Reconfirmation of *Culiseta (Allotheobaldia) longiareolata* (Macquart 1838) (Diptera: Culicidae) in Austria. The first sequence-confirmed findings in northeastern Austria

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During a two-year mosquito monitoring program in Danubean wetlands (Lower Austria) from 2012 to 2013 the newly introduced culicid species *Culiseta (Allotheobaldia) longiareolata* (Macquart 1838) was detected. This species invaded southern Austria from Italy and Slovenia in 2012 and is now reconfirmed in an artificial water storage container in October and November 2013 in northeastern Austria (Orth an der Donau, Lower Austria) at 150 m.a.s.l., N48°8'40.68", E16°41'57.85". A total of 100 larvae, three pupae and 37 adults (14 males and 23 females) were sampled. Species were determined morphologically and genetically by barcode methods. Biometrical data such as larval head capsule widths and body lengths were measured in order to define the four larval instars for lifecycle reconstruction. This species had one generation emerging in October/November 2013. Despite intensive monitoring, development stages of *Cs. longiareolata* were exclusively found in artificial, but not in natural breeding habitats.

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Während eines zweijährigen Stechmücken-Monitorings in den Donauauen bei Niederösterreich von 2012 bis 2013 wurde die kürzlich erstmals in Österreich nachgewiesene Stechmückenart *Culiseta (Allotheobaldia) longiareolata* (Macquart 1838) entdeckt. Diese Art wanderte 2012 von Italien und Slowenien ins südliche Österreich ein und wurde im Oktober/November 2013 erstmals im Nordosten Österreichs (Orth an der Donau, Niederösterreich) bei 150 *m ü.* A, N48°8'40.68", E16°41'57.85" in einem artifiziellen Wasserbehälter bestätigt. Es wurden 100 Larven, drei Puppen und 37 Adulte (14 Männchen und 23 Weibchen) gesammelt. Die Artbestimmung erfolgte sowohl mit morphologischen als auch genetischen Techniken (Barcoding). Biometrische Daten, wie larvale Kopfkapselbreiten und Körperlängen wurden vermessen um eine Differenzierung der 4 Larvenstadien zu ermöglichen, sowie den Lebenszyklus zu rekonstruieren. Larven von *Culiseta longiareolata* wurden im Untersuchungsgebiet trotz intensiven Monitorings ausschließlich in artifiziellen und nicht in natürlichen Bruthabitaten gefunden.

Keywords: *Culiseta longiareolata*, Culicidae, species inventory, phenology, barcoding, Austria.

Introduction

Systematic large field studies on the current mosquito species inventory had been neglected over a long time period in Austria. Therefore information on the indigenous mosquito species composition is scarce. At present, the mosquito species inventory of Austria consists of 39 species belonging to six genera (*Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Culiseta* and *Uranotaenia*) according to the Fauna Aquatica Austriaca (Mohrig & Car 2002). Since then, five more species were reported in Austria: *Ochlerotatus (Ochlerotatus) nigrinus* (Eckstein 1918), *Aedes (Stegomyia) albopictus* (Skuse 1894), *Anopheles (Anopheles) hyrcanus* (Pallas 1771), *Aedes (Finlaya) japonicus japonicus* (Theobald 1901) and *Culiseta (Allotheobaldia) longiareolata* (Macquart 1838) (Seidel 2011; Seidel et al. 2012, 2013a, 2013b; Lebl et al. 2013). *Cs. longiareolata* is one of six previously reported *Culiseta* species

