

Ecological preferences of the putative West Nile virus vector *Uranotaenia unguiculata* mosquito with description of an original larval habitat

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Abstract. The mosquito species *Uranotaenia unguiculata* has recently been described as a potential vector for a novel genetic lineage of West Nile virus (WNV), but our knowledge about the distribution and ecology of this mosquito is still rudimentary. Here, we summarize the literature regarding the ecology and behavior of mosquito *Ur. unguiculata*, and we provided novel data on the species breeding habit by DNA barcoding mosquito larvae from a trash deposit. Based on literature survey, *Ur. unguiculata* is a widespread species, reported from 25 different countries throughout the Mediterranean region, including Europe, southwest Asia and the Middle East. The immature mosquitoes occur mainly in floodplain forests, marshes, as well as in human-related habitats like suburban or rural areas around cities. The favoured larval breeding sites of *Ur. unguiculata* is similar to other mosquito species, namely ground waters with vegetation, mainly in shaded localities, however, its breeding in containers in a trash deposit is reported here for the first time. *Ur. unguiculata* females feed mainly on amphibians and reptiles, less often on birds and mammals such as domestic animals, and on very rare occasions they bite humans as well. Since several WNV strains have been described in *Ur. unguiculata* wild caught mosquitoes from multiple geographic locations, its role in the enzootic cycle of West Nile virus is highly presumable. Revealing the ecological background of *U. unguiculata* can possibly assist the development of laboratory strains, which could facilitate further studies, such as vector competence experiments.

Key words: summary, *Culicidae*, host preferences, suburban habitat, breeding habit.

Introduction

Mosquitoes transmit several important pathogens such as viruses (West Nile virus, Dengue virus, Chikungunya virus, etc.), parasites (*Dirofilaria spp.*) or even bacteria (*Francisella spp.*) (Hubálek 2008, Lundström et al. 2011, Kemenesi et al. 2015). Most publications dealing with mosquito-borne diseases in Central Europe focus on detection and/or characterization of different pathogens from mosquitoes, and only few studies have investigated changes in composition of mosquito faunas, the distribution of mosquitoes or the characteristics of their larval habitats in general (e.g. Beier et al. 1987, Sulesco et al. 2013). However, these data could provide important information for mosquito-borne disease control strategies.

Although mosquito species that were previously described as important viral vectors have been well characterised (e.g. Becker et al. 2010), there are some less-known species like *Uranotaenia unguiculata* (Edwards 1913) which might also play an important role in the lifecycle of different viruses. *Ur. unguiculata* species has already been de-

scribed as a potential vector for West Nile virus (WNV) by hosting different genetic lineages of WNV (Kemenesi et al. 2014, Pachler et al. 2014, Dinu et al. 2015), but our knowledge about the distribution and ecology of this mosquito is still rudimentary (Becker et al. 2010).

The goal of the current study is to summarize and evaluate the literature regarding the ecology of mosquito *Ur. unguiculata*. Additionally, novel data is also provided by reporting a previously undescribed larval habitat of the species in pond in urban related trash deposits.

Material and methods

Mosquito collection

Mosquito larval samples were collected within the framework of a monitoring programme supporting a mosquito control programme in southwest Hungary (Baranya county) in 2015. During the mosquito breeding season (from May to September) marshlands in the suburban area of the city Pécs were visited, and larvae from potential breeding sites, e.g. small, stagnant pools in shaded areas, were surveyed to determine characteristic

species. Larvae collection was conducted with Pasteur-pipettes directly from the water. Samples were transported to laboratory and sorted individually. Sample homogenization and nucleic acid preparation were fulfilled as described before (Szentpáli-Gavallér et al. 2014). For mosquito species identification, DNA barcoding was implemented following a previously published protocol (Kumar et al. 2007). PCR amplicons were directly sequenced bidirectionally (BigDye Terminator v1.1 Cycle Sequencing Kit) in ABI Prism 310 DNA Sequencer instrument (Applied Biosystems). Barcoding sequences were deposited in GenBank database under accession numbers: KU308382; KU308383; KU308384.

Literature search strategy

Systematic literature survey was carried out using the Web of Science and PubMed databases collecting published reports on mosquito *Ur. unguiculata*, until 5 December 2015. An initial list of potential references was established with database searches for topic keyword: "*Uranotaenia unguiculata*", which yielded a total of 25 publications. We manually searched through the title and abstracts of each article, then through the full text of each potentially relevant article to decide whether the article matched our selection criteria. Additionally, references cited in these articles and relevant books were also checked.

