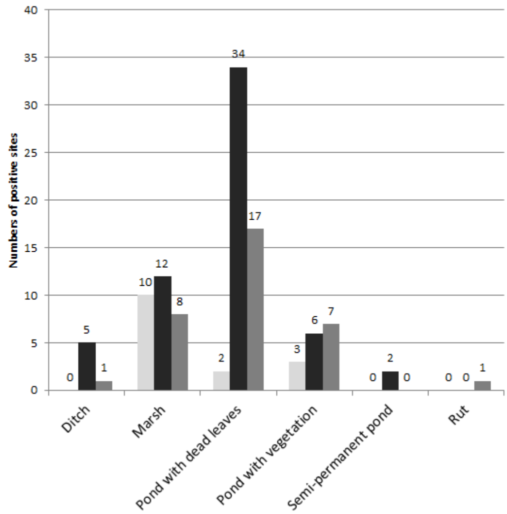


Fig. 71: Types of larval habitats in which *Aedes annulipes* (black), *Aedes annulipes/cantans* (deep grey) and *Aedes cantans* (light grey) were observed, Luxembourg, 2019-2021 (104 sites).

The females are day biters with a crepuscular activity; they feed on mammals and frequently on humans. When abundant they can generate some nuisance which justifies control campaigns. They have been naturally infected by Tahyna, West Nile and myxomatosis viruses and by *Setaria* nematodes (Schaffner et al. 2001).

Few literature occurrence data exist (n=10). Both species were first collected in 2001 (Beck et al. 2001, 2003) and two other studies reported precise presence data (Muller & Reye 2012; Schaffner

Fig. 72: Larval habitats of *Aedes annulipes* (a,b) and *Aedes cantans* (c,d) in Luxembourg: a. Marsh (site ATL081, Godbrange, Junglinster, 11/04/19); b. Pond with dead leaves (site ATL337, Bergem, Mondercange, 19/05/20, in association with *Anopheles maculipennis* s.l. and *Culiseta annulata*); c. Ditch (site ATL047, Roudenhaff, Echternach, 07/04/19, in association with *Aedes cinereus/geminus* and *Aedes rusticus*); d. Pond with dead leaves (site ATL368, Berdorf, 12/03/21, in association with *Aedes rusticus*). Photos: Francis Schaffner.

2022; Tab. 2). Our Atlas field study allowed us to show the presence of *Ae. annulipes/cantans* at 104 sites. In total (114 sites), the species is known from 61 5x5 km grid squares (out of 137; 44.5%) and 54 communes (out of 102; 52.9%) distributed over all 12 cantons, but mainly in the Gutland (Fig. 73). The species can be considered as common in the Grand Duchy.

In our study we collected *Ae. annulipes/cantans* in 119 larval samplings and 25 adult catches (Fig. 30). A vast majority of the larval samples was collected in spring months (n=114 samples; 95.8) and a few samples in summer months (n=4; 3.4%; Fig. 70). A single finding in winter (11/12/2019; 1 larva of *Ae. cantans*) was subsequent to egg hatching following autumnal flooding of a breeding site, in which *Ae. rusticus* was also developing. In total, we collected 2,930 larvae and 63 pupae of *Ae. annulipes/cantans*, and we caught 148 females and 66 males. The latter adults were caught in resting catches in the vegetation, human landing catches and CO_2-baited traps (n=16, 6 and 3 samplings, respectively) at, or in the proximity of, larval habitats, at different dates between May and August.

Almost all immature samples were collected in stagnant temporary water bodies (n=101; 97.1%), while two samples originated from (semi-) permanent water bodies (2 ponds) and one from a semi-natural water body (a rut without vegetation) (Figs 71; 72). Among the stagnant temporary water bodies, undergrowth ponds with dead leaves were the most frequently inhabited (n=52 out of 101, 51.5%), followed by marshes and ponds with vegetation (n=28 and 15; 27.7 and 14.9%, respectively). The remaining sites were ditches (n=6; 5.9%).

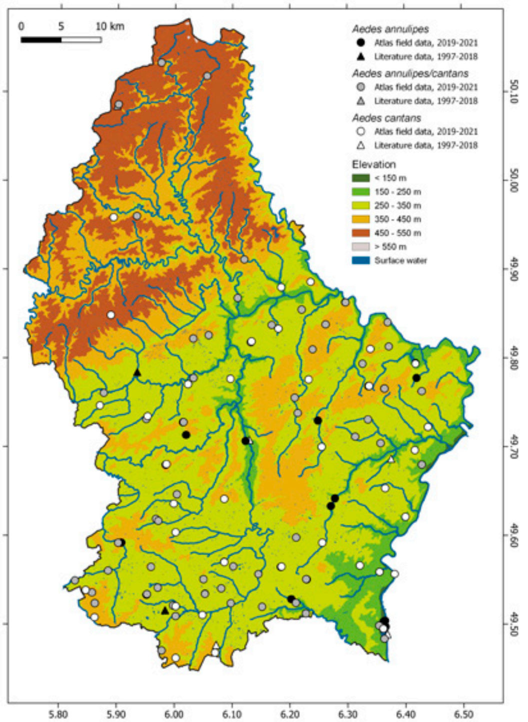


Fig. 73: Distribution of *Aedes annulipes* (black), *Aedes annulipes/cantans* (grey) and *Aedes cantans* (white) in Luxembourg.

Identification to the species level (based on examination of male genitalia) could be performed for about half of the positive sites (n=50 out of 104; 48.1%). Both *Ae. annulipes* and *Ae. cantans* were observed in sympatry at 4 sites, while they were observed singly at 14 and 32 sites, respectively. Comparing larval habitats of both species did not reveal any specific preferences in our study (Fig. 71).

Both *Ae. annulipes* and *Ae. cantans* have a western Palaearctic distribution and are widespread across the European continent. They are more abundant in the central parts of the continent, and *Ae. annulipes* is rare in southern Europe (e.g. absent from the Iberian Peninsula).

4.2.7 *Aedes (Ochlerotatus) communis*

Two species from Communis Group are reported in Luxembourg to date: *Ae. communis* and *Ae. sticticus*.

Fig. 74: Adult female of *Aedes communis*. Photo: Anders Lindström.

Aedes (*Och.*) *communis* (De Geer 1776)

= *Ochlerotatus (subgenus uncertain) communis* (de Geer 1776), sensu Reinert et al. (2009)

Informal names: en: Communal European pointy mosquito; de: Gewöhnliche Spitzstechmücke; lb: Gewéinlech Iergermustik; fr: Moustique pointu commun.

Adults of *Ae. communis* are medium-sized mosquitoes (Fig. 74). The proboscis and palps are dark-scaled; rarely, a few pale scales are scattered on the palps. The scutum is covered with yellow and dark scales; the latter form a broad median stripe and posterior submedian stripes, separated by narrow stripes of pale scales. The thoracic pleura have patches of pale scales. The femora, tibiae and tarsomere I of all legs are mostly dark-scaled dorsally, with a few whitish scales ventrally, and the remaining tarsomeres are entirely dark-scaled (Becker et al. 2020). The wing veins are covered with dark scales, and a few pale scales are scattered at the base of the costa and radius. The terga are dark-scaled with broad basal bands of white scales, often larger laterally. Larvae have a moderately long siphon, with a regular pecten.

Aedes communis is a snowmelt mosquito, whose larvae are the first to pupate in spring, along with *Ae. punctor*. The species is univoltine but hatchings can be observed later within the season, particularly after a dry spring. Larvae of this sylvatic species hatch at the end of the winter, and develop mainly in ponds and ditches with dead leaves at the bottom, under a cover of *Alnus* sp., *Betula* sp., *Quercus* sp., *Salix* sp., or of conifers, but also in

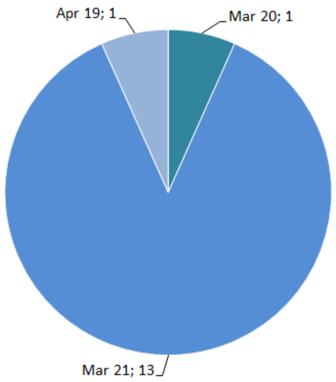


Fig. 75: Temporal distribution of *Aedes communis* immature samples, Luxembourg, 2019-2021 (16 samples). Blue: spring months.

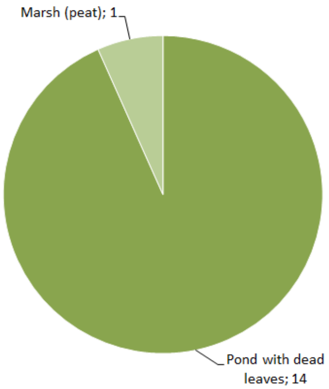


Fig. 76: Categories of larval habitats in which *Aedes communis* was observed, Luxembourg, 2019-2021 (15 sites). *Aedes communis* has a Holarctic global distribution. The species is found from Northern Europe to the Mediterranean region, but is absent from the Iberian Peninsula, North Africa and the Middle East (except some historical records from Turkey) (Robert et al. 2019).

breeding sites with aquatic vegetation (e.g. *Phragmites* sp., *Carex* sp.) (Schaffner et al. 2001; Becker et al. 2020). They can also be found in peat bogs and rock holes. Adults are present in spring and summer. Females do not disperse far from their larval habitat, and lay their eggs on the dried-out ground. Eggs are overwintering.

Females of *Ae. communis* feed on birds and mammals, humans included. They bite in the shade and after sunset or in the first hours of the day. Considering their usual low densities, females do not generate a particular nuisance problem. The

Fig. 77: Typical larval habitats of *Aedes communis* in Luxembourg: pond with dead leaves (site ATL575, Stegen, Vallée de l'Ernz, 16/03/21, in association with *Aedes annulipes/cantans* and *Aedes rusticus*). Photo: Francis Schaffner.

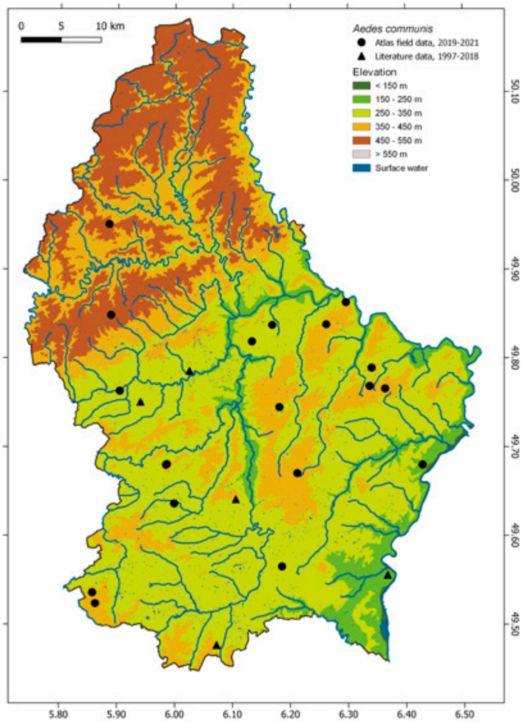


Fig. 78: Distribution of *Aedes communis* (black) in Luxembourg.

species is suggested to act as a vector of Sindbis virus. Some females have been found to be naturally infected by Batai and Inkoo viruses, as well as by tularaemia bacteria. In the laboratory, they can transmit Tahyna virus (Schaffner et al. 2001).

Aedes communis was first detected in Luxembourg in adult trappings performed in 2011 (Muller & Reye 2012; Tab. 2), with five different positive sites. Our field study yielded the finding of the species at 18 additional sites. In total (23 sites), the species is known from 22 5x5 km grid squares (out of 137; 16.1%) and 20 communes (out of 102; 19.6%) distributed over 10 cantons out of 12, but mainly in the Gutland (Fig. 78). The species can be considered as common in the Grand Duchy.

Aedes communis was found in 15 larval samplings and 4 adult catches (Fig. 30). Immature samples were collected exclusively during spring months (March and April; Fig. 75) and adult catches (1 human landing catch and 5 resting catches) were positive in May only (2020 and 2021), all these close to the larval habitat. In total, we collected and identified 230 larvae and 30 pupae of *Ae. communis* and we caught 6 females.

All immature samples were collected in stagnant temporary water bodies, i.e. ponds with dead leaves (n=15 out of 15; 93.3%), while the species was also observed to breed in one peat bog marsh (Figs 76; 77).

4.2.8 *Aedes (Ochlerotatus) punctor*

Aedes punctor is the only species from the Punctor Group that is reported from Luxembourg to date.

Aedes (*Och.*) *punctor* (Kirby 1837)

= *Ochlerotatus (subgenus uncertain) punctor* (Kirby 1837), sensu Reinert et al. (2009)

Informal names: en: Provocative Canadian pointy mosquito; de: Provozierende Spitzstechmücke; lb: Provokant Iergermustik; fr: Moustique pointu provocateur.

Adults of *Ae. punctor* are medium-sized mosquitoes (Fig. 79). The proboscis and palps are dark-scaled. The scutum is covered with yellowish-brown scales, usually with a median stripe of dark-brown scales, and the posterior submedian areas with dark-brown scales (Becker et al. 2020). The thoracic pleura have patches of pale scales. The wing veins are almost entirely dark-scaled. The femora, tibiae and tarsomeres are mostly dark-scaled. The abdominal terga are dark-scaled with basal bands of white scales, which are distinctly narrowed in the middle or even interrupted on the more anterior terga.

Aedes punctor is a snowmelt mosquito that seems to be univoltine. Larvae hatch during the snowmelt, thus are mainly found in winter and early spring, but additional egg hatchings occur later in the season until autumn, after heavy rain and larval habitat flooding (Schaffner et al. 2001). Immatures develop in swampy forests, preferably in acid boggy waters with *Sphagnum* sp. Adults emerge in the second half of April, a bit later than those of *Ae. communis*, but earlier than *Ae. annulipes/cantans* (Becker et al. 2020). The adults prefer sheltered terrain and seldom migrate out of the forest.

Females of *Ae. punctor* bite all warm-blooded vertebrates, mainly at dusk or in strongly shaded areas; they can be troublesome even during daytime (Becker et al. 2020). Females have been naturally infected by tularaemia bacteria, Batai and Inkoo viruses. It is a vector of Tahyna and West Nile viruses in the laboratory (Schaffner et al. 2001).

Aedes punctor was first detected in Luxembourg in 2001 (Beck et al. 2001, 2003) and later reported in two studies (Muller & Reye 2012; Schaffner 2022; Tab. 2), with three different positive sites in total. Our Atlas field study allowed us to locate the species at 12 additional sites. In total (15 sites), the species is known from 13 5x5 km grid squares (out of 137; 9.5%) and 9 communes (out of 102; 8.8%) distributed over 6 cantons out of 12, in both the Oesling and the Gutland (Fig. 83). The species can be considered as common in the Grand Duchy.

Aedes punctor was detected in 11 larval samplings and 2 adult catches (Fig. 30). Similarly to *Ae. communis*, immature samples of *Ae. punctor* were collected mainly during spring months (April-May; n=8 samples; 72.7%) but also in winter (March; n=3; 27.3%) (Fig. 80). In total, we collected and identified 123 larvae and 4 pupae of *Ae. punctor* and we caught 4 females. These adult catches (1 human landing catch and 1 resting catch) were positive in May only (2020), close to the larval habitat.

All immature samples were collected in stagnant temporary water bodies, mainly marshes (n=7 out of 11; 63.6%), of which two were peat bogs (Figs 81; 82).

This circumpolar species has a distribution area extending to the whole of Europe, and parts of North Africa and the Middle East (Anatolia, Turkey; Robert et al. 2019). Very common in Scandinavia, the species is rarer in other regions, except in peaty areas at higher altitude (Schaffner et al. 2001).

Fig. 79: Adult female of *Aedes punctor*. Photo: Anders Lindström.

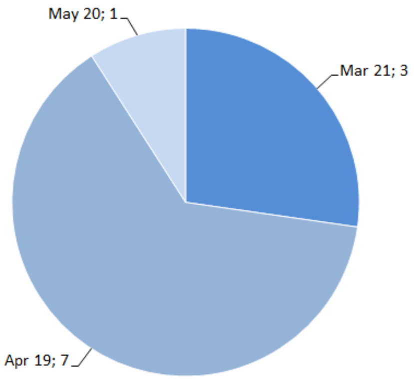


Fig. 80: Temporal distribution of *Aedes punctor* immature samples, Luxembourg, 2019-2021 (11 samples). Blue: spring months.

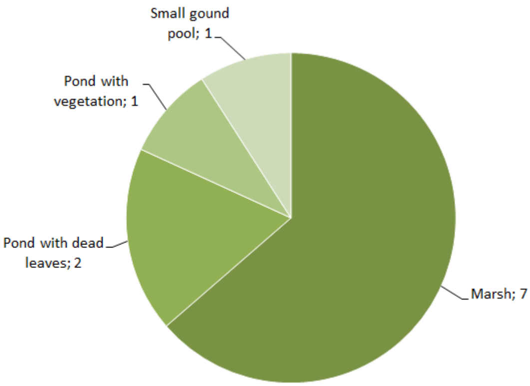


Fig. 81: Categories of larval habitats in which *Aedes punctor* was observed, Luxembourg, 2019-2021 (11 sites).

Fig. 82: Typical larval habitats of *Aedes punctor* in Luxembourg: peat marsh (site ATL050, Wilwerdange-Wemperhaardt, Troisvierges, 08/04/19, in association with *Culiseta morsitans*). Photo: Francis Schaffner.

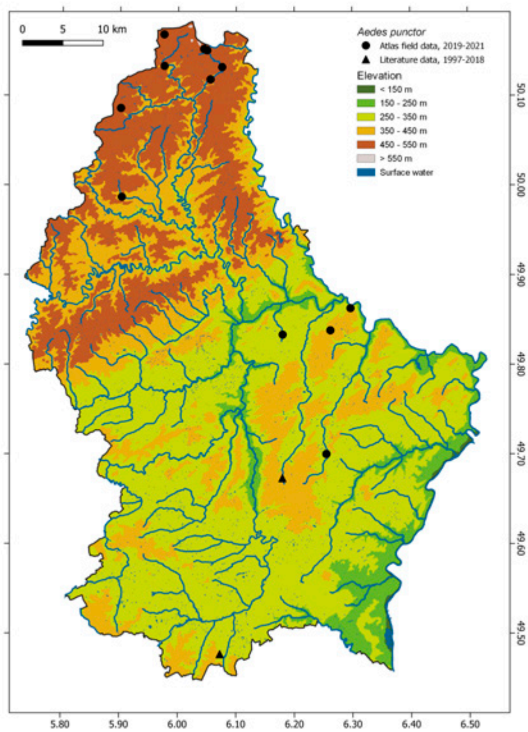


Fig. 83: Distribution of *Aedes punctor* (black) in Luxembourg.

4.2.9 *Aedes (Ochlerotatus) sticticus*

Aedes sticticus is the second species from the Communis Group that is reported in Luxembourg to date.

Aedes (*Och.*) *sticticus* (Meigen 1838)

= *Ochlerotatus (subgenus uncertain) sticticus* (Meigen 1838), sensu Reinert et al. (2009)

Informal names: en: Spotted German pointy mosquito; de: Gefleckte Spitzstechmücke; lb: Getëppelt Iergermustik; fr: Moustique pointu tacheté.

Adults of *Ae. sticticus* are medium-sized brownish mosquitoes, with the proboscis and palps dark-scaled (Fig. 84). The scutum has a dark, median longitudinal stripe and lateral parts are pale-scaled. Thoracic pleura have well-developed patches of white scales. The legs are dark-scaled dorsally and mostly pale-scaled ventrally, without pale rings. The wing veins are usually entirely dark-scaled. The abdominal terga are dark-scaled, terga II–IV have pale basal bands distinctly constricted in the middle, and on the following terga, the basal bands are interrupted, forming triangular pale patches at the lateral sides (Becker et al. 2020). The extremity of the abdomen is pointed and cerci are elongated. Larvae have a medium-sized siphon, bearing a pecten with evenly spaced teeth.

Aedes sticticus is a multivoltine and summer species. The larvae are present from spring to the end of summer depending on the floods. The species is abundant in the cold regions of Europe and present in lower densities in freshwater areas of the Mediterranean coast. Immatures develop in flood plain freshwaters, typically under trees (riparian forest), and occasionally in meadows, ponds and ditches. The species is commonly associated with *Ae. vexans* and *Ae. cinereus/geminus*. Females can disperse considerable distances when searching for a blood meal, e.g. 20 km or more. The eggs are laid in drained dips. They are overwintering and remain viable for 2-3 years in the ground (Becker et al. 2020).

Females of *Ae. sticticus* preferentially bite mammals and can generate severe nuisance to humans, outdoors and at sunset, when developing in huge numbers after spring and summer floods. The species is not known as a significant vector species, but females have been found in nature infected by various pathogens such as Inkoo, Tahyna and West Nile viruses or *Setaria tundra* nematodes and tularaemia bacteria; they are also laboratory competent to transmit Tahyna virus (Schaffner et al. 2001; Czajka et al. 2012).

Aedes sticticus was first detected in Luxembourg in 2001 (Beck et al. 2001, 2003) and later reported in a single data set (Zittra et al. 2021; Tab. 2), with four different positive sites in total. Our atlas field study located the species at four additional sites. In total (six sites), the species is known from only four 5x5 km grid squares (out of 137; 2.9%), five communes (out of 102; 4.9%; Bettembourg, Biwer, Leudelange, Luxembourg/Kockelscheuer, Rumelange) and four cantons of the Gutland only (Fig. 56). Similarly to *Ae. vexans*, the species can be considered as rare in the Grand Duchy.