(Mohrig & Car 2002; Seidel et al. 2013). While *Culiseta (Culiseta) annulata* (Schrank 1776) is widely distributed throughout Europe (Becker et al. 2010), *Culiseta (Culiseta) morsitans* (Theobald 1901), *Culiseta (Culiseta) alaskaensis* (Ludlow 1906), *Culiseta* (*Culiseta*) *glaphyroptera* (Schiner 1964) and *Culiseta (Culiseta) subochrea* (Edwards 1921) are occasionally found as a result of their limited distribution areas and low population densities in Austria and bordering countries, e.g. Germany (Kampen et al. 2013), Hungary (Mihályi & Gulyás 1963) and former Czechoslovakia (Trpiš 1962) *Culiseta* mosquitoes are suspected as essential vectors in the transmission cycle of the eastern equine encephalitis and potentially the West Nile virus (Cupp et al. 2003; Molaei and Andreadis 2006; Lubelczyk et al. 2014) as well as western equine Encephalomyelitis (Muul et al. 1975) and Sindbis Virus (Melaun et al. 2014). Members of the genus *Culiseta* have also been documented as vectors of *Dirofilairia immitis*, a parasite primarily infecting domestic and wild canines as definite hosts (Huang et al. 2013). In Europe, *Cs. longiareolata* is widely spread in the Mediterranean region (Becker et al. 2010) and was reported to have invaded southern Austria (Gamlitz, Styria) from Italy and Slovenia in 2012 (Seidel et al. 2013). The larvae of this species are primarily found in any kind of artificial breeding sites, e.g. rain barrels and wells, but rarely occur in natural water bodies (Becker et al. 2010, Seidel et al. 2013). Generally, females of this species avoid ovipositing in water containers with predators present, e.g. *Notonecta glauca* (Blaustein et al. 2004). The predaceous behaviour of this mosquito species itself is barely investigated. Predation against *Culex (Culex) pipiens* (Linnaeus 1958) had been reported for the first time by Kirkpatrick (1925). It was documented that late-instar larvae of *Cs. longiareolata* prey on tadpoles (Blaustein and Margalit 1994) and fourth instar larvae on younger instars of *Culex* (Culex) *quinquefasciatus* (Say 1823) (Al-Saadi and Mohsen 1988), *Ochlerotatus (Ochlerotatus) caspius* (Pallas 1771) *Anopheles (Cellia) multicolour* (Cambouliu 1902) and *Cx. pipiens* (Shaalan 2012). However, there is a lack of information on ecological patterns and distribution of *Cs. longiareolata* in Austria.

Material and methods

Cs. longiareolata was detected during a mosquito monitoring program carried out from May 2012 to November 2013 in the National Park Donauauen, Lower Austria. Mosquito development stages were sampled at 44 breeding habitats once a week using the catch per unit effort (CPUE) method. For this, a water column defined by a 1 m^2 surface area was sampled for one minute with a handnet (20 cm in diameter, mesh size: 200 µm) at each study site (Krebs 1989). In addition, three artificial water storage containers situated in Orth an der Donau, a village bordering the danubean wetlands in Lower Austria, were sampled once a week. Sampled specimens were preserved in vials containing ethanol (75%). Adults were sampled using three BG-Sentinel traps (Biogents, Germany) in Orth an der Donau (Lower Austria). The traps, equipped with CO_2 and BG-Lure™ (Biogents) as an additional attractant, were installed once a week for a 24 hour time period. In addition, mosquitoes were sampled manually with hand-held aspirators and tubes. At the mosquito breeding sites, dissolved oxygen, pH, conductivity and water temperature were measured using a calibrated Oxi 330i (WTW), pH 315i (WTW) and a LF 196 (WTW) probe. The specimens sampled (pre-imaginal stages and imagines) were identified morphologically using the keys of Cranston et al. (1987) and Becker et al. (2010) as well as genetically by barcoding (Folmer et al. 1994) using previously described protocols (Werblow et al., 2013). Genera and subgenera abbreviations follow Reinert (2001). Biometrical data, larval head widths and body lengths were measured to define the four larval instars. The head capsule widths were measured at the widest section of the head and body lengths dorsally from the anterior end of head capsule to the end of the eighth abdominal segment. Measurements were made to the nearest 0.001 mm using a binocular microscope (Stereo Lumar.V12, Zeiss). Dyar's rule was used to ensure instar definition (Dyar 1980). Therefore we analysed the relationship between instar number and the *logarithmus naturalis* of head width. The growth ratio was determined by dividing mean head capsule widths of adjacent instars (McDonald et al. 1977).

