First records of the thermophilic mosquito *Culiseta longiareolata* (Macquart, 1838) in Austria, 2012, and in Slovenia, 2013

Bernhard Seidel, [1, 2] Norbert Nowotny, [3, 4] Darja Duh, [5] Alexander Indra, [6] Peter Hufnagl, [6] Franz Allerberger [6]

[1] *Technical Office of Ecology and Landscape Assessment, Persenbeug, Austria*
[2] *Department of Theoretical Biology, University of Vienna, Vienna, Austria*
[3] *Institute for Veterinary Public Health, University of Veterinary Medicine,Vienna, Austria*
[4] *Department of Microbiology and Immunology, Sultan Qaboos University, Muscat, Oman*
[5] *Laboratory of Clinical Molecular Diagnostic, Maribor, Slovenia*
[6] *Austrian Agency for Health and Food Safety, Vienna, Austria*
**corresponding author: Bernhard.Seidel@univie.ac.at*

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Abstract: During a West Nile virus surveillance programme, *Culiseta longiareolata* (Macquart, 1838) was discovered for the first time in Austria. Two larvae and one female imago were collected from an artificial water storage container in the wine-growing village of Gamlitz (province of Styria) on July 12, 2012 at 450 m.a.s.l., 46°4058,2´N, 15°3151,4´E. Additional specimens of *Cs. longiareolata* were found on September 8, 2012, when numerous larvae were detected in used car tyres about 160 km westward, in the region of Finkenstein (province of Carinthia). A third site containing larvae of *Cs. longiareolata* was discovered on October 10, 2012, near Althofen about 60 km northeast of the Finkenstein location. Previously, in May 2012, the latter two sites were negative for *Cs. longiareolata.* In autumn 2013 *Cs. longiareolata* was reconfirmed in Carinthia and the first record detected for Slovenia with larvae collected from a rain barrel in a location approximately 6 km north-northeast of the city of Maribor, on September 1, 2013 at 288 m.a.s.l., 46°3959,8´N, 15°3942,2´E. It is proposed that *Cs. longiareolata* was not passively introduced along a major transportation route, but has either actively invaded from neighbouring Italy and Slovenia into the south of Austria within the last few decades and/or has remained undetected owing to its cryptic occurrence on account of its belated activity at the end of summer and during autumn. ***Journal of the European Mosquito Control Association*** **31: 17-20, 2013**

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Introduction

It is widely accepted that climate change will impact the spread of vector-borne communicable diseases in Europe (ECDC, 2012a; ECDC, 2012b) by affecting the distribution of vectors, in particular those expanding in their latitudinal and altitudinal limits (Focks *et al.*, 1995). On October 23, 2012, the Austrian Council of Ministers adopted the Austrian action plan for adaptations to climate change (APA0216 5 CI 0365 WI/II Di). As certain effects of climate change are no longer preventable, the council stressed the need to prepare health care systems for the arrival of new vectors and diseases. The actual role of climate change as a trigger for the invasion of exotic vectors is still a controversial discussion point.

In Central Europe several mosquito species have been introduced in recent years (Pluskota *et al.*, 2008; Schaffner *et al.*, 2009; Becker *et al.*, 2011; Becker & Hoffmann, 2011; Werner *et al.*, 2012). The Japanese rock pool mosquito *Aedes (Finlaya) japonicus japonicus* (Theobald, 1901) [also referred to as *Hulecoeteomyia japonica*] and the Asian tiger mosquito *Aedes albopictus* (Skuse, 1895) [also referred to as *Stegomyia albopicta*] originate initially from the Far East but have now been recorded from several European countries (Werner *et al.*, 2012), including Austria and Slovenia (Seidel *et al.* 2012). This paper reports on the first findings of the Mediterranean thermophilic species *Culiseta longiareolata* (Macquart, 1838) in southern Austria and northern Slovenia. This species is established from Eastern Europe to the Far East and from Italy and France to the African continent (Kirkpatrick, 1925; Edwards, 1941).

Materials and Methods

Before an Austrian West Nile virus (WNV) surveillance programme commenced in 2011 a few studies provided some data relating to activity of putative mosquito vectors of Japanese Encephalitis group viruses such as Usutu Virus (USUV) and WNV (Seidel, 2011). These studies used immature mosquito sampling and aspirator-sampling of adult mosquitoes in the field and collected hibernating female-mosquitoes.

