

## Mosquito species distribution in mainland Portugal 2005-2008

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### Abstract

Information on distribution and relative abundance of the mosquito fauna of mainland Portugal has been collected by several surveillance programmes and projects over a long period. The work presented here documents additional information provided by capture of adult mosquitoes by the use of CDC light traps baited with CO<sub>2</sub> in 48 localities in 13 districts in different parts of mainland Portugal during the period 2005-2008, and by larval surveys using dippers. More than 150,000 adult mosquitoes and 3,000 larvae belonging to 16 species were identified at the National Institute of Health / Centre for Vectors and Infectious Diseases Research. The three commonest species were *Ochlerotatus caspius*, *Culex pipiens*, and *Cx. theileri*. The most widespread species was *Cx. pipiens*, followed by *Culiseta longiareolata*. The composition and abundance of the different mosquito populations varied between geographical locations. Only larvae of two species, *Cx. hortensis* and *Cx. laticinctus*, were found. Some effects of collection methods on a complete picture of mosquito distribution and abundance are discussed.

### Keywords

Mosquito distribution, vector surveillance, CDC Light Traps, Portugal

### Introduction

An essential component of a mosquito-borne disease control programme includes a comprehensive mosquito surveillance plan that identifies the geographical distribution and relative abundance of potential vector populations (Ryan *et al.*, 2004). In Portugal, as in other European countries, important ecological changes due to future climate conditions are expected (Santos *et al.*, 2002). Portugal is currently recognized as a high risk country for the introduction of *Stegomyia albopicta* Skuse (Almeida *et al.*, 2008), a vector of several arboviruses, which is already present in Spain and other Mediterranean countries (Aranda *et al.*, 2006, Dalla Pozza & Majori, 1992). Furthermore another invasive species, *St. aegypti* L. was present in Portugal until 1956 (Ribeiro & Ramos, 1999) after which it was no longer detected on the mainland, but was reported in Madeira in 2005 (Margarida *et al.*, 2006).

To date a total of 40 mosquito species, 10 anopheline and 30 culicine, have been reported in mainland Portugal (Ribeiro *et al.*, 1988). *Culex pipiens* sensu lato L., *Anopheles maculipennis* s.l. Meigen, *Cx. theileri* Theobald, *An. claviger* s.l. Meigen and *Culiseta longiareolata* Macquart were found to be the most widespread mosquitoes in a country-wide survey carried out over the period 2001-2004 (Almeida *et al.*, 2008).

In the framework of arbovirus surveillance programmes the National Institute of Health has been studying the mosquito fauna of several Portuguese provinces to ascertain potential infection risks to the human population. The data included herein provide information about the distribution and abundance of mosquito species detected over the period 2005-2008. The aim of this report is to contribute with updated information on the mosquito fauna and on abundance of potential arbovirus vectors in mainland Portugal.

## **Material and Methods**

### **Study area and surveillance programmes**

A total of 48 localities belonging to 13 districts were surveyed between 2005 and 2008, mainly from May to October (Table 1). In 2005 and 2006 mosquitoes were collected from the districts of Setúbal and Faro. Both geographic areas are wetlands and bird sanctuaries, characterized by fresh and salt water marshes with small islands, dunes and beaches, as well as rice fields and reed plantations. In 2007 the survey took place only in five localities in the district of Faro. All of these sites are coastal wetlands rich in avifauna and include a multitude of habitats as described above. In 2008 the study was extended to 13 districts of mainland Portugal, where 48 localities were surveyed. In each locality one to 10 collection sites were set. The ecological characteristics of these collection sites exhibit a wide variation, ranging from rural to urban habitats.

### **Mosquito collections**

Adult mosquitoes were caught with CDC light traps baited with CO<sub>2</sub> and set for a minimum of 12 hour periods covering sunset to sunrise. The traps were set at the same collection site over 2 to 3 nights at monthly intervals. Mosquitoes were inactivated by placing them in a 4°C refrigerator and identified under a stereomicroscope, on a chill table, using the identification keys of Ribeiro & Ramos (1999) and Schaffner *et al.* (2001). Larvae were collected with a dipper in the same localities and districts, transported to the laboratory and identified using the same identification keys.

## **Results**

From 2005 to 2008 a total of 169,586 adult mosquitoes and 3,337 larvae were caught in 1,299 trap-nights and 143 positive larvae surveys (Table 1). The most abundant species during 2005-2008 was *Ochlerotatus caspius* Pallas, especially in 2005 when this species constituted 99% of the total mosquito collection, and was followed by *Cx. pipiens* s.l. and *Cx. theileri*. In 2008 numbers of these three common species were more similar and a total of 14 species were reported. In this year (2008) 13 districts of mainland Portugal were included in the surveillance programme and the collection period extended throughout the season when mosquitoes are active (Table 1).

