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First record of *Culiseta longiareolata* (Macquart) for Germany

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Abstract

For decades the German Mosquito Control Association (KABS) has conducted mosquito surveillance as part of a comprehensive mosquito control programme. During the summer of 2011 hundreds of dead birds, especially blackbirds (*Turdus merula*), were found infected with the tropical Usutu occurred to investigate whether nulliparous mosquitoes carry the virus. More than 6.000 breeding sites were investigated most of which were infested with *Culex pipiens/ Cx. torrentium* virus (USUV, Flaviviridae) which is mainly transmitted by ornithophilous mosquito species such as *Culex pipiens*. Developmental stages of mosquitoes are collected in areas where dead birds populations and some with the invasive species *Ochlerotatus japonicus japonicus.* However, in one water container, in an open allotment-garden located near Karlsruhe and the French border, third and fourth instar larva and five pupae of *Culiseta longiareolata* were found. Thus the number of mosquito species in Germany increases to 49 established species.

Key words: *Culiseta longiareolata*, Usutu virus, mosquitoes of Germany

Introduction

Mosquitoes are at the centre of worldwide entomological research primarily because of their medical importance as vectors of dangerous diseases like malaria and arboviroses (Becker *et al*., 2010). However, floodwater mosquitoes e.g. *Aedes vexans* and *Ochlerotatus caspius* can also significantly reduce life quality as they are nuisance species due to their mass development in, for example, river systems, rice fields, lakes or salt marshes with water level fluctuations. In Central Europe *Culex pipiens* can cause substantial nuisance problems especially in settlements in rural areas and is also known as the main vector for West-Nile viruses and other arboviruses. Nowadays, the invasive species *Aedes albopictus* is a serious problem in many Mediterranean countries and was involved in the first known transmission of Chikungunia virus in Italy 2007 and the first autochthonous dengue cases in Southern France and the Adriatic coast of Croatia (La Ruche *et al*., 2010; Schmidt-Chanasit *et al*., 2010).

In southwestern Germany hundreds of dead birds, especially blackbirds (*Turdus merula*) were found during the summer of 2011. Researchers of the German Mosquito Control Association (KABS) collected the dead birds and in cooperation with the Bernhard-Nocht-Institute (Hamburg) the birds were examined for various pathogens. It was believed that the birds died because of an infection with the tropical Usutu virus (USUV) which belongs to the family Flaviviridae and is mainly transmitted by ornithophilous mosquito species such as *Culex pipiens*. In 2010 we could already isolate the virus from *Culex pipiens pipiens* which indicated that the virus was already circulating in the mosquito-bird cycle as in Austria, Italy, Hungary, Switzerland and Spain (Weissenböck, 2007; Weissenböck *et al*., 2009; Pecorari *et al*., 2009; Jöst *et al*., 2010).

When the first cases of dead birds were recorded in July 2011, the public was asked by a media campaign by KABS to report any dead birds. As a result, almost two hundred dead birds were collected in an area of approximately 4000 km^2 for further research. A total of 168 birds from 18 species were examined. Positive to USUV were 66 black birds (*Turdus merula*), three starlings (*Sturnus vulgaris*), one house sparrow (*Passer domesticus*), two canaries (*Serinus canaria domestica*), six great grey owls (*Strix nebulosa*) and, for the first time in Europe, two kingfishers (*Alcedo atthis*).

Currently investigation is being carried out on whether USUV can be transmitted transovarially and is able to overwinter in the mosquito population. Therefore, developmental stages of mosquitoes were collected in areas where dead birds occurred to investigate whether nulliparous mosquitoes carry the virus.

