European Mosquito Bulletin 29 (2011), 13-21
Journal of the European Mosquito Control Association
ISSN 1460-6127; w.w.w.e-m-b.org
First published online 3 March 2011

Mosquito (Culicidae) voucher specimens in the RBINS collection: remnants of a past glory or hidden treasure?

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Abstract

In the beginning of the last century mosquitoes were collected extensively all over Belgium. A great part of this material was however never identified and stored in supplements of the Royal Belgian Institute of Natural Sciences (RBINS) collection. The Belgian Culicidae collection at the RBINS was subjected to a new investigation in the framework of the MODIRISK project (an inventory of native and invading mosquito species in Belgium in relation to global change and modelling 2007-2010). The identification and records of all voucher specimens of the collection were evaluated and digitised. One new species for the Belgian fauna was discovered in previously unexplored collection: *Culiseta subochrea*. None of the currently collected invasive and exotic species were discovered in the collection. Together with many records from other recent inventory projects carried out at the RBINS, a newly established database CULIBEL was generated that included all Belgian mosquito collection records. The present paper demonstrates the importance of extensive voucher specimen collections to basic invertebrate inventory.

Keywords: Culicidae, faunistics, mosquitoes, voucher specimens, re-evaluation of collections

Introduction

Until recently the distribution and diversity of Culicidae in Belgium was poorly known. The checklist from 1991 of the Belgian Culicidae named 24 species, which was the number of identified species found in the Royal Belgian Institute of Natural Sciences (RBINS) collection and additional species mentioned in the card-indexes (Gosseries & Goddeeris, 1991). These authors estimated the total number of species in Belgium to be approximately 50. However since 1991 only a few mosquito species were added to the Belgian fauna e.g. *Culex hortensis* (Versteirt *et al*., 2009) and *Culiseta ochroptera* (Schaffner pers. com.).

Ongoing eco-climatic changes and increasing worldwide transport of people and goods create suitable conditions for the introduction of exotic mosquito species as well as the (re)emergence of mosquito-borne diseases in Europe. Invading and exotic species, together with many native species, can act as vectors of arboviruses (viruses transmitted by arthropods). Recent outbreaks of mosquito-born diseases in nearby countries in western Europe (e.g. Benedict *et al*. (2007) Lancelot *et al*. (2009) Moutailler *et al*. (2008) and Romi *et al*. (2004) for the Mediterranean region), show the need for a better knowledge of the taxonomic and functional biodiversity of both native and invading vector species. In this context, a four year project (MODIRISK) was started in 2007 to survey and map the mosquito populations present in Belgium, with focus on both invading and exotic as well native species (Van Bortel *et al*., 2007).

Within the framework of the MODIRISK project, the Belgian mosquito collection at the RBINS was revised. A large proportion of the mosquito collection contains unidentified specimens (16 boxes, called the supplements). This revision could reveal previously undiscovered hidden treasures, e.g. new or rarely recorded species for Belgium or confirm the earlier presence of certain recent exotic and or invading species. The first confirmed record of an exotic mosquito species in Belgium dates back to 2000 when *Aedes albopictus* [*Stegomyia albopicta* of Reinert *et al*., 2009] larvae were discovered in a second hand tyre store in Vrasene (Schaffner *et al*., 2004). A second exotic mosquito *Aedes japonicus* [*Hulecoeteomyia japonica* of Reinert *et al*., 2009] was recorded in 2002 and its establishment was confirmed in 2007-2008 during the MODIRISK project (Versteirt *et al*., 2009). During this project, a third exotic species *Aedes koreicus* [*Hulecoeteomyia koreica* of Reinert *et al*., 2009] was found in an old sand quarry near the national park Hoge Kempen and close by the industrial area of Maasmechelen (Versteirt *et al*., unpublished). However, it is very likely that invasive or exotic species have been present in Belgium before 2000 and there are chances that specimens of these exotic species were collected and stored in the supplements of the mosquito collection.