Results and Discussion

Based on our literature survey there is very little information about the ecology of *Ur. unguiculata*. Even so altogether, we found 30 observations, which are summarized here. Most papers reported only faunistic data of single regions or first occurrences of the species. Based on the processed data, *Ur. unguiculata* is a frequent species throughout the Mediterranean region. In Europe, the species has been detected in Germany (Becker & Kaiser 1995, Becker et al. 2010), Austria (Pachler et al. 2014), Hungary (Tóth 2004, Tóth 2011, Kemenesi et al. 2014), Czech Republic (Ryba et al. 1974, Sebesta et al. 2010), as well as in France (Harant et al. 1952, Doby 1955, Mouchet & Rageau 1965), Italy (Ascoli et al. 2006), Cyprus (Violaris et al. 2009), Greece (Lytra & Emmanouel 2014), Albania and Macedonia (Schaffner et al. 2001). In Eastern Europe, *Ur. unguiculata* can be found in Moldova (Sulesco et al. 2013), and at Danube delta in Romania (Velehorschki et al. 1990, Dinu et al. 2015). Further occurrence is described in southwest Asia, at Volga delta and Volgograd in Russia (Shopenskaya et al. 2008), Azerbaijan (Bagirov et al. 1997), in the Middle East, e.g. Jordan (Amr et al. 1997), Israel (Braverman et al. 1991) to Iran and Pakistan (Azari-Hamidian et al. 2009, Moosa & Kazemi

2009) as well in North Africa, such as Morocco, Algeria, Tunisia, Libya and Egypt (Vermeil 1953, Abdel-Malek 1956, Beier et al. 1986, 1987, Brunhes et al. 2000). Based on previously published checklists on European distribution of *Ur. unguiculata*, further occurrences were recorded from Portugal, Spain, Slovakia, Ukraine, Croatia, Serbia, Bulgaria and Turkey (Ramsdale & Snow 2001, Schaffner et al. 2001). For detailed occurrence locations see the map on Figure 1.

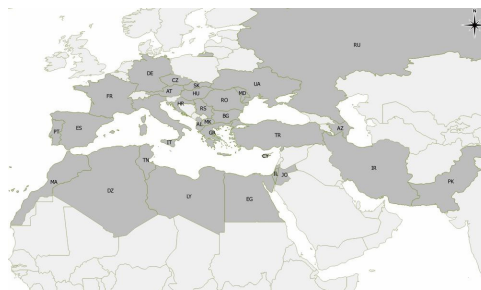


Figure 1. Distribution map of mosquito *Ur. unguiculata*. The two-letter country codes (ISO a2) show occurrence locations of the species, for source data see Table 1.

Interestingly, only a few papers contain information on the breeding biology of *Ur. unguiculata*, namely breeding habitats or sites where not only adult specimens but also larval stage was found. The species usually occurs in floodplain forests (Sebesta et al. 2010, Sulesco et al. 2013, Kemenesi et al. 2014); nonetheless, it was described from a farmed rice field as well (Lytra & Emmanouel 2014). The favoured larval breeding sites of this species are pools, ditches, or canals with stagnant or slow-flowing water and a rich growth of aquatic vegetation (Becker et al. 2010). They are also common in shallow shores and in coastal vegetation (e.g. reeds) of lakes (Ryba et al. 1974, Becker et al. 2010, Sulesco et al. 2013), mainly in shaded localities. The larvae prefer freshwater and are only occasionally found in water with a slight salinity (Rioux et al. 1975, Becker et al. 2010). Although larvae were found in animal sheds (Beier et al. 1986) and in water accumulated in hoof-prints (Sulesco et al. 2013), they have not been described from urban-related breeding sites so far. Urban-related breeding sites, such as containers in garbage deposits were already described as potential or even attractive breeding sites for several mosquito species, such as the common *Culex pipiens* and the invasive species *Aedes albopictus* and *Ae. japonicus* (Simard et al. 2005, Becker et al.

2010, Medlock et al. 2015). These artificial containers can provide sufficient breeding sites even during arid climatic periods. Here we describe for the first time artificial containers as breeding site for *Ur. unguiculata* (Table 1.). There are three factors contributing to our suggestion that *Ur. unguiculata* may be considered a public health risk. First, the mosquito species was described to feed on humans (Bagirov et al. 1997, Tóth 2011). Second, the currently discussed breeding habit may introduce the species to urban areas. Third, *Ur. unguiculata* was multiple times identified as a potential WNV vector (Kemenesi et al. 2014, Pachler et al. 2014).

Larvae of *Ur. unguiculata* can be found from May to early October with a peak in August (Becker et al. 2010), which is also supported by our observations. They often occur together with larvae of *Cx. pipiens*, *Culex modestus* (Ryba et al. 1974, Beier et al. 1986, Amr et al. 1997, Becker et al. 2010, Sebesta et al. 2010, Dinu et al. 2015), *Culex theileri* (Becker et al. 2010), *Anopheles claviger* (Ryba et al. 1974), *Anopheles hyrcanus* and *Anopheles sacharovi* (Becker et al. 2010, Sebesta et al. 2010). Our results also support these previous findings, since the barcoded larvae species were *Ur. unguiculata*, *Cx. pipiens* and *An. claviger* (GenBank: KU308382; KU308383; KU308384). Furthermore, in the Middle East they were observed together with larvae of *Culex perexiguus*, *Culex judaicus*, *Culex univittatus*, *Culex antennatus*, *Culiseta longiareolata*, *Anopheles sergentii*, *Anopheles superpictus* and *Aedes caspius* (Beier et al. 1986, Amr et al. 1997).

