Mosquito monitoring (2011 – 2018) in Germany – an update

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Zusammenfassung: Seit 2011 führen das Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF), Müncheberg, und das Friedrich-Loeffler-Institut (FLI), Bundesforschungsinstitut für Tiergesundheit, Greifswald, Projekte zur Erfassung der einheimischen Stechmückenfauna (Diptera: Culicidae) durch. Diese Erhebungen sind dringend notwendig, da Einzelfälle und Ausbrüche Stechmücken-assoziierter Krankheiten eine zunehmende Bedrohung für die Gesundheit von Mensch und Tier in Europa erlangen, während aufgrund der jahrzehntelang vernachlässigten Forschung auf diesem Gebiet grundlegende Daten zum Vorkommen und zur Verbreitung von Stechmücken in Deutschland fehlen. Mithilfe speziell konzipierter Stechmückenfallen, aber auch manuell sowie insbesondere durch das Citizen Science-Projekt ‚Mückenatlas', werden Stechmücken aller Entwicklungsstadien an möglichst vielen Standorten in Deutschland gesammelt. Bis 2018 erbrachte das Monitoring den Nachweis von 53 Culicidenspezies aus sechs Gattungen (*Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta, Uranotaenia*), von denen 50 als heimisch bzw. etabliert anzusehen sind. Bei vier der 50 Arten handelt es sich um nicht-endemische invasive Arten. Ein Pathogen-Screening führte zum Nachweis von Sindbis-Virus, Batai-Virus, Usutu-Virus sowie der Filarien *Dirofilaria repens, D. immitis* und *Setaria tundra*.

Keywords: mosquito monitoring, distribution, Germany, invasive species, Mückenatlas, citizen science, pathogen screening

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Introduction

The Culicidae have gained significant attention again as vectors of disease agents by European public and animal health authorities, caused by recent cases and outbreaks of mosquito-borne diseases in several EU states. Globalization and changing environmental conditions are major reasons of these (Tatem & al. 2012): mass transportation of humans and animals, climate warming, population migration and refugee issues, changes in landscape structures and management, urbanization, spread of settlements into natural areas, collapse of infrastructures and financial cuts in public health systems pave the way for the global displacement, establishment and spread of vectors and the pathogens they transmit. What is discussed as contributing to insect loss and die-off, is also causative for substantial changes in the composition, dynamics and distribution areas of insect faunas as well as in incidences of infectious diseases associated with haematophagous arthropods.

Before the background of mosquito research in Germany having been neglected for decades, baseline data systematically collected in the field, e.g. in the form of large-scale monitoring activities, are urgently needed prior to targeting scientific problems related to disease agent transmission by this group of dipterans. Consequently, the Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, and the Federal Research Institute for Animal Health, Friedrich-Loeffler-Institut (FLI), Greifswald, have been collecting mosquito distribution data all over Germany since 2011. Mosquitoes are primarily trapped, but data collection is supported by netting and aspirating adults and dipping immature aquatic stages, as well as by means of the citizen science project 'Mückenatlas' (www.mueckenatlas.com) which was launched in April 2012. In the first place, the data are meant to allow preparing mosquito distribution maps and performing future risk assessments for mosquito-borne diseases.

Materials and methods

Mosquito collection by trapping

To collect mosquitoes, BG sentinel traps (Biogents, Regensburg, Germany) were set up at stationary sites including marshes, flood plains, swamps, forests, meadows and urban areas all over Germany. Traps were also operated at possible ports of entry for exotic mosquitoes, i.e. along public transportation routes close to borders with neighbouring countries in southern Germany. All traps were equipped with BG Lure (Biogents) and a CO_2 source as attractants and were run for 24 hours per week annually from April to October. At numerous additional flexible sites, which were sampled for several weeks or months only, EVS (encephalitis virus surveillance) traps (Bioquip, Rancho Dominguez, CA, USA), gravid traps (Bioquip) and ovitraps (Bleu Line, Forlì, Italy) were used. Furthermore, approximately 1,300 manual collections were carried out until 2018 by netting adults in the vegetation, dipping larvae and pupae from their breeding places and aspirating adults in animal stables and winter shelters.

Mosquito collection by citizen science

To further enlarge the data pool, the citizen science project 'Mückenatlas' was brought to life in 2012 (Walther & Kampen 2017) where people are requested to collect mosquitoes in their private surroundings and make them available for scientific purposes. Detailed information about the procedure of participation, background and project aims are available on its homepage (www.mueckenatlas.com).

Mosquito identification

All mosquitoes were identified morphologically in the adult stage (i.e. larvae and pupae were reared in the laboratory until adult emergence) using the identification keys of Schaffner & al. (2001) and Becker & al. (2010). If genetic identification was necessary in the case of damaged specimens and cryptic species, the material was analysed by species-specific PCR assays (Rudolf & al. 2013, Proft & al. 1999, Kronefeld & al. 2014a) and CO1 barcoding (Ibáñez-Justicia & al. 2014).

Pathogen screening

A subset of the collected mosquitoes was screened for pathogens (Togaviridae, Orthobunyaviridae, Flaviviridae, filarial worms) of human and veterinary relevance (Kronefeld & al. 2014b, Scheuch & al. 2018, Heym & al. 2019).

Data management

All mosquito collections, plus supplemental data, are entered into the German mosquito database Culbase to be available for subsequent scientific analyses.

Important results

From 2011 to 2018, seven new mosquito species were detected in Germany (Kampen & al. 2017), as compared to the most recent German mosquito inventory which listed 46 species (Dahl & al. 1999): *Aedes albopictus, Ae. japonicus, Ae. koreicus, Anopheles petragnani* and *Culiseta longiareolata* which are now considered established, and *Ae. aegypti, Ae. berlandi* and *Ae. pulcritarsis,* which did not succeed in establishing. *Aedes albopictus, Ae. aegypti*, *Ae. japonicus* and *Ae. koreicus* represent potential vector species, although the latter two have so far not been shown to play important roles in the field.

