



## Relative abundance and geographical variation of *Culex pipiens* and *Culex torrentium* (Diptera: Culicidae) in CO<sub>2</sub>-baited traps in Denmark

Byriel, David B.; Kristensen, Birgit; Klitgaard Schou, Kirstine ; Bødker, Rene

*Published in:*  
Entomologica Fennica

*Publication date:*  
2018

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Byriel, D. B., Kristensen, B., Klitgaard Schou, K., & Bødker, R. (2018). Relative abundance and geographical variation of *Culex pipiens* and *Culex torrentium* (Diptera: Culicidae) in CO<sub>2</sub>-baited traps in Denmark. *Entomologica Fennica*, 29(3), 112-118. <https://journal.fi/entomolfennica/article/view/77278/38395>

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Relative abundance and geographical variation of *Culex pipiens* and *Culex torrentium* (Diptera: Culicidae) in CO<sub>2</sub>-baited traps in Denmark

David B. Byriel, Birgit Kristensen, Kirstine Klitgaard & René Bødker

Byriel, D. B., Kristensen, B., Klitgaard, K. & Bødker, R. 2018: Relative abundance and geographical variation of *Culex pipiens* and *Culex torrentium* (Diptera: Culicidae) in CO<sub>2</sub>-baited traps in Denmark. — Entomol. Fennica 29: 112–118.

European *Culex pipiens* and *Culex torrentium* are morphological fairly similar mosquito species with potentially different vector competences for pathogenic viruses. The relative abundance of the two species is therefore important for quantifying the potential for disease transmission in Denmark. Mosquitoes were sampled from 74 different sites in Denmark with CO<sub>2</sub> and octenol-baited suction traps. A total of 285 *Culex* specimens were identified to species using a restriction enzyme assay. *Culex pipiens* was the dominating species with 220 (77%) specimens caught at 22 different sites, while 65 (23%) specimens were identified as *C. torrentium* and only caught at 4 sites. The ratio of the two species differed significantly between sites with *C. torrentium* dominating in just a single location. Both mosquito species were predominantly caught late in the Danish mosquito season, from mid-August and onwards.

D. B. Byriel, DTU National Veterinary Institute, Bülowvej 27, 1870 Frederiksberg C, Denmark; E-mail: daby@ign.ku.dk

B. Kristensen, DTU National Veterinary Institute, Bülowvej 27, 1870 Frederiksberg C, Denmark

K. Klitgaard, DTU National Veterinary Institute, Bülowvej 27, 1870 Frederiksberg C, Denmark

R. Bødker, DTU National Veterinary Institute, Bülowvej 27, 1870 Frederiksberg C, Denmark

Received 8 February 2017, accepted 15 September 2017

## 1. Introduction

*Culex pipiens* Linnaeus, 1758 and *Culex torrentium* Martini, 1925 (Diptera: Culicidae) are morphologically fairly similar species with an overlapping range in northern Europe (Hesson *et al.* 2014). Accurate morphological identification of the two species can only be done on the basis of male hypopygial characters (Service 1968),

whilst wing morphology can be used to discriminate between female adults with an accuracy of more than 90% (Börstler *et al.* 2014). *Culex pipiens* and *C. torrentium* are both perennial species that hibernate as adults. The larvae of the two species often occur together, and are found in semi-permanent waters such as edges of slow running streams, in vegetation at borders of lakes, semi-permanent pools, marshy areas, man-made

containers and reservoirs of sewage plants (Becker *et al.* 2010). Females of the two species are both anautogenous and ornithophilic and rarely bite humans (Becker *et al.* 2010).

