

## Three rarely encountered and one new *Culiseta* species (Diptera: Culicidae) in Germany

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**Abstract:** With few exceptions, systematic large-scale field studies on the mosquito fauna have not been performed in Germany for decades. Thus, up-to-date data on the occurrence and distribution of the indigenous mosquito species are lacking. In particular, there is no information on whether once rare and restrictively occurring species are still present. Here we describe the recent finding of four *Culiseta* species rarely encountered in Germany: *Cs. alaskaensis*, *Cs. glaphyoptera* and *Cs. ochroptera*, which have always been considered endemic but were seldom found and last reported a long time ago, and *Cs. longiareolata*, which has recently been found to have established in southern Germany. *Journal of the European Mosquito Control Association* 31: 36-39, 2013

**Keywords:** *Culiseta alaskaensis*, *Culiseta glaphyoptera*, *Culiseta longiareolata*, *Culiseta ochroptera*, Germany

### Introduction

Fifty mosquito species are considered to be indigenous to Germany, including *Aedes japonicus japonicus* [= *Hulecoeteomyia japonica japonica* sensu Reinert et al., 2009] and *Culiseta longiareolata*, which have only recently been found established. As a matter of fact, the number of species listed for a given region is just an accumulation of species detected in the course of time. It is not clear whether all species ever demonstrated to occur are still present at the time the list is published, given that routine monitoring or regular and region-wide appropriate studies are lacking and that drastic environmental and ecological changes such as drainage of wetlands and deforestation have taken place. Except for the Upper Rhine Valley in southwestern Germany where mosquito control is performed on a regular basis and comprehensive data on the mosquito fauna have existed for the last 30 years or so, up-to-date data on the occurrence and distribution of mosquito species are missing for Germany. Since 2011, several mosquito monitoring programmes have contributed to updating Germany's culicid species inventory. Thus, *Ae. j. japonicus* has been demonstrated to be widespread and well-established (Becker et al., 2011; Huber et al., 2012; Kampen et al., 2012; Werner & Kampen, 2013) and *Anopheles daciae* of the Maculipennis Group of species has been found to belong to the German mosquito fauna (Kronefeld et al., 2012; Weitzel et al., 2012). Only recently, Krüger & Tannich (2013) reported the re-discovery of *An. algeriensis* after more than half a century. We here describe four *Culiseta* species recently found in Germany, including three species that had not been caught for many years and one species that is new to Germany.

### Materials and Methods

Mosquitoes were collected in 2011 and 2012 in the framework of a nationwide mosquito monitoring programme by BG Sentinel traps (Biogents, Germany) equipped with BG Lure™ (Biogents) and a CO<sub>2</sub> source as attractants, or manually by hand-held aspirators and tubes carefully put over the mosquitoes. 120 traps were operated from April to October for 24 hours weekly all over Germany while hand collections were performed intensely both by scientists and private persons requested via the media to contribute to mosquito mapping in Germany (www.mueckenatlas.de). All specimens referred to herein were captured as adults. They were

morphologically determined to species using the identification keys by Schaffner et al. (2001) and Becker et al. (2010) and genetically by COI barcoding. Briefly, a COI-PCR using the primers LCO1490 and HCO2198 was performed on DNA extracted from single mosquito legs by means of the QIAamp DNA Mini Kit (Qiagen, Germany) as described by Folmer et al. (1994). After agarose gel electrophoresis, amplicons were recovered by the QIAquick Gel Extraction Kit (Qiagen) and subjected to bidirectional cycle sequencing using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Germany) and one of the PCR primers. Cycle sequencing products were purified by SigmaSpin Sequencing Reaction Clean-Up Columns (Sigma Aldrich, Germany) and analysed by a 3130 Genetic Analyser (Applied Biosystems). Forward and reverse DNA sequences were aligned for each specimen and consensus sequences were compared to GenBank (<http://blast.ncbi.nlm.nih.gov>) and Barcode of Life Database (<http://www.boldsystems.org>) entries for species identification.