Aedes sticticus was collected in four larval samplings and four resting catches (Fig. 30). Immature samples were collected in spring only (May 2021), while adults were caught by human landing catches in summer, i.e. June 2019 and August 2021 (2 catches each, also with resting catches in August). Larvae were collected in temporary water bodies: flooded forest (n=2) and meadows (n=1), as well as in a group of puddles and ruts with little vegetation (Fig. 85). In total, we collected 61 larvae and we caught 68 females. The species was collected together with *Ae. cinereus/geminus* and *Ae. vexans*, among others, in both larval samplings and adult catches.

This Holarctic species is widespread in Europe, from Scandinavia to the Mediterranean and to Siberia in the east (Becker et al. 2020).

Fig. 84: Adult female of *Aedes sticticus*. Photo: Anders Lindström.

Fig. 85: Larval habitats of *Aedes sticticus* in Luxembourg: a. Flooded forest (site ATL111c, Kockelscheuer, Luxembourg, 28/05/21, in association with *Culiseta annulata*); b. Flooded grassland (site ATL601, Kockelscheuer, Luxembourg, 27/05/21, in association with *Culex pipiens*). Photos: Francis Schaffner.

4.2.10 *Aedes (Rusticoidus) refiki*

The subgenus *Rusticoidus* Shevchenko & Prudkina 1973 comprises the nine species that were formerly listed as a morphological group of the subgenus *Ochlerotatus* (Becker et al. 2020; Wilkerson et al. 2021). Six species occur in the western Palaearctic region: *Ae. lepidonotus* Edwards 1920, *Ae. quasirusticus* Torres Cañamares 1951, *Ae. rusticus*, *Ae. refiki*, *Ae. subdiversus* Martini 1926 and *Ae. krymmontanus* Alekseev 1989. Adults of that subgenus have a postprocoxal area (thoracic pleura) with a patch of broad dark scales as part of the classical patch of narrow curved pale scales. The larvae are also easily distinguished from all other *Aedes* by having several pairs of setae inserted dorsally on the siphon and a small branched setae located laterally, in addition to the siphonal tuft.

Aedes (*Rus.*) *refiki* Medschid 1928

= *Ochlerotatus* (*Rusticoidus*) *refiki* (Medschid 1928), sensu Reinert et al. (2009)

Informal names: en: Refik Turkish pointy mosquito; de: Refiki-Spitzstechmücke; lb: Refik-Iergermustik; fr: Moustique pointu turc de Refik.

Adults of *Ae. refiki* are medium-sized mosquitoes. The proboscis and palps are predominantly dark-scaled but have scattered pale scales. The integument of the scutum is blackish-brown and covered with yellowish scales and a median stripe of dark scales. The thoracic pleura have large patches of pale scales. The wing veins are covered with dark scales, but with scattered pale scales at the base of the costa, subcosta and radius veins. The femora are predominantly pale-scaled, the tibiae and tarsomeres I have mostly dark scales, and tarsomeres II–V are entirely dark-scaled. The abdominal terga are often covered with dark scales and indistinct pale basal bands not widened in the middle, and scattered pale scales at the distal part of the terga. Scales never form a longitudinal stripe in the middle, in contrast to *Ae. rusticus* (Becker et al. 2020). Larvae have a relatively short siphon with an irregular pecten that ends before the insertion of the siphonal tuft.

Fig. 86: Larval habitats of *Aedes refiki* in Luxembourg: marsh with willows and reeds (site ATL034, Stadtbredimus, 19/03/21, in association with *Aedes annulipes*, *Aedes cantans* and *Aedes rusticus*, also with *Anopheles claviger* s.s., *Culex pipiens/torrentium* and *Culiseta morsitans* on 06/04/19). Photo: Francis Schaffner.

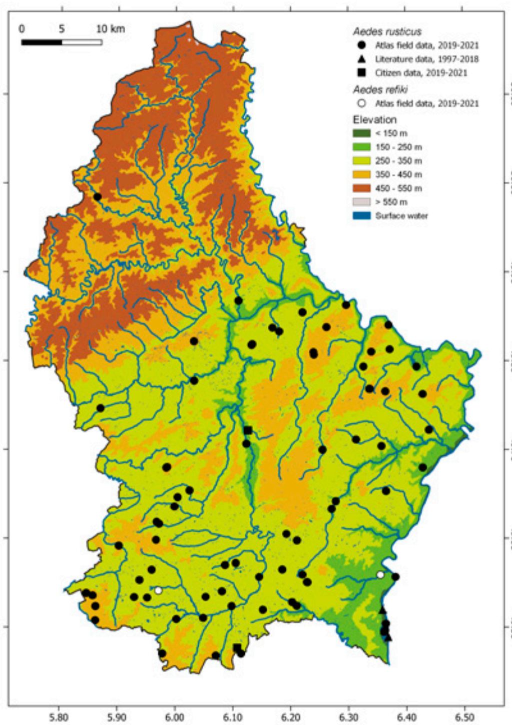


Fig. 87: Distribution of *Aedes refiki* (white) and *Aedes rusticus* (black) in Luxembourg.

Aedes refiki is a univoltine snowmelt mosquito. The larvae can hatch early in autumn or during a mild and rainy winter, and overwinter in the larval stage even when the breeding sites are covered with ice. However, the majority of the population overwinters in the egg stage, and hatching takes place during the snowmelt early in the year. Typical larval habitats are temporary water bodies in swampy woodlands, e.g. with *Alnus* sp. or *Salix* sp., more occasionally open marshes (with *Carex* sp.) or flooded meadows. Adults occur from spring to early summer; they do not disperse and prefer to stay close to their breeding sites in shaded areas (Schaffner et al. 2001; Becker et al. 2020).

Females of *Ae. refiki* bite humans and mammals even during daytime but preferably in shaded places, with a peak of activity at dusk (Becker et al. 2020). They are not known to carry any pathogen of human or animal relevance.

This study provides the first reports of the species in Luxembourg, but *Ae. refiki* was rarely collected, with three larval samplings collected at two different sites from the Gutland only, in two 5x5 km grid squares (out of 137; 1.5%), two communes and two cantons (Mondercange and Stadtbredimus, cantons of Esch-sur-Alzette and Remich, respectively) (Fig. 87). Immature samples (60 larvae and 5 pupae) were collected in spring months only (March 2021 and April 2019; n=1 and 2 samples, respectively). They were collected in temporary water bodies: one ditch with dead leaves at the bottom, and one marsh with reeds and *Salix* sp. (Fig. 86). The species was collected together with *An. claviger* s.s., *Ae. annulipes*, *Ae. cantans*, and *Ae. rusticus*.

This Palaearctic species is distributed mainly across central Europe, but considered a rare species (Becker et al. 2020). It is known from scarce locations in France (personal observations) and from Germany (Kuhlisch et al. 2017) and not known to occur in Belgium and the Netherlands. It is absent from both North Africa and the Middle East except where the species was first described (Turkey, Anatolia; Robert et al. 2019).

4.2.11 *Aedes (Rusticoidus) rusticus*

The taxon *Ae. rusticus* comprises the nominotypical *Ae. rusticus rusticus* and the subspecies *Ae. rusticus subtrichurus* Martini 1927 which is known to occur in Turkey only (Wilkerson et al. 2021).

Aedes (*Rus.*) *rusticus rusticus* (Rossi 1790)

= *Ochlerotatus* (*Rusticoidus*) *rusticus* (Rossi 1790), sensu Reinert et al. (2009)

Informal names: en: Rustic Italian pointy mosquito; de: Rustikale Spitzstechmücke; lb: Rustikal Iergermustik; fr: Moustique pointu rustique.

Adults of *Ae. rusticus* are large-sized mosquitoes (Fig. 88). The proboscis and palps are dark-scaled, with a few pale scales located at the base of the proboscis. The integument of the scutum is blackish-brown and covered with golden-bronze scales and a median stripe of dark scales, usually divided by a narrow stripe of pale scales, and with cream-coloured scales on the lateral parts. The thoracic pleura have large patches of pale scales. The wing veins are predominantly covered with dark scales, with scattered pale scales at the base of the costa, and on the subcosta. The femora have pale yellowish scales on the ventral surface and dark scales on the dorsal surface, the tibiae and tarsomeres I have pale and dark scales intermixed, and tarsomeres II–V are almost entirely dark-scaled. The abdominal terga II-VII are dark-scaled with pale basal bands that are usually widened in their middle and show a tendency to form a longitudinal stripe. Dark parts of the terga often have scattered pale scales (Becker et al. 2020). Larvae have a relatively short siphon with an irregular pecten that ends beyond the insertion of the siphonal tuft.

Aedes rusticus is another univoltine snowmelt mosquito. Larvae are able to hatch following heavy rainfalls in autumn when the larval habitat is flooded. These larvae enter into diapause as second and third larval instars when temperatures are decreasing in autumn, and their development restarts when these temperatures are rising again (around 10°C). Larvae also usually hatch from hibernating eggs in early spring during the snowmelt, in particular if these breeding sites were not flooded in autumn (Schaffner et al. 2001). Larvae of *Ae. rusticus* are often associated with those of *Cs. morsitans* and with overwintering larvae of *An. claviger*, but also with other snowmelt mosquitoes such as *Ae. communis* and *Ae. punctor*. Immatures develop in swampy woodlands, more rarely in floodplains, ditches, or ponds with dead leaves or vegetation, e.g. *Carex* sp. or *Phragmites* sp. Although the larvae of *Ae. rusticus* are the first *Aedes* larvae to occur in the season, adults appear after those of *Ae. communis*, usually in April (Becker et al. 2020). They are present until mid-summer and do not fly far away from the original breeding sites, usually not more than 2 km. Overwintering happens at larval and egg stages.

Fig. 88: Adult female of *Aedes rusticus*. Photo: Anders Lindström.

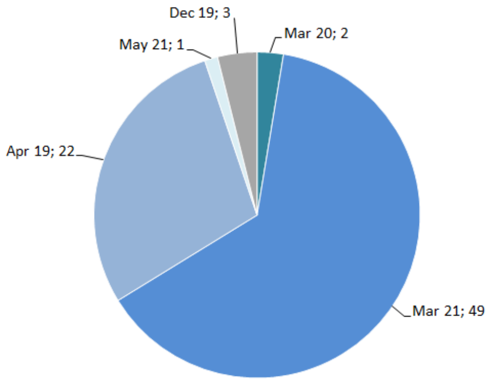


Fig. 89: Temporal distribution of *Aedes rusticus* immature samples, Luxembourg, 2019-2021 (77 samples). Blue: spring months; Grey: winter months.

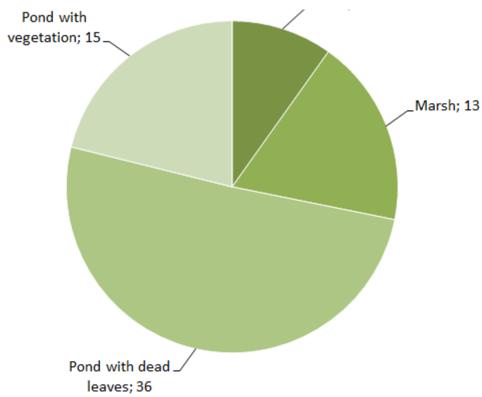


Fig. 90: Categories of larval habitats in which *Aedes rusticus* was observed, Luxembourg, 2019-2021 (71 sites).

Females of *Ae. rusticus* are anthropophilic, mammophilic and ornithophilic. They bite outdoors, mainly at dusk or in shaded areas, and can cause significant nuisance. Thus the species is sometimes targeted by control campaigns in peri-urban forest areas. Females are not known to carry any pathogen of human or animal relevance.

Aedes rusticus was first detected in Luxembourg in 2001 (Beck et al. 2001, 2003) and later reported in two data sets (Muller & Reye 2012; Schaffner 2022; Tab. 2), with three different positive sites in total. In addition, two citizen records are registered (Fig. 20). In our field study we report 71 additional sites for the species. In total (76 sites), the species is known from 49 5x5 km grid squares (out of 137; 35.8%), 44 communes (out of 102; 43.1%) and 10 cantons out of 12, of the Gutland mainly (Fig. 87). The species can be considered as common in the Grand Duchy.

Aedes rusticus was found in 77 larval samplings and 9 adult catches (Fig. 30). The scarcity of the species in the Oesling is probably due to the scarcity of the preferred larval habitats, i.e. swampy woodlands. Unsurprisingly, immature samples were collected during spring (n=74 samples; 96.1%) and winter (n=3; 3.9%) months (Fig. 89). In total, we collected and identified 1,036 larvae and 66 pupae of *Ae. rusticus* and we caught 71 females and 60 males. These adult catches (6 resting catches and 3 human landing catches) were positive in May (2020, 2021) and June (2019) only, close to the larval habitat.

Fig. 91: Typical larval habitats of *Aedes rusticus* in Luxembourg: pond with dead leaves (site ATL547b, Goeblange, Koerich, 23/03/21, in association with *Aedes annulipes/cantans*, *Aedes communis*, and *Culiseta morsitans*). Photo: Francis Schaffner.

Almost all immature samples were collected in stagnant temporary water bodies (n=70 out of 71; 98.6%), with a single one from a (semi-)permanent pond with vegetation. Positive larval habitat types were mainly ponds with dead leaves (n=36; 50.7%) and ponds with vegetation (n=15; 21.1%) (Figs 90; 91).

This Palaearctic species is widely distributed across Europe. It also occurs in North Africa and in Turkey, but not in other parts of the Middle East (Robert et al. 2019).

4.3 Genus *Culex* Linnaeus 1758

Members of the genus *Culex* are usually small- to medium-sized, poorly ornamented mosquitoes. The proboscis is usually dark, sometimes with scattered pale scales, and the length of the female palps is much shorter than the proboscis, while the palps of the males are longer than the proboscis. The scutellum is distinctly trilobed and the thorax pleura have pleural scaling. The wings have narrow unicolour scales on all veins. The legs are usually entirely dark-scaled. The abdomen has tergal plates with coloured scales producing species-specific patterns, is blunt-ended with short, oval cerci (Fig. 31). Larvae have a long or very long siphon. They develop in any standing water body, i.e. artificial and natural containers, (semi-)permanent or temporary water bodies. Eggs are laid in batches forming a raft, and are not resistant to desiccation. Adult females are overwintering at sheltered places. They bite any warm-blooded or cold-blooded host, preferentially at night, indoors or outdoors. Several tropical *Culex* species from Asia and Africa are well known for the transmission of lymphatic filariasis and arboviruses. *Culex* species are also considered the main vectors of West Nile and Usutu viruses, and contribute to the transmission of canine filariasis and tularaemia (Schaffner et al. 2001; Martinet et al. 2019). Some *Culex* species (e.g. the northern house mosquito) are also responsible for severe nuisance, indoors and at night.

The genus *Culex* with more than 750 described species from 24 subgenera worldwide is represented by only a few species in temperate areas. In the European region, species of the subgenera *Barraudius*, *Culex*, *Maillotia* and *Neoculex* occur, and most of them have a Mediterranean and/or Central European distribution (Becker et al. 2020).

4.3.1 *Culex (Barraudius) modestus*

The subgenus *Barraudius* Edwards 1921 comprises only four species of which two occur in the western Palaearctic region: *Cx. modestus* and *Cx. pusillus* Macquart 1850. The second species has a very limited distribution in Europe (Greece only), but is widespread throughout North Africa and the Middle East (Robert et al. 2019).

Culex (*Bar.*) *modestus* Ficalbi 1890

Informal names: en: Modest Italian typical mosquito; de: Bescheidene Stechmücke; lb: Bescheide Mustik; fr: Moustique typique modeste.

Adults of *Cx. modestus* (Fig. 92) appear brownish and are of medium size. The proboscis is dark brown, paler on its ventral surface from the base to the middle and slightly swollen at the apex. The palps and antennae are dark brown. The scutum is brown (both integument and scales). The thoracic pleura (lateral side of the thorax) are pale brown, with small patches of pale scales. The legs are mainly dark brown, with femora and tibia pale-scaled on their ventral surfaces, and tarsomeres entirely dark-scaled. The hind tarsomere I is shorter than the hind tibia (which is the inverse for *Cx. pipiens*). The wings are entirely dark-scaled. The abdominal terga are dark-brown-scaled, transverse pale bands are absent, but lateral pale patches usually form a continuous pale border on either side of the abdomen (Becker et al. 2020). Larvae have a medium-sized siphon, with setae 1-S consisting of 10–12 tufts arranged in a more or less ventral zigzag row.

Fig. 92: Adult female of *Culex modestus*. Photo: Anders Lindström.

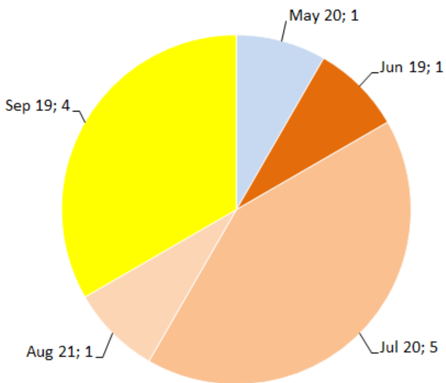


Fig. 93: Temporal distribution of *Culex modestus* immature samples, Luxembourg, 2019-2021 (12 samples). Blue: spring months; Orange: summer months; Yellow: autumn months.

The larvae occur from spring until late autumn, with at least two successive generations. They show a preference for shallow sunlit habitats and are frequently found in meadows, irrigation channels, inundation areas of rivers overgrown by reeds, or rice fields. Other common breeding waters are ground pools, ponds and marshes with rich vegetation, and the water may be fresh or slightly saline (Becker et al. 2020). In Central Europe, adults are more abundant from the beginning of July to late September. Diapausing females overwinter within the vegetation (Rudolf et al. 2020). Females readily bite humans outdoors in the daytime, but mainly at dusk or at night. They sometimes create a serious nuisance for

Fig. 94: Larval habitats of *Culex modestus* in Luxembourg: marsh with reeds and *Chara* sp. (site ATL039d, Haff Réimech, Wintrange, Schengen, 22/09/19, in association with *Anopheles daciae*, also on 05/07/20). Photo: Francis Schaffner.

humans and other mammals in the immediate surroundings of the larval habitats. The females disperse little (less than 1 km).

The species is considered an efficient vector of West Nile virus, and has been found naturally infected with tularaemia bacteria, myxomatosis, Batai, Sindbis, Tahyna and Usutu viruses, and *Dirofilaria immitis* helminths (Schaffner at al. 2001; Scheuch et al. 2018; Becker et al. 2020; Vilibic-Cavlek et al. 2020). Due to its patchy distribution and its limited dispersal ability, the vector role to humans is reduced compared to an enzootic and bridge vector role in nature and rural environments.

Our study provides the first observation of *Cx. modestus* in Luxembourg where the species is rare (Fig. 95). We found it at a single location (two sites), the Haff Réimech nature reserve (Schengen, canton of Remich) in the Moselle valley. Immatures were collected from temporarily-flooded marshes and pond borders with vegetation (e.g. reeds) (Fig. 94), between May and September (Fig. 93). The 12 immature samplings yielded a total of 110 larvae and 9 pupae, and adult catches gathered 4 females.

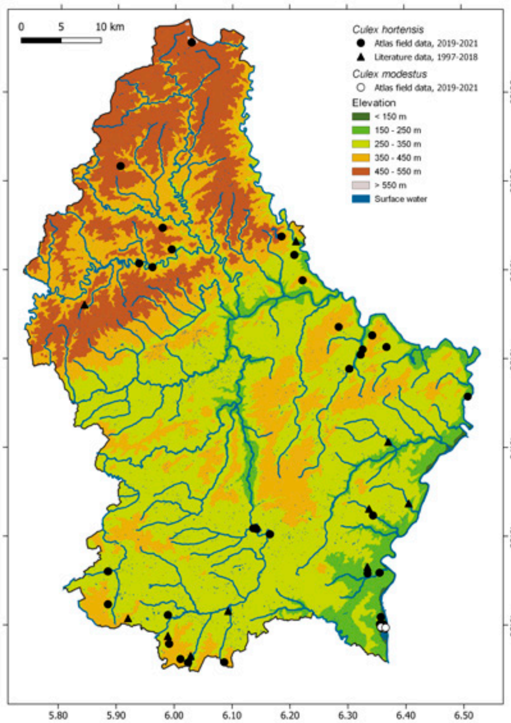


Fig. 95: Distribution of *Culex hortensis* (black) and *Culex modestus* (white) in Luxembourg.

The latter were caught in CO_2-baited traps at the same location on 24/06/2019 and 05/07/2020 (n=2 in both trappings). Resting catches performed in December 2019 and March 2020 in a wood shelter and in stone, wood and vegetation piles remained negative for that species.

Culex modestus is widely distributed in the Palaearctic region from England to Southern Siberia. It is recorded from middle and southwest Asia, northern India and North Africa (Becker et al. 2020). In Europe it is a common species in the southern and central countries, with a patchy distribution in other regions. Recent findings of the species in England (Hernandez-Triana et al. 2020), Denmark (Bødker et al. 2014), Sweden (Lindström & Lilja 2018) and very recently in Belgium (de Wolf et al. 2021), suggest a geographical northwards spread or/and a growth of populations where the species was historically present but poorly or not detected.

4.3.2 *Culex (Culex) pipiens/torrentium*

Culex pipiens/torrentium is a group of species that are hardly distinguishable by morphology, at both larvae and adult stages. In the western Palaearctic region, this group comprises the *Cx. pipiens* complex (*Cx. pipiens* sensu lato) and *Cx. torrentium*.