Material and Methods

In the vicinity of places where infected birds were found, developmental stages of *Culex pipiens* and associated mosquitoes were sampled e.g. in rainwater containers and small water collections like vases in cemeteries. A sufficient number of mosquito larvae was collected with a plankton net in single breeding sites and kept in 2-litre glass vessels filled with water from the breeding sites to assist the successful rearing to the adult stage. Each vessel was carefully marked with information about the breeding site. In the laboratory each single mosquito population was transferred into mosquito breeders (BioQuip) and maintained at 28°C. The larvae were fed with powdered fish (TertaTabimin) and the emergent adults on pads soaked with a 10% sugar-solution until a sufficient number of adults occurred in the rearing chamber. The rearing chamber was transferred into a container with dry ice to kill the adults. At least 50 adults from each breeding site were killed and kept at -20°C for further investigation in order to check for the presence of viruses by Real-time PCR. Some adults were mounted horizontally by the left pleuron on the tip of a triangular piece of cardboard with clear nail polish. This was located on a 38mm pin to which a cardboard label was attached with information on the sampling location, type of breeding site and species name (Bohard & Washino, 1978; Becker *et al.*, 2010).

The water parameters of a selection of breeding sites were determined by means of a "Compact laboratory for water testing" (Aquamerck) and a digital indicator (PCE-PHD 1). The following parameters were assessed: pH, conductivity, oxygen content, carbonate hardness, ammonium, nitrite and nitrate.

Results

Mosquito larvae were collected from 312 breeding-sites in the States of Baden-Württemberg and Rheinland-Pfalz, South-West Germany. The breeding sites comprised: 218 rainwater containers and 6.547 vases. From each breeding site 50 larvae and adults were determined to species level.
All positive breeding sites were inhabited by all developmental stages of *Culex pipiens/ Cx. torrentium*. In one water barrel (60cm high and 55cm diameter) 11 third and fourth instar larvae and 5 pupae of *Culiseta longiareolata* were found. The water barrel was one of 21 found in an open allotment-garden located in Hagenbach near Karlsruhe and the French border (Figure 1).