As BG-Sentinel traps (Biogents, Germany) have become a standard method for obtaining field data on mosquito species, this technique was introduced into the Austrian WNV surveillance programme; the programme covers all nine Austrian provinces and northern parts of Slovenia. The BG-Sentinel trap system (Meeraus *et al.*, 2008) baited with CO_2 has been chosen owing to its reported success with collecting adults of endemic as well as exotic mosquitoes.

Since the discovery of larvae of *Ae. japonicus*, and owing to the observed spread in its range in southern Austria and northern Slovenia, immature sampling was instigated, with samples preserved in 70% ethanol. The rapid success of ongoing *Ae. japonicus* monitoring and the advantages of immature sampling to produce enhanced ecological and behavioural observations (e.g., data on aquatic site type, hydrological data of aquatic site, approximate time of egg deposition and metamorphosis, size of growing generation, etc.) led to a reduction in the adult mosquito trapping protocol to just four trap locations per province each year.

The recent investigation area included a) the southeast of Carinthia (except mountainous areas above 800 metres above sea level [m.a.s.l.]), b) the southeast of Styria, c) the south of

Burgenland, d) a small area of Slovenia from the Austrian border to the city of Maribor following the river Drava/Drau westward to the city of Dravograd and e) a small region of Hungary bordering the Austrian province of Burgenland.

Species determination was performed using the keys of Mihályi & Gulyás (1963), Schaffner *et al.* (2001) and Becker *et al.* (2010).

Results

On July 12, 2012, two large water containers (a concrete cube with a height of 110 cm and a surface area of 150 cm x 85 cm, and a plastic barrel with a height of 130 cm and a diameter of 110 cm) used to store rainwater for irrigation were sampled near the village of Gamlitz in the Steinbach Valley, 450 m.a.s.l., (46.4058,2´N, 15.3151,4´E). They contained several hundred immature stages of the invasive mosquito *Ae. japonicus*, as well as *Culex pipiens* Linnaeus (1758). Two fourth-instar larvae were identified as *Cs. longiareolata*. Around these sites one adult female of *Cs. longiareolata* was caught by a mechanical aspirator together with two adult *Ae. japonicus* and 14 *Cx. pipiens*.

On September 8, 2012, numerous larvae of *Cs. longiareolata* were detected in used car tyres stored in a backyard in the region of Finkenstein (province of Carinthia, 46.5650,5´N, 13.8648,7´E, 510 m.a.s.l.). This site had previously tested negative for *Cs. longiareolata* on May 19, 2012.

On October 10, 2012, the inspection of an vehicle service station specialising in tyres for heavy vehicles, at Mölbling-Unterbergen (46.5234,9´N, 14.2702,3´E, 606 m a.s.l.) yielded *Cs. longiareolata*. The site was previously inspected on May 17, 2012, and car, truck, and tractor tyres were sampled, revealing numerous immature stages of *Cx. pipiens* and *Dahliana geniculata* (Olivier, 1791) but no *Cs. longiareolata*. In October 2012 some of the larger tyres contained numerous larvae of *Cs. longiareolata.* A small number of *Cx. pipiens* larvae were found exclusively in normal car tyres. *Dahliana geniculata* larvae were absent.

In September and October 2013 the three positive sites for *Cs. longiareolata* were again rechecked and confirmed positive. A fourth site was also positive with larvae of *Cs. longiareolata* found inside rain water barrels in the village of Hart near the city of Eberndorf at 512 m.a.s.l. on August 31, 2013 at 46.3429,7´N, 14.3928,9´E (Figure 1, yellow dot in Austria).

On September 1, 2013, nine larvae of *Cs. longiareolata* were collected from a rain barrel, about 6 km north of the city of Maribor, 288 m.a.s.l., 46.3959,8´N, 15.3942,2´E (Figure 1, yellow dot in Slovenia) – the first record from Slovenia. The Austrian site of the first finding is located 2.5 km from the Austrian-Slovenian border and approximately 9 km from the site of the recent record in Slovenia.

Within the relevant investigation area *Cs. longiareolata* was absent in two dozen trapping attempts for the ongoing WNV surveillance program using BG-lure and CO_2 as attractants.