Results and Discussion

A total of 100 larvae, three pupae and 37 adults (14 males and 23 females) of *Cs. longiareolata* were collected from October to November 2013. Morphological characteristics of larvae of *Cs. longiareolata* comprise 7–13 short teeth occupying up to 80% of the length of the siphon and a saddle which does not surround the anal segment. In addition, dense short spines at the posterior margin of the saddle were clearly visible from the second to the fourth instar (Becker et al., 2010). Females differ from other European *Culiseta* species by their distal longitudinal pale stripes on the scutum and by pale scales aggregated into conspicuous spots or stripes on the femora and tibiae (Cranston et al. 1987; Becker et al. 2010). Furthermore, the identity of *Cs. longiareolata* was confirmed by barcode methods. The Austrian samples revealed a 100% identity within the mitochondrial cytochrome c oxidase

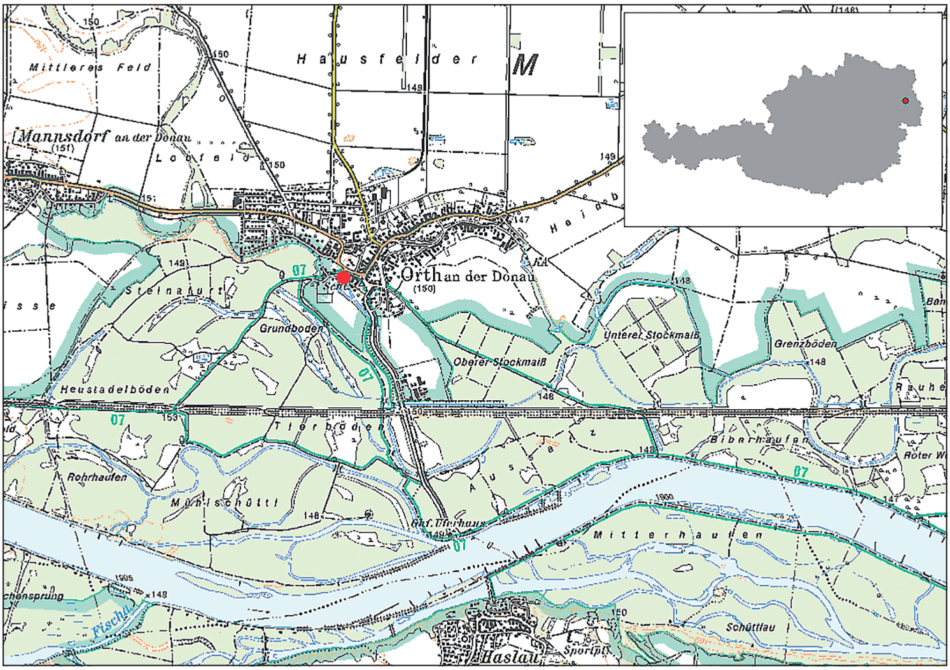

Fig. 1: Occurrence of *Culiseta longiareolata* in northeastern Austria (red dot) at the artificial water storage container in October and November 2013. – Abb. 1: Vorkommen von *Culiseta longiareolata* im nordöstlichen Österreich (rot gekennzeichnet) im artifiziellen Wasserbehälter im Oktober und November 2013.

subunit I (COI) gene to a German isolate (JQ388785) and an isolate from Kabul, Afghanistan (HG931139) and were deposited in GenBank under accession numbers KJ124849 and KJ124850. Developmental stages of *Cs. longiareolata* were exclusively detected in one artificial water storage container (150 m.a.s.l., N48°8'40.68", E16°41'57.85"; Fig. 1) and not at natural breeding sites. These findings confirm existing data which indicate that *Cs. longiareolata* mainly favours man-made and predator free artificial water containers and temporary waters with low predation risk for their offspring (Stav et al. 1999, Kiflawi et al. 2003) when compared with *Cs. annulata* which is frequently found in natural water bodies in Danubean wetlands (Zittra & Waringer, 2013).

The breeding habitat of *Cs. longiareolata* was characterized by a higher electric conductivity (1,605 μScm^{-1}) and a higher temperature (14.4 °C) than at the other artificial water containers (Tab. 1). Larvae and pupae of *Cs. longiareolata* shared their habitat with specimens of the *Culex pipiens* complex and *Cs. annulata*. The remaining artificial water storage containers were colonised by the *Culex pipiens* complex exclusively. In this study, it was not possible to detect adult specimens with the CO_2 baited BG-Sentinel traps; however, sampling with the hand-held aspirators and tubes near the breeding site was successful. Further-

Tab. 1: Habitat characteristics of the three artificial breeding habitats at Orth an der Donau (Lower Austria) measured in October/November 2013. – Tab. 1: Habitat Charakteristika der drei untersuchten artifiziellen Bruthabitate in Orth an der Donau (Niederösterreich).