### Results and Discussion

Except for *Cs. longiareolata*, a species new to Germany, the *Culiseta* species dealt with here were listed as species indigenous to Germany by Schumann et al. (1999). In their checklist of Germany's diptera, a total of seven *Culiseta* species are included: *Cs. (Culicella) fumipennis*, *Cs. (Culicella) morsitans*, *Cs. (Culicella) ochroptera*, *Cs. (Culiseta) alaskaensis*, *Cs. (Culiseta) annulata*, *Cs. (Culiseta) glaphyoptera* and *Cs. (Culiseta) subochrea*. While *Cs. annulata* is quite common in Germany and might even be a pest species regionally, the other endemic *Culiseta* species occur widespread but much less frequently (*Cs. fumipennis*, *Cs. morsitans*, *Cs. subochrea*), or are seldom encountered due to limited distribution areas and low population densities (*Cs. alaskaensis*, *Cs. glaphyoptera*, *Cs. ochroptera*). The latter, consequently, have produced few historic collection reports, the corresponding collection areas of which are marked in Figure 1 as long as they could be narrowed down with some precision by means of the original literature.

Among tens of thousands of mosquito specimens and 40-44 mosquito species (some specimens of very closely related species could neither morphologically nor genetically be reliably assigned to species) collected during the mosquito

monitoring programme, *Cs. annulata* was present in high numbers and at numerous places while *Cs. morsitans* was quite frequent as well. *Culiseta subochrea* was occasionally collected according to morphological identification. In several cases, where morphological features suggested *Cs. subochrea* but were not very well preserved, the COI barcode locus was not informative enough to allow differentiation between *Cs. subochrea* and *Cs. annulata*. *Culiseta fumipennis* was not collected in this study at all.

The four *Culiseta* species reported here stand out from all other mosquito species collected during the monitoring programme due to being considered rare and captured with few specimens only.

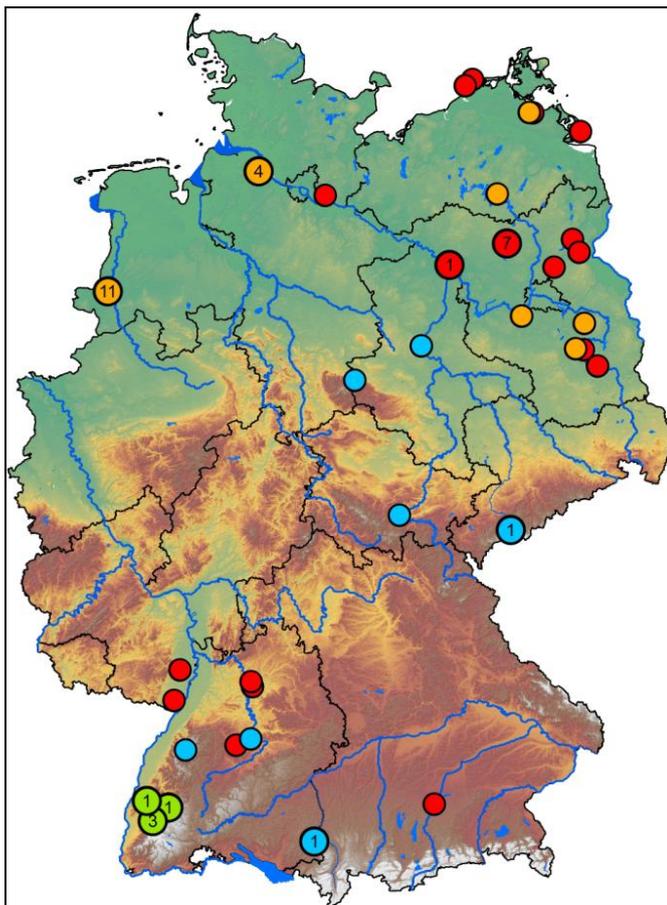


Fig. 1: New and historic collection sites of the *Culiseta* species discussed in this paper (red dots: *Cs. alaskaensis*, orange dots: *Cs. ochroptera*, blue dots: *Cs. glaphyoptera*, green dots: *Cs. longiareolata*; numbers in dots represent the numbers of specimens collected in the present study in 2011 and 2012 while smaller dots without numbers mark historic collection sites).