The first aim of this paper was to update and revise the Belgian mosquito collection of the RBINS, including the unidentified specimens. Secondly, the collections were searched for the presence of exotic or invasive mosquito species earlier than 2000.

Material and Methods

Voucher specimens

The most representative and rich collections of Belgian Diptera were collected by the renowned dipterologists M. Goetghebuer and M. Bequaert and these have been preserved for over a century at the RBINS (Grootaert *et al*., 1991). There are four sub-collections in the RBINS collection: a general collection, two sub-collections of Bequaert and Goetghebuer, and a sub-collection of unidentified specimens i.e. the supplements. The Bequaert sub-collection has 135 voucher specimens from 1912-1958. The Goetghebuer sub-collection contains 269 specimens and was collected between 1909-1946 (mainly between 1910 and 1930). A total of 241 specimens are present in the general collection, all of them collected between 1878-1967 (mainly between 1880 and 1925). The supplements are the largest sub-collection: 737 specimens collected between 1892 and present, with the majority of specimens

collected between 1920 and 1960. All specimens present in the four collections were collected by net sweeping and hand sampling.

All records (i.e. species, collection date, locality) were recorded to 10 x 10 km grid cells of the UTM (Universal Transverse Mercator) projection, hereafter called grid cells.

Identification

Identification of all voucher specimens from the available collections was done using the keys of Schaffner *et al*. (2001). Major changes within the tribe Aedini were recently published by Reinert *et al.*, (2009, and their earlier works). With the exception of separate recognition for *Aedes* and *Ochlerotatus* (Reinert, 2000), we use the traditional names in this publication as there is no consensus on the other major nomenclatural changes and to increase accessibility and readability by non-taxonomists working with aedine species. Morphological differentiation between *Aedes cinereus* and *Aedes geminus* was not possible and these two species will be referred to as *Aedes cinereus* sensu lato. All voucher specimens belonging to the *Anopheles maculipennis* species complex were not identified to the species level, i.e. *Anopheles atroparvus*, *Anopheles messeae* and *Anopheles maculipennis* except for some microscopic preparations from eggs and larvae from *An. atroparvus* in the sub-collection from Goetghebuer.

Results

General results

Identification of all the specimens in the RBINS collections was revised and all 1381 specimens belonging to 24 species were digitised (Table 1). Most of the specimens (77%) were collected between 1910 and 1960 (Figure 1), and the majority were collected between 1940 and 1950. The intensity of research and mosquito-sampling fluctuated during this period, as revealed by the number of voucher specimens per decade (Figure 1). The oldest specimens (collected in 1878) are deposited in the general collection.

Sixteen species are present in the general collection, 18 in the Bequaert sub-collection, 21 in the Goetghebuer sub-collection and 20 species were counted in the supplements.

Culex pipiens and *Culiseta annulata* were the most abundant species in the collections. Also many voucher specimens of *Ochlerotatus punctor* were present.