Culex (*Culex*) *pipiens pipiens* and *Culex* (*Culex*) *torrentium*

The globally distributed *Culex pipiens* complex (or *Culex pipiens* assemblage sensu Harbach 2012) consists of several taxa: the nominate taxon *Cx. pipiens pipiens* and *Cx. quinquefasciatus* Say 1823, *Cx. pipiens pallens* Coquillett 1899, and *Cx. australicus* Dobrotworsky & Drummond 1953 (Harbach 2012). While *Cx. pipiens pipiens* and *Cx. quinquefasciatus* are cosmopolitan species, in temperate and (sub-) tropical regions, respectively, the other taxa, *Cx. pipiens pallens* and *Cx. australicus*, have distributions that are restricted to eastern Asia and Australia, respectively. The degree of separation between these taxonomic entities is still the subject of debate.

The *Cx. pipiens pipiens* taxon includes two behaviourally and genetically distinct forms ('f.'), *Cx. pipiens* f. Pipiens, which is anautogenous, eurygamous and diapausing, and *Cx. pipiens* f. Molestus, which is autogenous, stenogamous and non-diapausing (Wilkerson et al. 2020). These forms do not differ morphologically and are able to hybridise in areas of coexistence (Zittra et al. 2016).

Fig. 96: Adult female of *Culex pipiens*. Photo: Francis Schaffner.

Fig. 97: Adult female of *Culex torrentium*. Photo: Anders Lindström.

The taxon *Cx. torrentium* is a sibling species of *Cx. pipiens*. Because of the similarities and their occurrence in sympatry, we hereafter discuss their morphology and biology simultaneously, highlighting the divergences.

Culex (*Cux.*) *pipiens pipiens* Linnaeus 1758

Informal names: en: Piping Swedish typical mosquito, Northern house mosquito; de: Gemeine Stechmücke, Nördliche Hausmücke; lb: Nërdlech Haus-Mustik; fr: Moustique typique domestique.

In this atlas we use the name *Cx. pipiens* to refer to the European populations of *Cx. pipiens pipiens*, including both Pipiens and Molestus forms.

Culex (Cux.) torrentium Martini 1925

Informal names: en: Torrential German typical mosquito; de: Strömungsliebende Stechmücke; lb: Stroum-Mustik; fr: Moustique typique torrentiel.

Adults of *Cx. pipiens* (Fig. 96) and *Culex torrentium* (Fig. 97) are medium-sized, light-brown mosquitoes with few distinguishing characteristics (Wilkerson et al. 2020). The palps are mainly black-scaled, and the proboscis has cream-coloured scales ventrally. The scutum has delicate golden-brown scales. The thoracic pleura have small pale scale patches. Prealar scales are absent or rare (1-2 scales), while *Cx. torrentium* adults always have one or more prealar scales. The hind femora have mostly whitish scales, whilst the tibiae and tarsi (Fig. 31) are dark-scaled. Wings are entirely dark-scaled. The abdominal terga are predominantly dark-scaled, and terga III–VII have whitish or yellowish narrow basal bands which expand laterally (Becker et al. 2020). Whilst the observation of prealar scales is elusive on field-caught females, adult males can be differentiated based on morphological differences in the genitalia. Larvae have a relatively long and slender siphon, and no morphological feature allows the accurate separation of these species at this stage.

Both species are multivoltine and abundant during the summer and the autumn. Larvae are present from the beginning of spring to autumn. Diapausing females of *Cx. pipiens* f. Pipiens and of *Cx. torrentium* overwinter in cellars, cowsheds, caves and other natural shelters. In a hypogean environment, *Cx. pipiens* f. Molestus develops all year long, with autogenous egg-laying (i.e. without

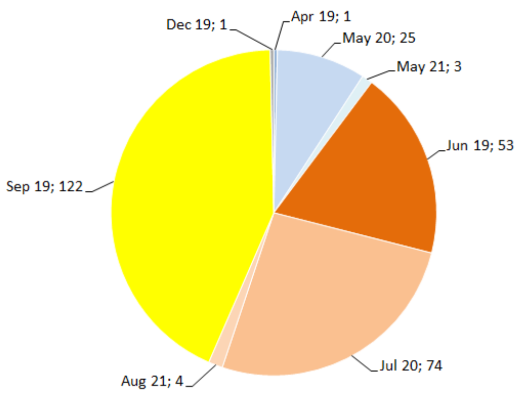


Fig. 98: Temporal distribution of *Culex pipiens/torrentium* immature samples, Luxembourg, 2019-2021 (283 samples). Blue: spring months; Orange: summer months; Green: autumn months; Grey: winter months.

any blood meal). The eggs are laid at the water surface, as rafts of 150 to 240 eggs following each blood meal, or as a single batch of 30 to 80 eggs only in the case of autogenous egg-laying. Immatures of *Cx. pipiens* f. Molestus typically grow in waters polluted with a high rate of organic matter (waste-water drainage ditches, underground cesspits or flooded ventilation space) while those of *Cx. pipiens* f. Pipiens typically develop in clear-water sites, artificial (rainwater casks, basins, containers, drainage holes) or natural (ponds, ditches, lake borders, rice fields, puddles and flooded areas). For both species, the water can also be brackish, very dirty, or rich in iron oxide (Schaffner et al. 2001; Becker et al. 2020).

Females of both species bite warm-blooded vertebrates at night. The *Cx. pipiens* f. Molestus feeds on mammals and is highly anthropophilic, mostly indoors, while the f. Pipiens is predominantly ornithophilic. Hybrid forms are potentially good bridge vectors, e.g. becoming infected by a first blood meal on an infectious bird, and transmitting the acquired pathogen to a mammal a few days later during the following blood meal. In certain cities where wastewater is poorly managed, *Cx. pipiens* f. Molestus can generate considerable nuisance, requiring control campaigns. *Culex pipiens* is a vector of Sindbis, Usutu and West Nile viruses, of *Dirofilaria immitis* helminths and of bird plasmodiums; it is also receptive to Tahyna virus and has been naturally contaminated by tularaemia bacteria (Schaffner et al. 2001; Scheuch et al. 2018; Wilkerson et al. 2020). Females of *Cx. torrentium* are predominantly ornithophilic; they are considered to be the main enzootic vector of Sindbis virus in Sweden (Hesson et al. 2011) and have been found naturally infected by Usutu virus and tularaemia bacteria (Scheuch et al. 2018; Vilibic-Cavlek et al. 2020).

Culex pipiens was the first species to be reported from Luxembourg (Weinachter 1911; Sunnen 1918; Massard 2019; Tab. 2). The literature revealed up to 263 precise locations for at least one taxon of the *Cx. pipiens/torrentium* group, and citizen reports provided four additional locations (Fig. 20). In addition, our atlas field study lists 400 additional sites for the species group. In total (667 sites), the species is known from almost all 5x5 km grid squares (132 out of 137; 96.4%), the six remaining being all partial squares (located at the country borders). The taxon is reported from 99 communes

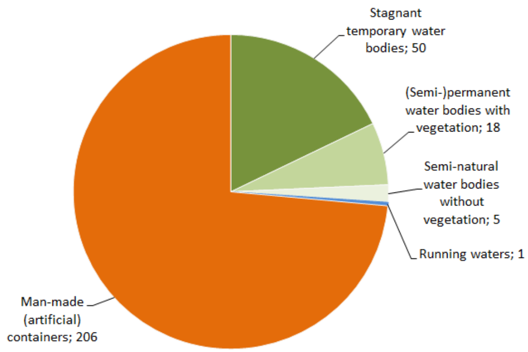


Fig. 99: Categories of larval habitats in which *Culex pipiens/torrentium* was observed, Luxembourg, 2019-2021 (280 sites).

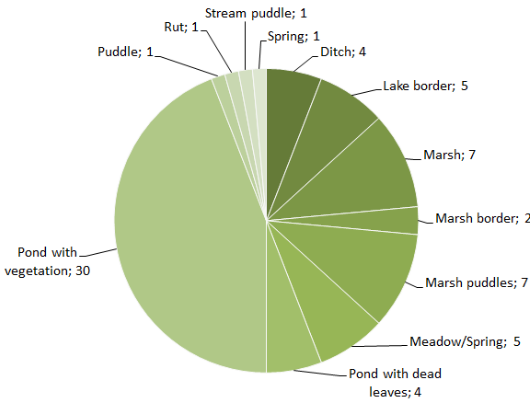


Fig. 100: Types of natural larval habitats (categories 'stagnant temporary water bodies' and '(semi-)permanent water bodies with vegetation') in which *Culex pipiens/torrentium* was observed, Luxembourg, 2019-2021 (68 habitats).

(out of 102; 97.1%; all except Schieren, Strassen and Vichten) and all cantons (Fig. 104). The *Cx. pipiens/torrentium* taxon is the most common one in the Grand Duchy.

In our study, more than half of the investigated sites proved positive for *Cx. pipiens/torrentium* (n=288; 51.5%; Fig. 30) and more than 300 samples out of 735 (41.5%), including 283 immature samplings and 22 resting catches, yielded at least one individual of *Cx. pipiens/torrentium*. Immatures were found mainly in summer (n=131 samples; 46.3%) and autumn (n=122; 43.1%) months, but also in spring (n=29; 10.2%) and even early winter (n=1) months (Fig. 98). The first positive sample in

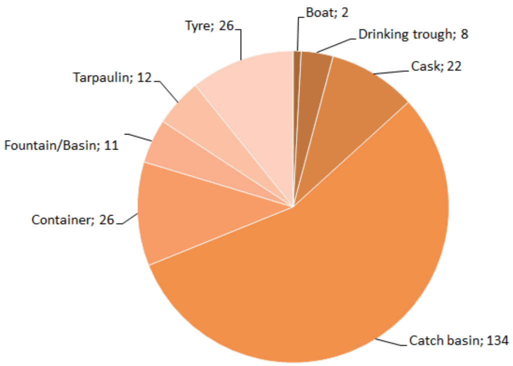


Fig. 101: Types of artificial larval habitats (category 'man-made containers') in which *Culex pipiens/torrentium* was observed, Luxembourg, 2019-2021 (241 habitats; many sites had more than one habitat type).

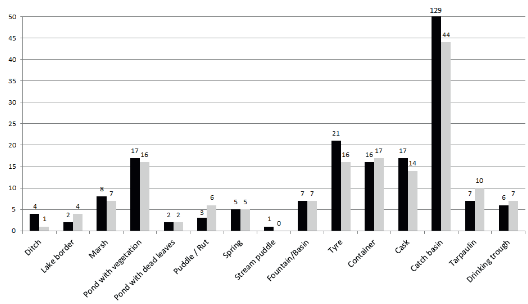


Fig. 102: Types of larval habitats in which *Culex pipiens* (black) and *Culex torrentium* (grey) were observed, Luxembourg, 2019-2021 (315 habitats).

the season was observed in April (06/04/2019) with the collection of an egg raft in a reed marsh, and the latest in December (12/12/2019) with the collection of one larva in a plastic bucket. A total of 140 eggs, 6,889 larvae and 1,852 pupae were observed. Adults (171 females and 48 males) of *Cx. pipiens/torrentium* could be caught by resting catches in the vegetation at/near larval habitats in spring and summer; both females and males could also be caught in shelters during these seasons. During autumn and winter, females were found in sheltered places, i.e. indoor barns, cellars and caves (Tab. 5). Positive larval habitats mainly belonged to man-made containers (n=206; 73.6%). Additionally, stagnant temporary water bodies were also found to be positive (n=50; 17.9%) as well as (semi-)permanent water bodies (n=18; 6.4%), a few semi-natural water bodies (n=5; 1.8%) and one running water (a spring with little

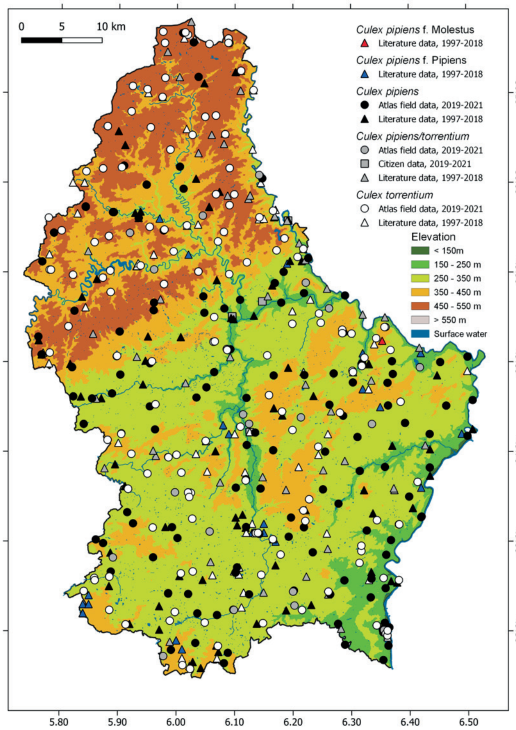


Fig. 104: Distribution of *Culex pipiens/torrentium* (grey), *Culex pipiens* (black: form not identified) form Molestus (red) and form Pipiens (blue), and *Cx. torrentium* (white) in Luxembourg.

flow) (Fig. 99). The positive natural larval breeding sites were ponds with vegetation (n=30 out of 68; 44.1%), besides a large variety of other habitats (Figs 100; 103d-e). The more frequently positive artificial containers were catch basins (at 134 sites out of 206; 65.0%) as well as tyres, rain water casks and a panel of other containers (Figs 101; 103a-c,f).

Identification to the species level could be performed for a high proportion of sites (n=267 out of 288; 92.7%). Both *Cx. pipiens* and *Cx. torrentium* were observed in sympatry at 102 sites, while they were observed singly at 129 and 36 sites, respectively. Comparing larval habitats of both species did not reveal any specific preferences (Fig. 102).

While *Cx. pipiens* is widely distributed throughout the entire Holarctic region, *Cx. torrentium* occurs only in the Palearctic region. The latter seems to be more abundant in northern regions and restricted to uplands in southern regions (Schaffner et al. 2001), but also more prevalent than *Cx. pipiens* in northern and central Europe (Hesson et al. 2014).

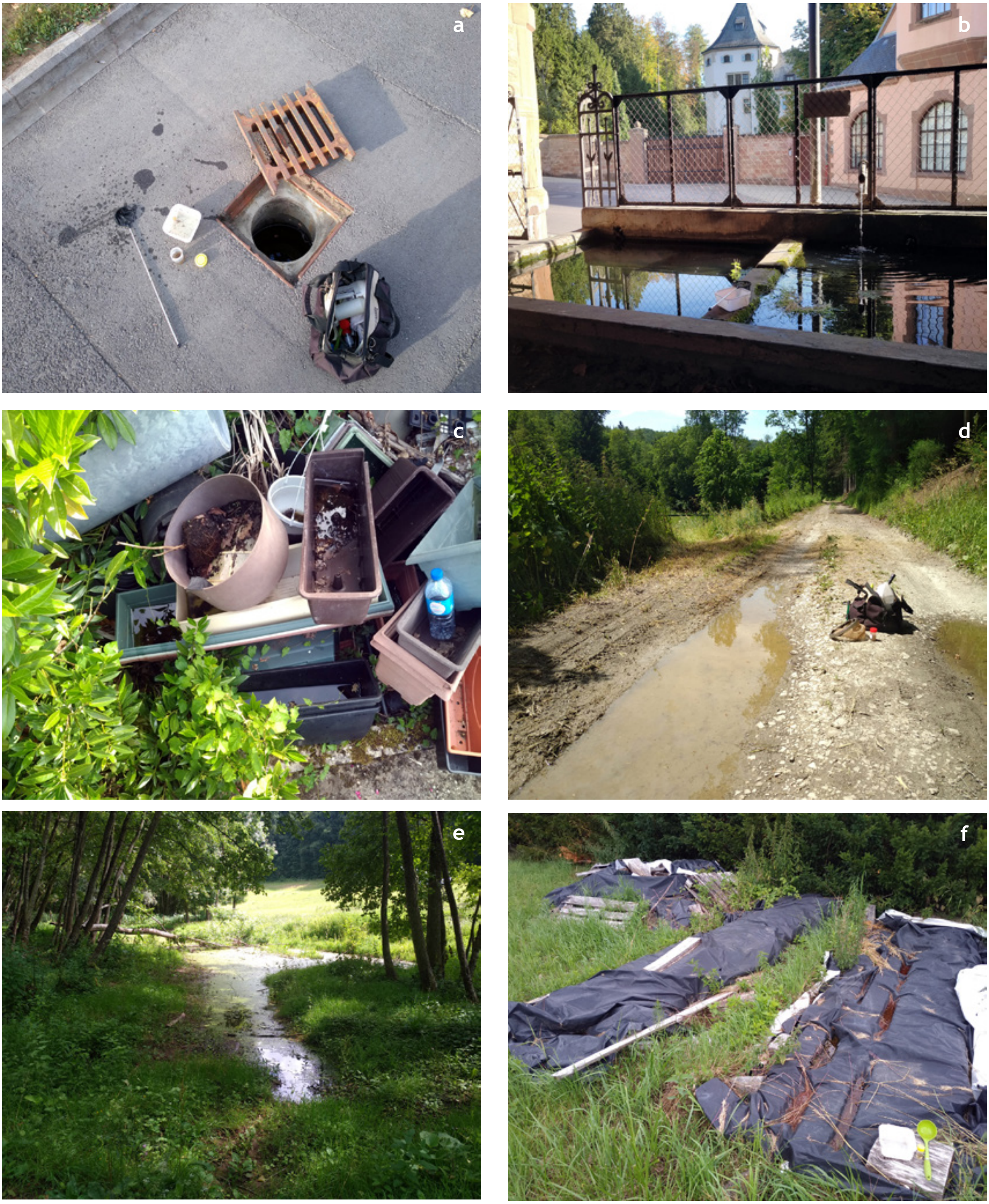


Fig. 103: Typical larval habitats of *Culex pipiens* (a,b,c) of *Culex torrentium* (a,b,d,e,f) in Luxembourg: a. Catch basin (site ATL181, Fentange, Hesperange, 16/09/19); b. Basin (site ATL199, Berg, Colmar-Berg, 20/09/19, in association with *Anopheles claviger* s.s.); c. Plastic containers (site ATL109, Luxembourg city, 18/06/19); d. Ground rut (site ATL153, Holtz, Rambrouch, 25/06/19, in association with *Anopheles maculipennis* s.s.); e. Pond with vegetation (site ATL507, Keiwelbach, Vallée de l'Ernz, 11/07/20, in association with *Anopheles maculipennis* s.s., *Culex territans* and *Culiseta annulata*); f. Tarpaulins (site ATL452, Berdorf, 06/07/20, in association with *Culex hortensis*). Photos: Francis Schaffner.

Tab. 5: Results of adult catches positive for *Culex pipiens/torrentium*, Luxembourg, 2019-2021. BT: CO_2-baited trap; HL: Human landing catch; RC: Resting catch; F: Female; M: Male.

Method	Season	Sampling period	Site ID	Date	Canton	Commune	Type of site	Results
RC	Winter	Mar 2020	ATL315	13/03/2020	Remich	Schengen	Indoor wooden shelter	1 F *Cx. pipiens* 1 F *Cx. torrentium*
RC	Winter	Mar 2021	ATL176d	09/03/2021	Esch-sur-Alzette	Pétange	Indoor abandoned building	3 F *Cx. pipiens*
RC	Winter	Mar 2021	ATL541b	12/03/2021	Vianden	Tandel	Indoor abandoned building	8 F *Cx. pipiens*
BT	Spring	May 2020	ATL141b	20/05/2020	Remich	Schengen	Wetland	3 F *Cx. pipiens*
RC	Spring	May 2020	ATL395	26/05/2020	Mersch	Colmar-Berg	Indoor service building	3 F, 2 M *Cx. pipiens*
RC	Spring	May 2020	ATL040b	28/05/2020	Remich	Schengen	Wetland vegetation	1 F, 1 M *Cx. pipiens*
HL	Spring	Jun 2019	ATL101b	19/06/2019	Esch-sur-Alzette	Bettembourg	Wetland	1 F *Cx. pipiens*
RC	Summer	Jul 2020	ATL040b	05/07/2020	Remich	Schengen	Wetland vegetation	1 F *Cx. pipiens*
RC	Summer	Jul 2020	ATL450b	06/07/2020	Echternach	Berdorf	Vegetation around pond	15 F, 15 M *Cx. pipiens*
RC	Summer	Jul 2020	ATL460b	07/07/2020	Clervaux	Parc Hosingen	Grassland around pond	15 F, 13 M *Cx. pipiens*
RC	Summer	Jul 2020	ATL040b	11/07/2020	Remich	Schengen	Wetland vegetation	1 M *Cx. pipiens*
RC	Summer	Aug 2021	ATL111b	18/08/2021	Luxembourg	Luxembourg	Forest near lake	6 F, 6 M *Cx. pipiens*
RC	Summer	Sep 2019	ATL227	21/09/2019	Grevenmacher	Wormeldange	Indoor animal shelter	30 F, 10 M *Cx. pipiens*
RC	Autumn	Dec 2021	ATL308	09/12/2019	Echternach	Berdorf	Indoor sandstone cave A	1 F *Cx. pipiens*
RC	Autumn	Dec 2021	ATL309	09/12/2019	Echternach	Berdorf	Indoor sandstone cave B	1 F *Cx. pipiens* 1 F *Cx. torrentium*
RC	Autumn	Dec 2021	ATL312	09/12/2019	Echternach	Berdorf	Indoor sandstone cave C	1 F *Cx. pipiens*
RC	Autumn	Dec 2021	ATL313	09/12/2019	Echternach	Berdorf	Indoor sandstone cave D	28 F *Cx. pipiens* 1 F *Cx. torrentium*
RC	Autumn	Dec 2021	ATL315	10/12/2019	Remich	Schengen	Indoor wooden shelter	4 F *Cx. pipiens*
RC	Autumn	Dec 2021	ATL316	10/12/2019	Remich	Schengen	Indoor wooden shelter	1 F *Cx. pipiens*
RC	Autumn	Dec 2021	ATL318a	12/12/2019	Luxembourg	Luxembourg	Hollow in rock face	12 F *Cx. pipiens/torrentium* 2 F *Cx. pipiens* 2 F *Cx. torrentium*
RC	Autumn	Dec 2021	ATL318b	12/12/2019	Luxembourg	Luxembourg	Indoor fortress cellar	9 F *Cx. pipiens/torrentium* 2 F *Cx. pipiens* 2 F *Cx. torrentium*
RC	Autumn	Dec 2021	ATL319	13/12/2019	Mersch	Mersch	Indoor animal shelter	1 F *Cx. pipiens/torrentium*
RC	Autumn	Dec 2021	ATL320	13/12/2019	Mersch	Mersch	Indoor barn and cellar	11 F *Cx. pipiens/torrentium*

4.3.3 *Culex (Maillotia) hortensis*

The subgenus *Maillotia* Theobald 1907 comprises nine species only (Wilkerson et al. 2021) of which three occur in the western Palaearctic region: *Cx. arbieeni* Salem 1938, *Cx. deserticola* Kirkpatrick 1925 and *Cx. hortensis*. The first two species have a very limited distribution in Europe (Canaries and Continental Spain, respectively) but are widespread in North Africa and the Middle East (Robert et al. 2019). The second comprises two subspecies: *Cx. hortensis maderensis* Mattingly 1955 which is endemic to Madeira (Portugal) and the Canary islands (Spain), and the nominal taxon *Cx. hortensis hortensis* (Schaffner et al. 2001).