Artifical water storage container	1	2	3
lenght × width × height (cm)	80 × 48.5 × 58	67 × 38 × 29	53 × 53 × 82
Water temperature (°C)	14.40	12.80	13.30
Conductivity (μScm^{-1})	1,605.00	425.00	833.00
O_2 (mgl^{-1})	10.38	9.09	5.56
O_2 (%)	88.60	92.50	60.00
pH	7.37	8.01	8.04
Culicidae species	*Cs. longiareolata* *Cs. annulata* *Cx. pipiens* complex	*Cx. pipiens* complex	*Cx. pipiens* complex

Tab. 2: Range of larval head capsule widths (mm) and growth ratios of *Culiseta longiareolata* (n=100) collected from October to November 2013 at one artificial water storage container in Orth an der Donau, Lower Austria. – Tab. 2: Größenordnung der larvalen Kopfkapselbreiten (mm) sowie Wachstumsraten von *Culiseta longiareolata* (n=100), die von Oktober bis November 2013 in Orth an der Donau (Niederösterreich) gesammelt wurden.

instar	range (mm)	±SD(mm)	growth ratio
2	0.40-0.50	0.04	
			0.54
3	0.71-1.03	0.06	
			0.65
4	1.18-1.50	0.08	

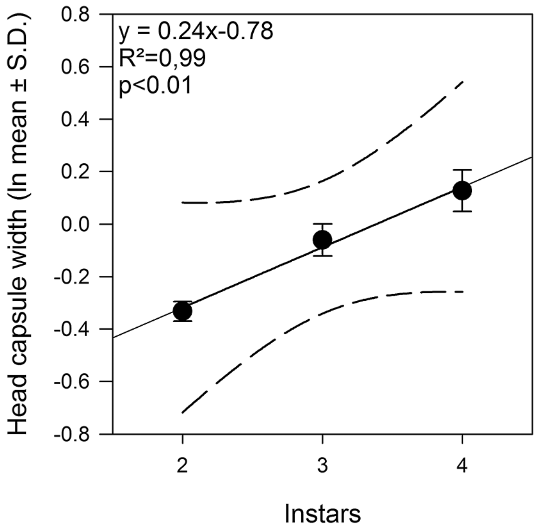

Fig. 2: Dyar's rule was applicable for instar versus ln head width regressions in *Culiseta longiareolata* (n=100). – Abb. 2: Abhängigkeit des natürlichen Logarithmus der Kopfkaspelbreite vom Entwicklunsgzustand (Larvenstadium) bei *Culiseta longiareolata* Larven. Diese Beziehung illustriert die Anwendbarkeit der Dyarischen Regel bei Gelsenlarven.

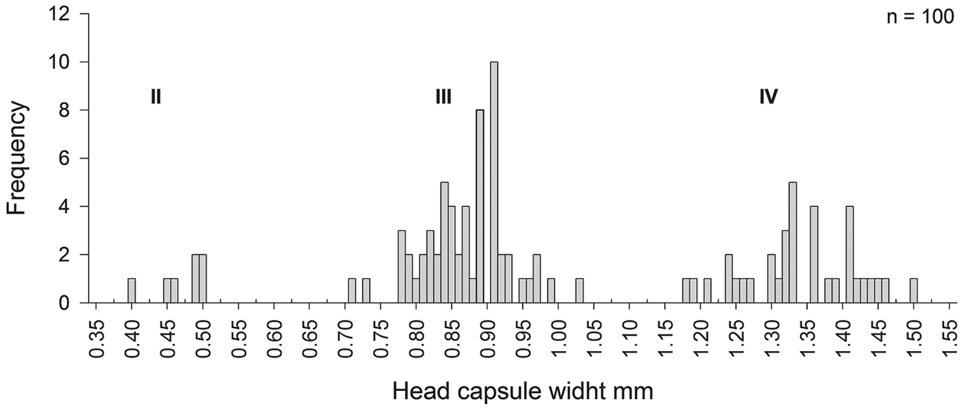

Fig. 3: Size-frequency histograms of larval head capsule width measurements of *Culiseta longiareolata* collected at Orth an der Donau, Lower Austria, in October/November 2013. Roman numerals indicate the larval instars. A total of seven larvae were second instar, 58 third instar and 35 larvae were fourth instar. – Abb. 3: Histogramm der Kopfkapselbreiten der *Culiseta longiareolata* Larven, die in Orth an der Donau, Niederösterreich im Oktober und November 2013 gesammelt wurden. Römische Zahlen bezeichnen die jeweiligen Larvalstadien. Insgesamt wurden sieben Individuen des zweiten Larvenstadiums, 58 Individuen des dritten und 35 Individuen des vierten Larvenstadiums gefangen.

