First monitoring of mosquito species (Diptera: Culicidae) in the Caffarella Valley, Appia Antica Regional Park, Rome, Italy

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Abstract: This study reports the results of the first entomological investigation focused on mosquitoes in Caffarella Valley, the inner part of Appia Antica Natural Reserve in Rome, carried out between 2012 and 2013. A total of 1173 mosquitoes were collected, with 9 species, belonging to 4 different genera, identified: *Culex pipiens*, *Anopheles maculipennis* sensu stricto (s.s.), *Anopheles claviger*, *Culiseta annulata*, *Culiseta longiareolata*, *Aedes albopictus*, *Culex hortensis*, *Culex territans* and *Anopheles plumbeus*. The monitoring of this area, bordering natural and urban environments, contributes to the knowledge on the culicid fauna of Rome.
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Introduction

The family Culicidae includes about 3500 mosquito species distributed worldwide (WRBU, 2017), 64 of which, belonging to nine genera, have been reported for Italy (Severini *et al*., 2009). The northern house mosquito *Culex pipiens* Linnaeus, 1758 and the Asian tiger mosquito *Aedes albopictus* (Skuse, 1854) are widely spread throughout the country and are proven vectors of several human disease agents (Gratz, 2004; Fortuna *et al*., 2015; Di Luca *et al*., 2016a). Moreover, two invasive species, *Aedes* (*Finlaya*) *koreicus* (Edwards, 1917) and *Aedes* (*Hulecoeteomyia*) *japonicus japonicus* (Theobald, 1901), that have recently been introduced into Italy (Capelli *et al*., 2011; Seidel *et al*., 2016), are also potential vectors of arboviruses. These invasive species coexist with humans, thriving in human-made containers in urban and rural areas. The importance of these mosquitoes as vectors of animal and human pathogens and their role in pathogen transmission remains to be defined in many temperate regions. For these reasons, a deeper knowledge concerning their distribution, ecology and behaviour is needed to better understand the transmission dynamics of vector-borne diseases and therefore to facilitate the implementation of effective control strategies, especially in densely populated areas.

As the studies on the mosquito fauna of Rome are quite fragmentary and date back some time (Romi *et al*., 1997), the objective of this study is to contribute to the knowledge of the mosquito composition and distribution in the city within a public health context.

This contribution presents the first checklist of the mosquito fauna in Caffarella Valley in the Appia Antica Regional Park, one of the most important areas for the conservation of natural environments in the centre of Rome.

Materials and Methods

Study Area

The study area is the Caffarella Valley (41°51′ N, 12°30′ E, Fig. 1), extending to 200 hectares from the Via Latina on its northern side to the Via Appia on its southern limit and representing the inner part of the Appia Antica Regional Park of Rome (Mancini *et al*., 2007). In this part of the park, the vegetation consists mainly of meadows surrounded by scattered wooded areas. The Caffarella Valley is traversed longitudinally by the Almone River and by two parallel ditches that give rise to different aquatic environments such as springs, temporary puddles and a small artificial lake, which was only recently established. The area hosts many species of birds, reptiles and amphibians, as well as several wild mammals, such as foxes, hedgehogs, hares, weasels, stone martens, porcupines and different rodents. Furthermore, there are three farms in the park where cattle, poultry, sheep, and rabbits are bred; dogs and cats are also present. The park is also intensely frequented by people for open-air activities (Parco Regionale dell'Appia Antica, 2017).

Mosquito collection and identification

From April 2012 to April 2013, 14 surveys were carried out in the Caffarella Valley in order to assess the presence and relative abundance of mosquito species. Larval catches were carried out using a standard 350 ml dipper (Service, 1993), collecting larval samples from 20 different water bodies, both natural (water puddles, marshes, springs, ponds, rivers, streams) and artificial (canals, cans, pots, etc.). A further larval breeding site, placed outside the Caffarella Valley, but inside the Park of Aqueducts (Fig. 1) and characterised by a flooded area with spring water, was routinely inspected. Some of these sites represented temporary water bodies, which dried out completely during the summer season.

Mosquito collections were performed in the Vaccareccia, the largest farm in the park (Fig. 1) by using both CDC light traps and BG Sentinel® traps equipped with BG lure as chemical attractant. The traps were set up outside the sheep shelters and were operated for one day from dusk to dawn every two weeks.