*Culex pipiens* and *C. torrentium* can maintain transmission of zoonotic viruses between birds. *Culex pipiens* can serve as a vector of several arboviruses including Japanese encephalitis, Sindbis virus, Usutu virus and West Nile virus (WNV) (Francy *et al.* 1989, Farajollahi *et al.* 2011). Less is known about the vector capability of *C. torrentium*, probably due to it mistakenly being confused with *C. pipiens*, its holarctic distribution, and its late recognition (Harbach 1985). However, *C. torrentium* is a known vector of Sindbis virus (Lundström *et al.* 1990) and was recently also found susceptible to WNV (Leggewie *et al.* 2016). During the last 40 years, both Sindbis virus and WNV have been isolated from wild-caught female mosquitoes in Europe (Lundström 1994, Nicolescu 1998, Jöst *et al.* 2010, Hesson *et al.* 2015a) and outbreaks of Sindbis virus and WNV in humans have occurred (Lundström 1999, A Sirbu *et al.* 2011). *Culex torrentium* appears to be a more efficient vector of Sindbis virus than *C. pipiens* (Lundström 1990), and recently *C. torrentium* was found to be highly susceptible for dissemination of WNV even at low temperatures, and with higher infection rates than *C. pipiens* (Leggewie *et al.* 2016). Because of the potential differences in vector competences, research related to their distribution, ecology and genetics has been conducted in European countries, and different molecular methods have been developed to separate females of *C. torrentium* from *C. pipiens* (Hesson *et al.* 2010, Rudolf *et al.* 2013).

In Denmark, the prevalence of *C. pipiens* and *C. torrentium* is relatively unknown, because they were previously considered one species. *Culex torrentium* was first discovered in Denmark in 1971 (Iversen 1971). Since then, little research on the distribution and occurrence of *C. torrentium* has been done. Hesson *et al.* (2014) conducted a study on the larval prevalence of the two species throughout Europe and included six artificial larval sampling sites in Denmark. The study found both species occurring together in some artificial containers and separately in others, but they were on average equally abun-

dant. Hesson *et al.* (2014) found that *C. torrentium* dominates in northern Europe whereas *C. pipiens* dominates south of the Alps. Contrary, another study found that less than 5% of the *Culex* population belonged to *C. torrentium* and 95% to *C. pipiens* in the northern part of Germany close to Denmark (Rudolf *et al.* 2013).

Exotic mosquitoes are spreading in Europe and recently the competent WNV bridge vector *Culex modestus* Ficalbi, 1947 was discovered in England and Denmark (Medlock *et al.* 2012, Bødker *et al.* 2014). Denmark is located on the migration route of many Palaearctic-African migratory birds and antibodies against WNV have been found in migratory birds in Denmark, with 30 seropositive songbirds among 1,056 examined from 2011 to 2015 and with positive birds detected in confirmatory tests of all five years (Lohse *et al.* 2016). In the same period of five years, 3,037 *Culex* mosquitoes from the Danish vector surveillance program ([www.myggetal.dk](http://www.myggetal.dk)) were all tested negative for WNV (Lohse *et al.* 2016).

Here we present the first study of the relative abundances of adult *C. pipiens* and *C. torrentium* and their geographical variation in Denmark. This study is targeting adult mosquitoes collected in private gardens, in public parks, and on animal production farms. The temporal and spatial abundance of the adult vectors on these locations are assumed to be the main drivers of the potential for disease transmission from birds to humans and to production animals in Denmark.

## 2. Materials and methods

Mosquitoes were collected in 2012 using Mosquito Magnet Independence CO<sub>2</sub>-baited traps with octenol (Mosquito Magnet, Lititz, PA, USA) from a total of 74 sites of randomly generated X–Y coordinates from three types of land use. The land use categories were urban gardens, recreational land use, and agricultural sites, which were further divided into four categories, pig, poultry, cattle and horse farms. At agricultural sites, the stable building or animal grazing fold nearest to the random coordinate was selected, and the trap was placed either fully outside or in the opening of a stable. Two of the 74 sites were

