

*Short Communication***First finding of the West Nile virus vector  
*Culex modestus* Ficalbi 1889 (Diptera; Culicidae) in Sweden**Anders Lindström<sup>1</sup>, Tobias Lilja<sup>1</sup><sup>1</sup> National Veterinary Institute, Uppsala, Sweden

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In recent years the mosquito *Culex modestus* Ficalbi 1889 appears to have spread in northern and central Europe (Votýpka *et al.*, 2008; Golding *et al.*, 2012, Bødker *et al.*, 2014). Due to its catholic feeding habits and vector competence, *Cx. modestus* has been identified as one of the main bridge vectors of West Nile virus between birds and mammals (Hubálek & Halouzka, 1999; Fyodorova *et al.*, 2006; Balanghien, 2007).

Since the reappearance of *Cx. modestus* in the UK (Golding *et al.*, 2012) and the subsequent report from Denmark (Bødker *et al.*, 2014) the authors have been aware that the species might show up in Sweden as well. Mosquito Magnet™ traps have been set up in Skåne, Southern Sweden, in locations deemed suitable for the species, but up until July 2016 no specimens had been caught. On July 1<sup>st</sup>, in central Simrishamn (55°33'23.6"N 14°20'59.9"E ±250m), South Eastern Skåne, one of the authors (AL) collected a mosquito that landed on his arm to blood feed. The mosquito was identified as *Cx. modestus*.

The specimen was determined to species using the keys in Becker *et al.* (2010) and Schaffner *et al.* (2001). While *Cx. modestus* is rather easy to identify on morphological characters, since it was a first observation for the country molecular

confirmation of the species through barcoding was also performed for six specimens.

The area around the city of Simrishamn is dominated by agricultural landscape, and in an effort to localise the breeding areas, potential breeding areas were located by studying maps and 21 localities in and around Simrishamn were visited. A Mosquito Magnet® trap was run for the rest of the season, i.e. until mid-September, in the garden where the specimen was caught but no further specimens were caught nor were any breeding grounds located. In 2017 a trap was run in Falsterbo from July 27<sup>th</sup> to September 14<sup>th</sup> and another one in Simrishamn, which ran from August 26<sup>th</sup> to September 7<sup>th</sup>. In Falsterbo 103 *Cx. modestus* were trapped between 27/7 and 11/9 2017. In the Simrishamn trap 3 *Cx. modestus* were caught between 30/8 and 4/9 2017 (Fig. 1). The composition of mosquito species and number of individuals caught in the traps are shown in Table 1. Another 53 localities were visited, and human landing catch, i.e. standing close to the water for 20 minutes, to attract *Cx. modestus* was attempted. In only one of those localities the attempt was successful, in Skanör on the Falsterbo Peninsula, where 1 *Cx. modestus* tried to blood feed.

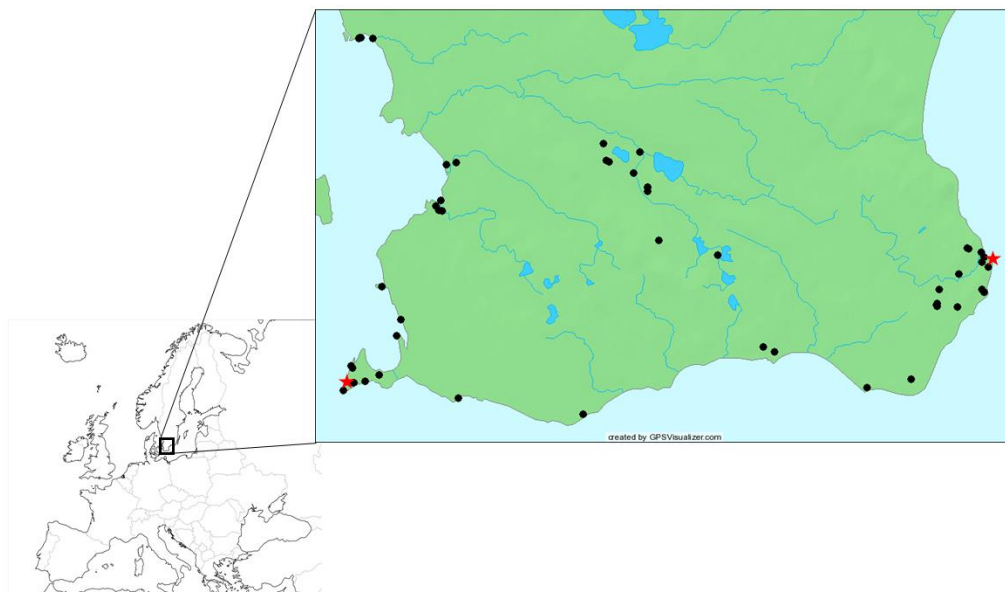


Figure 1: The sampling localities in Skåne. Black points (●) show negative localities, red stars (★) show where *Culex modestus* was found.

Table 1: Mosquitoes trapped during the project.

Another 238 specimens could not be identified to species.