Culex (*Mai.*) *hortensis hortensis* Ficalbi 1889

Informal names: en: Garden Italian typical mosquito; de: Garten-Stechmücke; lb: Gaarde-Mustik; fr: Moustique typique de jardin.

Adults of *Cx. hortensis* are medium-sized and appear globally light brown (Fig. 105). The proboscis is usually entirely dark-scaled, sometimes with scattered pale scales on the ventral surface. The scaling of the palps is variable, often with pale scales at the apex forming a ring on the palpomere V. The thoracic pleura have a few pale scale patches. The femora are mainly white-scaled but the hind femur has dark scales dorsally. White knee spots are present, and the tibiae and tarsomeres are dark with a few white scales on their ventral surfaces. The apex of the hind tibia has a white spot. The wing veins are entirely dark-scaled, except for the base of the costa which has pale scales. Abdominal terga have pale apical bands with a median widening on terga I–III; this character differentiates the adults from other *Culex* species in Europe (Becker et al. 2020). Larvae have a very long and slender siphon, and the setae 2-S are long and curved in the form of a hook, allowing the larvae to fix themselves to immersed plants or to the container inside (Schaffner et al. 2001).

Fig. 105: Adult female of *Culex hortensis*. Photo: Anders Lindström.

Culex hortensis is a multivoltine species, with larvae present from mid-spring throughout autumn and disappearing at the first frosts. Diapausing females overwinter in shelters, caves, cellars or ruins. They lay their eggs in rafts of e.g. 300 eggs on the water surface. The larval breeding sites are very varied,

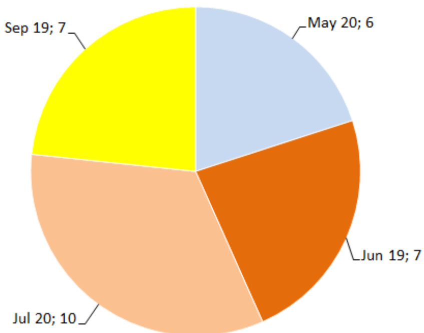


Fig. 106: Temporal distribution of *Culex hortensis* immature samples, Luxembourg, 2019-2021 (30 samples). Blue: spring months; Orange: summer months; Yellow: autumn months.

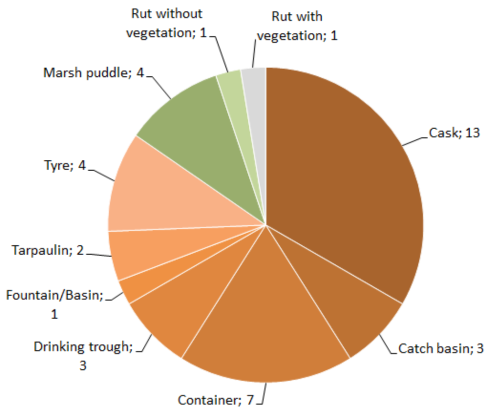


Fig. 107: Types of larval habitats in which *Culex hortensis* was observed, Luxembourg, 2019-2021 (39 larval habitats at 30 sites; more than one type could be investigated at a single site).

from small vegetation-free water bodies (rock pools, fountains, puddles in a torrent bed, rain water casks) to sites with algae and rich vegetation (pond sides, drain pipes). The water can be clear or rich in organic matter; it is generally fresh, sunlit and stagnant. A clear preference has been reported for artificial sites in lowlands and for natural sunlit sites in uplands (Schaffner et al. 2001).

The females do not feed on humans or mammals and are not suspected to transmit any pathogen of public or animal health relevance.

Culex hortensis was first detected in Luxembourg in 2005 (Schaffner 2022) and found in a study targeting container-inhabiting species (Schaffner & Ries 2019; Tab. 2), yielding ten precise locations for the species. Our field study allowed us to identify 35 additional sites where the species occurs. In total (45 sites), the species is known from 29 5x5 km grid squares (out of 137; 21.1%), 26 communes (out of 102; 25.5%) and 10 cantons out of 12 across the Grand Duchy (Fig. 95). The species can be considered as common in Luxembourg.

Culex hortensis was found in 30 immature samplings and 6 adult resting catches (Fig. 30; 173 larvae and 45 pupae observed). Immatures were found mainly in summer months (n=17 samples; 56.7%) but also in autumn (n=7; 23.3%) and spring (n=6; 20.0%) months (Fig. 106). Larval habitats mainly belonged to man-made containers (n=24; 80.0%; Fig. 108a) and a few stagnant temporary water bodies were found positive for the species (n=5; 16.7%), as well as one semi-natural water body (a large rut without vegetation). The more frequently positive artificial containers were rain water casks (at 13 sites out of 30; 43.3%). The positive natural larval breeding sites comprised four marshland puddles and one rut with vegetation (Figs 107; 108b). Positive resting catches were collected at 5 different locations, in December 2019 and March 2021 (Tab. 6; 10 females caught; Fig. 109).

This Palaearctic species is widely distributed around the Mediterranean Basin, in Caucasus and in the Middle East, but is absent from Northern Europe (Robert et al. 2019; Wilkerson et al. 2021).

Fig. 108: Larval habitats of *Culex hortensis* in Luxembourg: a. Basin/fountain (site ATL005, Esch-sur-Alzette, 19/06/19, in association with *Culex pipiens* and *Culex torrentium*); b. Rut with vegetation (site ATL148, Beaufort, 24/06/19, in association with *Anopheles claviger* s.s., *Culex territans* and *Culex torrentium*). Photos: Francis Schaffner.

Tab. 6: Results of adult resting catches positive for *Culex hortensis*, Luxembourg, 2019-2021. All adults caught were females.

Sampling period	Date	Canton	Commune	Type of site	No. of females
Dec 2019	09/12/2019	Echternach	Berdorf	Sandstone cave	1
Dec 2019	10/12/2019	Remich	Schengen	Wooden shelter	2
Dec 2019	12/12/2019	Luxembourg	Luxembourg	Hollow in rock face, semi open	2
Dec 2019	12/12/2019	Luxembourg	Luxembourg	Fortress cellar	1
Mar 2021	09/03/2021	Esch-sur-Alzette	Pétange	Abandoned building	3
Mar 2021	18/03/2021	Clervaux	Troisvierges	Tunnel gallery	1

Fig. 109: Overwintering resting site of adults of *Culex hortensis* in Luxembourg (site ATL586, Goedange, Troisvierges, 18/03/21, in company of *Culex pipiens*). Photo: Francis Schaffner.

4.3.4 *Culex (Neoculex) europaeus/territans*

The subgenus *Neoculex* Dyar 1905 consists of 27 species of which five occur in the western Palearctic region: *Cx. europaeus*, *Cx. impudicus* Ficalbi 1890, *Cx. judaicus* Edwards 1926, *Cx. martini* Medschid 1930, and *Cx. territans*. While *Cx. martini* has a broad but scarce distribution, *Cx. impudicus* is limited to but common in the Mediterranean region and *Cx. judaicus* is reported only from the Middle East.

The taxon *Cx. territans* is a widely distributed Holarctic species but da Cunha Ramos et al. (2003) suggested that the European populations differ from the Nearctic ones. Thus they renamed the European taxon *Cx. europaeus*, and subsequently other authors also consider that new species to occur all over Europe, including in Luxembourg (Martinet et al. 2019; Wilkerson et al. 2020). Concretely, in addition to the Portuguese populations originally studied (da Cunha Ramos et al. 2003), only populations from Extremadura, Spain, have been confirmed to belong to *Cx. europaeus* by morphological analysis (Bravo-Barriga et al. 2017). However elsewhere on the continent, i.e. in Turkey (Gunay et al. 2015), molecular investigations of *Cx. europaeus/territans* specimens revealed mitochondrial COI sequences that differ from those of Nearctic *Cx. territans*. To our knowledge, no other investigation of this taxon was performed outside these countries and an in-depth study based on both morphological and molecular methods remains necessary to clarify the affiliation of the European populations.

During our study, we collected several samples of *Cx. europaeus/territans* and we carefully analysed male genitalia from 15 locations (out of 67; 22.4%), and none of them showed the characters described for *Cx. europaeus*. Therefore we consider here that all populations from Luxembourg belong to the same taxon, under the name *Cx. territans*.

Culex (*Ncx.*) *europaeus* da Cunha Ramos, Ribeiro, & Harrison 2003

Informal names: en: Europa Portuguese typical mosquito; de: Europäische Stechmücke; lb: Europäesch Mustik; fr: Moustique typique européen.

Culex (*Ncx.*) *territans* Walker 1856

Informal names: en: Terrifying American typical mosquito; de: Unheimliche Stechmücke; lb: Onheemlech Mustik; fr: Moustique typique terrifiant.

Fig. 110: Adult female of *Culex territans*. Photo: Anders Lindström.

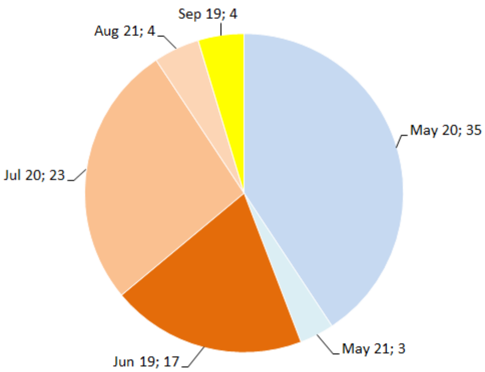


Fig. 111: Temporal distribution of *Culex territans* immature samples, Luxembourg, 2019-2021 (86 samples). Blue: spring months; Orange: summer months; Yellow: autumn months.

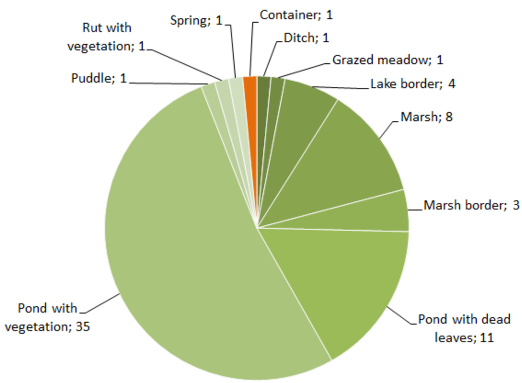


Fig. 112: Types of larval habitats in which *Culex territans* was observed, Luxembourg, 2019-2021 (67 sites).

Adults of *Cx. territans* (Fig. 110) are medium-sized brownish mosquitoes. The proboscis and palps are dark-scaled, and the proboscis is slightly swollen at the apex. Both the integument of the scutum and the scales are light brown. The thoracic pleura are brown with patches of broad whitish scales. The femora are dark-scaled on the anterior surface and pale posteriorly, with small pale knee spots. The hind tibia is without a pale spot. The tarsi are entirely dark-scaled. The wings are entirely covered with narrow dark scales. Abdominal terga are dark-brown-scaled with evenly narrow apical bands of pale scales (Becker et al. 2020). Larvae have a very long and slender siphon.

Culex territans produces several generations per year, but the populations are mainly present in the summer. The larvae occur from mid-spring to the first frosts in the autumn. Females overwinter in protected shelters, either natural or artificial (e.g. hollow tree trunk, caves, cellars). Immatures grow in varied breeding sites such as permanent marshes, lake borders, drainage ditches overcrowded with plants (including *Lemna* sp.), ponds and peaty ditches, and slow current streams. Larvae have also been found in drinking troughs, barrels and containers. A preference is observed for pure, cool, sunny waters in cold regions and shady in the hot regions, but the species can adapt to dirty waters (Schaffner et al. 2001).

Females predominantly feed on amphibians and reptiles. The species transmits batrachian's filariasis helminths and is not of medical relevance.

Culex territans was first collected in 2001 as larvae (Beck et al. 2001, 2003), and later another study that included larval sampling reported its presence in Luxembourg (Schaffner 2022; Tab. 2), yielding six precise locations for the species. Our field study provides 67 additional sites where the species occurs. In total (73 sites), the species is known from 51 5x5 km grid squares (out of 137; 37.2%) and 35 communes (out of 102; 34.3%) distributed in all cantons across the Grand Duchy, but mainly in the Gutland (Fig. 114). The species can be considered as common in Luxembourg.

We found *Cx. territans* in 86 immature samplings (Fig. 30; 458 larvae and 61 pupae observed), collected mainly in summer (n=44 samples; 51.2%) and spring (n=38; 44.2%) months, but also in autumn (n=4; 4.7%) months (Fig. 111). Larval habitats mainly belonged to stagnant temporary

Fig. 113: Larval habitats of *Culex territans* in Luxembourg: a. Temporary pond with vegetation (site ATL331b, Aspelt, Frisange, 19/05/20, in association with *Anopheles claviger* s.s.); b. Permanent lake border (site ATL456, Beringen, Mersch, 06/07/20, in association with *Anopheles claviger* s.s.and *Anopheles maculipennis* s.s.). Photos: Francis Schaffner.

water bodies (n=67; 77.9%) and semi-natural water bodies were also frequently positive for the species (n=18; 20.9%). Surprisingly, the species was found in one man-made container: a black plastic container overgrown by grasses. The presence of that vegetation makes the habitat resemble a natural one; it was also colonised by Ephemeridae species. The more frequently positive larval habitats were ponds with vegetation (temporary or (semi-)permanent; n=36; 43.3%), followed by marshes (n=24; 27.9%) and ponds with dead leaves (i.e. almost without aquatic vegetation; n=13; 15.1%) (Figs 112; 113).

The Holarctic taxon *Cx. territans* is widely distributed throughout the western Palaearctic region but absent from warm areas of the Mediterranean region, where it is replaced by *Cx. impudicus*.

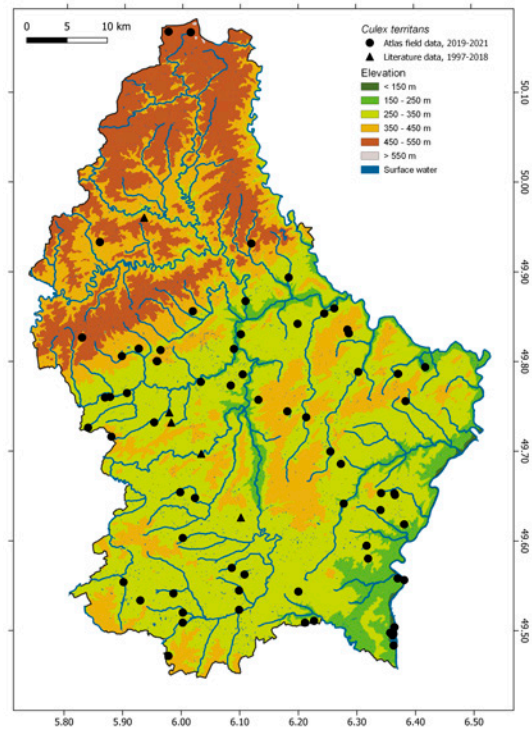


Fig. 114: Distribution of *Culex territans* (black) in Luxembourg.

4.4 Genus *Culiseta* Felt 1904

Mosquitoes of the genus *Culiseta*, formerly known under the name *Theobaldia* Neveu-Lemaire 1902, are of medium to large size. The females have a straight proboscis and short palps. Their abdomen is blunt-ended, with short and rounded cerci (Fig. 31). Some *Culiseta* species are known to feed exclusively on birds, but others, in particular those of the subgenus *Culiseta*, readily attack man and other mammals and are known to be severe biters. The genus includes species considered as significant vectors of viruses, bacteria and protozoa.

The *Culiseta* genus comprises approximately 40 valid species and subspecies, which are spread over 7 subgenera. Throughout the European continent, 10 species of three subgenera, *Allotheobaldia*, *Culicella* and *Culiseta*, are recorded (Becker et al. 2020). These three genera exhibit major differences in morphology, as well as in larval and adult behaviour. In the single species of the subgenus *Allotheobaldia*, females have a conspicuous colouration pattern on the scutum, and the palps of the males are shorter than the proboscis and distinctly swollen at the apex. Larvae have a rather short (*Culiseta*) or long (*Culicella*) siphon. They develop often in artificial containers. Eggs are laid in rafts on the water surface. Species of the subgenus *Culicella* (four species in Europe) usually have wings without spots, and indistinct short tarsal pale rings. Larvae develop in temporary natural water bodies. Eggs are deposited singly on the dried out ground, similarly to females of the genus *Aedes*. Species of the subgenus *Culiseta* (five species in Europe) have wings which bear dark spots that are more or less distinct. The palps of the males are as long as or longer than the proboscis, and the last two segments are distinctly swollen. If the tarsi are pale ringed, the rings are distinct and large but confined to the basal parts of each tarsomere. The females of the subgenus hibernate mainly as adults, occasionally also as larvae. They deposit the eggs in boat-shaped egg rafts on the surface of the water like the species of the genus *Culex* (Becker et al. 2020).

4.4.1 *Culiseta (Allotheobaldia) longiareolata*

The subgenus *Allotheobaldia* Brolemann 1919 involves the single species *Cs. longiareolata* (Wilkerson et al. 2021).

Culiseta (*All.*) *longiareolata* (Macquart 1838)

Informal names: en: Long Spanish cool mosquito; de: Langzellige Kaltstechmücke; lb: Laangzelleg Keeltmustik; fr: Moustique du froid allongé.

Adults of *Cs. longiareolata* are among the largest mosquitoes in Europe. The species can easily be distinguished from all other European species by its distinct longitudinal pale stripes on the scutum, forming a lyre in shape, and the femora and tibia with pale scales aggregated into conspicuous spots and stripes (Fig. 115). The wing veins are covered with dark scales that are aggregated at some cross veins and furcations, forming distinct dark spots. The scales of the terga vary in colour but usually form broad white basal bands. A mixture of yellowish and brown scales is more frequently found on the last terga (Becker et al. 2020).

The *Cs. longiareolata* species is multivoltine. It shows a continuous development in warm countries, but it can show a winter diapause at the larval stage of temperate regions and females may overwinter in cold regions (Schaffner et al. 2001). Adults are present the whole year round with a maximal density in spring and autumn.

Breeding sites are quite varied (barrels and cisterns, drinking troughs, catch basins, rock holes; more rarely ponds, canals), but water is always stagnant and generally rich in organic matter. These breeding sites can be either permanent or temporary, shaded or sunny, with fresh or brackish water, and with clean or polluted water. Such a wide spectrum of characteristics explains the wide distribution and abundance of this species (Schaffner et al. 2001).

Fig. 115: Adult female of *Culiseta longiareolata*. Photo: Anders Lindström.

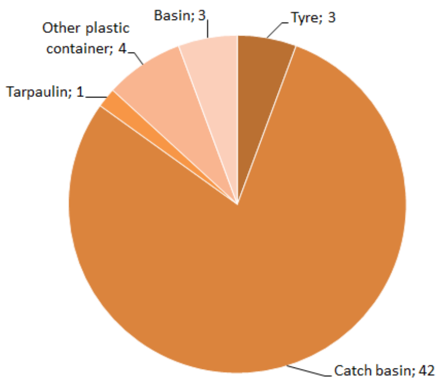


Fig. 116: Categories of larval habitats in which *Culiseta longiareolata* was observed, Luxembourg, 2019-2021 (48 samples; some samples were taken from more than one breeding site).

Fig. 117: Larval habitats of *Culiseta longiareolata* in Luxembourg: a. Basin (site ATL238, Dalheim, 22/09/19, in association with *Culex pipiens* and *Culex torrentium*); b. Tyres (site ATL017, Garnich, 04/04/19). Photos: Francis Schaffner.