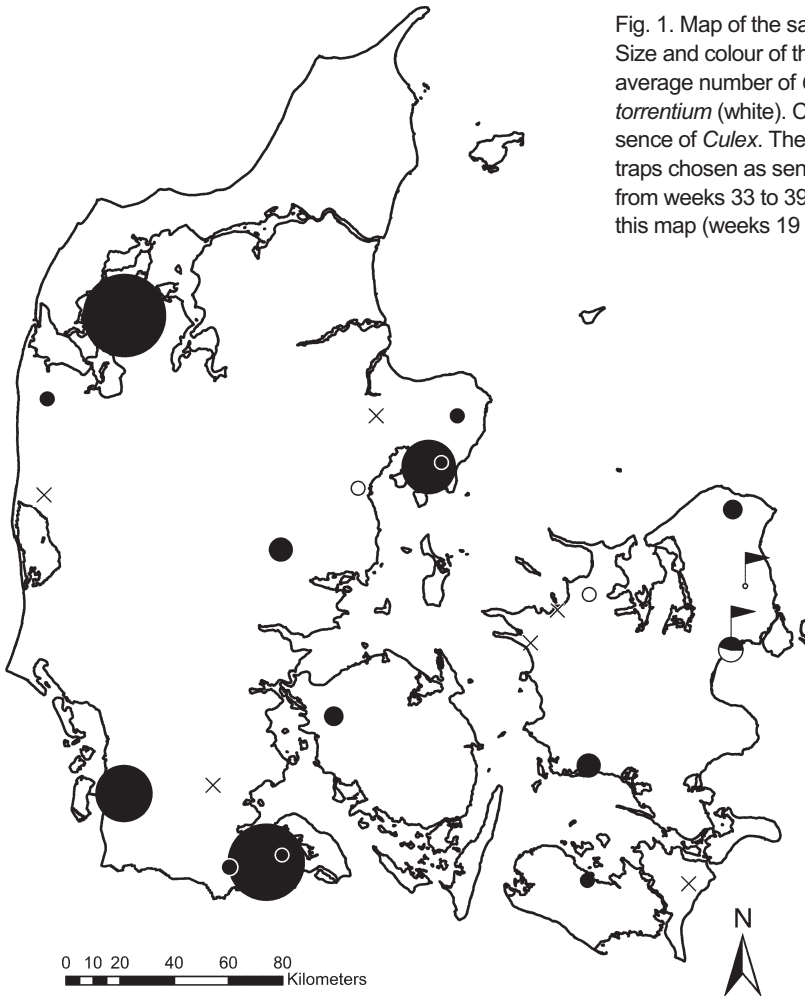


Fig. 1. Map of the sampling sites in Denmark. Size and colour of the dots represent the daily average number of *C. pipiens* (black) and *C. torrentium* (white). Crosses represent an absence of *Culex*. The two flags represent the traps chosen as sentinels. Only the catches from weeks 33 to 39 (272 exx.) are included in this map (weeks 19 to 32 with only 13 exx.).

selected as sentinel sites. The southern sentinel site was sampled daily from week 18 to 39 and the northern one weekly from week 21 to 39. These sentinel sites were therefore sampled more than the other 72 sites and these two therefore represented 29% and 26% of all collection nights. Because of a limited number of traps and the aim to sample a large number of different areas of Denmark, the traps were moved between the remaining sites every week (temporary traps). This was done in a total of 72 different temporary sites from week 21 to 37, where traps were left at each site for three consecutive days, constituting the remaining 45% of all collection nights. Each temporary collection site was only sampled once, and between three and seven new collection sites were sampled each week. Species were identified

using the identification key by Becker *et al.* (2010).

Species of the morphological fairly similar *C. pipiens* and *C. torrentium* were separated by a restriction enzyme assay according to Hesson *et al.* (2010) with modifications. DNA extraction was performed with Qiagen DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany) using the manufacturer's protocol for insect DNA extraction. Mosquito legs and the lower part of thorax were used for DNA extraction, and a microtube pestle was used to homogenize the sample, as this procedure yielded the best results. The PCR reactions consisted of: 6  $\mu$ L DNA, 5  $\mu$ L Buffer (10 x mM  $(\text{NH}_4)_2\text{SO}_4$ ), 5  $\mu$ L  $\text{MgCl}_2$  (2.0 mM), the primer pair 0.5  $\mu$ L TL2-N-3014 and 0.5  $\mu$ L C1-J-2183 (Simon *et al.* 1994), 0.5  $\mu$ L DNTP