The adults of *Ur. unguiculata* are most abundant in late summer (Ascoli et al. 2006, Azari-Hamidian et al. 2009), and were found repeatedly in the cellar of a school-building from autumn to spring, also with overwintering females (Tóth 2004), which may lead to the presumption that the species hibernates and overwinters as an adult stage (Rudolf et al. 2015). We have very few information not only on breeding biology of the species, but also about feeding preferences. It seems likely that females of *Ur. unguiculata* do feed on blood, the species is strictly an amphibian-reptile feeder (Beier et al. 1986, 1987, Dinu et al. 2015), but is prone to bite birds as well (Petrishcheva 1962, Ryba et al. 1974). Generally, it is thought that these mosquitoes never bite humans or domestic animals even though they may be capable of doing so, since it was observed feeding on horses (Braverman et al. 1991). However, Tóth (2011) was able to catch only one single female specimen of *Ur. unguiculata*, using human landing collection

method in South-western part of Hungary (Mecsek Hills). This is the simplest method of collecting host-seeking mosquito females, it can be performed for estimating landing rates and it is the most direct and rapid way for estimating a nuisance, hereby procure important information about which species are involved in human biting nuisance (ECDC 2012). Furthermore, Bagirov et al. (1997) have demonstrated the attractiveness of *Ur. unguiculata* to humans and found daily changes in the activity of these mosquitoes, with maximums in the evening and morning, and minimums at night and during daytime. Data regarding ecological preferences discussed in the manuscript along with the year of information are summarized in Table 1.

Based on blood meal identification of other species within the genus, *Uranotaenia* are known to feed on cold-blooded animals, especially amphibians and reptiles, but not on warm-blooded animals (Toma et al. 2014). However, Molaei et al. (2008) found that *Uranotaenia sapphirina* feed predominantly upon mammalian hosts such as the white-tailed deer (*Odocoileus virginianus*) and humans. However, findings should be interpreted cautiously, where only a limited number of specimens were examined, and further blood-meal analyses are needed to prove these presumptions. Further long-term investigations are needed to resolve this contradiction regarding feeding preferences of the species.

Disease transmission role of *Ur. unguiculata* had been barely discussed before. Genetically distinct lineages of WNV were described from different geographic regions in *Ur. unguiculata* mosquito samples. Lineage 4 of WNV was detected in Russia, Volgograd region (Shopenskaya et al. 2008) as well as in Romania, Danube delta region (Dinu et al. 2015). Similar strains have already been reported by Russian authors from Volga Delta (Lvov et al. 2004). Furthermore, a novel genetic variant of WNV (WNV lineage 9) has been recently described from Hungarian *Ur. unguiculata* samples in the Southern Transdanubian region (Kemenesi et al. 2014). A closely related WNV strain was also detected collaterally in Austria, and was named WNV lineage 4c (Pachler et al. 2014). It is not clear whether the natural geographic segregation of different WNV genetic variants can explain the above mentioned geographic distribution between different WNV lineages or can be presumably connected to different vector competence capabilities of different *Ur. unguiculata* populations. Further

Table 1. List of references involved in the synthesis. Country and letters in parentheses after the country mean ISO a2 country codes indicate occurrence locations of *Ur. unguiculata* on the map (see Figure 1).