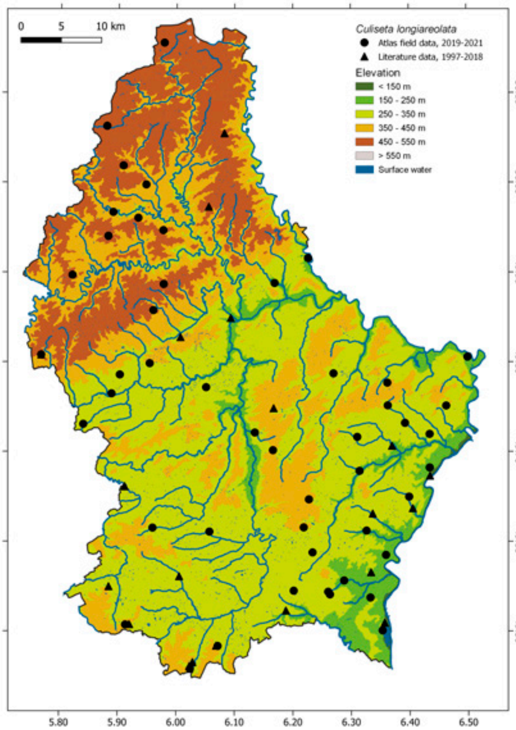


Fig. 118: Distribution of *Culiseta longiareolata* (black) in Luxembourg.

Females rarely bite humans outdoors but are regarded as vectors of avian malaria parasites, and they are laboratory competent to transmit West Nile virus (Schaffner et al. 2001).

The species *Cs. longiareolata* was first recorded in Luxembourg during our survey of the distribution of *Ae. japonicus* (Schaffner & Ries 2019; Tab. 2). During that field study (2018), the species was recorded at 20 sites. Our atlas field study revealed 48 additional sites. In total (68 sites), the species was observed in 55 5x5 km grid squares (out of 137; 40.1%) from 52 communes (out of 102; 51.0%) distributed in all cantons across the Grand Duchy (Fig. 118). The species appears to be common in Luxembourg.

All our 48 data are based on immature samplings (Fig. 30). One sample was taken in spring (April 2019; a single egg raft of 40 eggs) while all 47 others were taken in autumn (September 2019; for a total of 290 larvae and 95 pupae collected and identified). All positive larval habitats were classified as man-made (artificial) water containers, and catch basins were the most frequently positive breeding site observed (87.5% of the positive sites; Figs 116; 117).

This warm-climate species is widespread in the south of the western Palaearctic region, as well as in middle and southwest Asia and in the Afrotropical region. The recent findings of the species in northern France (1994, pers. obs.), northern Switzerland (Schaffner et al. 2009), Austria (Seidel et al. 2013), Germany (Kampen et al. 2017), Luxembourg (Schaffner & Ries 2019), Belgium and the Netherlands (Deblauwe et al. 2021) suggest a northwards spread of the species with a warming climate.

4.4.2 *Culiseta (Culicella) morsitans*

Four species of the subgenus *Culicella* Felt 1904 occur in the European region (*Cs. fumipennis* (Stephens 1825), *Cs. litorea* (Shute 1928), *Cs. morsitans*, and *Cs. ochroptera* (Peus 1935)), but only one *Cs. morsitans* is recorded in Luxembourg to date.

Culiseta (*Cuc.*) *morsitans* (Theobald 1901)

Informal names: en: Biting British cool mosquito; de: BissigeKaltstechmücke; lb: Bësseg Keeltmustik; fr: Moustique du froid piquant.

The adults of *Cs. morsitans* are rather large. The wing veins are entirely dark-scaled, without spots, and the scales are narrow and dark brown. Pale knee spots are well-developed, and all tibiae have short pale rings at their apices (Fig. 119). The tarsi are dark with weak pale rings around both ends of the joints. The terga are dark with narrow pale basal bands. Females are difficult to distinguish from the other *Culicella* species, i.e. *Cs. fumipennis*, *Cs. litorea* and *Cs. ochroptera* (Becker et al. 2020).

Culiseta morsitans is univoltine and overwinters as larvae. Adults emerge at the end of spring and disappear in summer. The eggs, resistant to desiccation, are laid individually in depressions littered with dead vegetation and liable to flooding, or on banks of drying pools. They hatch in autumn or in early spring, when heavy rainfall leads to their flooding. During wintertime, the larval development is slowed down. Larvae breed in pools, temporary ponds, swampy woodlands, as well as ditches overgrown with vegetation and with slowly running water (Fig. 122). These breeding sites can be shady or sunny, their bottom is often littered with dead leaves. The water of breeding sites is generally fresh, more rarely brackish.

Females seem to feed essentially on birds, and do not attack mammals and humans. The species is suggested to act as a vector of Sindbis virus. Females have been found naturally infected by West Nile virus and *Dirofilaria* helminths; they are competent to transmit the latter in the laboratory. However, according to its trophic preferences, the risk of parasitic transmission to humans is insignificant (Schaffner et al. 2001).

This Holarctic species is very common in central and northern Europe, but it becomes sparse in southern regions (Schaffner et al. 2001).

Fig. 119: Adult female of *Culiseta morsitans*. Photo: Anders Lindström.

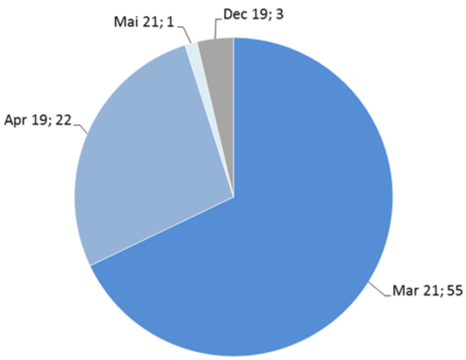


Fig. 120: Temporal distribution of *Culiseta morsitans* immature samples, Luxembourg, 2019-2021 (81 samples). Blue: spring months; Grey: winter months.

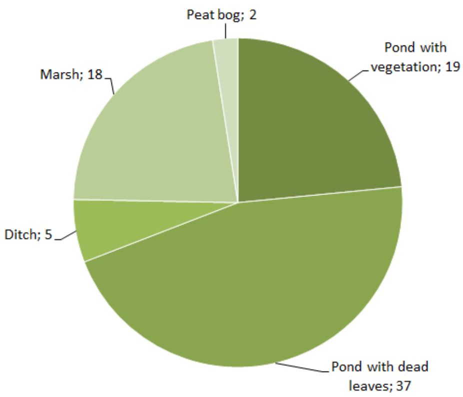


Fig. 121: Categories of larval habitats in which *Culiseta morsitans* was observed, Luxembourg, 2019-2021 (81 samples).

In Luxembourg, *Cs. morsitans* was first collected in 2003 (Schaffner 2022; Tab. 2), and only four locations are known from the literature (Fig. 123). In our study, we identified 76 additional sites. In total (79 sites), the species was observed in 56 5x5 km grid squares (out of 137; 40.9%), 51 communes (out of 102; 50.0%) distributed in all cantons across the Grand Duchy over all cantons except Vianden. The species appears to be common in the Gutland, more rare in the Oesling due to the scarcity of typical larval habitats (Fig. 123).

A total of 81 immature samplings yielded *Cs. morsitans* specimens (Fig. 30; 409 larvae and 13 pupae collected and identified). These were mainly collected in spring months (n=78 samples; 96.3%), with only a few collected in winter (December 2019; n=3, 3.7%) (Fig. 120). In addition, 2 resting catches gathered one adult female in spring (28/05/2020) and one adult male in summer (04/07/2020).

Among the positive larval breeding sites, most of them were classified as 'stagnant temporary water bodies' (n=77, 95.1%) and a few as '(semi-) permanent water bodies with vegetation (n=4; 4.9%). Besides these types, they can be grouped into five categories (Figs 121; 122). Immatures were most frequently found in forest ponds (local name: mardelles) littered with dead leaves and almost without aquatic vegetation (45.7% of the positive sites), but ponds with a significant part colonised by aquatic vegetation also frequently yielded the species (23.5%). In addition, marshes were found positive, as well as ditches and peat bogs (22.2, 6.2 and 2.5%, respectively). The latter are also among the typical breeding sites of the species, but rare in Luxembourg.

Fig. 122: Larval habitats of *Culiseta morsitans* in Luxembourg: a. Ditch (site ATL016, Fingig, Käerjeng, 04/04/19, in association with *Aedes annulipes/cantans* and *Aedes rusticus*); b. Pond with dead leaves (site ATL579, Grevenmacher, 17/03/21, in association with *Aedes annulipes/cantans*, *Aedes communis* and *Aedes rusticus*). Photos: Francis Schaffner.

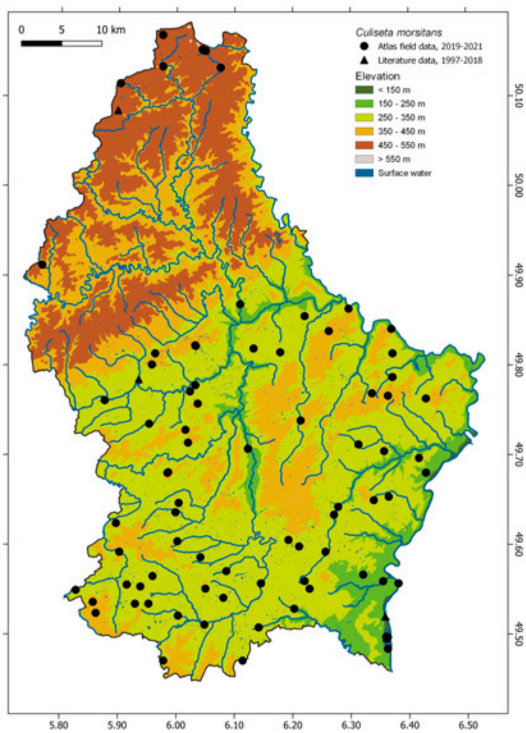


Fig. 123: Distribution of *Culiseta morsitans* (black) in Luxembourg.

4.4.3 *Culiseta (Culiseta) annulata*

Six species of the subgenus *Culiseta* Felt 1904 occur in the European region. They can be listed in two groups: a first group of 4 species with annulated tarsi comprising *Cs. alaskaensis alaskaensis* (Ludlow 1906), *Cs. annulata*, *Cs. atlantica* (Edwards 1932), and *Cs. subochrea* (Edwards 1921), and a second group of 2 species with uniform dark tarsi including *Cs. bergrothi* (Edwards 1921) and *Cs. glaphyroptera*. One species from each of these groups is recorded in Luxembourg to date.

Culiseta (*Cus.*) *annulata* (Schrank 1776)

Synonyme: *Culex annulatus* F., used in Sunnen (1918).

Informal names: en: Ringed Austrian cool mosquito; de: Geringelte Kaltstechmücke, Ringelmücke, Große Hausmücke; lb: Geréngelt Keeltmustik; fr: Moustique du froid annelé.

The adults of *Cs. annulata* are large dark brown mosquitoes with whitish markings on the abdomen and the legs. The wing veins are dark-scaled, some of which are aggregated to form distinct dark spots at some vein furcations. The femora are predominantly dark-scaled with scattered pale scales, distinct white subapical rings and pale knee spots. Tarsomeres I have a noticeable white ring in the middle and white rings are also present at the bases of tarsomeres I–IV. Tarsomeres V of all the legs are entirely dark-scaled (Fig. 124). The terga are dark with narrow whitish basal bands. Females can be difficult to distinguish from *Cs. subochrea*, which has terga scattered with more or less numerous yellowish scales, also forming indistinct yellowish basal bands (Becker et al. 2020).

In Central Europe larvae of *Cs. annulata* usually occur from early spring onwards. The species may have at least two generations per year in Luxembourg. Adults emerging at the end of the summer overwinter in cellars, natural caves or tree holes (Schaffner et al. 2001; Zittra et al. 2021). The females of *Cs. annulata* can often be found inside houses even during the day from early autumn on, when they search for their winter habitats (Becker et al. 2020). Larvae can still be found during winter if the meteorological conditions are mild. They breed in a wide variety of sites, natural (ponds, flooded meadows, ditches, puddles) or artificial (polluted pools, cisterns, drinking troughs, barrels, used tyres) (Fig. 126).

Fig. 124: Adult female of *Culiseta annulata*. Photo: Anders Lindström.

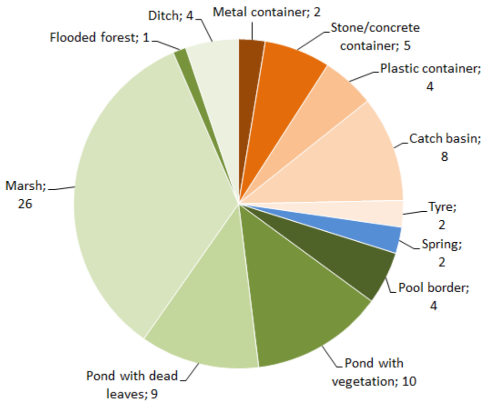


Fig. 125: Categories of larval habitats in which *Culiseta annulata* was observed, Luxembourg, 2019-2021 (75 samples; some samples were taken from more than one breeding site).

High larval densities frequently occur in nitrogen-rich waters, and they tolerate brackish water.

Females bite all warm-blooded vertebrates but preferably birds. Generally nocturnal, they do not hesitate to enter houses and cowsheds for blood-feeding. The species is involved in the transmission of avian plasmodiums. It is also a possible vector for Tahyna virus, and females have been found naturally infected by Usutu virus and *Setaria* nematodes (Schaffner et al. 2001; Martinet et al. 2019).

In Luxembourg, *Cs. annulata* is one of the first two recorded mosquito species, in the early 20th century (Sunnen 1918; Tab. 2), and 33 precise

Tab. 7: Numbers and distribution of samplings positive for Culiseta annulata and stages collected, Luxembourg, 2019-2021. All adults caught by resting catches. L: larvae; P: pupae; F: females; M: males.

Sampling period	Apr-19	Jun-19	Sep-19	Dec-19	May-20	Jul-20	Mar-21	May-21	Aug-21
No. (%) of pos. larval samplings	3 (4.0%)	20 (26.7%)	11 (14.7%)	4 (5.3%)	16 (21.3%)	10 (13.3%)	2 (2.7%)	5 (6.7%)	4 (5.4%)
No. of pos. adult catches	-	1	-	2	2	2	1	-	1
Stages collected	L, P	L, P, M	L, P	L, P, F	L, P, F, M	L, P, F, M	L, P, F	L, P	L, P, F

Fig. 126: Larval habitats of *Culiseta annulata* in Luxembourg: a. Concrete basin / drinking trough (site ATL323, Gostingen, Flaxweiler, 05/07/20, in association with *Culex hortensis*, *Culex pipiens* and *Culex torrentium*); b. Grey water semi-permanent pond border (site ATL460, Holzthum, Parc Hosingen, 07/07/20, in association with *Culex pipiens* and *Culex torrentium*). Photos: Francis Schaffner.

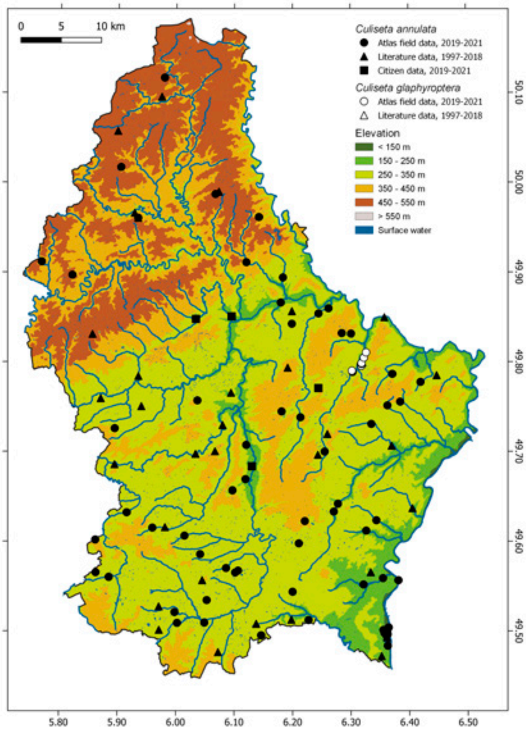


Fig. 127: Distribution of *Culiseta annulata* (black) and *Culiseta glaphyroptera* (white) in Luxembourg.

locations are known from the literature (Fig. 127). In addition, four citizen data are reported for that species (Fig. 19). In our atlas field study, we identified 63 additional sites. In total (100 sites), the species was observed in 64 5x5 km grid squares (out of 137; 46.7%), 59 communes (out of 102; 57.8%) distributed in all cantons across the Grand Duchy. The species can be considered as common in Luxembourg (Fig. 127).

A total of 75 immature samplings yielded *Cs. annulata* specimens (547 larvae and 137 pupae collected and identified). *Culiseta annulata* is the only species which was collected as immatures during all seasons and all sampling periods (Tab. 7). However most of them were collected during summer (n=34 samples; 45.3%) and spring (n=26; 34.7%) months. The remaining samples were collected in autumn (n=11; 14.7%9 and winter (n=4; 5.3%) months. The discovery of larvae in December and early March suggest overwintering of immatures is possible in Luxembourg. Nine adult resting catches proved positive for the species throughout the year, yielding 15 adult females and 5 males (Tab. 7), including overwintering females caught in a cave (1 female, 09/12/2019) and in a wood shelter (9 females, 10/12/2019).

Unsurprisingly, the positive larval breeding sites were classified as 'stagnant temporary water bodies' (n=49, 65.3%) or '(semi-)permanent water bodies with vegetation (n=6; 8.0%), but also as 'man-made (artificial) containers' (n=19; 25.3%). In more detail, they are all grouped into 12 categories (Fig. 125). Immatures were most frequently found in marshes and ponds (both with dead leaves and with aquatic vegetation). Within artificial breeding sites, catch basins were positive most frequently.

This Palaearctic species is widely distributed throughout Europe, from northern regions to the Mediterranean, and extends into North Africa, the Middle East and Southwest Asia (Robert et al. 2019; Becker et al. 2020).

4.4.4 *Culiseta (Culiseta) glaphyroptera*

Culiseta (*Cus.*) *glaphyroptera* (Schiner 1864)

Informal names: en: Elegant Austrian cool mosquito; de: Elegante Kaltstechmücke; lb: Elegant Keeltmustik; fr: Moustique du froid élégant.

The adults of *Cs. glaphyroptera* are large dark brown mosquitoes (Fig. 128). The wing veins are dark-scaled and aggregated scale spots are very light or absent. The legs are dark-scaled, and the posterior surface of the femur and tibia have light scales, which sometimes form a longitudinal stripe, and the knee spot is distinct and yellowish white. The tarsomeres are entirely covered with dark brown scales, and pale rings are absent. The terga are dark brown with indistinct bands of light brown to pale scales in the basal third of each tergum (Becker et al. 2020). The species is hard to distinguish from *Cs. bergrothi*, which has a more northern distribution.

The *Cs. glaphyroptera* species has one or two generations per year. Larvae are present from the end of spring until the end of autumn. They usually occur in the beds of small mountain streams, rock pools

Fig. 128: Adult female of *Culiseta glaphyroptera*. Photo: Anders Lindström.

or puddles. They also inhabit artificial containers (e.g. barrels, tyres, plastic buckets). These breeding sites are always small and shady; vegetation is absent or sparse; the bottom is stony or littered with dead vegetation (e.g. pine needles). Females have a diapause and overwinter in sheltered places such as caves (Schaffner et al. 2001).

Little is known about the feeding behaviour of the females. It seems likely that they feed on birds or small mammals which inhabit the forest. No parasitic transmission involving this species has been reported to date (Schaffner et al. 2001; Martinet et al. 2019).

The *Cs. glaphyroptera* species was first collected in Luxembourg in 2007, during a study of subterranean fauna (Weber 2013; Zittra et al. 2021; Tab. 2). Overwintering adults were collected from three sites, all located in the Müllerthal nature park (Berdorf and Waldbillig, canton of Echternach). To confirm the occurrence of the species and identify larval habitats, we intensified our sampling in that region. As a result, we located two positive larval habitats: a concrete basin littered with dead leaves (1 pupa, 24/06/2019; Fig. 129a) near the village of Müllerthal (Waldbillig), and a small forest pond littered with a few dead leaves (Berdorf, 32 larvae, 23/05/2020; Fig. 129b). In addition, we performed resting catches of overwintering females in five caves of the same area (Berdorf; 16 females, 09/12/2019; Fig. 10b). A total of 33 larvae and one pupa was collected, and 16 adult females were caught. No other larval sampling of adult resting catch proved positive for the species elsewhere in the Grand Duchy, suggesting that the species is

Fig. 129: Larval habitat of *Culiseta glaphyroptera* in Luxembourg: a. Concrete basin (site ATL142, Müllerthal, Waldbillig, 24/06/19, in association with *Culex pipiens* and *Culex torrentium*); b. Small forest pond (site ATL366, Berdorf, 23/05/20, in association with *Culex hortensis*). Photos: Francis Schaffner.

rare and localised in that hilly and rocky region (Fig. 127). It is to date reported from a single 5x5 km grid square (out of 137; 0.7%), 2 communes (out of 102; 2.0%) and one canton of the Grand Duchy.

This Palaearctic species' distribution is limited to Central and Eastern Europe, up to southern Scandinavia. It is classically found in mountainous regions (e.g. the Vosges mountains in France, the Black forest in Germany; first author personal observations). It can be considered as a rare species in western Europe, where it has only been reported in France and Germany so far (Kampen et al. 2013; Robert et al. 2019).

4.5 Genus *Coquillettidia* Dyar 1905

The genus *Coquillettidia* comprises three subgenera of which solely the subgenus *Coquillettidia* Dyar 1905, which contains 44 species, is represented in the western Palaearctic region (Wilkerson et al. 2021).

The palps of females are short, 1/4 the length of the proboscis or shorter, while those of the male are about as long as the proboscis. The abdomen of the female is truncated, and the cerci are short and blunt. The wing veins are covered with a mixture of pale and dark broad scales.