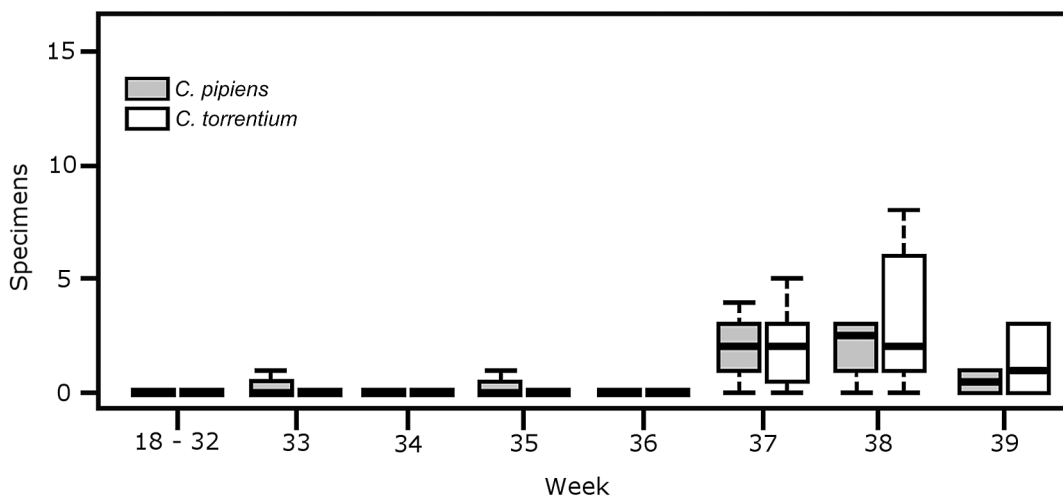


Fig. 2. Box and whisker plots of the daily catches of two *Culex* species from the southern sentinel CO<sub>2</sub>-baited collection site. Catches are based on seven collection nights per week. Number of specimens with minimum, maximum, median, 25<sup>th</sup> and 75<sup>th</sup> percentiles are shown for each week.

(0.1 μM per sample), 0.20 μL Taq (0.02 U per μL), and 32.3 μL H<sub>2</sub>O. Thermocycler conditions were: 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 46 °C for 30 s, 72 °C for 1 min, and final extension of 72 °C for 7 min. Restriction enzyme digestion consisted of: 10 μL PCR reaction mixture, 18 μL nuclease-free water, 1 μ 10 X Buffer tango, 0.1 μL FspBI (Thermo Scientific, Waltham, MA, USA). After a gentle mixing, it was incubated at 37 °C for 3 h, followed by an inactivation of FspBI by incubation at 65 °C for 20 min. Restrictions were run on a 2% agarose E-Gel® (Invitrogen, Carlsbad, CA, USA).

Statistical analyses and box and whisker plots were made with R-Studio (R Core Team 2013). Statistical analyses were performed on weekly accumulated mosquito catches. Box and whisker plots were made as the daily catches of *C. torrentium* and *C. pipiens* (based on seven nights for the southern sentinel trap and three nights for the temporary traps) accumulated for each week. The map was made in ArcMap 10.3 (ESRI).

### 3. Results

A total of 285 female *Culex* specimens from the entire season were analyzed using the restriction enzyme assay. Of these, 220 specimens were identified as *C. pipiens* and 65 as *C. torrentium*.

From week 19 to 32 a total of 10 *C. pipiens* and 3 *C. torrentium* were collected from 7 out of 50 sites. While this represents 2/3 of the collection period, it only constitutes 4.6% of the collected *Culex* specimens. During the last 1/3 of the collection period from week 33 to 39 a total of 210 specimens of *C. pipiens* were caught from 15 out of 24 different sites, and 62 specimens of *C. torrentium* were caught from 4 out of 24 different sites (Fig. 1). The spatial variation in the ratio of the two species differed significantly with Fisher's exact test between the collection sites at week 34 ( $p = 0.032$ ,  $df = 4$ ), 35 ( $p = 0.012$ ,  $df = 6$ ), 36 ( $p = 0.025$ ,  $df = 3$ ) and 37 ( $p < 0.0001$ ,  $df = 5$ ), but not at week 33 ( $p = 1.0$ ,  $df = 1$ ) and 38 ( $p = 1.0$ ,  $df = 1$ ). At week 39, mosquitoes were only captured in one of the collection sites. During the entire collection period, *C. pipiens* was caught at 22 different sites, significantly more than *C. torrentium* caught at just four sites (74 sites examined; Fisher's exact test:  $p = 0.0001$ ,  $df = 1$ ).