#### *Culiseta (Culiseta) alaskaensis* (Ludlow)

*Culiseta alaskaensis* is a holarctic species, typical for tundra and boreal zones. Its southern distribution extends towards the Alps. Being univoltine in the north, it is multivoltine in the south. It is supposed to refrain from human settlements and to dwell in humid forest areas. By contrast, the species readily feeds on humans if chance arises, and it requires two blood meals to lay eggs. The larvae are found in semi-permanent water bodies such as marshes and swamps, and adults already occur in early spring. *Culiseta alaskaensis* hibernates as adult females and is not known to be involved in the transmission of pathogens.

The species resembles *Cs. annulata* and *Cs. subochrea* except that the subapical white ring on the femora and the median white ring on tarsomeres I of the legs is absent (Mohrig, 1969; Becker *et al.*, 2010).

The occurrence of *Cs. alaskaensis* in Germany was first recognised by Martini (1924a) and later confirmed by various other authors. According to Mohrig (1969), the species is widely distributed in Germany, although nowhere frequent. Most historic findings were reported from the northern German lowlands (e.g. Martini, 1924a; Peus, 1929; Mohrig, 1965; Müller, 1965). Mohrig (1969) described *Cs. alaskaensis* to be common at some places on the Baltic Sea coast in alder marsh forests behind the dunes. Only exceptionally were individuals found in southern Germany, such as in the northern part of the federal state of Baden-Württemberg (Vogel, 1933), in the Upper Rhine Valley (Becker & Ludwig, 1981) and in southern Bavaria (Kühlhorn, 1954).

In the present study, eight females were collected by hand-held aspirator in June 2012 in the Prignitz-Havelland region, northwest of Berlin in the German federal state of Brandenburg (Fig. 1), both in a sheep stable in a forested region with swampy areas nearby (n=7) and outdoors in a rural human settlement (n=1).

#### *Culiseta (Culiseta) glaphyoptera* (Schiner)

*Culiseta glaphyoptera* is a montane species that occurs in Central and southeastern Europe. It is quite common in the southwestern Czech Republic (Dvořák, 2012). Overwintering takes place as adult females and one or two generations per year are produced. Larvae prefer cold clear water, such as present in rock or spring pools, in shaded environments. The species feeds on birds and mammals but is not known to play a role as a vector of disease agents.

Morphologically, *Culiseta glaphyoptera* may be confused with *Cs. bergrothi*, which also has entirely dark tarsomeres. However, in contrast to *Cs. bergrothi*, the maxillary palps of *Cs. glaphyoptera* are dark. In addition, *Cs. bergrothi* only occurs in northern European regions (Mohrig, 1969; Becker *et al.*, 2010).

*Culiseta glaphyoptera* larvae were collected in Germany in the Thuringian Forest (Martini, 1924b), in the Harz Mountains (Peus, 1929) and in the Black Forest (Vogel, 1933, 1940). To the best of our knowledge, the last report from Germany dates back to 1968 when three larvae were found close to Magdeburg in the federal state of Saxony-Anhalt (Schuster & Mohrig, 1971). Our manual collections from 2012 included two females of *Cs. glaphyoptera*, one from the East German Erz Mountains collected in late October and one from the South German Allgäu region collected in late November (Fig. 1).

#### *Culiseta (Culicella) ochroptera* (Peus)

*Culiseta ochroptera* is a rare tyrphophilous species associated with bogs. Its distribution covers the forest zones from Sweden to Romania and China. The species is multivoltine and its larvae develop in bog waters, marshes and ditches. Hibernation takes place in the larval or egg stage, or as adult females. *Culiseta ochroptera* preferentially feeds on birds and amphibians, and has no known medical or veterinary importance.

The morphology of *Culiseta ochroptera* is similar to that of *Cs. morsitans* but it is more brownish and the tibiae of its forelegs are more yellowish-grey. In particular, uniform bronze-brownish scales on the anterior part of the mesonotum, on the propleuron and on the proepimeron are characteristic for *Cs.*

*ochroptera* whereas these thoracic sclerites appear to be greyer in *Cs. morsitans* due to interspersed bright scales (Mohrig, 1969; Becker *et al.*, 2010).