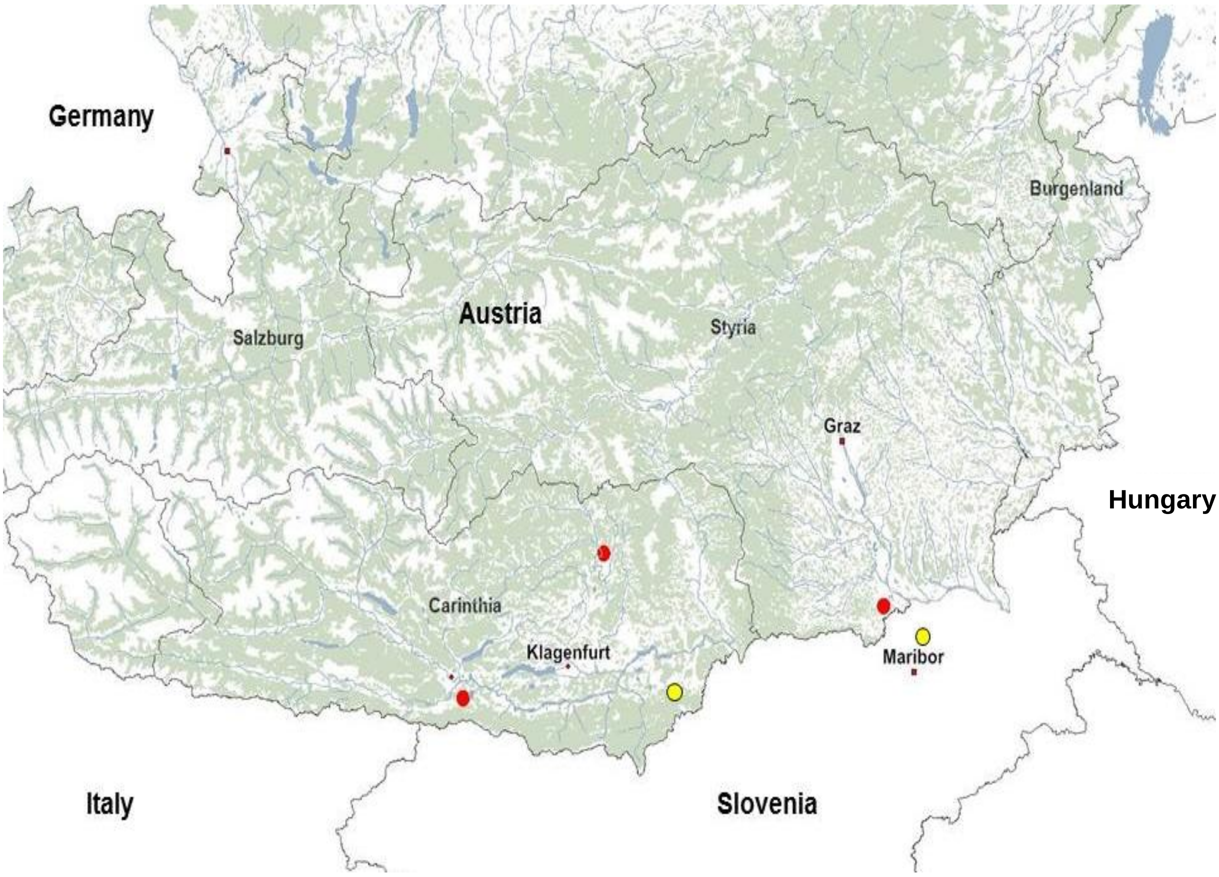

Figure 1: Evidence of *Culiseta longiareolata* in southern Austria in 2012 (red dots) and in Austria and Slovenia in 2013 (yellow dots).

Discussion

Culiseta longiareolata was not previously known to occur in either Austria or Slovenia (Becker *et al.*, 2010; Becker & Hoffmann, 2011). In Italy *Cs. longiareolata* is considered to occur everywhere (Severini *et al.*, 2009) but its distribution has been explained as "occurring undiscovered over long periods of time and can seldom be found" (Fabrizio Montarsi, pers. comm.). This scenario is reminiscent of the current situation now possibly occurring in southern Austria and Slovenia. However in contrast to extensive efforts in Italy, studies in Austria have not used a targeted method for this species nor conducted field studies during the appropriate season, owing largely to the considered absence of this species. Clearly ongoing WNV surveillance activities were likely to overlook *Cs. longiareolata*.

