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DIVERSITY OF BLOOD-SUCKING DIPTERAN IN URBAN AREAS OF NORTHERN SPAIN

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Abstract The diversity and abundance of different blood-sucking insects was studied in the three main cities of the Basque Country region (northern Spain). Sampling took place in one green area and one cemetery from each city, where two CDC-light traps baited with CO_2 were placed fortnightly from May to October 2019. Immature stages around CDC sites were also sampled periodically and reared in the laboratory until adult emergence. Fourteen species of native mosquitoes (Culicidae), seventeen biting midges (Ceratopogonidae), undetermined black fly species (Simulidae) and one sand fly (Psychodidae) species were catched. *Culex pipiens* s.l. and *Culiseta longiareolata* species were the most trapped both at urban areas and cemeteries. A few specimens of *Phlebotomus mascittii* and *Simulium* spp. were also trapped in a cemetery and in a forested green area, respectively. Based on DNA barcoding, blood-engorged mosquitoes and biting midges fed primarily on urban birds. These urban entomological studies are useful to increase the knowledge of blood-sucking Diptera composition and abundance, as well as their habitat and blood-feeding preferences, in order to assess the potential risk of vector-borne disease transmission.
Key Words Mosquitoes, biting midges, blood meals, green areas, cemeteries, avian hosts

INTRODUCTION

Blood-sucking Diptera have an important role in public health not only as annoying biting pests but also due to their capacity to transmit pathogens that affect both humans and animals. The landscapes of urban and periurban areas can affect the abundance and diversity of these groups of insects. Urbanization processes have a major impact on mosquito communities by decreasing species richness and increasing the abundance of some mosquito species well adapted to urban ecosystems, such as *Aedes* spp. invasive mosquitoes (Wilke et al., 2021). Besides, in urban areas some native mosquito species may find more suitable habitats in different types of artificial containers (Ferraguti et al., 2016). Moreover, urban green spaces provide benefits to humans and are important biodiversity hotspots (Aronson et al., 2017). The management and type of urban green areas has an impact on biodiversity. Areas with complex and heterogeneous vegetation support rich wildlife communities (Aronson et al., 2017). Other vectors of pathogens, such as sand flies, black flies and biting midges, have received little attention in urban environments in Europe. Most studies on biting midges of the genus *Culicoides*, vectors of Bluetongue and Schmallenberg viruses, have been mainly focused on rural settings with domestic animals (Möhlmann et al., 2018). Therefore, it is of great importance to know the distribution and abundance of these species in urbanised environments.

Information on vector-host interactions and their transmission dynamics are essential for risk assessment as well as the control of the diseases. Thus, to identify the host affinity of arthropod vector species under field conditions is fundamental. Host feeding is not random; depending on the species of mosquito, the host preferences vary, although most of the species follow opportunistic behaviours. The objective of the present work was to study the flying hematophagous Diptera diversity, abundance, and feeding preferences in urban settings of the Basque Country (northern Spain), an under-studied region in relation to these insects.

MATERIAL AND METHODS

The study sites were in the three main cities of the Basque Country, a region in the North of Spain. Sampling was performed fortnightly from 1-May to 30-October 2019 in one green area and one cemetery per city, deploying two CDC-miniature traps (model 1212) in each sampling site, equipped with incandescent light and baited with ca. 1.5 kg of dry ice (CO_2). Traps were set up early in the morning and recovered 24 h later. Mosquito breeding sites were searched and sampled once per month in a radius of 200 m around CDC traps. Immature stages were collected with a dipper, transported to the laboratory, and kept in mosquito Breeders (Bioquip Products, U.S.A.) until adult emergence. The type of larval site and presence of water was recorded.

All the insects collected in traps as well as those reared in the laboratory were separated into different blood-feeding groups, i.e., biting midges, mosquitoes, sand flies and black flies. Culicidae mosquitoes were morphologically identified by using taxonomic keys (Becker et al. 2020) and *Culicoides* with local morphological keys (González and Goldazarena, 2011). Sand flies were both morphologically and molecularly determined as reported (Alarcón-Elbal et al. 2021). Black fly species were determined at genus level. Doubtful specimens of Diptera were mounted on slides and/or molecularly identified based on COI barcoding. Ecoforms of *Culex pipiens* s.l. and members within *An. maculipennis* complex were also determined (González et al. 2021a). Abdomens of blood-fed biting-midges and mosquito specimens were classified following the Sella scale (II-VII) (Martinez de la Puente et al., 2013) and analysed to determine their vertebrate host species following the procedures previously described (González et al. 2021b).