Species	Number
<i>Culex pipiens/torrentium</i>	1034
<i>Aedes (Och) caspius</i>	1010
<i>Aedes (Och) detritus</i>	321
<i>Culex (Ncx) modestus</i>	106
<i>Aedes (Aed) vexans</i>	90
<i>Anopheles claviger</i>	57
<i>Coquillettidia richiardii</i>	38
<i>Aedes cinereus</i>	8
<i>Aedes (Och) annulipes</i>	7
<i>Culiseta annulata</i>	5
<i>Aedes (Och) sticticus</i>	3
<i>Aedes (Och) cantans</i>	2
<i>Aedes (Och) flavescens</i>	2
<i>Anopheles maculipennis</i>	2
<i>Culiseta subochrea</i>	2
<i>Aedes (Fin) geniculatus</i>	1

For DNA barcoding, three mosquito legs were used for DNA isolation by homogenization in 30 µl Prepman Ultra (Life Technologies, Carlsbad, CA, USA) (Ander, 2013). The sample was then lysed at 100°C for 10 min. Tissue debris was removed by centrifugation at 12,000 x g for 2 min after which 20 µl of the supernatant was transferred to a fresh tube and used as template in PCR reactions. The COI region was amplified using a previously published primer pair LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer, 1994) which amplify the 5'-part of the gene. PCR reactions were performed using AmpliTaq DNA polymerase (Invitrogen, Thermo Fischer Scientific, Waltham, MA USA). The PCR-product were purified with Agencourt AMPure XP beads (Beckman Coulter, Pasadena, CA, USA) and sent to Macrogen (Seoul, South Korea) for sequencing in both directions using the same primers as in the PCR.

The resulting sequence (Genbank accession number MF537266, MG844175-MG844179) was identified as *Culex modestus* using the BOLD database and identification ([www.barcodinglife.org](http://www.barcodinglife.org)). Sequences were aligned using ClustalW as implemented in MEGA6 (Tamura *et al.*, 2013) and phylogenetic clustering was performed using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). All six sequences clustered together with European *Culex modestus* with high bootstrap values in a phylogenetic tree of publicly available COI sequences from *Culex* mosquitoes. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

With the discovery of *Cx. modestus* populations and the rediscovery of *Culex pipiens* bf molestus, also in 2016 (Lindström, 2017), Sweden now has all the vector components necessary for spread of the West Nile virus. In 2005-2006 Jourdain *et al.* (2011) analysed blood samples collected from migratory birds captured in south-eastern Sweden for antibodies against WNV. Only two birds out of 1935 were positive. However, since then West Nile virus has become endemic in both Greece and Italy and it would be reasonable to imagine that a higher percentage of the birds migrating through those areas become infected by WNV and transport the virus northwards.

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## References

- Ander, M., Troell, K. & Chirico, J. (2013). Barcoding of biting midges in the genus *Culicoides*: a tool for species determination. *Medical and Veterinary Entomology*, **27**, 323-331.
- Balenghien, T., Vazeille, M., Reiter, P., Schaffner, F., Zeller, H. & Bicout, D.J. (2007). Evidence of laboratory vector competence of *Culex modestus* for West Nile virus. *Journal of the American Mosquito Control Association*, **23**, 233-236.
- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M. & Dahl, C. (2010). Mosquitoes and Their Control. SpringerVerlag Berlin Heidelberg.
- Bødker, R., Klitgård, K., Byriel, D. & Kristensen, B. (2014). Establishment of the West Nile virus vector, *Culex modestus*, in a residential area in Denmark. *Journal of Vector Ecology*, **39**, 1-3.
- Fyodorova, M.V., Savage, H.M., Lopatine, J.V., Bulgakova, T.A., Ivanitsky, A.V., Platonova, O.V., & Platonov, A.E. (2006). Evaluation of potential West Nile vectors in Volgograd Region, Russia, 2003 (Diptera: Culicidae): Species composition, bloodmeal host utilization, and virus infection rates of mosquitoes. *Journal of Medical Entomology*, **43**, 552- 563.
- Golding N., Nunn, M.A., Medlock, J.M., Purse, B.V., Vaux, A.G.C., & Schäfer, S.M. (2012). West Nile virus vector *Culex modestus* established in southern England. *Parasites and Vectors*, **5**, 32.
- Hubálek Z. & Halouzka, J. (1999). West Nile fever - a reemerging mosquito-borne viral disease in Europa. *Emerging Infectious Diseases*, **5**, 643-649.
- Jourdain, E., Olsen, B., Lundkvist, Å., Hubálek, Z., Šikutová, S., Waldenström, J., Karlsson, M., Wahlström, M., Jozan, M. & Falk, K. (2011). Surveillance for West Nile Virus in wild birds from Northern Europe. *Vector-borne and Zoonotic Diseases*, **11**, 77-79
- Lindström, A. (2017). History of human-biting *Culex pipiens* in Sweden and Scandinavia. *Journal of the European Mosquito Control Association*, **35**, 10-12
- Schaffner, F., Angel, G., Geoffroy, B., Hervy, J.P., Rhaiem, A. & Brunhes, J. (2001). The Mosquitoes of Europe: An Identification and Training Programme. Montpellier, France: IRD.
- Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution*, **9**, 678-687.
- Tamura K., Stecher G., Peterson D., Filipowski A., & Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, **30**, 2725-2729.
- Votýpka, J., Seblová, V., & Rádrová, J. (2008). Spread of West Nile vector *Culex modestus* and the potential malaria vector *Anopheles hyrcanus* in central Europe. *Journal of Vector Ecology*, **33**, 269-277.