

Blood-feeding Diptera (Culicidae and Ceratopogonidae) in an urban park of the city of Vitoria-Gasteiz (Basque Country, Spain)

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Abstract: Haematophagous and synanthropic Diptera are responsible for the transmission of several pathogenic agents to humans, domestic and wild animals, causing diverse vector-borne diseases of paramount importance. The presence of these blood-feeding insects is well reported in many areas of the world; however, the distribution in anthropogenic zones has not been studied so far across Spain. In order to gather information on this entomofauna in urban environments, adult stages were collected using an automatic entomological aspirator during the summer season in street lamps of a public park located in the city of Vitoria-Gasteiz (Basque Country, Spain). A total of 333 culicids from four species were collected in a two-year sampling period: *Culex pipiens* (91.3%), *Culiseta longiareolata* (8.1%), *Culiseta litorea* (0.3%) and *Culex hortensis hortensis* (0.3%). Moreover, 225 *Culicoides* specimens belonging to six different species were trapped: *Culicoides punctatus* (61.3%), *Culicoides obsoletus* s.s. (16.8%), *Culicoides dunningtoni* (13.3%), *Culicoides kibunensis* (6.2%), *Culicoides dewulfi* (1.7%) and *Culicoides clastrieri* (0.4%). The first data of *Culicoides* species distribution in urban areas and the first record of *C. litorea* are recorded for the Basque Country region. *Journal of the European Mosquito Control Association* 33: 10-14, 2015

Keywords: *Culicidae*, *Culicoides*, mosquitoes, biting midges, urban environment, Basque Country, Spain.

Introduction

Mosquitoes (Diptera, Culicidae) are involved in the transmission of pathogens that cause serious diseases in humans (e.g. malaria, yellow fever, dengue, chikungunya, among others) and animals to a lesser extent (Spielman & D'Antonio, 2001). Culicids are commonly found in a wide range of habitats distributed all over the world except in a few islands and Antarctica (Rueda, 2008). There are some species, such as those included in the *Culex pipiens* complex (*Cx. pipiens* Linnaeus, 1758, *Cx. quinquefasciatus* Say, 1823, and/or hybrids), *Aedes aegypti* (Linnaeus, 1762) and the Asian tiger mosquito *Aedes albopictus* (Skuse, 1894), which show a strong association with anthropogenic areas and a great ability for breeding in urban environments (Robinson 2005). Although adjacent provinces surrounding the Basque Autonomous Community (Northern Spain) have been monitored for the detection of culicids (Alarcón-Elbal *et al.*, 2012, Bueno Marí 2012), this region is still poorly studied in the field. However, there has been a recent interest in this area due to the high risk of colonisation of the invasive species *Ae. albopictus*, which has been recently reported in the northeast side of the Basque Country (Delacour *et al.*, 2015). The family Culicidae is represented by 64 species in Spain, whereas in the Basque Country region only twelve have been recorded (Cirujano *et al.*, 2003; de Castro, 2004; Bueno Marí *et al.*, 2012; Delacour *et al.*, 2015).

Moreover, the fearsome reputation of biting midges (Diptera, Ceratopogonidae) is due to their role as vectors of internationally important arbovirus of livestock (Mellor *et al.*,

2000), but *Culicoides* midges have only rarely been implicated as human disease vectors except as the primary agents of two tropical diseases to or between humans (Carpenter *et al.*, 2013). In particular, only three genera feed on warm-blooded vertebrates (*Leptoconops*, *Forcipomyia* subgenus *Lasiohelea* and *Culicoides*). The latter is the most widespread and abundant both in numbers and species (Kettle 1962). Furthermore, *Culicoides* midges can be found preferably associated with livestock on farms and small holdings as well as in wildlife environments in low numbers (González *et al.*, 2013a). *Culicoides* biting midges became the focus of attention in Spain due to the African horse sickness (1966, 1987-1990) (Rodríguez *et al.* 1992) and Bluetongue disease (1956-1969, 2000-onwards) (Pérez de Diego *et al.*, 2014). The genus *Culicoides* represents 81 species in Spain (Alarcón-Elbal & Lucientes 2012) and 52 of them have so far been reported associated with livestock and wild environments in the Basque Country region (González *et al.*, 2013a). However, there are scarce records for urban *Culicoides* species and a lack of knowledge about the ability of biting midges to inhabit metropolitan environments.