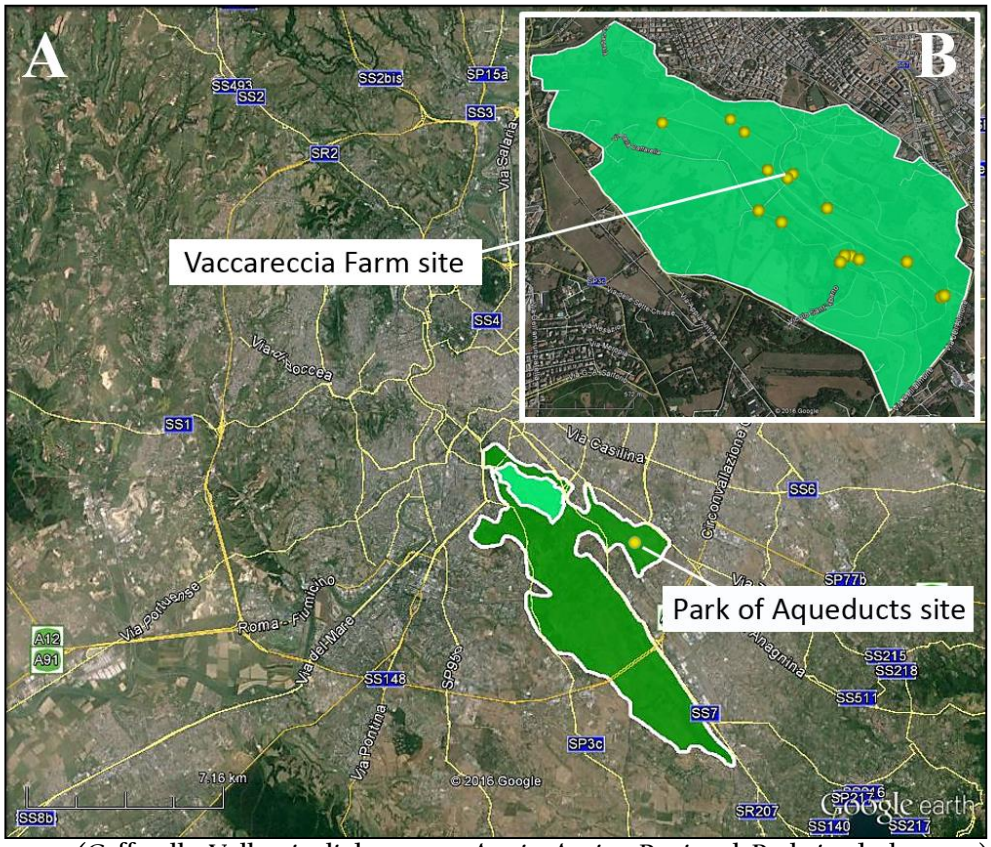

Figure 1: The study area (Caffarella Valley in light green, Appia Antica Regional Park in dark green) located in the southwestern part of Rome. B) Details of Caffarella Valley with collection sites (yellow dots). Source: Google Earth V 7.1.8.3036 (October 28, 2016). Rome, Italy. 41°52'35.58''N, 12°31'21.89''E, Eye alt 30.57 km. @2016 Google, Image Landast/Copernicus.

All collected larvae were transported alive to the laboratory and reared until adulthood. Both larvae and adult mosquito specimens were identified according to the morphological key by Severini *et al.* (2009).

Molecular analyses were carried out on sub-samples of mosquitoes belonging to the *Culex pipiens* and *Anopheles maculipennis* complexes. ACE and CQ11 targets were used to discriminate *Cx. pipiens* from *Culex torrentium* Martini, 1925 and to identify the two biological forms, *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus* (Smith and Fonseca 2004; Bahnck and Fonseca 2006). Ribosomal internal transcribed spacer 2 (ITS-2) was used as marker to identify the members of the *An. maculipennis* complex, according to Proft *et al.* (1999).

Diversity analysis

In order to characterise the species diversity in the study area, the Shannon's diversity index (H) was used. The Shannon index is a mathematical measure of species diversity in a community, assuming that all species are represented in a sample and that they are randomly sampled (Suhasini & Sammaiah, 2014).

Results

During the study period, a total of 1173 mosquitoes (larvae and adults) were collected, and 12 out of 20 breeding sites inspected were found positive for mosquito larvae (Fig. 1). Nine taxa belonging to four genera were morphologically identified: *Culex pipiens* (49.1%, n=574), *Anopheles maculipennis* s.l. Meigen, 1818 (17.7%, n=207), *Anopheles claviger* (Meigen, 1804) (13.7%, n=160), *Culiseta annulata* (Schrank, 1776) (8.9%, n=104), *Culiseta longiareolata* (Macquart, 1838) (6.9%, n=81), *Aedes albopictus* (2.5%, n=29), and *Culex territans* Walker, 1856, *Culex hortensis* Ficalbi, 1889 and *Anopheles plumbeus* Stephens, 1828 (these latter three species comprising less than 1%, n=9, 6, 3, respectively) (Fig. 2).