Culiseta longiareolata has been recorded in Slovakia (Trpiš, 1962), Hungary (Mihályi & Gulyás, 1963) and Switzerland (Becker *et al.*, 2010). In Germany a small number of immatures were recently found at one site near the French border (Becker & Hoffmann, 2011), and it is assumed that the field capture was carried out in the middle or late summer season. It is proposed that the peak of activity for this species occurs in the autumn coinciding with the reduction in the intensity of surveillance activity; in particular immature surveillance. This may explain why immature activity has frequently been missed each year. After this species was incidentally discovered in Austria in 2012, the lack of published recordings from Slovenia highlighted that perhaps it may also be present in Slovenia, and therefore it would also appear in larval samples.

The aquatic stages of *Cs. longiareolata* are known to tolerate a high degree of water pollution and the species shows considerable propensity for utilising human-made habitats (Becker *et al*., 2010). The larvae are filter feeders, but are also capable of predatory feeding, including cannibalism (Maslov, 1967), as well as feeding on mosquito larvae of other species (Shaalan, 2012). The late stages of *Cs. longiareolata* larvae have been reported to attack tadpoles of the toad *Bufo viridis* (Laurenti, 1768) (Amphibia: Anura) (Blaustein & Margalit, 1994). Such a situation can be reconstructed in the laboratory on captive toads but is not likely in the wild in Carinthian or Central European conditions where Anuran tadpoles have either reached an enormous body mass or have already metamorphosed in the late summer and early autumn whilst the *Culiseta* larvae reach a relevant size that is large enough to predate tadpoles. Tadpoles of Anuran species which do not metamorphose in summer and hibernate, like *Pelobates fuscus* (Laurenti, 1768), may reach a body mass at the end of summer that could be thirty times greater than any *Culiseta* larvae (Schramm cited in Seidel 1996). This predatory behaviour does however explain cannibalism among this species, and that may suggest that they may feed on other mosquito larvae too and may explain why they were the only mosquito species found in some of the positively tested Carinthian aquatic breeding sites in 2012 and 2013.

So far there is no evidence to suggest any vector role for *Cs. longiareolata* in Austria. The species is known to be ornithophagous and, therefore, a potential vector of the "avian viruses" WNV and USUV, both of which have been frequently documented in eastern Austria since 2000 (Bákonyi *et al*., 2008; Seidel, 2011; Bákonyi *et al*., 2013).

Until recently there had been little evidence of active habitat expansion of new mosquito species into Austria. However, that has now changed since an Austria-wide WNV surveillance program was implemented by the Austrian Agency for Health and Food Safety (AGES) in 2011. The presence of a large population of *Ae. japonicus* in southern Styria was immediately reported (Seidel *et al.*, 2012). The population covered the whole investigation area in the Slovenian Drava/Drau valley, and it reported a small cluster restricted to the narrow valley of river Drava/Drau probably after having crossed the Slovenian border into Carinthia in 2011. The Styrian part of the population meanwhile also colonised southern Burgenland, and a similar spread was reported into the Carinthian region. An invasion into Hungary and Italy from the Austrian territory seems probable. In 2012, two findings of *Ae. albopictus* were reported (Seidel *et al*. 2012), one from the Tyrol province (western Austria) and another from Burgenland province (southeastern Austria). During the same year the presence of *Anopheles hyrcanus* (Pallas 1771) was also reported in Burgenland (Ponçon et al., 2007; Seidel *et al.*, 2013a; Seidel *et al*., 2013b). Therefore, *Cs. longiareolata* is the fourth thermophilic or invasive culicid species to be discovered in Austria within the last three years.

Despite a routine adult trapping programme all *Cs. longiareolata* specimens were detected as larvae, except for one adult female caught with an aspirator on the surface of a rain water container. Therefore, the documentation of eggs, larvae, and pupae is important alongside the monitoring of adults to ensure that all species are surveyed. *Culiseta longiareolata* was not found in the proximity of the two capital cities of Carinthia and Styria, Klagenfurt and Graz, nor along the main traffic routes in the valleys of the rivers Mur and Drau. The massive larval occurrence at several sites in Carinthia led us to assume that *Cs. longiareolata* has actively invaded Austria some time previously and was not passively nor recently introduced along a major transportation route, as had been reported in one case for south-western Germany (Becker & Hoffmann, 2011). From the four species newly found in Austria, *Cs. longiareolata*, as well as *Ae. japonicus* and *An. hyrcanus* are already widely distributed. It is postulated that all three species have actively invaded Austria from the neighbouring countries of Italy, Hungary, or Slovenia. Only *Ae. albopictus* was documented in two separate, rather small and isolated foci which strongly suggests a new passive introduction by international traffic or by local business activities.

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