In total, 95% of all *C. torrentium* were collected at the southern sentinel collection site. This trap constituted 29% of all collection nights of the 74 traps. The southern sentinel collection site collected both species, with 61 specimens of *C. torrentium* and 51 of *C. pipiens*. This allowed for a comparison of the phenology for the two species, which showed fairly even seasonal dynamics at this site with an abundance peak from week

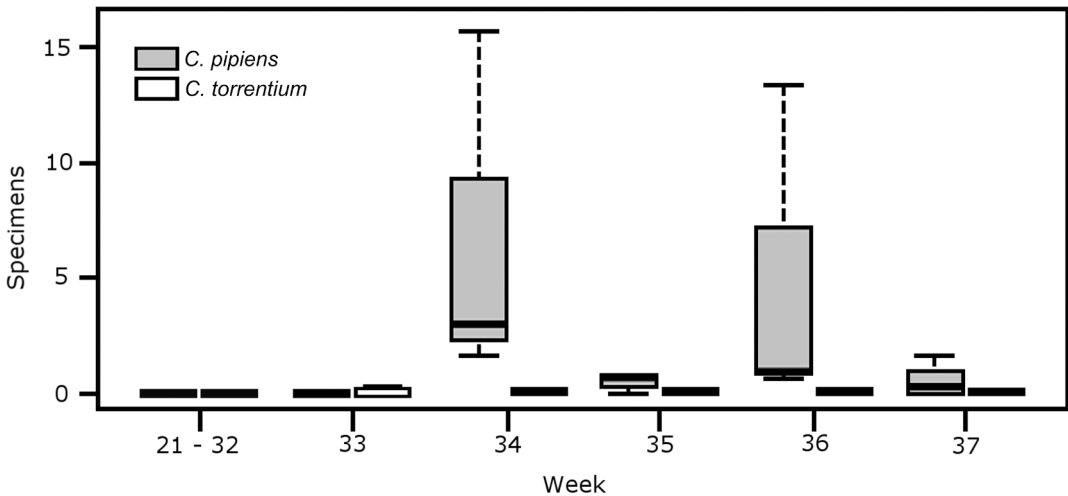


Fig. 3. Box and whisker plots of the average daily catches of two *Culex* species from three to seven weekly temporary trap sites. Each weekly catch consist of 3 consecutive collection nights. Number of specimens with minimum, maximum, median, 25<sup>th</sup> and 75<sup>th</sup> percentile are shown for each week.

37 to 39 (Fig. 2). The 210 specimens of *C. pipiens* collected at the temporary sites were primarily found in weeks 34 and 36 (Fig. 3) but showed a large geographical variation in abundance each week.

#### 4. Discussion

Constituting 77% of all *Culex* specimens, this study found *C. pipiens* to be the dominant species in Denmark in octenol and CO<sub>2</sub>-baited traps. This is consistent with the findings from the northern part of Germany, where *C. pipiens* was found to be the dominant species (Rudolf *et al.* 2013), but not with *C. torrentium* being the dominant species in northern Europe as suggested from a larger European study (Hesson *et al.* 2014). While *C. pipiens* was collected at 22 different Danish sites, *C. torrentium* was only collected at four sites. At the southern sentinel site *C. torrentium* was the dominant species with 54% during weeks 32 to 39, and this site collected 95% of all specimens of *C. torrentium*. Thus, while *C. pipiens* on average dominated nationally in both average abundance and geographical distribution, one hot spot was dominated by *C. torrentium* as expected for Northern Europe (Hesson *et al.* 2014). In general, the ratio of the two species varied significantly between the collection sites and the coincidental

inclusion of an apparently rare *C. torrentium* hotspot in the survey as the sentinel site with relatively many collection nights increased the proportion of *C. torrentium* to 23%. The true national average of *C. torrentium* in Denmark is therefore likely to be lower than 23%; indeed, excluding this sentinel site lowers the prevalence of *C. torrentium* to just 1.4%. Therefore, the true national prevalence depends on the frequency of such hot spots in Denmark.

Species ratios may also be strongly influenced by sampling design with *C. pipiens* being more prevalent than *C. torrentium* in CO<sub>2</sub>-baited traps compared to larval sampling and gravid traps (Weitzel *et al.* 2011, Hesson *et al.* 2015b). The reason for this remains unknown, but it has been speculated that *C. torrentium*, being strictly ornithophilic, spends its time around bird nests in high trees and only visits the ground to oviposit (Petrić *et al.* 1999). Both *C. pipiens* and *C. torrentium* were found to occur in high numbers late in the season. *Culex pipiens* hibernates as adults from August and slowly depletes the fat reserves and never takes blood during hibernation (Sulaiman & Service 1983). While determining *Culex* under a stereo microscope we found that many of them had developed fat reserves. This observation together with information on their hibernation biology, suggests that these mosquitoes may not have been attracted to CO<sub>2</sub>-baited traps

in search of blood meals, but rather as a result of exploring desired places for hibernation. If this is the case, differences in their hibernation biology and/or possibly phenology could also explain the low number of *C. torrentium* caught in CO<sub>2</sub>-baited traps.