RESULTS

A total of 462 blood-sucking insects were trapped by baited-CDC traps (265 mosquitoes, 181 biting midges, 11 black flies and 5 sand flies). Thirteen species of mosquitoes were identified (Table 1). *Culex pipiens* s.l. (including *Cx. torrentium*) was the most captured taxa accounting for 60.8% of the total mosquito catches. Based on molecular analyses, we identified *Anopheles atroparvus,* as the unique species within *An. maculipennis* complex, and two ecoforms (*Cx. pipiens pipiens* and *Cx. pipiens molestus*) belonging to the *Culex pipiens* complex. Seventeen species of *Culicoides* were determined (Table 1), the *Obsoletus* complex the most trapped (43.1%). The green urban area of Donostia was the most productive site. *Culicoides begueti* is reported for the first time in the Basque Country region.

Eight mosquito species emerged from immature collections, 317 specimens from green areas and 129 specimens from cemeteries (Table 2). Pots and drainage systems were the most prolific mosquito larval places in green areas whereas flowerpots accounted for most of the collections in cemeteries. Among the 490 breeding sites examined, 303 retained water and only 30 harboured immature mosquito stages. *Culex pipiens* and *Culiseta* spp. bred in a wide variety of artificial and natural water-holdings whereas *Aedes geniculatus* preferred to rear exclusively on tree-holes. The study of larval habitats allowed the identification of *Cx. territans,* which was not collected with the CDC traps.

Blood-meal host identification yielded 58.3% (14/24) of the total specimens. Higher amplification success was recorded in biting-midges (60.0%, 6/7) compared to mosquitoes (47.1%, 8/17). Female mosquitoes that failed in identifying DNA host were categorised within advanced Sella stages. *Culex pipiens* s.l. fed on seven species of urban birds (Table 3) and four species of biting midges fed on avian hosts, mostly belonging to the *Turdus* species (Table 3).

DISCUSSION

This work highlights the existence of 14 species of mosquitoes and 17 species of *Culicoides* inhabiting urban environments in northern Spain with remarks on their feeding habits. The higher number of catches of Culicidae when compared to *Culicoides* could be explained by the differences in suitable microhabitats required by each dipteran group. Culicidae need aquatic environments for their development, while biting midges deposit their eggs on semi-aquatic and moist substrates (Mullen and Durden, 2009). Urban spaces could provide more permanent water holding artificial containers than wet organic soils, benefiting the development of mosquitoes (Ferraguti et al. 2016). Despite the lower abundance, this study provides new and interesting data on *Culicoides* species in urban environments.

Table 1. Culicidae and *Culicoides* trapped by baited CDC traps in the six urban environments from the Basque Country (northern Spain).

Culicidae	**Green areas**			**Cemeteries**			**Total**
	Bilbao	Donostia	Gasteiz	Bilbao	Donostia	Gasteiz	
Cs. annulata	0	5	5	0	1	1	12
Cs. longiareolata	6	4	3	0	31	3	47
Cs. subochrea	0	0	1	0	0	0	1
Cx pipiens s.l./*Cx. torrentium*	47	67	7	7	27	6	161
Cx. hortensis	0	0	0	2	6	0	8
An. claviger s.l.	0	0	9	0	0	0	9
An, atroparvus	0	0	1	0	0	0	1
An. plumbeus	0	4	0	0	2	0	6
Ae vexans	0	0	2	0	0	0	2
Ae geniculatus	0	2	0	0	0	0	2
Oc rusticus	0	0	6	0	0	0	6
Co buxtoni	0	4	0	1	0	0	5
Unidentified species	0	0	0	0	2	0	2
Total	54	88	34	10	69	10	265
Culicoides	Bilbao	Donostia	Gasteiz	Bilbao	Donostia	Gasteiz	**Total**
C. obsoletus/C. scoticus	2	40	12	9	6	9	78
C. minutissimus	3	0	0	0	0	0	3
C. kibunensis	0	26	4	9	0	0	39
C. begueti	0	3	0	0	0	0	3
C. alazanicus	0	15	5	1	0	2	23
C. punctatus	0	6	0	0	1	0	7
C. festivipennis	0	3	2	0	0	0	5
C. pallidicornis	0	1	0	0	0	0	1
C. achrayi	0	0	0	1	0	0	1
C. brunnicans	0	1	1	1	0	1	4
C. cataneii	0	0	0	2	0	0	2
C. circumscriptus	0	0	0	1	0	1	2
C. lupicaris	0	5	1	0	0	0	6
C. duddingstoni	0	0	0	0	0	2	2
C. poperhingensis	0	0	1	0	0	0	1
C. clastrieri	0	0	1	0	0	0	1
Unidentified species	0	0	0	3	0	0	3
Total	5	100	27	27	7	15	181

Table 2. Culicidae species found in breeding sites in the six urban environments studied in the Basque Country (northern Spain).