Species of the genus *Coquillettidia* have an original biology, since larvae and pupae attach themselves to the submerged part of emergent aquatic macrophytes and get oxygen from the aerenchymal channels of roots. Larvae and pupae penetrate the plant tissues with their modified and specialised siphon or trumpets, respectively. Females lay their eggs on the water surface, aggregated in a raft.

Coquillettidia species are associated with several viruses (Wilkerson et al. 2021).

4.5.1 *Coquillettidia (Coquillettidia) richiardii*

In Europe, the subgenus *Coquillettidia* is represented by only two species: *Coquillettidia buxtoni* (Edwards 1923) and *Cq. richiardii*.

Coquillettidia (*Coq.*) *richiardii* (Ficalbi 1889)

Informal names: en: Richiardi Italian underwater mosquito; de: Richiardi-Unterwasserstechmücke; lb: Richiardi-Ënnerwaassermustik; fr: Moustique sous-marin de Richiardi.

Adults of *Cq. richiardii* are of moderate size. The genus characteristic of wing veins (Fig. 31) covered with a mixture of pale and dark broad scales is particularly visible in this species. The apex of the proboscis is slightly broader and darker than the preceding part. All tarsomeres have a pale-scaled ring on the basal part and an additional pale ring is present in the middle of tarsomere I of all the legs (similar as for *Cs. annulata*) (Fig. 130).

In temperate regions like Luxembourg, the species has a single generation per year. Imagoes hatch at the end of spring and they disappear at the end of summer. The larval development is slow and lasts from summer to the following spring. The larvae

Fig. 130: Adult female of *Coquillettidia richiardii*. Photo: Anders Lindström.

Fig. 131: Larval habitat of *Coquillettidia richiardii* in Luxembourg: permanent pond border (site ATL045, Jakobsberg, Bech, 29/05/20; in association with *Anopheles claviger* s.s. and *Culex territans* on 27/06/19). Photo: Francis Schaffner.

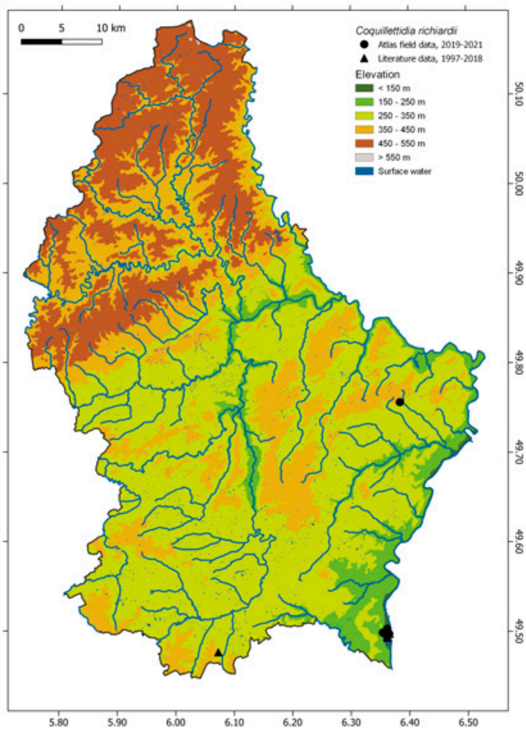


Fig. 132: Distribution of *Coquillettidia richiardii* (black) in Luxembourg.

overwinter underwater within the first centimetres of mud, remaining fixed on the plant roots, where they feed mainly on bacteria (Sérandour et al. 2006). They develop in permanent water bodies with vegetation such as *Phragmites*, *Typha* and *Juncus* spp.

Imagoes do not fly far from their breeding sites. Females feed mainly on mammals but also bite birds and amphibians. They bite at dusk and at night and they sometimes enter houses to bite humans. *Coquillettidia richiardii* is suggested as a vector of Sindbis virus, and may contribute to the transmission of *Dirofilaria* nematodes and of Batai, Tahyna and West Nile viruses. The species can be responsible for severe nuisance, particularly in close proximity of large breeding sites (Schaffner et al. 2001; Hubálek 2008; Becker et al. 2020).

The *Cq. richiardii* species was first reported in 2001 (Beck et al. 2001) from Haff Réimech nature reserve (Schengen, canton of Remich) from where it was collected again in 2016 (Ries et al. 2019). A second location was identified in Dudelange (canton of Esch-sur-Alzette) in 2011 (Muller & Reye 2012). During our study, we identified two positive locations only, including the Haff Réimech in the Moselle valley (6 sites), and a nature area near Jakobsberg (Geyershaff, canton of Echternach). Immatures were collected from the latter only, from a permanent large pond border (Fig. 131) on two occasions: one pupa on 27/06/2019 and 5 larvae on 29/05/2020. They are difficult to collect since they can only be detected if detached from the plants and this happens only when disturbing the roots they are attached to. Adults were caught at Haff Réimech by CO_2-baited traps (2 females in two trap-nights), resting catches (3 females and 3 males within five catches) and human landing catches (12 females within two catches). Catches were positive in June 2019, and May and July 2020. With a total of 9 positive sites only, from three 5x5 km grid squares (out of 137; 2.2%), three communes (out of 102; 2.9%) and three cantons all located in the Gutland, the species can be considered as rare in Luxembourg (Fig. 132).

Coquillettidia richiardii is widely distributed in the western Palaearctic region (Robert et al. 2019) and common in Europe (Becker et al. 2020).

4.6 Genus *Uranotaenia* Lynch Arribalzaga 1891

Mosquitoes of the genus *Uranotaenia* are generally small and dark, and characterised by having short palps in both sexes, a proboscis usually swollen at the tip, and very plumose antennae of the males. The wings exhibit a characteristic venation pattern with the anal vein sharply bent apically, ending before or at the same level as the furcation of the cubitus vein. The abdomen of the female is blunt-ended, and the cerci are short and rounded (Becker et al. 2020). Larvae are small and have a short and dark siphon. *Uranotaenia* is a relatively large genus, which occurs mainly in regions with tropical climates, and only a single species, *Ur. unguiculata*, occurs on the European continent. Little is known about the adult blood-feeding behaviour (some species are known to feed on amphibians) and they are not considered to play any significant role in pathogen transmission.

Fig. 133: Adult female of *Uranotaenia unguiculata*. Photo: Anders Lindström.

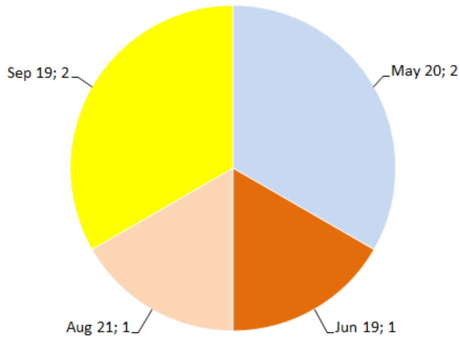


Fig. 134: Temporal distribution of *Uranotaenia unguiculata* immature samples, Luxembourg, 2019-2021 (6 samples). Blue: spring months; Orange: summer months; Yellow: autumn months.

4.6.1 *Uranotaenia (Pseudoficalbia) unguiculata*

Uranotaenia (*Pfc.*) *unguiculata unguiculata* Edwards 1913

Informal names: en: Fingernail Israeli heaven mosquito; de: Fingernagel-Himmelsstechmücke; lb: Fangernol-Himmelmustik; fr: Moustique-griffe merveilleux.

A second subspecies, *Ur. unguiculata pefflyi* Stone 1961, is described from Saudi Arabia. Only the nominal taxon occurs in Europe, hereafter given as *Ur. unguiculata*.

Adults of *Ur. unguiculata* are among the smallest mosquitoes in Europe (around 4 mm). They are easily identifiable by the two silvery white lines running across the lateral sides of the thorax (Fig. 133).

Larvae breed in relatively shallow herbaceous marshes, such as reed beds (*Phragmites* sp.) rich in organic matter of vegetal origin (Schaffner et al. 2001; Rudolf et al. 2015). (Fig. 135). The waters can be slightly brackish. Immature development is continuous in the south of Europe (Greece), but seasonal, from May to October, in colder regions. In that case, females overwinter in the vegetation or in aboveground or underground shelters (Zittra et al. 2021), which is also confirmed by our observations.

As with other *Uranotaenia* species, females may prefer amphibians and reptiles as blood hosts, but *Ur. unguiculata* has been occasionally reported to feed on birds and mammals, including humans.

Fig. 135: Larval habitat of *Uranotaenia unguiculata* in Luxembourg: marsh puddles (site ATL039e, Haff Réimech, Wintrange, Schengen, 21/05/20, in association with *Anopheles claviger* s.s., *Culex hortensis*, *Culex pipiens*, *Culex territans* and *Culiseta annulata*). Photo: Francis Schaffner.

They also seem to be autogenous. It is not known whether *Ur. unguiculata* is vector-competent for pathogens, although specimens collected in the field were found infected with West Nile virus and *Dirofilaria repens* (Tippelt et al. 2017).

Our data are the first evidence of the occurrence of *Ur. unguiculata* in Luxembourg. The species is rare in the Grand Duchy (Fig. 136; one 5x5 km grid squares, one commune and one canton); we found it at four sites from a single location (4 sites) in the Gutland, the Haff Réimech nature reserve (Schengen, canton of Remich), and in the Moselle valley. Immatures were collected from temporarily-flooded marshes and pond borders (Fig. 135), between May and September, equally distributed throughout spring, summer and autumn (six samples, 2 within each season; Fig. 134). A total of 20 larvae and 14 pupae were collected, and 2 adults caught. One adult male was caught in a CO_2-baited trap on 22/09/2019, and one hibernating female was caught in a wood shelter on 10/12/2019.

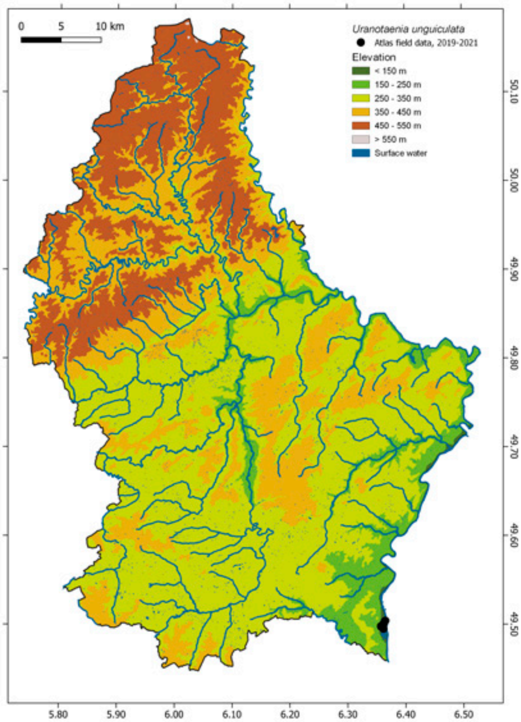


Fig. 136: Distribution of *Uranotaenia unguiculata* (black) in Luxembourg.

This warm-climate species is commonly found throughout the Mediterranean region up to middle Asia. Our finding in Luxembourg is the second northernmost record, the first being located in Germany, west of Berlin (Tippelt et al. 2017).

5 Analysis of the mosquito fauna of Luxembourg

5.1 Biogeography

5.1.1 Distribution ranges, frequency and biodiversity

Combining all available distribution data (atlas field study + citizen + literature data) reveals the taxon *Cx.pipiens/torrentium* to be the most widespread and frequently encountered, and probably the most abundant taxon in Luxembourg. This taxon, which

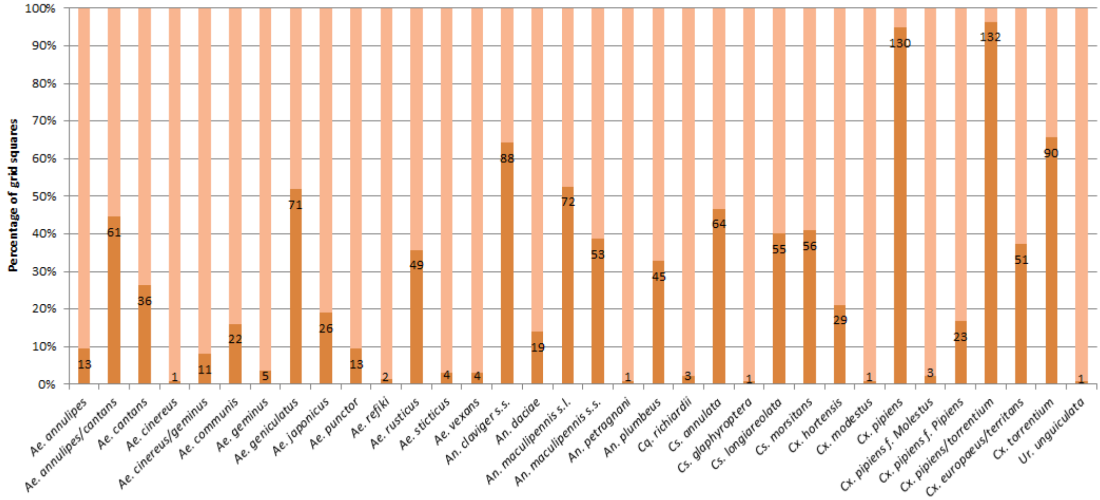


Fig. 137: Relative areas of distribution for every mosquito species recorded in Luxembourg, according to the percentage (vertical axis) and numbers (column values) of 5x5 km grid squares (total n=137) with at least one positive record, all data (atlas field study, citizen, literature), by January 2022. Grid squares area covered per taxon: median=17.9% (n=25); range 0.7-96.4% (n 1-132).

comprises all *Cx. pipiens* complex members and *Cx. torrentium*, is reported from 96.4% of the grid squares (132 out of 137). In detail, *Cx. pipiens* and *Cx. torrentium* have been reported from 94.9 and 65.7% of the 5x5 km grid squares, respectively; distribution data for the *Cx. pipiens* complex members, i.e. the forms Pipiens and Molestus, are more limited and based on the single study of Zittra et al. (2021), with records in 16.8 and 2.2% of the grid squares, respectively (Fig. 137). Overall, the frequency of findings of *Cx. pipiens/torrentium* fits with the taxa's unselective colonised larval habitats and their close proximity to humans. *Culex pipiens* is considered the most widespread species in Europe, and *Cx. torrentium* is also widespread in the more temperate part of the continent, i.e. north of the Alps (Hesson et al. 2014).

The other taxa that show a wide-ranging distribution are *An. claviger* s.s., *An. maculipennis* s.l., *Ae. geniculatus*, *Cs. annulata*, *Ae. annulipes/cantans*, *Cs. morsitans*, *Cs. longiareolata*, *An. maculipennis* s.s., *Cx. territans*, and *Ae. rusticus*, with occurrences recorded from a grid square area constituting between 35 and 65% (50 and 89 grid squares). They develop in different habitats that are all frequent in the Grand Duchy (i.e. semi-permanent water bodies, stagnant temporary water bodies, man-made containers, or tree holes). Some taxa have a very limited distribution range (reported in up to 7.5% of the squares only, i.e. 1 to 10 squares): *Ae. cinereus/geminus*, *Ae. sticticus*, *Ae. vexans*, *Cq. richiardii*, *Ae. refiki*, *An. petragnani*, *Cs. glaphyroptera*, *Cx. modestus* and *Ur. unguiculata*, while the larval habitats in which they develop are also widely present in Luxembourg, at least in the Gutland. Therefore, it would not be surprising to locate additional sites for these species across the country in upcoming surveys.

The number of mosquito taxa reported in every 5x5 km grid square varied from 0 to 25 (median=9). The grid square with the highest taxonomic diversity is #P9 with 25 species (Fig. 8). This grid square does not cover a completed surface (i.e. 25 km²) but it includes the Haff Réimech wetland nature reserve which yields a high mosquito diversity, and it has been investigated within several historical studies (Beck et al. 2013; Muller & Reye 2012; Ries et al. 2019; Schaffner & Ries 2019; Schaffner 2022). The other grid squares with the highest diversity, with 18 species each, are: #I9 which encompasses the Mullerthal nature park which was also well studied (Schaffner & Ries 2019; Zittra et al. 2021; Schaffner 2022) and where we intensified our investigations to confirm the occurrence of *Culiseta glaphyroptera* (14 sites in our atlas field study, our second highest value), #O3 which encompasses the communes of Mondercange and Sanem and their surroundings (investigated in 2 studies only (Muller & Reye 2012; Schaffner 2022) but at 11 sites in our atlas field study, our third highest value), and #N5 which encom-

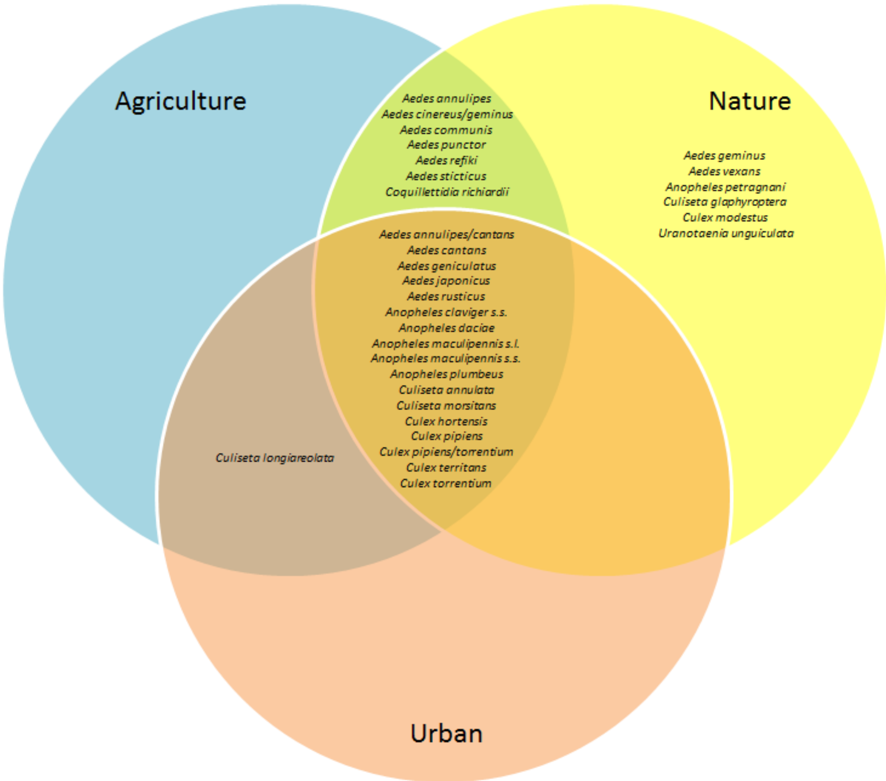


Fig. 138: Venn diagram of land cover categories, showing the absolute presence of mosquito species in Agriculture (blue), Nature (yellow), and Urban (orange) categories in our dedicated field study, Luxembourg, 2019-2021.

passes the Kockelscheuer nature area (investigated in 3 other studies (Ries et al. 2019; Schaffner & Ries 2019; Schaffner 2022) and at 9 sites in our Atlas field study, our seventh highest value). All four grid squares show a broad variety of larval habitats and are located in the Gutland. The grids with the lowest taxonomic diversity are #C1 (no data), D6, L1, Q1 and Q4 (a single taxon recorded in each), all four being located at the country borders and covering very reduced surfaces (Fig. 8).

Among the 28 species reported in the Grand Duchy, 17 (60.7%) occur in both the Oesling and the Gutland regions. The Oesling has one species that is not found in the Gutland, *An. petragnani*, though based on a single finding; while the Gutland has 10 species that were not found in the Oesling: *Ae. annulipes* (found in 13 grid squares) was not confirmed, while unresolved *Ae. annulipes/cantans* were found; *Ae. cinereus* (a single historical record), *Ae. geminus* (5 records), and additional unresolved Ae. cinereus/geminus (10 grid squares in total); *Ae. sticticus* (4 grid squares), *Ae. vexans* (4), *Cq. richiardii* (3), *Ae. refiki* (2), *Cs. glaphyroptera* (1), *Cx. modestus* (1), and *Ur. unguiculata* (1). This apparent lower taxonomic diversity in the Oesling can be explained partly by the absence of large (semi-)permanent wetlands and floodplain habitats for this region.

5.1.2 Faunal elements

- Mosquitoes and land cover categories

Among the 31 mosquito taxa collected in our study, 17 (54.8%) were found in all three land cover categories that we defined to select sampling locations (Fig. 138). Seven taxa were found in both Agriculture and Nature categories, and one was found in both Agriculture and Urban (*Cs. longiare-*

Fig. 139: Examples of mosquito habitats in the three different land cover environments: a. Pond in agriculture land cover (site ATL015, Limpach, Sanem, 04/04/19, *Aedes annulipes/cantans*, *Aedes rusticus*, *Culiseta morsitans*); b. Marsh in nature land cover (site ATL562, Kapweiler, Saeul, 15/03/21, *Aedes cantans*, *Culiseta morsitans*); c. Man-made containers in urban land cover (site ATL105; Steinfort, 17/06/19, *Culex pipiens*, *Culex torrentium*). Photos: Francis Schaffner.

olata). Six species were collected solely in the Nature category, but based on a maximum of just four 5x5 km grid squares, while no species was restricted to Agriculture or Urban only (Fig. 139).

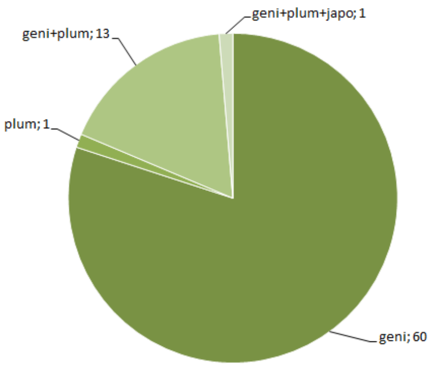


Fig. 140: Occurrences of mosquito species in tree holes in our dedicated field study, Luxembourg, 2019-2021 (75 sites). geni: *Aedes geniculatus*; japo: *Aedes japonicus*; plum: *Anopheles plumbeus*.