The taxonomic description of this species by Peus (1935) was based on specimens found in a peat bog in Lower Lusatia, Germany, in Silesia, Poland, and in the Riga district, Latvia. With regard to Germany, Mohrig (1969) described the occurrence of *Cs. ochroptera* in the Brandenburg and Mecklenburg regions. The westernmost finding was in Westphalia (Peus, 1950). Most recently, Dix (1974) collected an approaching *Cs. ochroptera* female in 1972 in the present-day Müritz National Park in the federal state of Mecklenburg-Western Pomerania.

In agreement with its association with bogs, 11 females were trapped in this study by BG sentinel traps between June and mid-August 2012 in the North German Emsland region close to the Dutch border. Four further females were caught outdoors with tubes in the vicinity of peatland in the Elbe estuary on one and the same day in mid-August 2012 (Fig. 1).

#### *Culiseta (Allotheobaldia) longiareolata* (Macquart)

*Culiseta longiareolata* is a multivoltine, thermophilic and mainly ornithophilic species. It usually breeds in small water bodies, mainly human-made, and adults may enter settlements and houses and occasionally attack humans (Maslov, 1967). The species has been implicated in the transmission of viruses, avian plasmodia and *Francisella tularensis* (Maslov, 1967; van Pletzen & van der Linde, 1981), and was highly susceptible to West Nile virus infection after microinjection into the hemocele (Hurlbut, 1956). In nature, it does not appear to have any epidemiological relevance for human or zoonotic diseases.

*Culiseta longiareolata* is a very eye-catching mosquito, easily distinguished from all other European *Culiseta* species. It is characterised by numerous white stripes and spots on the legs, the head, the maxillary palps and the thorax. Notably, white scales frame the posterior margins of the eyes, densely cover the costal veins of the wings and form lyre-shaped lines on the thorax (Becker *et al.*, 2010).

*Culiseta longiareolata* occurs throughout the Afrotropical region and parts of the Oriental and Palaearctic regions, including the Mediterranean. Rare historic records also exist from southern England (Cranston *et al.*, 1987). In France, the species is abundant in the south, but some observations are reported from more northern areas, up to Normandy, Île-de-France and Alsace (Moussiegt, 1986). In the recent past, the species was also found in northern Switzerland (Schaffner *et al.*, 2009) and was confirmed for Alsace (F. Schaffner, pers. comm.).

As described previously (Werner *et al.*, 2012), a female *Cs. longiareolata* was trapped in a BG Sentinel in mid-August 2011 in Freiburg. Using the same methodology, the authors collected further females in the months of September 2011 and 2012 in Freiburg (n=1) and Herbolzheim (n=1), respectively. Males were trapped in August 2011 (n=1) and August 2012 (n=2) in Freiburg. Also in summer 2011, Becker & Hoffmann (2011) found larvae and pupae in a water barrel close to Karlsruhe while a female had allegedly been collected already in late October 2007 near Heilbronn (A. Krüger, pers. comm.). All collection sites were located in southwestern Germany in the federal state of Baden-Württemberg, not far from the German-French border (see Figure 1). The reports of *Cs. longiareolata* from that area over several years and the discovery of developmental stages clearly indicate establishment.

#### Conclusions

Rare mosquito species are hard to detect and encounters are more or less random. Some of these species are restricted to particular geographical and/or ecological areas or to short activity seasons, others escape attention because they are not attracted to humans and commonly used trap systems. Mosquito monitoring programmes intended not only to detect invasive or vector species but also to collect data on the biodiversity and add to mosquito inventories are therefore dependent on various tools to collect mosquitoes and should target both pre-imaginal and adult stages in as many ecologically diverse sites as possible. Applying this strategy, this study found three particularly rare *Culiseta* species in Germany and discovered a newly established one. The four species are not considered nuisance pests and apparently are not involved in the transmission of disease agents, suggesting that control is not necessary. In contrast, they add to the indigenous mosquito fauna and to the biodiversity of the country.

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