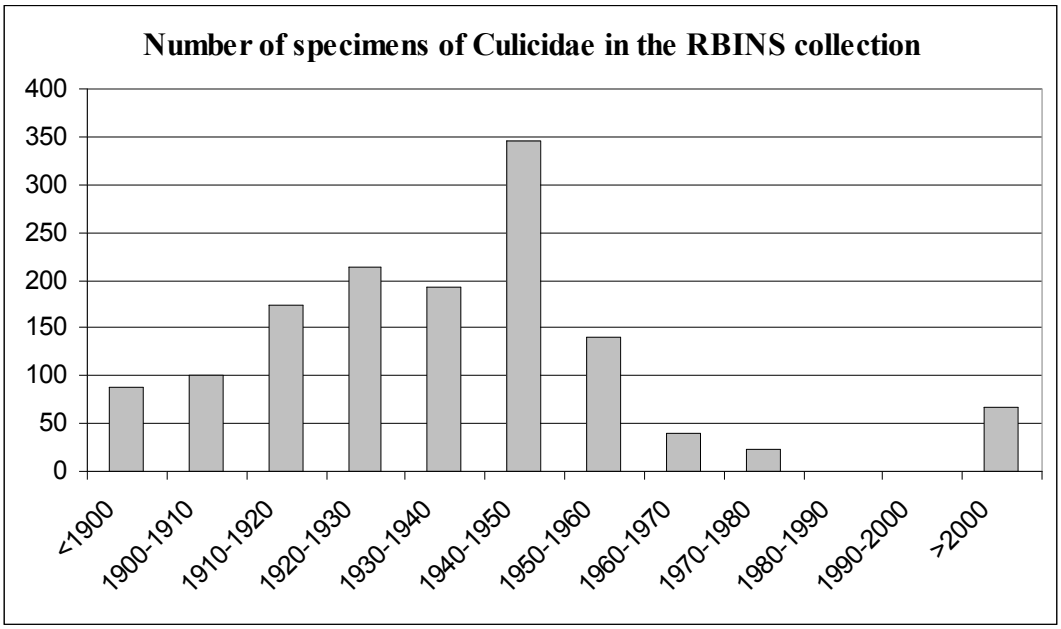

Figure 1: Number of identified voucher specimens for each considered period.

Mosquito records were present from 100 grid cells. Voucher specimens form RBINS collection reveal that in the past in some regions mosquitoes were more intensively sampled. In several grid cells near Brussels and the city of Ghent more than 7 species were collected before 1960 (Figure 2). Grid cells near Knokke and Blankenberge contain many historical records and high numbers of species.

Records of rare and interesting species

Culex hortensis was only recently added to the Belgian fauna (Versteirt *et al*., 2009), but a female was collected by Bequaert in Aywaille, Nonceveux on 6/7/1947. Other remarkable records are for *Culiseta fumipennis*, which was collected by Goetghebuer from two different sites: it was collected in Hockai near Stavelot on 11/6/1914 and 1/7/1920, probably in bogs and wet heathland vegetations of the Hautes Fagnes. It was also collected in Lovendem, Vinderhoute on 9/5/1920.

Of the mosquito species *Culiseta subochrea*, two specimens were found in the collection of Bequaert: one male at Destelbergen, Heusden (Prov. Eastern-Flanders), 2/8/1944 and one female in Blankenberge (Prov. Western-Flanders), 6/11/1955.

Table 1: Species list of the mosquitoes of Belgium to date and number of specimens of each species found in the RBINS mosquito collection ([1] Schaffner *et al*., 2004, [2] Versteirt *et al*., 2009, [3] Versteirt *et al*., unpublished [4], Boukraa *et al*., unpublished).

Species	Number of specimens in RBINS collection	Mentioned in checklist 1991
Aedes cinereus/geminus	56	Y
Aedes vexans Meigen	8	Y
Anopheles claviger (Meigen)	59	Y
Anopheles maculipennis s.l.	59	Y
Anopheles atroparvus Van Thiel	8	Y
Anopheles messeae Falleroni	/	Y
Anopheles plumbeus Stephens	22	Y
Coquillettidia richiardii (Ficalbi)	37	Y
Culex hortensis Ficalbi	1	N
Culex pipiens Linnaeus	414	Y
Culex torrentium Martini	1	N
Culex territans Walker	9	Y
Culiseta annulata (Schrank)	192	Y
Culiseta subochrea (Edwards)	2	N
Culiseta fumipennis (Stephens)	4	Y
Culiseta morsitans (Theobald)	72	Y
Culiseta ochroptera (Peus)	/	N
Ochlerotatus annulipes (Meigen)	36	Y
Ochlerotatus cantans (Meigen)	89	Y
Ochlerotatus caspius (Pallas)	7	Y
Ochlerotatus communis (De Geer)	16	Y
Ochlerotatus detritus Haliday	14	Y
Ochlerotatus punctor (Kirby)	114	Y
Ochlerotatus sticticus (Meigen)	18	Y
Ochlerotatus dorsalis (Meigen)	/	Y
Ochlerotatus flavescens (Muller)	/	Y
Ochlerotatus rusticus (Rossi)	55	Y
Aedes albopictus (Skuse)[1]	/	N
Aedes geniculatus (Olivier)	88	Y
Aedes japonicus (Theobald)[2]	/	N
Aedes koreicus (Edwards)[3]	/	N
Culex modestus Ficalbi [4]	/	N