- Mosquito species in phytotelma habitats

Phytotelma habitats are natural plant cavities that accumulate rainwater. Such an environment welcomes a specific faunistic community, of which many species develop exclusively in phytotelmata. In Europe, phytotelmata mainly consist of tree holes in hardwood tree species. The accumulated water is often rich in vegetal organic matter and tannins. The native European mosquito fauna comprises five *Aedes* species (*Ae. berlandi*, *Ae. echinus*, *Ae. geniculatus*, *Ae. gilcolladoi*, *Ae. pulcritarsis*), one *Anopheles* species (*An. plumbeus*) and one *Orthopodomyia* species (*Or. pulcripalpis*). In addition, some ubiquist species such as *Cx. pipiens* can occasionally be found in phytotelmata, as well as some invasive species (*Ae. albopictus*, *Ae. japonicus*). In our field study, tree holes were found at 75 sites across the country (Figs 140; 141). The species *Ae. geniculatus* was most frequently found alone (n=60; 80%), while *An. plumbeus* was found alone in a single site/sampling; both species were commonly associated (n=13; 17.3%), and both were also associated with *Ae. japonicus* in a single site/sample.

- Peat bogs mosquito fauna

Peat bogs in moorlands are naturally acidic wetland environments filled with decaying vegetation which is dominated by *Sphagnum* species (Fig. 143). Few such sites with stagnant water exist in Luxembourg (Felten 2012; Schneider 2013); we found six sites, of which five were positive for mosquito larvae. The *Cs. morsitans* species was the most frequently found, at all five sites, followed by *Ae. punctor* (3 sites out of 5); eight other species

Fig. 141: Typical phytotelmata mosquito habitats in Luxembourg: a. Beech tree hole at the ground/roots level (site ATL410, Steinfort, 27/05/20, *Aedes geniculatus*); b. Beech tree hole in a branch at 2.6 m height (site ATL587, Goedange, Troisvierges, 18/03/21, *Aedes geniculatus*). Photos: Christian Ries (b), Francis Schaffner (a).

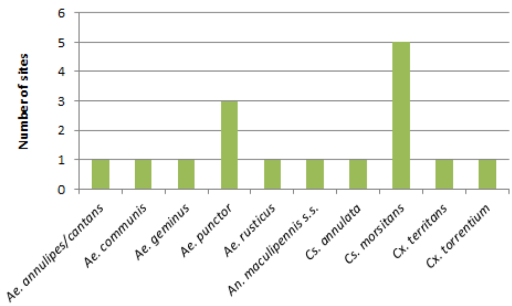


Fig. 142: Occurrences of mosquito species in peat bog sites in our dedicated field study, Luxembourg, 2019-2021 (5 sites).

Fig. 143: Typical peat bog mosquito habitat in Luxembourg (site ATL576, Hossenberg, Vallée de l'Ernz, 17/03/21, *Aedes annulipes/cantans*, *Aedes communis*, *Aedes punctor*, *Aedes rusticus*, *Culiseta morsitans*). Photo: Francis Schaffner.

Fig. 144: Typical floodland mosquito habitat in Luxembourg (site ATL563, Kuborn, Wahl, 15/03/21). Photo: Francis Schaffner.

Fig. 145: Typical snowmelt mosquito habitat in Luxembourg (site ATL558a, Birkenhaff, Nommern, 15/03/21, *Aedes cantans*, *Aedes communis*, *Aedes rusticus*). Photo: Francis Schaffner.

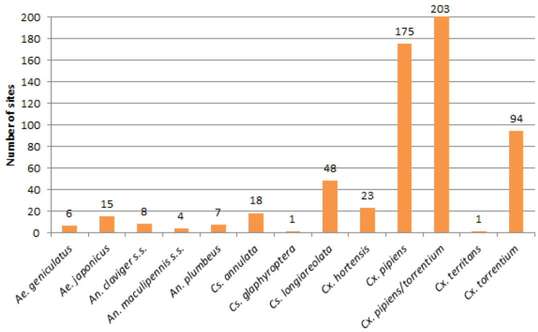


Fig. 146: Occurrences of mosquito species in man-made (artificial) containers in our dedicated field study, Luxembourg, 2019-2021 (213 sites).

were found at a single site (Fig.142). The two more frequent species found are known as typical species in peat bogs, but others, like *Ae. communis*, *Ae. pullatus* or *Cs. ochroptera* are missing here.

- Floodwater mosquito species

Typical floodwater mosquitoes develop in floodplains, usually along rivers or lakes with water fluctuations following heavy rains in early and mid-summer, and can generate severe nuisance since they generally emerge in huge numbers from the usually large surfaces of flooded areas (Fig. 144). In addition, these mosquitoes readily bite humans and can disperse far from their larval habitat to find hosts (Becker et al. 2020). These species are multivoltine, and the best developmental conditions for larvae occur between April and September, when high water temperatures enable rapid development. Taxa that belong to this category and are observed in Luxembourg are *Ae. cinereus/geminus*, *Ae. geminus*, *Ae. sticticus*, and *Ae. vexans*.

- Snowmelt mosquito species

Typical snowmelt mosquitoes develop in swampy woodlands, in pools that are formed by heavy rainfall in late autumn, after the snow melts, or rainfall in spring (Becker et al. 2020; Fig. 145). These species are univoltine; diapausing eggs will not hatch during the possible short-term summer flooding of these pools. Species that belong to this group and are present in the Grand Duchy are *Ae. annulipes*, *Ae. cantans*, *Ae. communis*, *Ae. punctor*, *Ae. refiki*, *Ae. rusticus*, and *Cs. morsitans*.

- Man-made container mosquito fauna

Man-made containers comprise a wide range of artificial containers that can hold rainwater when stored outdoors (Fig. 147). Many mosquito species can develop in such water bodies, given that they can lay their eggs on the water surface or on the inner side of these containers. During our atlas field study, 13 taxa were collected in man-made containers (Fig. 146). The taxon *Cx. pipiens/torrentium* was the most frequently found (n=203 out of 213; 95.3%) of which *Cx. pipiens* and *Cx. torrentium* were confirmed at 175 and 94 sites, respectively. Other species commonly found in these breeding sites were *Cs. longiareolata* (n=48; 22.5%), *Cx. hortensis* (n=23; 10.8%) and *Ae. japonicus* (n=15; 7.0%). Six additional species were only occasionally found to breed in such sites (between 1 and 8 sites, less than 3.8%).

- Invasive mosquito species

The European continent is currently facing the invasion of four exotic mosquito species: *Ae. (Stg.) aegypti aegypti* (Linnaeus 1762), *Ae. albopictus* (Fig. 149), *Ae. japonicus* and *Ae. koreicus* (Medlock et al. 2015). A fifth species, *Ae. (Georgecraigus) atropalpus* (Coquillett 1902), was present in Italy and in the Netherlands for some time, but has been

Fig. 147: Examples of man-made (artificial) water containers frequently inhabited by mosquito species in Luxembourg: a. Road drain / catch basin (site ATL181b, Fentange, Hesperange, 16/09/19, *Culex pipiens* and *Culex torrentium*); b. Rain water casks and other containers (site ATL393, Ettelbruck, 25/05/20, *Aedes geniculatus*, *Culex torrentium*); c. Tyres and tarpaulins (site ATL207b, Hovelange, Beckerich, 09/07/20, *Culex torrentium*); d. Fountain / basin (site ATL267, Baschleiden, Boulaide, 24/09/19, *Culex pipiens*, *Culex torrentium*, *Culiseta annulata*, *Culiseta longiareolata*); e. Drinking trough (site ATL382, Huldange, Troisvierges, 24/05/20, *Culex pipiens*); f. Boat (site ATL422, Linger, Pétange, 03/07/20, *Culex pipiens* and *Culex torrentium*). Photos: Francis Schaffner.

Fig. 148: Adult female of *Aedes japonicus*. Photo: Francis Schaffner.

Fig. 149: Adult female of *Aedes albopictus*. Photo: Francis Schaffner.

eradicated. During our field study, we detected only the invasive species *Ae. japonicus* (Figs 63; 148), despite the use of ovitraps at the two major points of entry, the A6 highway parking lots and the survey of larval habitats around the international airport.

5.1.3 Comparison with neighbouring countries

Whilst 28 taxa are now listed to occur in Luxembourg, there are 34 and 38 taxa reported from Belgium and the Netherlands, respectively, and 58 and 51 from France and Germany, respectively (Tab. 8).

No countrywide study was performed in France or the Netherlands, thus these species lists result from the compilation of various local scale studies. On the contrary, a countrywide inventory of the Culicidae fauna of Belgium was performed in 2007-2008, based mainly on adult trapping at randomly selected places across the country, in order to develop spatial mosquito distribution models (Versteirt et al. 2009, 2013). As a result, that study detected, for the first time in Europe, the presence of the exotic species *Ae. koreicus* (Versteirt et al. 2012) and 22 other species including the exotic *Ae. japonicus*, out of the 29 species reported to occur in the country at the time of the study (Boukraa et al. 2015). In Germany, a countrywide project combining active monitoring and citizen science has been running since 2011. By the end of 2019, this study recorded 49 species out of the 52 registered to occur to date, including six species not reported for decades and nine species that had never been documented before (Werner et al. 2020).

The Grand Duchy covers a much smaller territory than any of the neighbouring countries, but it shows the highest number of mosquito species per 1000 km^2 (n=10.83; n between 0.11 and 1.11 for the four neighbouring countries; Tab. 8). This indicates the mosquito diversity is not proportional to the size of a territory, but is most probably related to the diversity of habitats and climates that are encompassed by that territory. However, differences in sampling efforts may also influence the results, particularly for the detection of rare species.

The lower number of species in Luxembourg compared to Belgium and the Netherlands is related in particular to the occurrence in these countries of species breeding in coastal brackish waters (*Ae. caspius*, *Ae. detritus*, *Ae. dorsalis*, *Ae. flavescens*), other members of the *An. maculipennis* species complex (*An. atroparvus*, *An. messeae*, *An. melanoon*) and additional invasive species (*Ae. albopictus*, *Ae. koreicus*). The difference is greater with the fauna reported from France and Germany, which both have, on their very large territory, more diverse climatic and environmental conditions that are suitable to mosquitoes developing in e.g. coastal marshes or high altitude wetlands, and under warmer (southern France; e.g. *Ae. mariae*, *Ae. vittatus*, *An. hyrcanus*, *Cx. mimeticus*, *Cx. impudicus*, *Cx. theileri*) or colder (northern Germany; e.g. *Ae. excrucians*, *Ae. surcoufi*, *Cs. alaskaensis*) climates.

Tab. 8: Mosquito species reported from Luxembourg (this study included) and neighbouring countries. BEL: Belgium; DEU: Germany; FRA: France, continental; NLD: Netherlands; X: Listed species.

Mosquito taxa	LUX	BEL	DEU	FRA	NLD
Aedes (Acartomyia) mariae				X	
Aedes (Aed.) cinereus	X*	X	X	X	X
Aedes (Aed.) geminus	X		X	X	
Aedes (Aed.) rossicus			X	X	
Aedes (Aedimorphus) vexans	X	X	X	X	X
Aedes (Dahliana) geniculatus	X	X	X	X	X
Aedes (Fredwardsius) vittatus				X	
Aedes (Georgecraigus) atropalpus					Ø
Aedes (Hulecoeteomyia) japonicus	X	X2	X2	X2	X2
Aedes (Hul.) koreicus		X2	X2		X1
Aedes (Ochlerotatus) annulipes	X	X	X	X	X
Aedes (Och.) berlandi				X	
Aedes (Och.) cantans	X	X	X	X	X
Aedes (Och.) caspius s.l.		X	X	X	X
Aedes (Och.) cataphylla			X	X	
Aedes (Och.) coluzzii				X	
Aedes (Och.) communis	X	X	X	X	X
Aedes (Och.) cyprius			Ø		
Aedes (Och.) detritus		X	X	X	X
Aedes (Och.) diantaeus			X	X	
Aedes (Och.) dorsalis		X	X	X	X
Aedes (Och.) excrucians s.l.			X		X
Aedes (Och.) flavescens		X	X	X	X
Aedes (Och.) intrudens			X		
Aedes (Och.) leucomelas			X		X
Aedes (Och.) nigrinus			X*	X	X
Aedes (Och.) pulcritarsis				X	
Aedes (Och.) pullatus			X	X	
Aedes (Och.) punctor	X	X	X	X	X
Aedes (Och.) riparius			X		X
Aedes (Och.) sticticus	X	X	X	X	X
Aedes (Och.) surcoufi				X	
Aedes (Rusticoidus) refiki	X		X	X	
Aedes (Rusticoidus) rusticus	X	X	X	X	X
Aedes (Stegomyia) aegypti			Ø	Ø	Ø
Aedes (Stg.) albopictus		X1	X2	X2	X1
Anopheles (Anopheles) algeriensis			X	X	X
Anopheles (Ano.) atroparvus		X	X	X	X
Anopheles (Ano.) claviger s.s.	X	X	X	X	X
Anopheles (Ano.) daciae	X	X	X	X	
Anopheles (Ano.) hyrcanus				X	
Anopheles (Ano.) maculipennis s.s.	X	X	X	X	X
Anopheles (Ano.) melanoon				X	X
Anopheles (Ano.) messeae		X	X	X	X
Anopheles (Ano.) petragnani	X		X	X	
Anopheles (Ano.) plumbeus	X	X	X	X	X

Tab. 8: (cont.)

Mosquito taxa	LUX	BEL	DEU	FRA	NLD
Coquillettidia (Coquillettidia) buxtoni				X	
Coquillettidia (Coq.) richiardii	X	X	X	X	X
Culex (Barraudius) modestus	X	X	X	X	X
Culex (Culex) mimeticus				X	
Culex (Cux.) pipiens	X	X	X	X	X
Culex (Cux.) theileri				X	
Culex (Cux.) torrentium	X	X	X	X	X
Culex (Maillotia) hortensis	X	X	X	X	
Culex (Neoculex) europaeus/territans	X	X	X	X	X
Culex (Ncx.) impudicus				X	
Culex (Ncx.) martinii			X	X	
Culiseta (Allotheobaldia) longiareolata	X	X	X	X	X
Culiseta (Culicella) fumipennis		X	X	X	
Culiseta (Cuc.) litorea				X	
Culiseta (Cuc.) morsitans	X	X	X	X	X
Culiseta (Cuc.) ochroptera			X		X
Culiseta (Culiseta) alaskaensis			X	X	X
Culiseta (Cus.) annulata	X	X	X	X	X
Culiseta (Cus.) glaphyroptera	X		X	X	
Culiseta (Cus.) subochrea		X	X	X	X
Orthopodomyia pulcripalpis		X*		X	
Uranotaenia (Pseudoficalbia) unguiculata	X		X	X	
No. of species reported to currently occur	28	34	51	58	38
Countries' territory surface (km^2)	2,586	30,689	357,022	542,973	41,545
No. of species per 1000 km^2	10.83	1.11	0.14	0.11	0.91

* Uncertain, presence to be confirmed; 1 Introduced only; 2 Introduced and established; Ø Eliminated or extinct.
Sources: Robert et al. (2019); Verdonschot et al. (2019); Werner et al. (2020); De Wolf et al. (2021); Deblauwe et al. (2021); Hohmeister et al. (2021); Smitz et al. (2021); Teekema et al. (2022).
Exotic or European species for which introductions have not led to any local establishment in at least one of the listed countries are not listed in the table (e.g. Ae. (Ste.) flavopictus Yamada 1921, Cx. quinquefasciatus) (Scholte et al. 2010; Ibáñez-Justicia 2020).

5.1.4 Other species possibly occurring in Luxembourg

A number of mosquito species that occur in neighbouring countries (Tab. 8) may find suitable habitats for their development in Luxembourg. Although not yet recorded, some of the 22 species listed below could potentially be found in the Grand Duchy in the future.

5.1.4.1 Genus *Aedes* Meigen, 1818

According to their occurrence in neighbouring regions of France and/or Germany and to their ecological requirements, the following species could occur seldomly in Luxembourg: *Ae. (Aed.) rossicus* Dolbeskin, Gorickaja & Mitrofanova 1930, *Ae. cataphylla*, *Ae. diantaeus*, *Ae. excrucians*, *Ae. pullatus* and *Ae. surcoufi*. The two additional species *Ae. leucomelas* and *Ae. riparius* which occur in both Germany and the Netherlands may be added, as well as *Ae. nigrinus* which is reported from all three countries. Most of these species would develop in snowmelt pools, while *Ae. rossicus* and *Ae. nigrinus* would occur in floodland water bodies.

Two *Aedes* species which have a Mediterranean distribution, *Ae. berlandi* and *Ae. pulcritarsis*, have recently been detected in more northern regions of France (FS, unpublished data) and Germany

(Kampen et al. 2017). Both species breed exclusively in phytotelmata. Given the abundance of tree holes and the apparent northwards spread of the species, it can not be excluded that we will find these species in Luxembourg in the future.

Finally, the two invasive species *Ae. albopictus* and *Ae. koreicus* will probably be introduced into Luxembourg in the coming years. These species have nowadays established populations in neighbouring regions, of France and Germany for the former, Belgium and Germany for the latter, and are keen to disperse by means of ground vehicles (Eritja et al. 2017). Since established populations are increasing in number and size, the frequency of transport of these mosquitoes in vehicles across the region is increasing, as demonstrated by regular introductions into Belgium and the Netherlands (Deblauwe et al. 2015; Teekema et al. 2022).

5.1.4.2 Genus *Anopheles* Meigen 1818

Anopheles (Ano.) algeriensis Theobald 1903 mainly occurs in the Mediterranean region. However, isolated populations occur in northern regions, including in Germany and the Netherlands (Robert et al. 2019). A population of that species was recently described from Schleswig-Holstein, northern Germany, 55 years after the previous country record (Krüger & Tannich 2013; Werner et al. 2020). If present in Luxembourg, the species may be found in large wetlands with *Phragmites* sp. and *Carex* sp..

Among the Maculipennis species complex, two other members could occur in Luxembourg, since they are reported from neighbouring regions: *An. atroparvus* and *An. messeae*, reported from all four neighbouring countries (Tab. 8). Considering their known precise distribution, we expect above all *Ae. messeae* to be found in Luxembourg (Jetten & Takken 1994; Kampen et al. 2016; Smitz et al. 2021).

5.1.4.3 Genus *Culex* Linnaeus 1758

Among the genus *Culex*, one additional species, *Culex martinii* could possibly be found in Luxembourg. This rare species occurs in the neighbouring Grand Est region, France (Alsace, FS. unpublished data), and has recently been rediscovered in Thuringia, Germany, 44 years after the previous country record (Kuhlisch et al. 2018).

5.1.4.4 Genus *Culiseta* Felt 1904

To date, four Culiseta species are known to occur in Luxembourg. Four other species are seldom found in neighbouring regions and could possibly be found in the Grand Duchy: *Cs. alaskaensis*, *Cs. subochrea*, *Cs. fumipennis*, and *Cs. ochroptera*. While *Cs. subochrea* is reported from all four neighbouring countries, *Cs. alaskaensis* occurs in all but Belgium, *Cs. fumipennis* in all but the Netherlands, and *Cs. ochroptera* in Germany and the Netherlands only (Schaffner 1992; Kampen et al. 2013; Robert et al. 2019; Tab. 8).

5.1.4.6 Genus *Orthopodomyia* Theobald 1904

One species of the genus *Orthopodomyia, Or. pulcripalpis* (Rondani 1872), occurs in the western Palaearctic region. The species is mainly distributed in the Mediterranean region, but extends in Europe northwards to northern France, Belgium and Southern England (Moussiegt 1986; Robert et al. 2019). However, the Belgium record dates back to 1952 and is considered uncertain (Boukraa et al. 2015). The species inhabits phytotelmata, and it can not be excluded that the species could be found in the Grand Duchy.

5.2 Faunistic changes

5.2.1 Changes observed in Luxembourg

Due to the scarcity of historical data, little analysis can be performed on the apparent faunistic changes. Also, our field data are of qualitative and not quantitative nature, and thus can not detect changes in species' abundances compared to previous longitudinal field studies (Muller & Reye 2012; Ries et al. 2019). However cross-sectional qualitative data can reveal changes in spatial distribution patterns in terms of presence/absence.

During our atlas field study, we collected all species previously reported except *Ae. cinereus* (i.e. 22 / 23 species). In addition, we recorded five species new for the country: *Ae. refiki*, *An. daciae*, *An. petragnani*, *Cx. modestus*, and *Ur. unguiculata* (Tab. 4). Compared to earlier data, our field study does not indicate any species extinction or distribution range retraction.

Overall, the Luxembourgish Culicidae fauna does not include any presumed extinct or threatened mosquito species in the regional context. On the contrary, the territory has been colonised in recent years by two new species: *Ae. japonicus* and *Cs. longiareolata*. According to our personal observations in France and Germany (FS; unpublished data) and to published data (Kampen et al. 2017; Schaffner & Ries 2019), these species may have extended their distribution ranges from neighbouring regions. Whether the five species we report for the first time have also recently extended their distribution ranges from neighbouring regions, or if they were merely unnoticed is not clear. A special case refers to *An. daciae*, which was certainly present for a long time, if not always, and for which molecular identification enabled us to detect this taxon for the first time in Luxembourg.