Exploratory studies of the knowledge, prevention and control of the concerning mosquitoes and other biting midges are historic and infrequent. It is especially important to know whether vector or nuisance species are present in urban areas and their abundance, particularly with reference to the most relevant vectors. For this reason current work was conducted to establish the species composition of haematophagous Diptera (*Culicidae* and *Culicoides* spp.) in an urban park.

Materials and Methods

Vitoria-Gasteiz (42° 51' 0" N, 2° 40' 12" W; 525 m above sea level) is a city located in the province of Álava (Basque Country, Northern Spain) (Figs. 1A, 1B) with a population of 242,082 inhabitants. Álava is an inland territory and features a largely transitional climate between the humid, Atlantic neighboring Northern provinces and the dry and warmer lands south of the Ebro River basin.

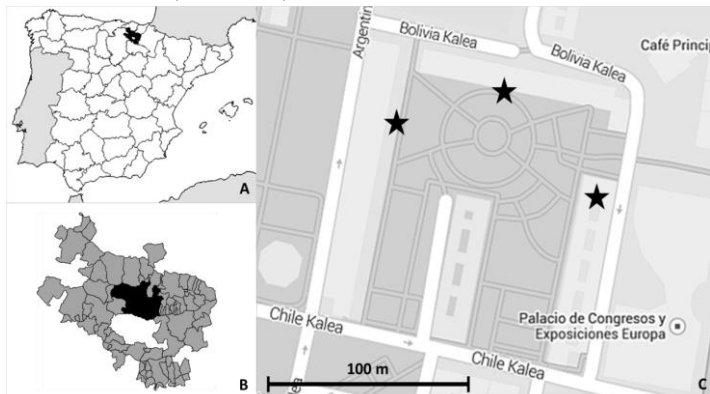


Figure 1: (A) Map of Spain indicating the province of Álava. (B) Map of Álava pointing out the city of Vitoria-Gasteiz. (C) Sampling area, where black stars correspond to the three points of capture within the park.

Up until the mid-twentieth century, agriculture and farming shaped the landscape of the territory, but the trend shifted gradually during the 1960s and 1970s on the grounds of growing industry. The climate is characterised by cold, wet winters and fairly cool and limited rainfall in summers, strongly influenced by the surrounding orography. The sampling area is a public park (210 x 140 m) surrounded by blocks of flats (Fig. 1C). The park is structured in different green areas with a wide variety of trees, bushes and lawns with patches of flowers. It also comprises a recreational area with walking trails, frequented by walkers with their domestic pets. Wild fauna includes mainly birds such as pigeons (*Columba livia*), blackbirds (*Turdus merula*) and other small Passeriformes.

Blood-sucking dipterans were sampled weekly in three equidistant lamps placed under roofs (Fig. 1C) during July, August and the first two weeks of September. A total of 60 collections were performed (three sampling sites for 10 nights) during 2013-2014. Sampling nights were selected in advance according to nocturnal temperature forecast of Euskalmet (Basque Agency of Meteorology). All the insects on lights and around the wall were aspirated with an automatic entomological aspirator (Hausherr's Machine Works, Inc. 186 Old Freehold Road Toms River, USA) for 10 minutes.

Culicoides midges and culicids were killed by freezing. *Culicoides* and males of culicids were preserved in a mixture of pure ethanol (75%) and water (25%), whereas female mosquitoes were stored dry to avoid damaging morphological features. Taxonomical identification was made following appropriate keys for culicids (Schaffner *et al.*, 2001) and *Culicoides* (González & Goldarazena, 2011). Both culicids and *Culicoides* females were categorised based on their abdominal conditions as unfed, blood-fed and gravid. Doubtful specimens of *Culicoides* and all male mosquitoes were mounted in slides with Hoyer's medium to be examined under a Leica DM4500B microscope.

Results

A total of 558 blood-fed Diptera (333 *Culicidae* and 225 *Culicoides* spp.) from ten species were collected (Tables 1 and 2). Within *Culicidae*, the most abundant species was *Cx. pipiens*, which accounted for 91.3% of the total collections, followed by *Culiseta longiareolata* (Macquart, 1838) (8.1%), *Culiseta litorea* (Shute, 1928) (0.3%) and *Culex hortensis hortensis* Ficalbi, 1889 (0.3%). *Culiseta litorea* is a new record for the Basque Country region. Males represented 12.0% of the captured specimens (Table 1). Regarding the genus *Culicoides*, six different species were identified. *Culicoides punctatus* (Meigen, 1804) was the most common species (61.3%), followed by *Culicoides obsoletus* s.s. (Meigen, 1818) (16.8%), *Culicoides dunningtoni* Kettle & Lawson, 1955 (13.3%), *Culicoides kibunensis* Tokunaga, 1937 (6.2%), *Culicoides dewulfi* Goetghebuer, 1936 (1.7%) and *Culicoides clastrieri* Callot, Kremer & Deduit, 1962 (0.4%). Males represented 18.2% of the collections (Table 2).

