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## **Establishment of the West Nile Virus Vector, *Culex modestus*, in a Residential Area in Denmark**

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## Scientific Note

### Establishment of the West Nile virus vector, *Culex modestus*, in a residential area in Denmark

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*Culex modestus* Ficalbi 1889 is a competent vector of West Nile virus (Balanghien 2007, Fyodorova et al. 2006, Hubálek and Halouzka 1999). Because *Cx. modestus* feeds on both birds and mammals and often bites during the day, it can be an important bridge vector between humans and West Nile virus circulating among birds (Balanghien et al. 2006). The ornitophilic *Cx. pipiens* and *Cx. torrentium* are both common in Europe and Scandinavia with the latter species more prevalent at northern latitudes (Hesson et al. 2013). *Cx. modestus* has never been reported from Scandinavia, but in the last decade both West Nile virus and *Cx. modestus* have been reported moving north in Europe (Balengien et al. 2007, Golding et al. 2012).

In response to the growing threat of invasive mosquitoes and potential transmission of insect-borne diseases countrywide, surveys for mosquitoes were conducted in Denmark in 2011, 2012, and 2013 at randomly selected locations in urban, agricultural, and recreational areas. Survey sites were sampled only once for three consecutive days in a season. Additionally, three sites in 2011 were selected as sentinels and sampled either three or seven days a week repeatedly through the seasons. Two of these sentinel sites were used again in 2012; one of which was a garden, situated in the municipality of Greve, 25 km south of Copenhagen. In 2013, a national weekly surveillance system was initiated based on the collections from five sentinel sites established in private gardens, including the garden in Greve. During the three years, 141 different sites were sampled for 1,574 nights, of which the Greve trap constituted 439 nights. All survey collections were done with Mosquito Magnet suction traps baited with CO<sub>2</sub> and Octenol. To allow rapid weekly web publishing, mosquitoes in 2013 were initially only determined to genus. Later in the autumn, all individuals were identified to species according to Becker et al. (2010) and Schaffner et al. (2001). For *Culex* specimens resembling *modestus*, the lengths of the two hind tibiae and the tarsomere I were measured and the mean ratio calculated. DNA was extracted and a 709 bp fragment of the cytochrome c oxidase subunit I (COI) was amplified (Folmer et al. 1994). The PCR products were sequenced by cycle sequencing on an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA). Analysis and phylogenetic-tree construction were performed with BioNumerics, version 7.1 (Applied-Math, Sint-Martens-Latem, Belgium). Daily minimum and maximum temperatures from a modeled spatial grid of Europe was obtained from MARS-Agri4cast, and four selected grid points for Greve, the Kent marsh area in the UK, Camargue

in France, and South Bohemia, Czech Republic, were used to calculate mean monthly temperatures for the period 2003-2013.

During species identification, three individuals of *Culex* morphologically resembling *Cx. modestus* were identified in collections from August 2, 4, and 25, 2013, at the garden used as sentinel all three years. The lateral terga of these specimens had clear triangular patches of pale scales. The ratio of tarsomere I to tibia on the hind legs ranged from 0.76 to 0.80, while the ratio for seven *Culex pipiens/torrentium* ranged from 0.95 to 1.03. Species identification of unsorted archived collections from the site in 2011 revealed two more specimens from August 16 and September 12. No collections remained from 2012. All five specimens were sequenced together with six *Culex* morphologically identified as *pipiens/torrentium* and two specimens previously identified by molecular methods as *Cx. pipiens* and *Cx. torrentium* (unpublished data). The sequencing of the COI gene confirmed the morphological identification of all five individuals as *Cx. modestus* (Figure 1).

These are the first recordings of *Cx. modestus* in Denmark. All five individuals were collected at the same coastal site in the densely populated coastal municipality of Greve, 25 km south of Copenhagen center. The Greve area is situated along the bird spring migration route to Scandinavia. The coastal habitat is coherent with Copenhagen Airport 20 km away with daily connections to endemic West Nile areas in Europe and overseas. The finding of *Cx. modestus* in both 2011, when Copenhagen was flooded after heavy rains in July, and in the dry summer of 2013, strongly suggests the species is established in the area. Since *Cx. modestus* was not identified until after the mosquito season in 2013, the breeding sites could not be identified. It is therefore not yet known whether the species was only present at low densities or if the sentinel site was in the periphery of a high abundance area.

These are the most northern recordings in Europe of the presumed thermophilic *Cx. modestus* (Votýpka et al. 2008). Greve has considerably lower temperatures and shorter summers than known *Cx. modestus* sites in central and southern Europe (Figure 2). This important West Nile vector may therefore also be present in other residential areas around the southern part of the Baltic Sea. So far, most mosquito surveys in southern Scandinavia have been conducted as ecological studies in natural habitats and consequently little is known of mosquito communities in residential areas, where the majority of mosquito-human interaction is likely to occur. *Cx. modestus* has been found as far north as Greifswald