Reference	Country (ISO a2)	Habitat	Larval/Associated species Imago	WNV genetic lineage detected	Host
Abdel-Malek 1956	Egypt (EG)				
Amr et al. 1997	Jordan (JO)		L Cx: <i>perexiguus</i> , Cx: <i>pipiens</i> Cx: <i>perexiguus</i> , Cx: <i>pipiens</i> , Cx: <i>judaiticus</i> , An: <i>sergentii</i> , An: <i>superpictus</i>		
Ascoli et al. 2006	Italy (IT)		I		
Azari-Hamidian et al. 2009	Iran (IR) Pakistan (PK)		L/I		
Bagirov et al. 1997	Azerbaijan (AZ)		I		human
Becker et al. 2010	Germany (DE)	pools, ditches, canals	L/I	An: <i>lyrcanus</i> , An: <i>sacharovi</i> , Cx: <i>pipiens</i> , Cx: <i>modestus</i> , Cx: <i>theileri</i>	amphibian
Beier et al. 1986	Egypt (EG)	inside houses, animal sheeds	L	Cx: <i>pipiens</i> , Cx: <i>univittatus</i> , Cx: <i>antennatus</i> , Ae: <i>caspius</i> , Cs: <i>longiarcolata</i>	amphibian, reptile
Beier et al. 1987	Egypt (EG)				
Braverman et al. 1991	Israel (IL)		I		horse
Brunhes et al. 2000	Morocco (MA) Algeria (DZ) Tunisia (TN) Libya (LY) Egypt (EG)				
Dinu et al. 2015	Romania (RO)		I	Cx: <i>pipiens</i> , Cx: <i>modestus</i>	WNV lineage 4 amphibian
Doby 1955	France (FR)				
Harant et al. 1952	France (FR)				
Kemenesi et al. 2014	Hungary (HU)		I		WNV lineage 9
Khoshdel-Nezamihia et al. 2014	Iran (IR)		L		
Lvov et al. 2004	Russia (RU)				WNV lineage 4
Lytra and Eimannouel 2014	Greece (GR)	farmed rice field	I		
Moosa and Kazemi 2009	Iran (IR) Pakistan (PK)		L		
Mouchet and Rageau 1965	France (FR)				
Pachler et al. 2014	Austria (AT)		I		WNV lineage 9
Petrishcheva 1962	Russia (RU)				bird
Ramsdale and Snow 2001	Portugal (PT) Spain (ES) Slovakia (SK) Croatia (HR)				

Reference	Country (ISO a2)	Habitat	Larval/Associated species Imago	WNV genetic lineage detected	Host
Rudolf et al. 2015	Serbia (RS) Ukraine (UA) Bulgaria (BG) Turkey (TR) Czech Republic (CZ) Austria (AT) Hungary (HU)		I		
Ryba et al. 1974	Czech Republic (CZ)	reed	I	<i>Cx. pipiens, Cx. modestus, An. claviger</i>	bird
Schaffner et al. 2001	Albania (AL) Austria (AT) Bulgaria (BG) Croatia (HR) France (FR) Germany (DE) Greece (GR) Hungary (HU) Italy (IT) Macedonia (MK) Portugal (PT) Serbia (RS) Slovakia (SK) Spain (ES) Romania (RO)				
Sebesta et al. 2010	Czech Republic (CZ)	floodplain forest	I	<i>Cx. pipiens, An. hyrcanus</i>	pigeon
Shpenskaya et al. 2008	Russia (RU)				
Sulesco et al. 2013	Moldova (MD)	hoofprints, coastal vegetation of a lake, floodplain	L/I		
Tóth 2004	Hungary (HU)	coastal vegetation of a lake	L/I		amphibian, reptile
Tóth 2011	Hungary (HU)			<i>An. messeae, Cx. territans, Cx. torrentium, Oc. dorsalis, Oc. sticticus</i>	human
Velehorskí et al. 1990	Romania (RO)				
Vermeil 1953	Tunisia (TN)				
Violaris et al. 2009	Cyprus (CY)				
Present study	Hungary (HU)	trash deposit	L	<i>Cx. pipiens, Cx. modestus, An. claviger</i>	

studies regarding the enzootic cycle of these locally circulating WNV strains are necessary to better understand the exact role of this particular species in natural WNV transmission. The significance of the species as a potential WNV vector can not be underestimated, although vector competence studies related to *Ur. unguiculata* are not available to date.

In summary, *Uranotaenia unguiculata* is the only species within the genus *Uranotaenia* occurring in temperate climate regions, although it is a widespread species, with reports from 31 different countries throughout the Mediterranean region, including Europe, southwest Asia and the Middle East. The immature stages of *U. unguiculata* utilise a range of habitats, mainly occurring in floodplain forests and marshes, as well as in human-related habitats, i.e. farmlands, domestic animal farms or deserted suburban and rural areas around cities. The favoured larval breeding sites of *Ur. unguiculata* are similar to other mosquito species, namely ground waters with vegetation, including swamps, ditches and temporary pools, mainly in shaded localities. The occurrence of the species in containers in trash deposits is reported here for the first time, which, in the light of recent publications about the potential role of the species as WNV vector (Kemenesi et al. 2014, Pachler et al. 2014), can suggest the species to be more relevant for human or veterinary health than previously estimated. The feeding preference of *Ur. unguiculata* is rarely discussed and available data indicate that mainly amphibians and reptiles, less often birds and mammals, serve as hosts. Furthermore, there is a debate on human biting of this species, so further blood meal analyses are needed to clarify this issue. Based on these limited data, long-term conclusions can not be drawn, and further studies are needed regarding the ecology of this species. The role of *Ur. unguiculata* as a disease vector is assumable, since several WNV strains have been described from different geographic regions. Revealing its ecological background can possibly assist the development of laboratory strains, which could facilitate further studies, such as vector competence experiments.

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