Table 1: Culicid collections between 2013-2014 in a public park of Vitoria-Gasteiz. (F= Females; M = Males).

Culicidae species	2013		2014		Total
	F	M	F	M	
<i>Culex pipiens</i>	156	22	112	14	304
<i>Culiseta longiareolata</i>	2	1	23	1	27
<i>Culiseta litorea</i>	0	1	0	0	1
<i>Culex hortensis hortensis</i>	0	0	0	1	1
	158	24	135	16	
Total		182	151		333

Table 2: *Culicoides* collections between 2013-2014 in a public park of Vitoria-Gasteiz. (F= Females; M = Males).

<i>Culicoides</i> species	2013		2014		Total
	F	M	F	M	
<i>Culicoides punctatus</i>	86	18	28	6	138
<i>Culicoides obsoletus</i>	15	3	18	2	38
<i>Culicoides dunningtoni</i>	14	1	12	3	30
<i>Culicoides kibunensis</i>	1	0	8	5	14
<i>Culicoides dewulfi</i>	0	0	2	2	4
<i>Culicoides clastrieri</i>	0	1	0	0	1
	116	23	68	18	
Total		139	86		225

The physiological (gonotrophic) status examination revealed that female culicids included 66.1% unfed, 11.2% gravid and 23.7% blood-fed, whereas *Culicoides* midges 92.4% were unfed, 7.1% gravid and 0.5% blood-fed. Other non-biting midges of the family Ceratopogonidae were also very common, such as genera *Forcipomyia*, *Dasyhelea*, *Atrichopogon* and to lesser extent members of tribe Palpomyiini. No representatives of other blood-feeding dipterans were found in the study area.

Discussion and conclusion

This study shows the presence of diverse species of blood-feeding dipterans in an urban environment and represents the first sampling trial conducted in an urban area in the Basque Country. With regards to *Culicoides*, the number of collected specimens was low in comparison with other surveys conducted in non-urban locations in the Basque Country (González, 2014). This might be attributable to: a) collections were performed in an area full of different types of urban light sources (light pollution disperse the population of mosquitoes and therefore reduce the number of specimens caught in each trap), b) no species-specific suction traps, lures or appropriate light sources designed to collect these groups were used (Ultraviolet light source is desired for *Culicoides* trapping and specific lures in combination with white light are required to increase collection of culicids), c) hypothetically there are no suitable breeding sites for both groups in the sampling area (culicids need a minimum layer of standing water to breed and *Culicoides* midges prefer diverse organic substrates with high humidity as breeding sites), d) animal host availability is low in comparison to studies performed in farms with permanent livestock.

In view of the diversity found in the survey, authors contemplate various critical questions: 1) What type of breeding sites are being used? 2) What are their main host preferences in urban areas? 3) What implications could entail the existence of potential vectors in the area to the human welfare?

1) It is well-known that sewers, gutters, small containers and irrigation heads could be the main sources of emerging sites of the dominant species *Cx. pipiens*, observations in accordance with Bueno Marí (2010). This important vector can be found in a fairly wide range of larval habitats but is generally associated with water that has a high organic content including natural environments (Alarcón-Elbal *et al.*, 2013a; Alarcón-Elbal *et al.*, 2013b). Both *Cx. pipiens* and *Cs. longiareolata* are the most abundant and well-distributed species in many regions of Spain (Bueno Marí *et al.*, 2012). In fact, larvae of *Cx. pipiens*, *Cx. hortensis hortensis* and *Cs. longiareolata* are usually found cohabiting in river banks, irrigation ditches and troughs for livestock (Encinas-Grandes, 1982). The species *Cs. longiareolata*, *Cs. litorea* and *Cx. hortensis hortensis* occupy a wide variety of habitats, however, the former two species are more commonly found in artificial devices (Chordá Olmos, 2014).

In contrast, *Culicoides* species do not require permanent water-source availability for their development, and they may breed in a wide range of different types of substrates dispersed over the ground (eg. rotting fallen leaves, organic matter, faeces, of humid soil of former puddles, etc.). Due to their plasticity in using different types of microhabitats (González *et al.*, 2013b, Harrup *et al.*, 2013) as breeding sites, any kind of soil is valid if it provides enough moisture and organic matter to allow the development of the larvae (Kettle, 1962).