more, cannibalism and predation on larvae belonging to the *Cx. pipiens* complex had been observed under laboratory conditions.

The relationship between instar number and the *logarithmus naturalis* of head width (mm) yielded a significant linear regression (Fig. 2). Size-frequency histograms for measured head capsule widths were clearly separated (Fig. 3) and did not overlap. The smallest head capsule width observed was 0.40 mm; the largest was 1.50 mm (Tab. 2). A total of seven larvae were second instar, 58 third instar and 35 larvae were fourth instar. This species is known to produce several times per year. However, within this study, only one generation emerging in October/November 2013 was observed at the artificial breeding site. After this, water suddenly froze in early December 2013. In order to fully elucidate the further distribution and establishment of this newly detected species, further long-term studies are necessary.

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References

Al-Saadi M. & Mohsen Z.H., 1988: Predatory and cannibalistic behaviour of *Culiseta longiareolata* (Macquart) (Diptera: Culicidae) in Iraq. Journal of Biological Science Research 19, 339–351.

Becker N., Pedric D., Zgomba M., Boase C., Madon M., Dahl C. & Kaiser A., 2010: Mosquitoes and their control. Springer, Heidelberg, Dordrecht, New York, 577 pp.

Blaustein L. & Margalit J., 1994: Mosquito larvae (*Culiseta longiareolata*) prey upon and compete with toad tadpoles (*Bufo viridis*). Journal of Animal Ecology. 63, 841–850.

Blaustein L., Kiflawi M., Eitam A., Mange M. & Cohen J.E., 2004: Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. Oecologia. 138, 300–305.

Cranston P.S., Ramsdale C.D., Snow K.R. & White G.B., 1987: Adults, larvae and pupae of British mosquitoes (Culicidae): A key. Scientific Publications of the Freshwater Biological Association 48, 152 pp.

Cupp E.W., Klinger K., Hassan H.K., VIguers L.M. & Unnasch T.R., 2003: Transmission of eastern equine encephalomyelitis virus in Central Alabama. American Journal of Tropical Medicine and Hygiene. 68, 495–500.

Dyar H.V., 1980: The number of molts of lepidopterous larvae. Psyche. 5, 420–422.

Folmer O., Black W., Hoeh W., Lutz R. & Vrijenhoeck R., 1994: DNA Primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3, 294–299.

Huang S., Smith D.J., Molaei G., Andreadis T.G., Larson & S.E. Lucchesi E.F., 2013: Prevalence of *Dirofilaria immitis* (Spirurida: Onchocercidae) Infection in *Aedes*, *Culex*, and *Culiseta* Mosquitoes from North San Joaquin Valley, CA. Journal of Medical Entomology. 50, 1315–1323.

Kampen H., Kronefeld M., Zielke D. & Werner D., 2013: Three rarely encountered and one new *Culiseta* species (Diptera: Culicidae) in Germany. Journal of the European Mosquito Control Association. 31: 36–39.

Kiflawi M., Blaustein L. & Mangel M., 2003: Oviposition habitat selection by the mosquito Culiseta longiareolata in response to risk of predation and conspecific larval density. Ecological Entomology. 28, 168–173.

Kirkpatrick T.W., 1925: The Mosquitoes of Egypt- Government Press. Cairo, Egypt 224 pp.

Krebs C.J., 1989: Ecological Methodology. Harper Collins Publishers, 654 pp.

Lebl K., Nichler E.M., Walter M., Brugger K. & Rubel F., 2013: First record of the disease vector *Anopheles hyrcanus* in Austria. Journal of the American Mosquito Control Association. 29, 59–60.