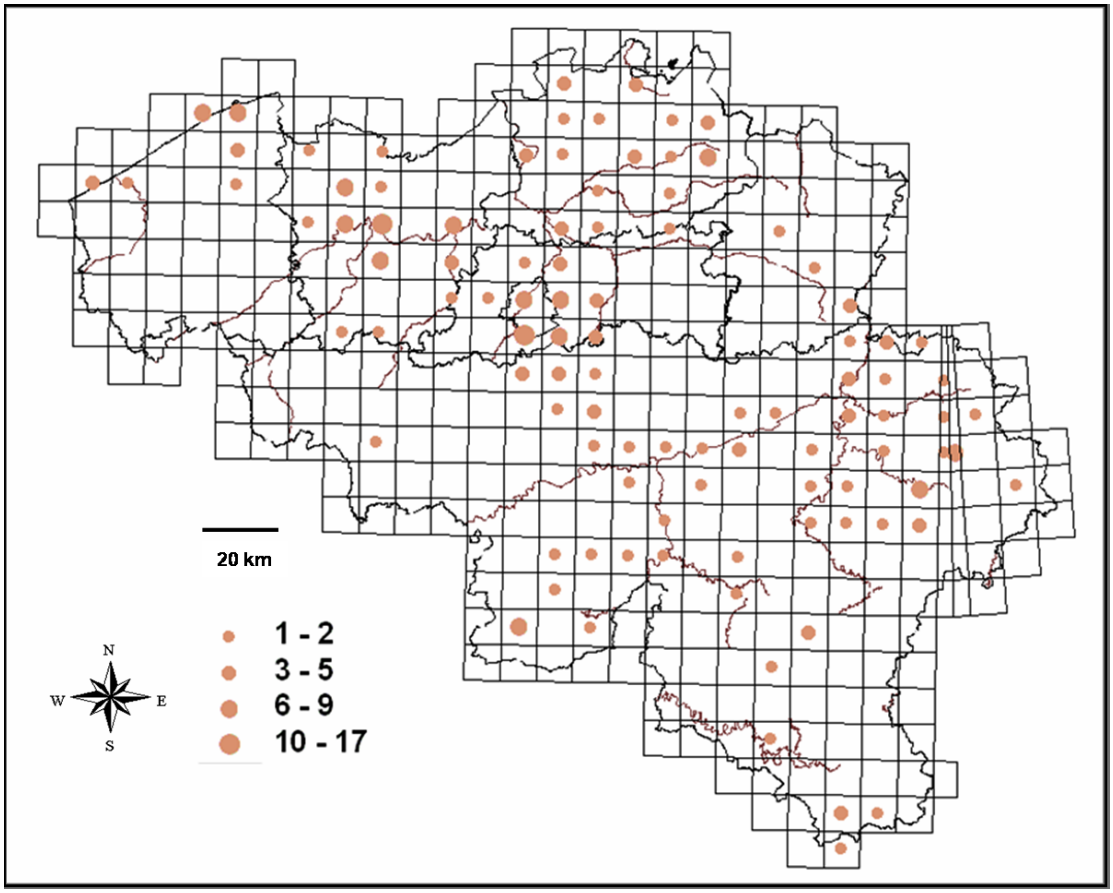

Figure 2: Number of mosquito species for each UTM 10km x 10km square discovered in the RBINS collection, in grid cells without dots no mosquitoes were sampled before 1960.