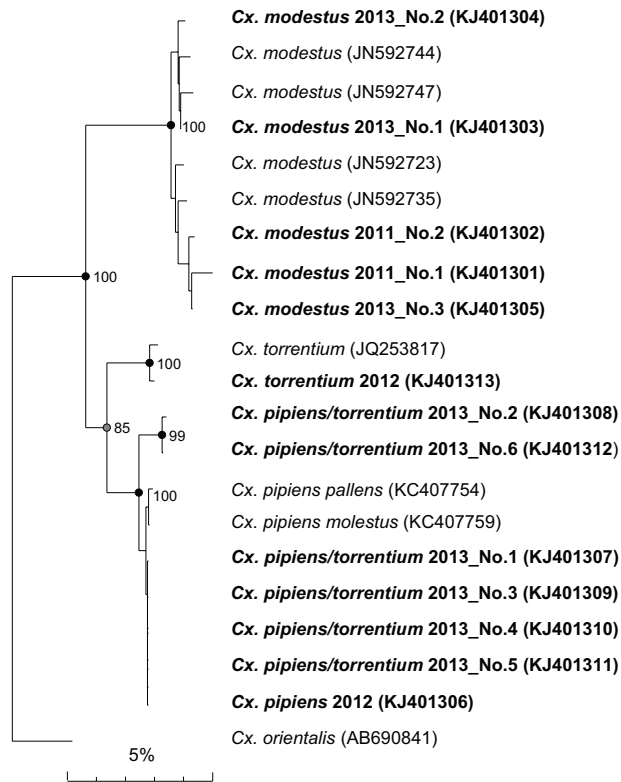


Figure 1. Phylogenetic tree based on COI gene sequence comparison over 658 aligned bases from Danish (DK) *Culex* species (specimens in boldface) and other representatives of the *Culex* genus downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/BLAST/>). The tree was constructed by the neighbor-joining method. The scale bars represent a 5% difference in nucleotide sequence. Bootstrap values are shown for the main clades only. Accession numbers in brackets.

in northern Germany in 1960 but disappeared from the area in the 1970s as a result of environmental changes caused by

deliberately lowering the groundwater table (Mohrig 1981). In the Camargue area, changes in the abundance of *Cx. modestus* from 1940s to after 2000 have been demonstrated to be highly determined by human-made environmental changes, notably by trends in rice cultivation and the associated pest control (Poncon et al. 2007). In recent years, the species has been reported to increase in abundance and to move north in Europe. Presently, the most northern established population of *Cx. modestus* appears to be southern England, where the species was reported in very low numbers around Portsmouth in 1944-1945 and then disappeared only to be rediscovered in 2011 in high densities in North Kent Marshes (Golding et al. 2012). It was suggested that the 2011 findings may be a result of a recent reintroduction originating from the high number of international shipping terminals in the area (Golding et al. 2012). In Czech Republic in central Europe, *Cx. modestus* is now widely distributed and a dominating species in light trap collections, after having been considered rare in the 1950s, 1970s, and 1980s (Votýpka 2008).

The species may be recently introduced in Greve and established as a result of environmental changes, or it may have remained unnoticed for years. Together with other European findings, where *Cx. modestus* went from rare or absent to become the dominating species, the results presented here suggest a need to monitor this important bridge vector for West Nile virus in northern Europe. The morphological identification proved to be easy and reliable, and the species was identified during the first year of the newly established surveillance system for mosquitoes in Denmark, thus underlining the usefulness of surveillance activities for insect vectors.

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The mosquito surveys in 2011-2013 and the vector

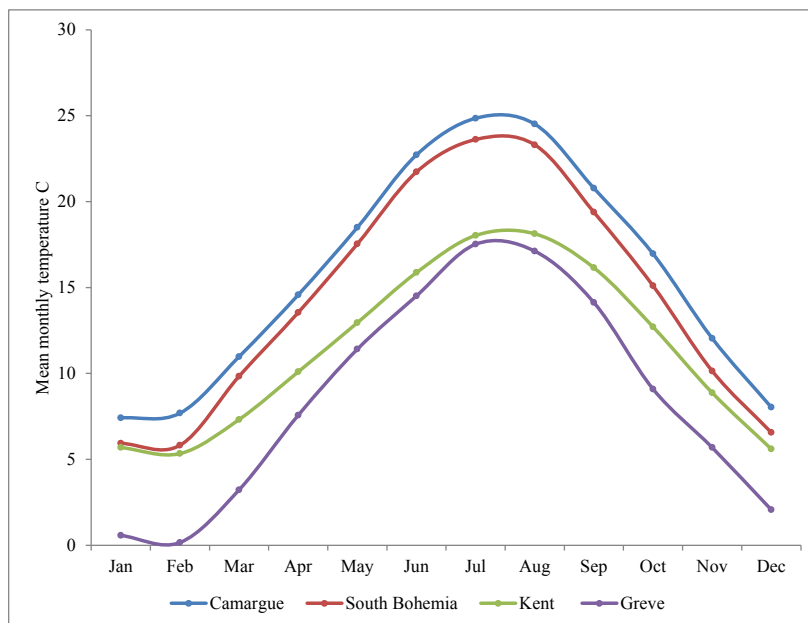


Figure 2. The average monthly temperatures in the ten-year period 2003 to 2012 were markedly lower at the new Danish *Culex modestus* site compared to Camargue and South Bohemia while July and August temperatures on average were just 0.5 and 1.0 C colder than an emerging *Cx. modestus* site in England. Winter temperatures in Greve were much colder than the three other sites and this may be important for *Cx. modestus* that survives winter in the adult stage.

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