Culex hortensis and *Cx. territans* were found exclusively in the Park of Aqueducts from late August to early October while the other species were collected in the Caffarella Valley throughout the whole study period.

The traps placed in the Vaccareccia farm collected a total of 72 adults (19 mosquitoes caught with BG sentinel trap and 53 with CDC trap, respectively) belonging to four genera and six taxa: *Aedes albopictus* (23.6%, n=17), *An. claviger* (1.4%, n=1), *An. maculipennis* s.l. (4.2%, n=3), *Cx. pipiens* (63.9%, n=46), *Cs. annulata* (2.8%, n=2) and *Cs. longiareolata* (4.2%, n=3). The limited number of suitable sites for adult mosquito sampling allowed the positioning of two traps only. Despite the limited number, the trap collections represented all taxa collected throughout the Caffarella Valley except *An. plumbeus* which was collected in the larval stage only (Fig. 2).

Molecular analysis on ten specimens belonging to the *Cx. pipiens* complex, collected in the Vaccareccia farm, allowed the identification of 7 *Cx. pipiens* f. *pipiens* and 3 hybrids; *Cx. pipiens* f. *molestus*; *Cx. torrentium* were absent from the samples.

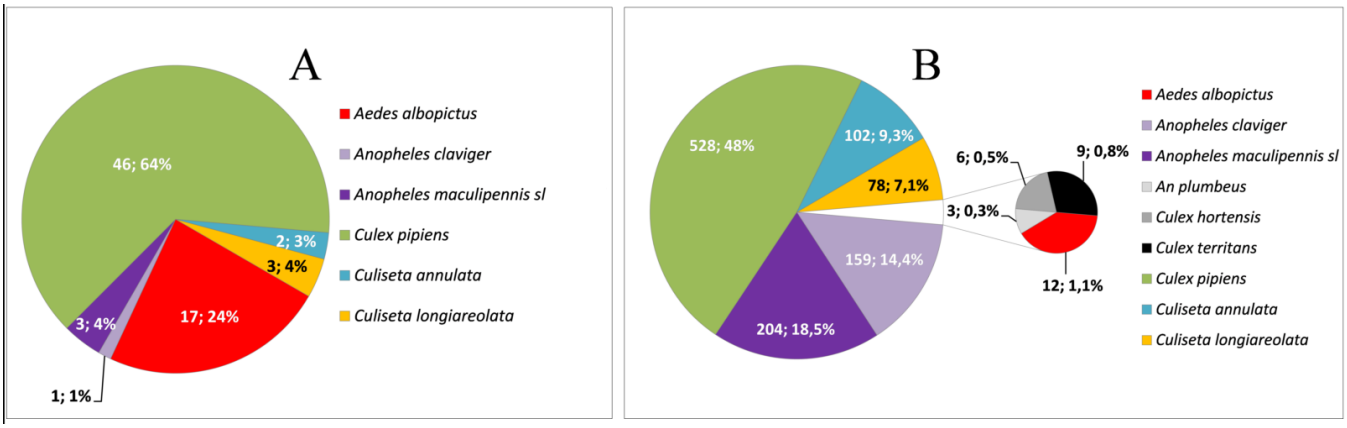

Figure 2: Total number of adult mosquitoes (A) and larvae (B) collected in the Caffarella Valley. Species with values lower than 1% are represented in the sub-pie of pie chart B.

A multiplex polymerase chain reaction (PCR) for ITS-2 conducted on ten *An. maculipennis* s.l. larvae identified all specimens as *An. maculipennis* s.s. Meigen, 1818.

The Shannon index evaluated for the Caffarella Valley was 1.50.

Discussion

The existence in the Caffarella Valley of different biotopes and many water sources (both natural and artificial, temporary and permanent) favouring numerous larval breeding sites, makes this environment a suitable area for the development of different mosquito species.

During the presented study, common and uncommon species of the Italian mosquito fauna were found.