The late occurrence of *C. pipiens* and *C. torrentium* is a problem in regard to the sampling design used in this study, which was intended to catch a broad variety of mosquitoes from as many different sites in Denmark as possible. Therefore, many sites sampled early in the season that did not catch any *Culex*, would most likely have been positive for one or both of the studied species, if the sites had been sampled later in the season. Based on the results presented here, we suggest that studies investigating the ratio of *C. pipiens* and *C. torrentium* should be carried out late in the season and supplementary traps not based on CO<sub>2</sub> and octanol should be included in future surveys. In the present study, we used a restriction enzyme assay for species identification of 285 *Culex* specimens. For future high throughput screening of *Culex* samples, a more suitable method could be the qPCR assay for differentiation between *C. pipiens* and *C. torrentium* developed by Rudolf *et al.* (2013).

*Acknowledgements.* The mosquito survey was funded by the Danish Food and Veterinary Administration. Data analysis was funded by the EMIDA ERA-NET supported project “VICE Risk based Surveillance for Vector Borne Diseases”. The authors are grateful to Dr. Jenny Hesson (University of Uppsala) for sending reference material to get started with the molecular identification, and to the anonymous reviewers for their valuable comments and suggestions to improve this paper.

## References

- A Sirbu, C. C., Panculescu-Gatej, R., Vázquez, A., Tenorio, A., Rebreanu, R., Niedrig, M., Nicolescu, G. & Pistol, A. 2011: Outbreak of West Nile virus infection in humans, Romania, July to October 2010. — *Eurosurveillance* 16(2): 2–7.
- Becker, N., Petrić, D., Zgomba, M., Boase, C., Minoo, M., Dahl, C. & Kaiser, A. 2010: Mosquitoes and their control. — Springer-Verlag, Heidelberg, Germany. 577 pp.
- Börstler, J., Lühken, R., Rudolf, M., Steinke, S., Melaun, C., Becker, S., Garms, R. & Krüger, A. 2014: The use of morphometric wing characters to discriminate female *Culex pipiens* and *Culex torrentium*. — *Journal of Vector Ecology* 39(1): 204–212.
- Bødker, R., Klitgård, K., Byriel, D. B. & Kristensen, B. 2014: Establishment of the West Nile virus vector, *Culex modestus*, in a residential area in Denmark. — *Journal of Vector Ecology* 39(2): 1–3.
- Farajollahi, A., Fonseca, D. M., Kramer, L. D. & Kilpatrick, A. M. 2011: “Bird biting” mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. — *Infection, Genetics and Evolution* 11(7): 1577–1585.
- Francy, D. B., Jaenson, T. G., Lundström, J. O., Schildt, E. B., Espmark, A., Henriksson, B. & Niklasson, B. 1989: Ecologic studies of mosquitoes and birds as hosts of Ockelbo virus in Sweden and isolation of Inkoo and Batai viruses from mosquitoes. — *The American Journal of Tropical Medicine and Hygiene* 41(3): 355–363.
- Harbach, R. E., Dahl, C. & White, G. B. 1985: *Culex (Culex) pipiens* Linnaeus (Diptera: Culicidae): concepts, type designations, and description. — *Proceedings of the Entomological Society of Washington* 87: 1–24.
- Hesson, J. C., Lundström, J. O., Halvarsson, P., Erixon, P. & Collado, A. 2010: A sensitive and reliable restriction enzyme assay to distinguish between the mosquitoes *Culex torrentium* and *Culex pipiens*. — *Medical and Veterinary Entomology* 24(2): 142–149.
- Hesson, J. C., Rettich, F., Merdić, E., Vignjević, G., Östman, Ö., Schäfer, M. & Scholte, E. J. 2014: The arbovirus vector *Culex torrentium* is more prevalent than *Culex pipiens* in northern and central Europe. — *Medical and veterinary entomology* 28(2): 179–186.
- Hesson, J. C., Ignell, R., Hill, S. R., Östman, Ö. & Lundström, J. O. 2015b: Trapping biases of *Culex torrentium* and *Culex pipiens* revealed by comparison of captures in CDC traps, ovitraps, and gravid traps. — *Journal of Vector Ecology* 40(1), 158–163.
- Hesson, J. C., Verner-Carlsson, J., Larsson, A., Ahmed, R., Lundkvist, Å. & Lundström, J. O. 2015a: *Culex torrentium* mosquito role as major enzootic vector defined by rate of Sindbis virus infection, Sweden, 2009. — *Emerging Infectious Diseases* 21(5): 875–878.
- Iversen, T. M. 1971: *Culex torrentium* Martini (Dipt. Culicidae) ny for Danmark, med en beskrivelse af larvebiotopen. — *Entomologiske Meddelelser* 39: 235–239. [In Danish.]
- Jöst, H., Bialonski, A., Storch, V., Günther, S., Becker, N. & Schmidt-Chanasit, J. 2010: Isolation and phylogenetic analysis of Sindbis viruses from mosquitoes in Germany. — *Journal of Clinical Microbiology* 48(5): 1900–1903.
- Leggiewie, M., Badusche, M., Rudolf, M., Jansen, S., Börstler, J., Krumkamp, R., Huber, K., Krüger, A., Schmidt-Chanasit, J., Tannich, E. & Becker, S. C. 2016: *Culex pipiens* and *Culex torrentium* populations from Central Europe are susceptible to West Nile virus infection. — *One Health* 2: 88–94.
- Lohse, L., Madsen, J. J., Huda, A., Bødker, R., Thorup, K., Polacek, C. & Bøtner, A. 2016: West Nile Fever – En