In western Europe, indications of changes in mosquito distribution exist, with the discovery of warm-climate species in temperate regions and the obvious introduction and spread of invasive species. Species considered as preferring warm climates, such as *An. petragnani* and *Cs. longiareolata* show a clear northwards spread. *Culex modestus* is suspected to do so, and is strengthening its population in temperate regions, where it was perhaps overlooked earlier (Hernandez-Triana et al. 2020). Since earlier studies at the Haff Réimech reserve did not detect the species (Beck et al. 2003), one can suspect the species as being undetected with a reduced population size at that time, if not absent. The recent northernmost findings of *Ur. unguiculata* in Luxembourg and Germany also suggest such a spread (Tippelt et al. 2017). If these changes are a result of climate changes, other species, known to be associated with cold climates, may show a distribution range retraction (from south to north). This is not yet reported but might be in the future if targeted field studies are implemented.

The most obvious change in mosquito fauna composition in Europe is the arrival of exotic species. The well-known spread of the tiger mosquito, *Ae. albopictus*, is now of concern for almost all European countries (Medlock et al. 2015; ECDC 2022). Here the available data are comprehensive, since almost all countries at risk are implementing targeted surveillance to detect its introduction and follow its spread, and most of the time to implement immediate control measures to reduce or avoid the species' spread and prevent pathogen transmission (Schaffner et al. 2013a; Gossner et al. 2018). Four invasive species are currently spreading on the European continent, and in addition to *Ae. japonicus* which is already present, at least two of them may colonise the Grand Duchy: *Ae. albopictus* and *Ae. koreicus*.

5.2.2 Changes observed in neigbouring regions

Cases of species extinctions exist in Europe with e.g. *Ae. aegypti* which invaded the whole Mediterranean Basin during the 18th century and disappeared by the mid-20th century due to reasons which are unclear (Schaffner & Mathis 2014; Wint et al. 2022). Additionally, in some neighbouring countries other species are considered extinct, e.g. *Ae. cyprius* in Germany (Werner et al. 2020). However, except for *Ae. aegypti*, these cases are always based on little data, if not single observations, and therefore these historical data remain questionable and generate uncertainty about changes in distribution ranges. As for *Ae. cyprius*, the observed change can be due to incorrect identification or to a distribution range retraction of a cold-climate species.

Obvious changes are related to invasive species, with in particular *Ae. albopictus* and *Ae. koreicus* being frequently introduced to neighbouring regions and in some cases establishing and spreading (Deblauwe et al. 2015; Ibáñez-Justicia 2020; ECDC 2022). As for Luxembourg, there is no environmental or climatic reason that *Ae. albopictus* will not establish populations (Ibáñez-Justicia et al. 2020; Wint et al. 2020).

5.2.3 Changes observed in Luxembourg for other insect orders

In Luxembourg, many faunistic changes are reported in other insect orders such as Coleoptera, Hymenoptera, Lepidoptera, and Odonata, with some species extinctions and distribution range retractions, but also expansions and even introductions (Vitali 2018; Schneider & Cungs 2022). The arrival of warm-climate hymenopteran species has recently been reported (Weigand & Herrera-Mesías 2020; Schneider & Cungs 2022). Some warm-climate species have increased their presence (Odonata) while others have decreased (Orthoptera, Lepidoptera: Papilionoidea). Species related to moorlands and humid habitats have often decreased (Coleoptera: Carabidae; Coleoptera: Hydrophilidae; Odonata; Lepidoptera: Papilionoidea). Among the reasons for species regression or extinctions, anthropisation is cited (Lepidoptera: Papilionoidea), changes in agriculture (Coleoptera: Curculionoidea), the loss of habitats (Odonata; in particular for species related to moorlands) and the deterioration of particular habitats, and changes in climatic conditions (Coleoptera: Cerambycidae). Some aquatic beetles and dragonflies related to moorlands have regressed or become locally extinct. In contrast, some warm-climate Odonata species have colonised or increased their presence. This suggests that aquatic insects, if not related to specific environments such as moorland, are not negatively affected by climatic change and are even favoured in the case of warm-climate species (Vitali 2018). Considering the climate, an increasing oceanicity of the Luxembourgish climate is observed, suggesting species related to a more continental climate will regress (Vitali 2018).

5.2.4 Future possible changes

As with many other insects, mosquito species are dependent on the presence of suitable environments for their development. Species sensitive to environmental changes are those that develop in specific environments that can regress, such as moorlands or floodlands. While during the 20th century there was a clear strategy to reduce wetlands, and floodlands in particular, for several reasons, including to reduce mosquito nuisance and infectious diseases transmission (Hackett 1937; Zimmermann 2001), there is nowadays a reversed trend for the restoration or creation of wetlands and ponds, in order to reduce flooding damages, but also favour aquatic fauna and amphibians (Dale & Knight 2008; Proess 2016; Natur&Ëmwelt 2018; Martinou et al. 2020). Thus, mosquito species may be globally favoured by the current environmental changes. Even in the case of anthropisation, which is always associated with the formation of artificial water bodies, some mosquito species will be favoured, in particular the common house mosquito *Cx. pipiens* which is sympatric to humans. Additionally, the invasive species, in particular the container-inhabiting *Aedes* species, are fostered by anthropisation similarly to *Cx. pipiens*, and the global increase of travel and trade is boosting their international transportation and dispersal. The arrival and local establishment of e.g. *Ae. albopictus* is a matter of time and will most probably not be avoided. Finally, climatic changes may favour the establishment and expansion of warm-climate species in Luxembourg (e.g. *An. petragnani*, *Ae. berlandi*), while cold-adapted species may be disfavoured and might show a retraction of their ranges (e.g. *Ae. communis*, *Cs. glaphyroptera*), according to the degree of changes that will occur in the future.

5.3 Sanitary and vectorial risk

Besides provoking hypersensitivity or allergies to mosquito bites, mosquitoes can transmit pathogens and parasites such as bacteria, nematodes, protozoans and viruses that can cause diseases in humans and animals. The transmission occurs during the mosquito bite, when the female mosquito injects her saliva that contains the pathogen, to facilitate the blood meal. Transmission can be mechanical or biological. While mechanical transmission is often associated with various insect species (e.g. Myxoma pox virus causing myxomatosis and *Francisella tularensis* bacteria causing tularaemia), biological transmission is insect family-specific and even species-specific. This transmission mode is more complex because it involves an obligatory period of replication and/or development of the pathogen or parasite in the insect vector (Becker et al. 2020).

The incidence of vector-borne diseases is a worldwide concern for human and animal (pets, livestock and wildlife) health (Jones et al. 2008), and changes in distribution and incidence of these diseases is determined by a complex set of environmental, demographic, and social factors in which global travel and trade and urbanisation play key roles (Kilpatrick et al. 2012). For a given pathogen or parasite, different mosquito species can play different roles, e.g. they can be primary or secondary vectors, they can be enzootic (i.e. transmitting between animal hosts only) or a bridge vector (i.e. transmitting between animal hosts and humans). Assessing this role, which can also vary between populations within a species, is not easy and requires the determination of the vectorial capacity of a given mosquito population in the local context. Data that allow the determination of the vectorial capacities include those obtained from the laboratory evaluation of the vector competence, those from the field assessment of the host preferences and the biting behaviour, and of the vector population abundance. However, as a primary assessment, the theoretical risk posed by mosquitoes can be assessed by ranking the species according to the available information about the species' role as observed in other countries, the laboratory competence, and the infection by a pathogen in nature. The latter shows that the species is at least able to acquire the given pathogen from an infected host in natural conditions.

Pathogens of primary human health relevance include human malaria parasites and eight arboviruses: Batai, chikungunya, dengue, Inkoo, Sindbis,

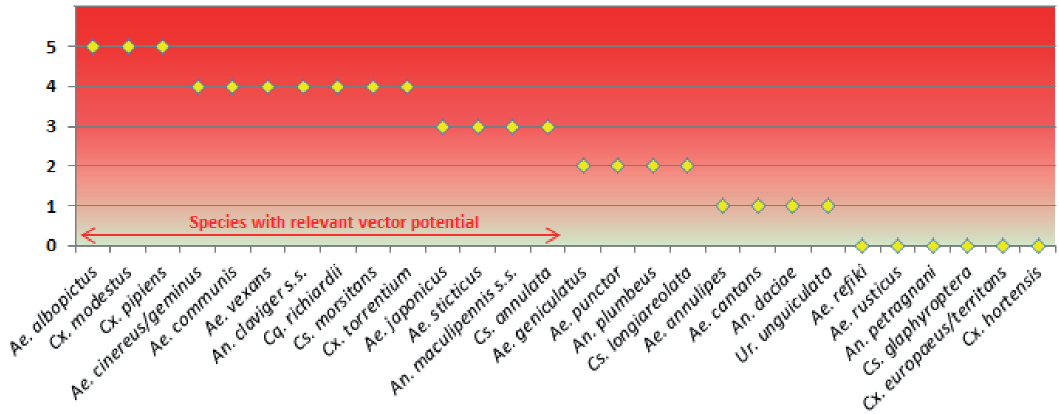


Fig. 150: Chart of vectorial risk for human malaria parasites and eight arboviruses of public health relevance (Batai, chikungunya, dengue, Inkoo, Sindbis, Tahyna, West Nile, Zika) and for all mosquito species observed to occur in Luxembourg plus *Aedes albopictus*. Ranking: 0: Species not implicated in any pathogen transmission or absence of information for the species; 1: Species infected in nature only; 2: Species competent in the laboratory only (at low, moderate or high level); 3: Species infected in nature and competent, for the same pathogen; 4: Species known as past/present vector in regions and countries outside Western Europe or, for malaria, secondary vector only; 5: Species known as vector in Western Europe.

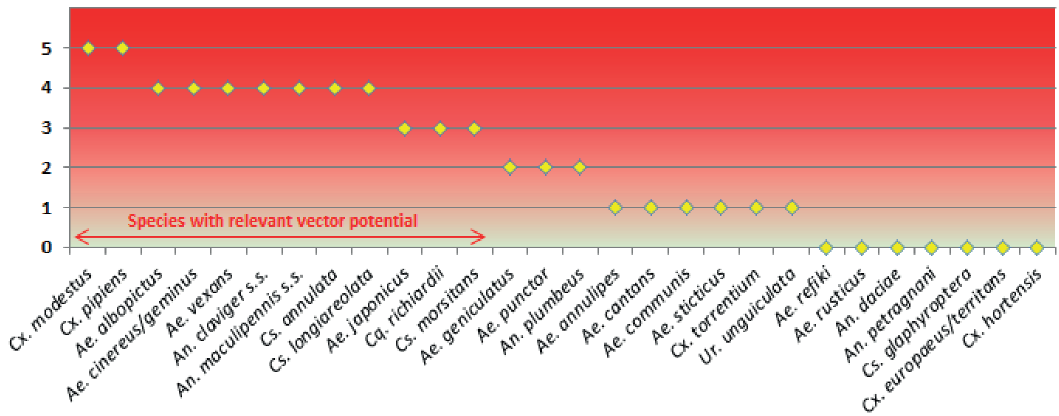


Fig. 151: Chart of vectorial risk for seven pathogens and parasites of animal health relevance (avian plasmodiums, myxoma pox virus, Usutu and West Nile viruses, *Dirofilaria* and *Setaria* nematode species, tularaemia bacteria) and for all mosquito species observed to occur in Luxembourg plus *Aedes albopictus*. Ranking: 0: Species not implicated in any pathogen transmission or absence of information for the species; 1: Species infected in nature only; 2: Species competent in the laboratory only (at low, moderate or high level); 3: Species infected in nature and competent, for the same pathogen; 4: Species known as vector in regions and countries outside Western Europe; 5: Species known as vector in Western Europe.

Tab. 9: Vector potential for pathogens of primary public health (bold) and animal health (regular) importance and/or causing nuisance, for the mosquito taxa occurring in Luxembourg plus the invasive species *Aedes albopictus*. Pathogens of zoonosis are underlined.

Mosquito taxa	**Batai virus**	**Chikungunya virus**	**Dengue viirus**	**Inkoo virus**	**Sindbis virus**	**Tahyna virus**	Usutu virus	**West Nile virus**	**Zika virus**	Myxoma pox virus	Avian malaria	**Human malaria**	*Dirofilaria* sp.	*Setaria* sp.	Tularaemia bacteria	**Can generate nuisance**
Aedes (Aed.) cinereus/geminus					4	1		1							4	P
Aedes (Adm.) vexans	1	2			0	4	1	3	3				3	4		P
Aedes (Dah.) geniculatus		2						2							1	O
Aedes (Hul.) japonicus		2	2				1	3	2							O
Aedes (Och.) annulipes						1				1						S
Aedes (Och.) cantans						1		1		1				1		S
Aedes (Och.) communis	1			1	4	2									1	O
Aedes (Och.) punctor	1			1		2		2							1	O
Aedes (Och.) sticticus				1		3		1	0					1	1	P
Aedes (Rus.) refiki																
Aedes (Rus.) rusticus																S
Aedes (Stg.) albopictus		5	5				3	3	5				4			P
Anopheles (Ano.) claviger s.s.	1					2				1		4	1	4	1	O
Anopheles (Ano.) daciae	1															
Anopheles (Ano.) maculipennis s.s.	3*	0			1*	0*		1*		1*		3	3	4		O
Anopheles (Ano.) petragnani																
Anopheles (Ano.) plumbeus								2				2				S
Coquillettidia (Coq.) richiardii	1				4	1		1					3			S
Culex (Bar.) modestus	1				1	1	1	5		1			4		1	O
Culex (Cux.) pipiens	1	0			5	3	5	5	0		5		4		1	P
Culex (Cux.) torrentium					4		1		0						1	
Culex (Mai.) hortensis																
Culex (Ncx.) europaeus/territans																
Culiseta (All.) longiareolata								2			4					
Culiseta (Cuc.) morsitans					4			1					3			
Culiseta (Cus.) annulata						3	1	0			4			1		O
Culiseta (Cus.) glaphyroptera																
Uranotaenia (Pfc.) unguiculata								1					1			

Empty cell: Absence of information for the species
Arboviruses: 0: Refractory to infection in the laboratory, 1: Species infected in nature only, 2: Species competent in the laboratory only (at low, moderate or high level), 3: Species infected in nature and competent, 4: Species known as vector in regions and countries outside Western Europe only; 5: Species known as vectors in Western Europe
Human malaria: 2: Species competent in the laboratory that could be an occasional vector, 3: Secondary vector only, 4: Primary vector outside Western Europe only, 5: Primary vector in Western Europe
Can generate nuisance: P: Primarily; S: Secondarily; O: Occasionally.
Western Europe: as defined by the United Nations geoscheme (Austria, Belgium, France, Germany, Luxembourg, Netherlands, Monaco, Switzerland).
* Described for *An. maculipennis* s.l. only.
Sources: Schaffner et al. (2001); Hubálek (2008); Schaffner et al. (2014); Elizondo-Quiroga et al. (2018); Scheuch et al. (2018); Martinet et al. (2019); Becker et al. (2020); Vilibic-Cavlek et al. (2020).

Tahyna, West Nile, and Zika viruses. Most infections appear to pass unnoticed or are present with transient mild febrile symptoms, commonly referred to as “summer flu” (Hubálek 2008). However, West Nile, chikungunya, and dengue infections have also been associated with fatal disease. Whilst most of these arboviruses are transmitted by native mosquito species, only the invasive Asian tiger mosquito, *Ae. albopictus*, is responsible for the recent outbreaks of chikungunya, dengue and Zika (Johnson et al. 2018; Giron et al. 2019). Pathogens of primary animal health relevance include Usutu and West Nile viruses, myxoma pox virus, avian malaria plasmodiums, *Dirofilaria* sp. and *Setaria* sp. nematodes, and tularaemia bacteria. Some of these pathogens are responsible for zoonosis, i.e. infections that affect both animals and humans: Batai, Sindbis, Tahyna, Usutu and West Nile viruses, and tularaemia bacteria.

A primary risk assessment performed for the mosquito species reported to occur in Luxembourg plus the invasive species *Ae. albopictus*, shows that at least 15 species could be involved in a pathogen or parasite transmission (risk rank between 3 and 5; Tab. 9). When sorting the pathogens, 14 taxa show a relevant vector potential of public health importance (human malaria plasmodiums and Batai, chikungunya, dengue, Inkoo, Sindbis, Tahyna, West Nile, and Zika viruses; Fig. 150) and twelve of animal health importance (avian plasmodiums, myxoma pox virus, Usutu and West Nile viruses, *Dirofilaria* and *Setaria* nematode species, tularaemia bacteria; Fig. 151). Among these taxa, three are relevant for public health only (*Ae. communis*, *Cx. torrentium*, *Ae. sticticus*), one for animal health only (*Cs. longiareolata*), and eleven for both (*Ae. albopictus*, *Cx. modestus*, *Cx. pipiens*, *Ae. cinereus/geminus*, *Ae. vexans*, *An. claviger* s.s., *An. maculipennis* s.s., *Cq. richiardii*, *Cs. morsitans*, *Ae. japonicus*, *Cs. annulata*). This ranking can be used when defining a vector-targeted surveillance, but may be fine-tuned according to other risk parameters (e.g. circulation of a pathogen in a neighbouring region or importation of infected hosts) (Braks et al. 2011; Schaffner et al. 2014). Considering the nuisance aspect, 18 species may have the potential to disturb humans, with five taxa that can be considered as primary nuisant species, i.e. *Ae. cinereus/geminus*, *Ae. vexans* and *Ae. sticticus* in outdoor conditions, mainly at dusk and in the neighbourhoods of wetlands; while *Ae. albopictus* may become a source of nuisance outdoors and day-long in urbanised areas, and *Cx. pipiens* indoors and during the night (Schaffner et al. 2001; Becker et al. 2020).

5.4 Conclusions and outlook

Within our study, we assembled two sets of data; firstly, 642 mosquito taxa occurrences from published or unpublished sources from the period 1997 to 2018, and secondly 22 citizen data and 735 original mosquito occurrences obtained from 560 sites distributed across the Grand Duchy and collected during our atlas field study, 2019-2021. All data are stored in a unique database that allows the production of updated mosquito distribution maps for the 28 species (31 taxa) reported to occur in Luxembourg to date, with one species, *Ae. cinereus*, for which the occurrence remains to be confirmed.

The development of this Atlas of the Culicidae of Luxembourg constitutes a basis for several potential follow-up activities. First, diversity and distribution data may be continuously updated given the steady contribution of citizen scientists and the incorporation of culicid specimens from national projects or from specific *Aedes* invasive mosquito surveillance activities. In doing so, the developed database and the related online maps may be regularly updated. Second, a comparable survey and biodiversity assessment could be performed after e.g. ten years time, analysing diversity and distribution changes of individual species in a standardised way. Third, the atlas data can be used as the frame for the development of a sophisticated surveillance programme of target species, e.g. distribution and abundance changes of *Aedes* invasive mosquitoes or native vector species. Fourth, distribution and diversity data of potential vector species also can serve to establish a surveillance scheme for vector risk assessment, e.g. by informing local authorities about the presence and adult activity of a given vector species so that specific pathogen screenings can be applied.

Overall, there is a clear need for additional monitoring and surveillance to further assess the biodiversity and the vectorial risk and to prepare possible mosquito control actions if needed. Specific measures could focus on searching for the species listed as possibly occurring in Luxembourg (cf. section 5.1.4), with a priority for the invasive Asian tiger mosquito

Ae. albopictus, in view of its high vector potential ranking. Studies may also focus on assessing field parameters of the vectorial capacities for the main vector species occurring in Luxembourg, i.e. *Cx. pipiens, Cx. modestus*, and the other species showing relevant vector potential (cf. previous section), and screen pathogens in these mosquitoes in a context of suspicion of pathogen circulation.

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- use n. sp., n. gen., n. fam., etc. for new taxa;
- use italicized words only for taxa of generic and subgeneric ranks;
- use lowercase characters for authority names
- references to illustrations and tables should be indicated as follows: (Fig. 1), (Fig. a, d), (Fig. 2a-d), (Figs 3; 6), (Figs 3-5; Tab. 2); (Tab. 1); for German texts use Abb. instead of Fig.
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Copies of all figures and tables should be included with the manuscript. They can be either included in the text at the correct locations with their legends or referenced in the text and included as annexes.

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References

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Høeg J. T. & Lützen J. 1985. - Comparative morphology and phylogeny of the family Thompsoniidae (Cirripedia: Rhizocephala: Akentrogonida) with description of three new genera and seven new species. Zoologica Scripta 22: 363-386.

Marshall C. R. 1987. - Lungfish: phylogeny and parsimony, in Bernis W. E., Burggren W. W. & Kemp N. E. (eds), The Biology and Evolution of Lungfishes, Journal of Morphology 1: 151-152.

Röckel D., Korn W. & Kohn A. J. 1995. - Manual of the Living Conidae. Volume 1: Indo-Pacific Region. Christa Hemmen, Wiesbaden, 517 p.

Schwaner T. D. 1985. - Population structure of black tiger snakes, *Notechis ater niger*, on off-shore islands of South Australia: 35-46, in Grigg G., Shine R. & Ehmann H. (eds), Biology of Australasian Frogs and Reptiles. Surrey Beatty and Sons, Sydney.

Gerecke R., Stoch F., Meisch C. & Schrankel I. 2005. - Die Fauna der Quellen und des hyporheischen Interstitials in Luxemburg unter besonderer Berücksichtigung der Milben (Acari), Muschelkrebse (Ostracoda) und Ruderfusskrebse (Copepoda). Ferrantia 41, Musée national d'histoire naturelle, Luxembourg, 140 p.

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