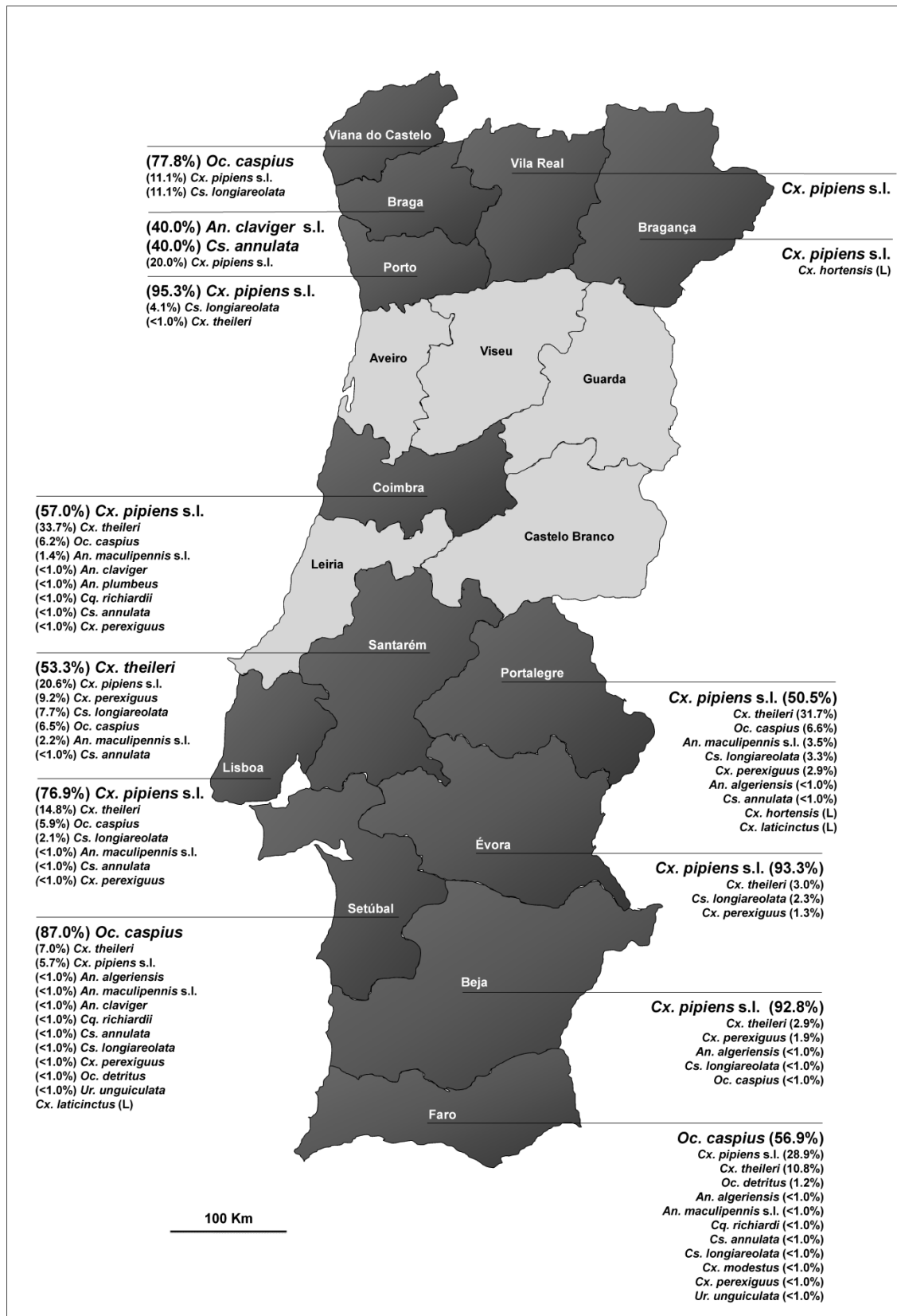
**Table 1.** Summary of Collections

Year	2005	2006	2007	2008
CDC Trap/nights (n=1299)	320	440	80	459
Adult mosquitoes (n = 169,586)	63,503	58,733	11,560	35,784
Larval collections	-	-	-	143
Larvae (n = 3,337)	-	-	-	3,337
Commonest species (%)	<i>Oc. caspius</i> (99.3)	<i>Oc. caspius</i> (78.6)	<i>Oc. caspius</i> (66.9)	<i>Oc. caspius</i> (36.2)
	<i>Cx. pipiens</i> (<1)	<i>Cx. pipiens</i> (19.4)	<i>Cx. pipiens</i> (22.6)	<i>Cx. theileri</i> (35.4)
	<i>Cx. theileri</i> (<1)	<i>Cx. theileri</i> (1.6)	<i>Cx. theileri</i> (2.7)	<i>Cx. pipiens</i> (25.0)
Genera/Species (n =6/16)	3/6	6/10	5/10	6/14
Localities/Districts	2/2	2/2	5/1	48/13

During the surveillance period a total of 16 species belonging to six genera were identified, representing 40% of the 40 known species in mainland Portugal (Ribeiro & Ramos, 1999) (Table 2). The overall number of mosquitoes varied considerably between collection sites, but the commonest species were *Oc. caspius* (129,907 specimens), *Cx. pipiens* s.l. (24,953 specimens) and *Cx. theileri* (14,223 specimens). *Culex pipiens* s.l. was the most widespread mosquito, being present in all districts sampled, and was followed by *Cx. theileri* and *Cs. longiareolata*, which were reported in 9 districts (Table 2; Figure 1).