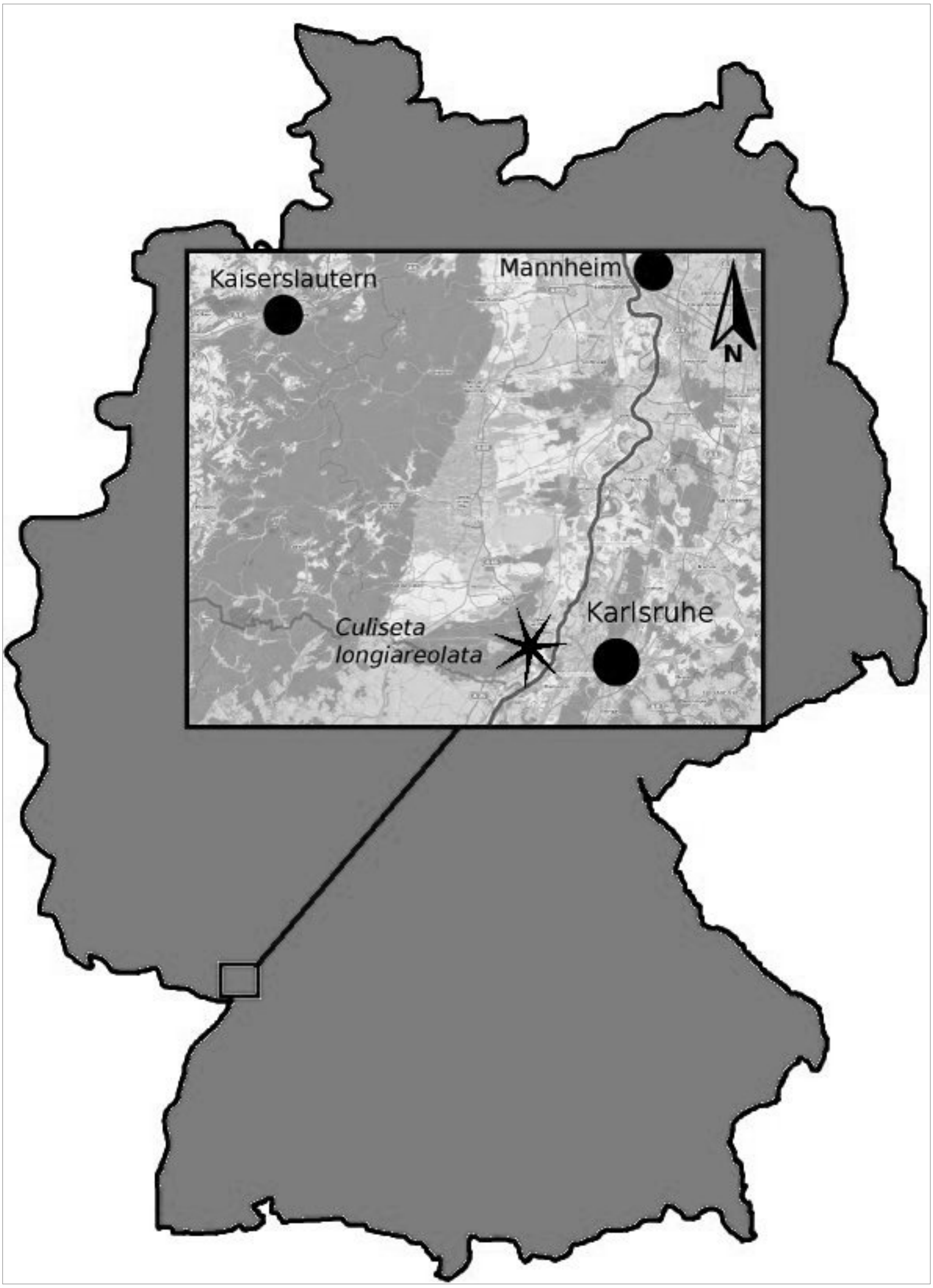

Figure 1: Location of *Culiseta longiareolata* in Germany (backroundmap inset ©OpenstreetMap and contributors 2011, CC-BY-SA)

The *Cs. longiareolata* larvae were associated with larvae of *Culex pipiens*. The ratio of *Culex*-*Culiseta* developmental stages was: 25 to 1. The other barrels contained either *Culex pipiens* larvae or no mosquito larvae.

The larvae could easy be recognized and divided from *Culex* larvae by their sluggish movement. Fourth instar larvae and pupae of *Cs. longiareolata* were significantly larger than those of *Culex pipiens/Cx. torrentium*.

Five fourth instar larvae were mounted and determined. The remaining 11 adults (3 females and 8 males) were also mounted and the hypopygia of the males transferred to Canada balsam. All stages were identified as *Cs. longiareolata*.

The water parameters of the breeding site are shown in Table 1.

pH	Temperature [°C]	Conductance [µS/cm]	Oxygen content [mg/l]	Sulphite [mg/l]	Ammonium [mg/l]	Carbonate hardness [°dH]	Nitrate [mg/l]	Nitrite [mg/l]
9.07	13.3	305	11.6	2	0	14	10	0

Table 1: water parameters of the breeding site.

Discussion

Recently *Ochlerotatus j. japonicus* was recorded as an established species in Germany (Becker *et al*., 2010). With the discovery of *Cs. longiareolata* the number of established mosquito species found in Germany increased to 49 (Table 2). Forty-nine species are established and one species namely *Aedes albopictus* can be found only sporadically but is not as yet established (Pluskota *et al*., 2009).

Species/Author	Eckstein (1920)	Scherpner (1960)	Becker/Kaiser (1995)	Becker *et al.* (2010)	Becker & Mohrig Total no. of species
Ae. vexans	++++	++	++++	++++	recorded
Oc. sticticus	+++	+++	+++	+++	recorded
Ae.cinereus	+++	++++	++	++	recorded
Ae. geminus	?	?	proven	proven	recorded
Ae. rossicus	?	?	++	++	recorded
*Ae. albopictus**	-	-	-	[(+)]	[not established]
Oc. caspius	-	(+)	+	(+)	recorded
Oc. dorsalis	+	-	-	(+)	recorded
Oc. detritus	-	-	(+)	(+)	recorded
Oc. nigrinus	-	-	(+)	(+)	recorded
Oc. leucomelas	-	(+)	+	+	recorded