Lubelczyk, C., Elias L.K., Albert J., Hansen S., Saxton-Shaw K., Macmillan K., Smith L.B., Eisen R., Swope B., Smith R.P. & Mutebi J.-P., 2014: Detection of Eastern Equine Encephalitis Virus Antibodies in Moose (*Alces Americana*), Maine, 2010. Vector Borne and Zoonotic Diseases. 14, 77–81.

McDonald G., Smith I.R. & Sheldon. G.P., 1977: Identification of instars of *Culex annulirostris* Skuse (Diptera: Culicidae).Journal of the Australian Entomological Society. 16, 359–360.

Melaun C., Werblow A., Busch M.W., Liston A. & Klimpel S., 2014: Bats as potential reservoir hosts for vector-borne diseases. In: Bats (Chiroptera) as vectors of diseases and parasites (eds Klimpel S., Mehlhorn H.). Springer, Parasitology Research Monographs. 5, 25–61.

Mihályi F. & Gulyás M., 1963: Mosquitoes of Hungary. Akademia Kiádo, Budapest. 229 pp

Mohrig W. & Car M., 2002 : Diptera: Culicidae.- Teil III, in Moog O. (Ed) (2002): Fauna Aquatica Austriaca, Lieferung 2002.- Wasserwirtschaftkataster, Bundesministerium für Land und Forstwirtschaft, Umwelt- und Wasserwirtschaft, pp. 1–9.

Muul I., Johnson B.K. & Harrison B.A., 1975: Ecological studies of *Culiseta melanura* (Diptera: Culicidae) in relation to eastern and western equine encephalomyelitis viruses on the eastern shore of Maryland. Journal of Medical Entomology. 11, 739–748.

Reinert J.F., 2001: Revist list of abbreviations for genera and subgenera of Culicidae (Diptera) and the notes on generic and subgeneric changes. Journal of the American Mosquito Control Association 17, 51–55.

Seidel B., 2011: Erstnachweis der Gelsenart *Aedes* (*Ochlerotatus*) *nigrinus* (Eckstein, 1918) (Diptera, Culicidae) in Österreich Wissenschaftliche Mitteilungen aus dem Niederösterreichischen Landesmuseum. 21, 407–414.

Seidel B., Duh D., Nowotny, N. & Allerberger F., 2012: Erstnachweis der Stechmücken *Aedes (Ochlerotatus) japonicus japonicus* (Theobald, 1901) in Österreich und Slowenien in 2011 und für *Aedes (Stegomyia) albopictus* (Skuse, 1895) in Österreich 2012 (Diptera: Culicidae). Entomologische Zeitschrift. 122, 223–226.

Seidel B., Nowotny N., Duh D., Indra A., Hufnagl P. & Allerberger F., 2013a: First records of the thermophilic mosquito *Culiseta longiareolata* (Macquart 1838) in Austria, 2012 and in Slovenia, 2013). Journal of the European Mosquito Control Association. 31, 17–20.

Seidel B., Hufnagl P., Indra A. & Allerberger F. (2013b) First record of *Anopheles hyrcanus* (Pallas, 1771) for Austria (Diptera: Culicidae). Entomologische Zeitschrift. 125, 39–41.

Shaalan E.A., (2012): Predation Capacity of *Culiseta longiareolata* Mosquito Larvae against Some Mosquito Species Larvae. Journal of Entomology. 9, 183–186.

Stav G., Blaustein L. & Margalith J., 1999: Experimental evidence for predation risk sensitive oviposition by a mosquito, Culiseta longiareolata. Ecological Entomology. 24, 202–207.

Trpiš M., 1962: First record of *Theobaldia (A.) longiareolata* Macq. 1838 (Diptera, Culicidae) in Czechoslovakia. Biológia. 17, 213–215.

Werblow A., Bolius S., Dorresteijn A.W.C., Melaun C. & Klimpel S., 2013: Diversity of *Culex torrentium* Martini, 1925 – a potential vector of arboviruses and filarial in Europe. Parasitology Research. 112, 2495–2501.

Zittra C. & Waringer J., 2013: Species inventory, ecology and seasonal distribution patterns of Culicidae (Insecta: Diptera) in the National Park Donau-Auen. 5th Symposium Conference Volume for Research in Protected Areas, Mittersill, Austria, p. 837–842.

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