2) *Culex pipiens* shows a plastic behaviour capable of feeding both humans and birds. The species is recorded as a very common mosquito in urban areas of Spain (Bueno Marí, 2010) showing a high rate of endophily, biting humans inside houses during the nights (Llave Correas & González Mora, 1996). On the contrary, *Cs. longiareolata* and *Cs. litorea* feed on blood from birds and rarely from humans (Becker *et al.*, 2003) whereas *Cx. hortensis hortensis* takes meals from batrachians and reptiles (Schaffner *et al.*, 2001). Until the recent arrival of *Ae. albopictus*

throughout the eastern Mediterranean region in the last decade (Alarcón-Elbal *et al.*, 2014), both *Cx. pipiens* and *Cs. longiareolata* had been recorded as the most common mosquitoes in urban environments (Bueno Marí, 2010; Chordá Olmos, 2014).

Furthermore, *Culicoides* biting midges have broad host preferences and most likely some species show opportunistic feeding behavior (Lefèvre *et al.*, 2009). Species like *C. dunningstoni* and *C. kibunensis* are avian feeders (Martínez-de la Puente *et al.*, 2009, Votýpka *et al.* 2009). However, there are many interpretations depending on the study area (farms, forest, suburban, wild habitats) and host availability. Consequently, *C. kibunensis* species has also been identified as a human feeder in suburban areas (Santiago-Alarcón *et al.*, 2012, 2013). While *C. obsoletus* and *C. dewulfi* are mostly mammalophilic, including humans, the dominant species *C. punctatus* is very plastic and takes meals from different types of vertebrates (Calvo *et al.*, 2012; Lassen *et al.*, 2012; Santiago-Alarcón *et al.*, 2012; 2013). According to their morphometric parameters, *C. clastrieri* species could match with an ornithophilic species (Braverman *et al.*, 2012; Isberg *et al.*, 2013), however, Santiago-Alarcón *et al.* (2013) found that this species fed either on birds or on humans in high numbers.

3) Some of the specimens collected have important implications in animal and public health. Within the Culicidae family, *Cx. pipiens* is the most common mosquito species worldwide, acting as a vector of multiple diseases such as West Nile fever, St. Louis encephalitis, Japanese encephalitis, Western equine encephalitis, Rift Valley fever, Sindbis fever or avian malaria, amongst others (Becker *et al.*, 2003). *Culiseta longiareolata* and *Cs. litorea* show a marked ornithophilic tendency, being important vectors of various avian malaria parasites, whereas *Cx. hortensis hortensis* has never been involved in the transmission of parasitic diseases to human (Schaffner *et al.*, 2001).

The public health importance of *Culicoides* biting midges in Europe is currently restricted to the biting nuisance caused by female adults (Carpenter *et al.*, 2013). Certain species have become notorious for this activity due to their vast population densities and persistent biting attacks, such as *Culicoides impunctatus* Goetghebuer, 1920 in Scottish Highlands (Blackwell, 2001) disturbing tourism and recreational activities. Although there is no evidence of such excessive populations near human presence in Spain, most recently molecular tools have allowed finding out a significant number of different *Culicoides* species that had human blood meals in many ecosystems, including urban forest/areas (Santiago-Alarcón *et al.*, 2012; 2013). Host generalist biting midges are of special interest because they are capable of feeding on different vertebrate groups, and thus, can facilitate the emergence of new diseases (Santiago-Alarcón *et al.*, 2012). Leaving aside their biting impact, current evidence demonstrates that the role of *Culicoides* in the transmission of known and unknown zoonotic arboviruses worldwide is unclear but thought to be limited (Carpenter *et al.*, 2013). Moreover, avian biting midges take part in the transmission cycle of the majority of avian parasites carrying different haemosporidian (Ferraguti *et al.*, 2013).

To conclude, although the risk of human disease transmission by mosquitoes and *Culicoides* is hypothetically very low in the Basque Country region, the presence of regional-scale entomological studies is required. In fact, the recent report of the presence of *Ae. albopictus* in the Basque

Country reinforces the need to raise awareness of these threats for human health. Citizens, entomologists, pest control operators and public health officials can justify that controlling haematophagous arthropods improves the quality of life of residential areas, even in the absence of pathogen transmission (Vaidyanathan, 2014). In this sense, we believe that our findings may be considered as a starting point for future urban surveillance studies, which should include different sampling methods in order to collect a wider range of potential vectors.

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