Oc. flavescens	-	-	(+)	(+)	recorded
Oc. intrudens	-	-	-	-	recorded
Oc. annulipes	(+)	+	+	++	recorded
Oc. cantans	++++	+++	++	++	recorded
Oc. cataphylla	-	+	(+)	(+)	recorded
Oc. excrucians	-	(+)	(+)	(+)	recorded
Oc. rusticus	+	+	+	++	recorded
Oc. refiki	-	-	-	-	recorded
Oc. communis	++	+++	+	+	recorded
Oc. cyprius	-	-	-	-	recorded
Oc. punctor	-	(+)	+	+	recorded
Oc. riparius	-	-	-	-	recorded
Oc. diantaeus	-	-	(+)	(+)	recorded
Oc. pullatus	-	-	-	(+)	recorded
Oc. geniculatus	+	+	(+)	(+)	recorded
Oc. japonicus	-	-	-	+	recorded
Cx. pipiens s.l.	++++	++++	++++	++++	recorded
Cx. p. biotype *pipiens*	?	[proven]	[proven]	[proven]	[recorded]
Cx. p. biotype *molestus*	?	[proven]	[proven]	[proven]	[recorded]
Cx. torrentium	?	+++	proven	proven	recorded
Cx. hortensis	-	-	-	-	recorded
Cx. martinii	-	-	-	-	recorded
Cx. modestus	-	-	++	++	recorded
Cx. territans	++	(+)	(+)	++	recorded
Cs.annulata	++	++	++	++	recorded
Cs.morsitans	++	+	+	+	recorded
Cs. subochrea	-	(+)	(+)	(+)	recorded
Cs. ochroptera	-	-	-	+	recorded
Cs. fumipennis	-	-	-	-	recorded
Cs. alascaensis	-	-	(+)	(+)	recorded
Cs. glaphyroptera	-	-	-	(+)	recorded
Cs. longiareolata	-	-	-	(+)	recorded
An. maculipennis s.l.	++++	[+++]	[++]	[+++]	[recorded]
An. messeae	*?*	+++	*proven*	*proven*	*recorded*
An. atroparvus	*?*	++	*proven*	*proven*	*recorded*
An. maculipennis s.s.	?	+	proven	proven	recorded
An. labranchiae	-	-	-	-	recorded
An. claviger	+++	++	+	++	recorded
An. algeriensis	-	(+)	-	-	recorded
An. plumbeus	+	+	+	++	recorded
Cq. richiardii	++	+	+	+	recorded
Ur. unguiculata	-	-	(+)	(+)	recorded
No. of species	**17**	**26**	**34**	**38**	**49**

Table 2: Recorded mosquito species from 1920 to 2010 in Germany (occurrence: ++++ = massive; +++ = abundant; ++ = frequent; + = regularly; (+) = rare; * species is found only sporadically; ? = uncertain; [ ] = not counted in the species lists.

The females of *Cs. longiareolata* rarely bite humans and appear to be ornithophilic, and are regarded as vectors of blood parasites in birds (Becker *et al*., 2010). Another ornithophilic species, *Uranotaenia unguiculata* Edwards, was recorded the first time in Germany in 1994 (Becker & Kaiser, 1995). The mild winters and warm summers over the last years favoured the development of these species which predominantly occur in the Mediterranean area. Neither are invasive species like *Ae. albopictus* and *Oc. japonicus*, which lay individual eggs in natural and artificial breeding sites such as used tires, vases or ornamental plants kept in water for the transportation.

The larvae within the eggs of these species can withstand desiccation for many months. *Culiseta longiareolata* and *Ur. unguiculata* lay egg rafts on the water surface and the larvae emerge immediately after completing embryogenesis. It can therefore be assumed that *Cs. longiareolata*, as well as *Ur. unguiculata*, have been established in Germany for a long time but were not detected because of their preferred ornithophilic biting habit and rare occurrence. However, *Cs. longiareolata* can occasionally be found in used tyres, so it is possible that this mosquito was imported to Germany through the international used tyre trade (Roiz *et al*, 2007).

Larvae of *Cs. longiareolata* can usually be found in rock pools or in any kind of artificial container e.g. wooden and metal barrels, tanks built of concrete and wells where they are usually associated with *Cx. pipiens*. Rarely do they occur in natural water bodies like pools or ditches. The larvae are able to tolerate a high degree of pollution. They are filter feeder but are also capable of predatory feeding including cannibalism (Maslov, 1967).