- virus sygdom der breder sig i Europa. — Dansk Veterinærtidsskrift 8: 10–13. [In Danish.]
- Lundström, J. O., Niklasson, B. & Francy, D. B. 1990: Swedish *Culex torrentium* and *Cx. pipiens* (Diptera: Culicidae) as experimental vectors of Ockelbo virus. — *Journal of Medical Entomology* 27(4): 561–563.
- Lundström, J. O. 1994: Vector competence of Western European mosquitoes for arboviruses: A review of field and experimental studies. — *Bulletin of the Society of Vector Ecology* 19: 23–36.
- Lundström, J. O. 1999: Mosquito-borne viruses in Western Europe: a review. — *Journal of Vector Ecology* 24: 1–39.
- Medlock, J. M., Hansford, K. M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H. & Bortel, W. V. 2012: A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. — *Vector-Borne and Zoonotic Diseases* 12(6): 435–447.
- Nicolescu, G. 1998: A general characterisation of the mosquito fauna (Diptera: Culicidae) in the epidemic area for West Nile virus in the south of Romania. — *European Mosquito Bulletin* 2: 13–18.
- Petrić, D., Zgomba, M., Bellini, R., Veronesi, R., Kaiser, A. & Becker, N. 1999: Validation of CO<sub>2</sub> Trap Data in three European Regions. — *Proceedings of the 3<sup>rd</sup> International Conference on Urban Pests*: 437–445. Prague, Czech Republic. 678 pp.
- R Core Team, 2013: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. — Available from <http://www.R-project.org/>
- Rudolf, M., Czajka, C., Börstler, J., Melaun, C., Jöst, H., von Thien, H. & Tannich, E. 2013: First nationwide surveillance of *Culex pipiens* complex and *Culex torrentium* mosquitoes demonstrated the presence of *Culex pipiens* biotype *pipiens/molestus* hybrids in Germany. — *PLoS One* 8(9): e71832/journal.pone.0071832.
- Service, M. W. 1968: The taxonomy and biology of two sympatric sibling species of *Culex*, *C. pipiens* and *C. torrentium* (Diptera, Culicidae). — *Journal of Zoology* 156(3): 313–323.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. — *Annals of the Entomological Society of America* 87(6): 651–701.
- Sulaiman, S. & Service, M. W. 1983: Studies on hibernating populations of the mosquito *Culex pipiens* L. in southern and northern England. — *Journal of Natural History* 17(6): 849–857.
- Weitzel, T., Braun, K., Collado, A., Jöst, A. & Becker, N. 2011: Distribution and frequency of *Culex pipiens* and *Culex torrentium* (Culicidae) in Europe and diagnostic allozyme markers. — *European Mosquito Bulletin* 29: 22–37.