Culex pipiens was the most abundant species and was detected during the whole study period in most of the collecting sites. *Culex pipiens* comprises two biotypes or biological forms, *pipiens* and *molestus*, differing in behavioural and physiological characteristics: the *pipiens* biotype, referred to as the rural form thriving in open spaces, requires a blood meal for egg development and bites mostly birds; the *molestus* biotype, the urban form, can be found in underground habitats, where it lays eggs without a blood meal, even if mammal blood hosts, including humans, are available. The two biotypes may interbreed producing hybrids that could act as West Nile virus bridge-vectors (Huang *et al.*, 2009). For this reason, the discrimination of the forms and hybrids and the evaluation on their involvement in the circulation of pathogens represent a focal point for public health strategies. Although the *molestus* form was not identified in the subsample analysed, the occurrence of hybrids suggests the presence of this anthropophilic fraction, probably related to the proximity to urban environments. The occurrence of the two different forms and their hybrids, with a predominance of the *pipiens* form in rural environments, are consistent with findings of a previous study on *Cx. pipiens* distribution in Italy (Di Luca *et al.*, 2016b).

Three different *Anopheles* species were found: *An. claviger*, *An. plumbeus* and *An. maculipennis* s.s., the last never found before in the urban area of Rome. *Anopheles maculipennis* s.l. is a complex of sibling species that includes former vectors of malaria in Italy such as *Anopheles labranchiae* Falleroni, 1926 and *Anopheles sacharovi* Favre, 1903, the latter having not been found in the country for many years (Bietolini *et al.*, 2006).

The occurrence in the Caffarella Valley of *An. maculipennis* s.s., which is considered mainly a zoophilic species within the complex, seems to be linked to the presence of sheep flocks in the area.

Two other widely distributed species, *Cs. longiareolata*, very common in urban environments, and *Cs. annulata*, typical in rural areas, were also frequently found during the study period. *Aedes albopictus*, now widespread in Rome and highly worrisome for its potential as a vector of several arboviruses, including chikungunya and Zika viruses (Di Luca *et al.*, 2016a), was found in association with *An. claviger* larvae in a spring of fresh water. This finding is noteworthy, because such a breeding site is quite uncommon for *Ae. albopictus*, a container-breeding mosquito, that thrives in completely different breeding sites in urban areas (Hawley, 1988; Li *et al.*, 2014). This observation highlights and confirms the high adaptability of this species to many different conditions (Knudsen *et al.*, 1996, Romi, 1995) as well as a contemporaneous capacity of re-colonising natural environments available in its distribution range (Benedict *et al.*, 2007). Interestingly, *Cx. territans* Walker, 1856 was found during this study, an uncommon species occurring in some regions of central-northern Italy which feeds mostly on birds and amphibians (Severini *et al.*, 2009). Not reported in a previous comprehensive study on the entomological fauna of Rome (Romi *et al.*, 1997), *Cx. territans* has recently been found in the Insugherata Natural Reserve in the northwestern part of Rome (Toma *et al.*, 2015).

In order to assess the degree of biodiversity of Caffarella Valley, data related to the collected species were compared with those of the "Bosco della Fontana", a natural reserve located near Marmirolo, about 5 km north-west of Mantova Lombardia (45°12'N, 10°44'E). Geographically far from urbanised centres, this reserve comprises about 2.3 square kilometres and is occupied by woodland, artificially flooded wetlands and meadows (Toma, 2004). The two compared areas showed similar Shannon index values, 1.50 for the Caffarella Valley and 1.56 for the Bosco della Fontana (nine species recorded in both areas). Caffarella Valley is surrounded by densely populated settlements, but its connection to the Appia Antica Regional Park, forming one of the main wildlife corridors of Rome, determines a high degree of naturalness and environmental preservation of the park, comparable to the Bosco della Fontana, as confirmed by equivalent Shannon index values.

In conclusion, the Caffarella Valley, although located within the city and bordering highly urbanised areas, has maintained a preserved environment, closely connected with the Park of the Aqueducts. In fact, its biodiversity clearly reflects the variety of biotopes and larval breeding sites. Besides providing a checklist of mosquito species, this entomological survey contributes to highlighting the

importance of field investigations for public health purposes. Indeed, the data collected in this study are appropriate to alert the responsible authorities about the presence of some mosquito species representing potential vectors of disease agents of humans and animals and the risk of introduction of new invasive mosquitoes. In fact, the contemporary presence of potential mosquito vectors such as *Ae. albopictus* and *Cx. pipiens* and possible reservoirs of pathogens such as migratory and resident birds inside a metropolitan context, makes this urban park a potential risk area of vector-borne disease transmission.

Moreover, the recent emergence in Italy of new mosquito species potentially affecting human health, in addition to mosquito vectors already present, prompts us to conduct further studies in other natural areas of the city to deepen the knowledge on the biology and distribution of species relevant for human health.

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