The distribution-map (Figure 2) indicates that *Cs. longiareolata* has its origin in tropical regions south of the tropic of Cancer. In Europe *Cs. longiareolata* is widely distributed in the Mediterranean region. It is possible that *Cs. longiareolata* spread northward as a result of global climate change. Another possibility is that larvae were transported to Germany and other European countries through the international used tyre trade.

Today *Cs. longiareolata* is distributed in Albania, Azores, Botswana, Bulgaria, Canary islands, Croatia, Cyprus, Djibouti, Egypt, southern England, Ethiopia, France as far north as Paris, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Lebanon, Lesotho, Madeira, Mauritania, Morocco, Namibia, Pakistan, Portugal, Romania, Russia, Slovakia, Somalia, South Africa, Spain, Sudan, Switzerland, Syria, Tajikistan, Tunisia, Turkey, Ukraine and the lower Volga area as far as the northern slopes of Caucasus as well as in Yemen (Systematic Catalog of Culicidae, 2011; Becker *et al*., 2010).

Culiseta longiareolata is a vector for brucellosis, avian influenza and West Nile encephalitis (Maslov, 1967).

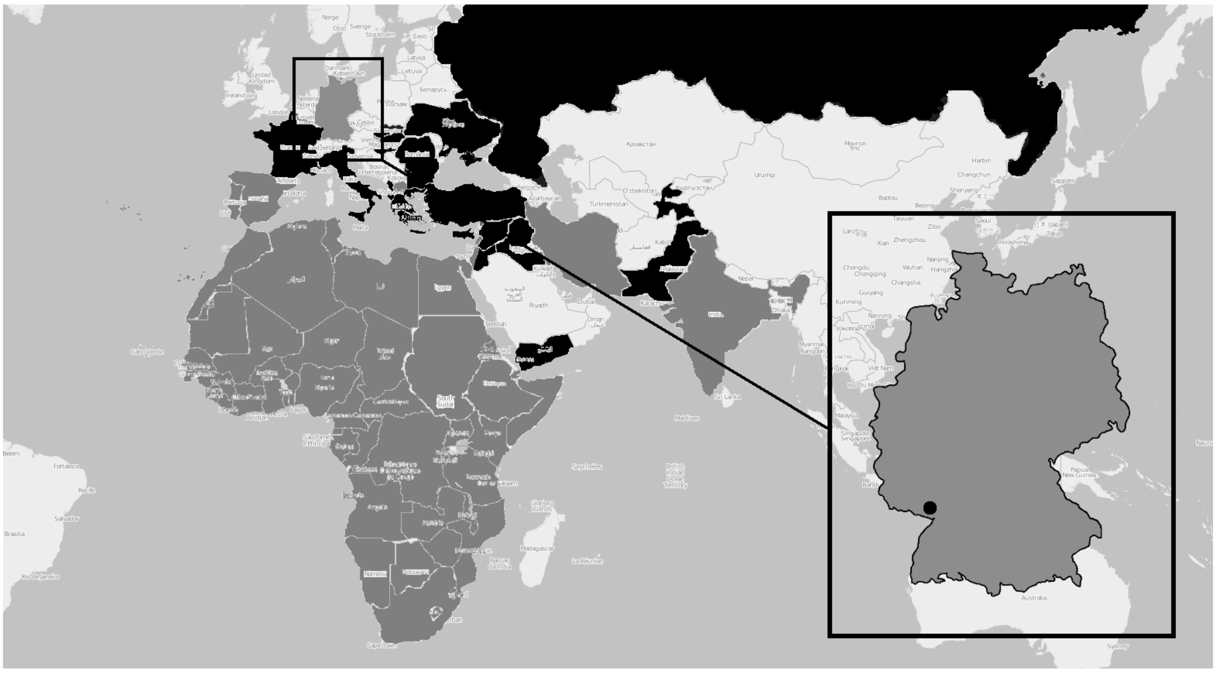

Figure 2: Grey: known distribution 1941 (Edwards, 1941), black: distribution 2011 (Systematic Catalog of Culicidae 2011), inset: first record for Germany (2011) [© OpenStreetMap and contributors, CC-BY-SA].

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