Culicidae species	Green areas			Cemeteries			Total
	Bilbao	Donostia	Gasteiz	Bilbao	Donostia	Gasteiz	
Cs. annulata		7	1				8
Cs. longiareolata		3	28		38		69
Cx. pipiens s.l./*Cx. torrentium*	21	112	118	30	43		324
Cx. hortensis					18		18
Cx. territans		19					19
An. claviger s.l.			1				1
Ae. geniculatus		7					7
Total	21	148	148	30	99	0	446

Table 3. Bloodmeal host identification in Culicidae and *Culicoides* in the six urban environments studied in the Basque Country (northern Spain).

City	Culicidae		
	No.[1]	**Species**	**Host DNA (No.)**
Bilbao	3	*Cx. pipiens* s.l.	*Turdus merula* (1)
Donostia	1	*Cs. annulata*	-
	4	*Cs. longiareolata*	-
	9	*Cx. pipiens* s.l.	*Turdus merula* (1) *Turdus philomelos* (1) *Anas platyrhynchos* (1) *Erithacus rubecula* (1) *Passer domesticus* (1) *Serinus serinus* (1) *Serinus canarius* (1)

City	*Culicoides*		
	No.[1]	**Species**	**Host DNA (No.)**
Donostia	1	*C. kibunensis*	*Turdus philomelos* (1)
	1	*C. begueti*	-
Gasteiz	3	*C. alazanicus*	*Troglodytes troglodytes* (1) *Turdus philomelos* (2)
	1	*C. festivipennis*	*Turdus philomelos* (1)
	1	*C. clastrieri*	*Turdus merula* (1)

[1] Total number of blood-fed specimens analized.

No invasive mosquitoes were identified in the current study. Among the Culicidae detected, *Culex pipiens* s.l. and *Cs. longiareolata* were the most abundant species, well adapted to breed in natural and artificial breeding sites in urban environments (Ferraguti et al. 2016). Differences in abundance and species richness found among cemeteries could be explained by the practices and management policies. In fact, two of the cemeteries sampled contained few water-holdings containers whereas the cemetery of Donostia was full of suitable artificial containers such flowerpots, pots, ashes urns, etc. Similarly, the differences found in green areas among the three sampling sites might be explained due to landscape use, i.e. the urban park of Donostia includes vegetated gardens, various naturalized ponds, waterholes, tree-holes, tanks etc. in contrast to Bilbao, which only supported small patches of gardens and a clean urban pond. It is known that urbanization degree reduces the number of larval predator and levels of inter-specific competition, thus changing the abundance and diversity of Diptera (Wilke et al., 2021).

Some mosquito species found in the current study are considered potential vectors of pathogens. It should be noted that *Culex pipiens* has an increasing role as a vector of arbovirus (West Nile virus, Usutu virus) in humans in Europe (Becker et al., 2020). In spite of the few specimens captured, also relevant is the detection of *An. atroparvus*, not identified previously in the study area, and a recognised vector of malaria (Bueno-Marí and Jiménez-Peydró, 2008). Blood-meal analysis is a fundamental tool to understand the ecology of mosquitoes and biting-midges. In this study, both Diptera groups showed a tendency to feed mostly on avian hosts in urban environments, even when other mammal hosts were available nearby, such as walkers and dogs. This feeding pattern of *Cx. pipiens* is in accordance with another work published in urban areas of the Basque Country (González et al. 2021b), where all the DNA hosts identified were urban birds. Blood-engorged *Culicoides* analysed in this study belong to the subgenus *Oecacta*, that have proven to be primarily avian feeders in agreement with previous results (Martinez de la Puente et al. 2015).

CONCLUSIONS

The study of the diversity and abundance of blood-sucking Diptera in urban areas, as well as their breeding sites and host feeding preferences, are key to understand the epidemiological cycles of both, vectors and pathogens. Our results indicate that *Cx. pipiens* sl. is the most common taxa of the *Culicidae* family in urban areas and showed an ornithophilic feeding preference. *Culicoides* belonging to the *Obsoletus* complex were present in low numbers in green areas. Some *Culicoides* species showed a preference to feed on avian hosts. A better understanding of the trophic behavior/preferences of these Diptera pests can show the transmission patterns of pathogens of public health interest.

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