Exotic and invasive species

No exotic or invasive/introduced species were discovered in the RBINS collection. The first confirmed record of exotic species in Belgium dates back to 2000 (Schaffner *et al*., 2004). To date, we have no evidence that exotic or invasive mosquitoes were noticed or specimens were collected before 2000.

Discussion and conclusions

Very interesting specimens and some long-time untouched treasures were (re)discovered during the revision of the RBINS mosquito collection. Many records from the beginning of the last century (before 1960) were listed and some faunistic important voucher specimens were present. The voucher specimens in these collections provide historical (pre-1980) information on more than 70% of the currently recorded mosquito species of Belgium. No exotic or invasive species were found in the collection.

One new species was left undetected for a long time in the Bequaert sub-collection. This demonstrates the importance of extensive voucher specimen collections to basic faunistic invertebrate studies. The storage of voucher specimens in Natural History Museums or other collections (whether they are identified or not), can yield important information on the past glory of one particular insect group if the specimens are appropriately stored and put as supplements in the right collection.

Some species were absent in the RBINS collection although their presence in Belgium was confirmed several times. *Ochlerotatus dorsalis* (once mentioned by Goetghebuer in 1909 (Goetghebuer, 1910)), *Ochlerotatus flavescens* (included in the 1991 checklist) and *Anopheles messeae* are lacking in the collections. The absence of the latter species is not surprising as it can only be distinguished form its sibling species *Anopheles atroparvus* by molecular analyses (Linton *et al*., 2002). The other two species were probably only added to the 1991 checklist based on literature.

Because there is a lack of records and voucher specimens from 1960-present, no invasive or exotic species have been collected and stored in the RBINS collection before 2000 (the first records of exotic mosquito species were *Aedes albopictus* in 2000 (Schaffner *et al*., 2004), and *Aedes japonicus* in 2002 (Versteirt *et al*., 2009). The lack of information about this period makes it very difficult to historically place the invasion and establishment of invasive or exotic species. Specimens of all recently discovered exotic species in Belgium have been added to the RBINS collection in a MODIRISK sub-collection.

The digitised (historical and recent) records of this revision were brought together in a new database CULIBEL (CULIcideaBELgium = all Belgian Culicidae records). It is possible that interesting faunistic records can still be discovered in private collections. This database will provide an opportunity to collect many other records of several rare native species and will be a useful tool to generate up-to-date maps of present and former distributions of mosquitoes in Belgium. This database will be kept up to date by the RBINS Departement of Entomology. Many records from other inventory projects carried out at the RBINS will also be integrated in this newly established database.

Some areas and time periods are sampled more intensively than others. For example, between 1940 and 1950, many mosquitoes were collected near Ghent and Brussels. This pattern is also found in other insect families, e.g. the up-dated red list of Carabidae (Desender *et al*., 2008). Hence, investigating trends in changing mosquito diversity in Belgium will only be appropriate for certain regions. The current mosquito diversity (MODIRISK) can be compared with past diversity (voucher specimens) in and around Ghent, in Brussels and the surrounding Sonien forest, and in the Hautes Fagnes region. Making a valid comparison between historical and present data is therefore a big challenge. Mapping biodiversity shifts is an integral part of understanding how species and communities have responded to past environmental change and predicting how they might respond to future environmental modifications. However, due to the limited early data of Culicidae in Belgium, such analyses will fail to take into account the whole study area and accurate estimation of earlier biodiversity will prove difficult. Nevertheless, these analyses will still represent general trends and allow us to visualise the importance of good collection management. Furthermore, they can stimulate a revision of voucher specimens in various museums (Shaffer *et al*., 1998, Schlick-Steiner, *et al*., 2003).

Acknowledgements
We would like to thank the technical staff of the RBINS collection for their help and assistance to visit the collections. This work was funded by the Belgium Government (Belspo; SD/BD/04A and SD/BD/04B).

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