The yellow fever mosquito *Ae. aegypti* was detected in Germany only once in late spring 2016, when an indoor population could develop after the introduction of eggs attached to plant seedlings by a returning traveller (Kampen & al. 2016). Elimination of the breeding sites led to the eradication of the population. By contrast, establishment of the Asian tiger mosquito *Ae. albopictus*, most probably continuously introduced by vehicular ground transport of adults from southern Europe (Becker & al. 2013, Kampen & al. 2013a), could not be prevented, resulting in several populations of this species now being present in Germany (Becker & al. 2017, Walther & al. 2017, Kuhlisch & al. 2018a). As opposed to these thermophilic species, *Ae. japonicus* is well adapted to Central European climatic conditions and had no difficulties in establishing and quickly spreading. It is now found throughout the southern half of Germany, although with much higher population densities in the west than in the east (Koban & al. 2019). Recently, also *Ae. koreicus* was shown to have become established in Germany (Pfitzner & al. 2018, Steinbrink & al. 2018), after a first specimen had been submitted to the 'Mückenatlas' in 2015 (Werner & al. 2016).

The invasive species *Cs. longiareolata* and *An. petragnani* are no known vectors but thermophilic species of Mediterranean origin, their establishment being indicators for ameliorating developmental conditions in Germany and probably climate warming.

The 'Mückenatlas' was involved in the detection of numerous specimens and almost all populations of invasive mosquito species in Germany. Its fast and sensitive notification of alarming developments in the mosquito fauna demonstrates the extraordinary value of this passive surveillance scheme for health threats posed by invasive vector species. Findings of invasive mosquitoes, as soon as considered relevant (e.g., in the case of local reproduction), trigger the prompt information of local and federal authorities in order to decide whether management should be implemented.

In addition to invasive species, the monitoring activities produced records of very rare species and species not documented for decades such as *An. algeriensis, Ae. refiki, Cx. martinii, Cs. glaphyroptera, Cs. ochroptera* and *Ur. unguiculata* (Kampen & al. 2013b, Tippelt & al. 2017, 2018, Kuhlisch & al. 2017, 2018b, 2019).

Screening of the mosquitoes for pathogens resulted in the detection of *Dirofilaria repens* (in *An. daciae, An. messeae*), *D. immitis* (in *Cx. pipiens* s.l.), *Setaria tundra* (in *Ae. annulipes* group, *Ae. vexans, An. daciae*), Batai virus (in *Ae. vexans*, *An. daciae, An. messeae*, *Cx. modestus, Cx. pipiens* s.l., *Culiseta* spec.), Sindbis virus (in *Cx. pipiens* s.l.), Usutu virus (in *Cx. pipiens* s.l.), and various haemosporidian species of the genera *Haemoproteus, Leucocytozoon* and *Plasmodium* (in *An. maculipennis* s.l., *Cx. pipiens* s.l.) (Kronefeld & al. 2014b, Scheuch & al. 2018, Heym & al. 2019).

Conclusion

Having checked some 400,000 mosquito specimens, distribution and phenological data of several frequent mosquito species occurring in Germany could be updated. As compared to previous inventories (e.g. Mohrig 1969, Dahl & al. 1999), substantial changes in the indigenous mosquito fauna have been demonstrated, with non-native species having established and thermophilic species having spread. Obvious reasons are globalization and climatic and environmental changes. Disease agents found to circulate in the German mosquito fauna are considered to be of low to moderate human and partly high animal pathogenicity. The first outbreak of West Nile fever in Germany in 2018, thought to be supported by an extended heatwave (Ziegler & al. 2019), added a mosquito-borne pathogen of high pathogenicity for both humans and animals. Mosquito surveillance should be continued in Germany as globalization and climate warming are proceeding, to be able to provide adequate and timely management of mosquitoes and prevent mosquito-borne diseases.

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Table 1: Mosquito species detected within the scope of active and passive monitoring (*: not considered established in Germany so far)

genus *Anopheles*	genus *Aedes*	genus *Coquillettidia*	genus *Culex*	genus *Culiseta*	genus *Uranotaenia*
An. algeriensis	*Ae. aegypti**	*Cq. richiardii*	*Cx. hortensis*	*Cs. alaskaensis*	*Ur. unguiculata*
An. atroparvus	*Ae. albopictus*		*Cx. martinii*	*Cs. annulata*	
An. claviger	*Ae. annulipes*		*Cx. modestus*	*Cs. fumipennis*	
An. daciae	*Ae. berlandi**		*Cx. pipiens*	*Cs. glaphyroptera*	
An. maculipennis	*Ae. cantans*		*Cx. territans*	*Cs. longiareolata*	
An. messeae	*Ae. caspius*		*Cx. torrentium*	*Cs. morsitans*	
An. petragnani	*Ae. cataphylla*			*Cs. ochroptera*	
An. plumbeus	*Ae. cinereus*			*Cs. subochrea*	
	Ae. communis				
	Ae. detritus				
	Ae. diantaeus				
	Ae. dorsalis				
	Ae. excrucians				
	Ae. flavescens				
	Ae. geminus				
	Ae. geniculatus				
	Ae. intrudens				
	Ae. japonicus				
	Ae. koreicus				
	Ae. leucomelas				
	*Ae. pulcritarsus**				
	Ae. pullatus				
	Ae. punctor				
	Ae. refiki				
	Ae. riparius				
	Ae. rossicus				
	Ae. rusticus				
	Ae. sticticus				
	Ae. vexans				

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