**Table 2.** Mosquito species collected in Portugal over 2005-2008 in order of adult abundance (A- adults; L- larvae)

	Districts (n=13)	Adults (A) / Larvae (L)	Adults (n=169,586) (%)	Larvae (n= 3,337) (%)	TOTAL (n= 172,923) (%)
<i>Ochlerotatus caspius</i>	8	A	129,907 (76.6)	0 (0)	129,907 (75.1)
<i>Culex pipiens</i> s.l.	13	A / L	23,302 (13.7)	1,651 (49.5)	24,953 (14.4)
<i>Cx. theileri</i>	9	A / L	14,201 (8.4)	22 (1.3)	14,223 (8.2)
<i>Oc. detritus</i> s.l.	2	A	615 (0.4)	0 (0)	615 (0.3)
<i>Cx. perexiguus</i>	8	A / L	517 (0.3)	66 (2.0)	583 (0.3)
<i>Anopheles algeriensis</i>	4	A	495 (0.3)	0 (0)	495 (0.2)
<i>Culiseta longiareolata</i>	9	A / L	212 (0.1)	1,258 (37.7)	1,470 (0.9)
<i>An. maculipennis</i> s.l.	6	A / L	136 (0.1)	4 (0.1)	140 (0.1)
<i>Uranotaenia unguiculata</i>	2	A / L	90 (0.1)	1 (<0.1)	91 (<0.1)
<i>Cs. annulata</i>	6	A	72 (<0.1)	0 (0)	72 (<0.1)
<i>Coquilletidea richiardii</i>	3	A	30 (<0.1)	0 (0)	30 (<0.1)
<i>An. claviger</i> s.l.	3	A	7 (<0.1)	0 (0)	7 (<0.1)
<i>An. plumbeus</i>	1	A	1 (<0.1)	0 (0)	1 (<0.1)
<i>Cx. modestus</i>	1	A	1 (<0.1)	0 (0)	1 (<0.1)
<i>Cx. hortensis</i>	2	L	0 (0)	40 (1.2)	40 (<0.1)
<i>Cx. lacticinctus</i>	2	L	0 (0)	295 (8.8)	295 (0.1)



**Figure 1.** Relative abundance by district of the adult mosquitoes collected in mainland Portugal over 2005-2008 (L- species collected only as larvae)

The less common species, *An. algeriensis* Theobald, *Uranotaenia unguiculata* Edwards and *Oc. detritus* s.l. Pallas were only found in the southernmost regions of Portugal. The most northerly findings of *An. algeriensis* were in the district of Portalegre, and of *Ur. unguiculata* and *Oc. detritus* s.l. in the district of Setúbal. *Anopheles maculipennis* s.l. was detected in the central district of Coimbra and southernmost regions of Setúbal and Faro, but the three widely separated districts from which *An. claviger* s.l. was collected were in the north, centre and south of the country. *Culex perexiguus* Theobald and *Cs. annulata* Schrank were present in eight and six widely separated districts, respectively. *Coquilletidia richiardii* Ficalbi was found in Coimbra, Setúbal and Faro districts. The rarest species, with just one adult specimen of each, were *An. plumbeus* Stephens (in Coimbra) and *Cx. modestus* Ficalbi (in Faro) (Table 2; Figure 1).

Neither *St. albopicta* nor *St. aegypti* were detected in this study.

The commonest larvae were those of *Cx. pipiens* s.l., and *Cs. longiareolata* (Table 2). Both species were found in a vast number of different breeding sites and throughout all districts surveyed. *Culex hortensis* Ficalbi, confined to the inner districts of Portalegre and to Bragança (Figure 1) and *Cx. laticinctus* Edwards were only collected as larvae.

## Discussion

*Ochlerotatus caspius*, *Cx. pipiens* s.l. and *Cx. theileri* were the most abundant and widespread species found in this survey followed by *Cs. longiareolata* and *Cx. perexiguus*, which were captured in more than eight of the districts surveyed. Only low numbers of *An. maculipennis* s.l. and *An. claviger* s.l., both considered to be abundant species in Portugal, were collected in this survey, which used CO<sub>2</sub> baited CDC traps and may misrepresent the relative numbers of species present and consequently the mosquito fauna of individual study areas. These results are in agreement with previous surveys in which it was observed that *An. maculipennis* s.l. seldom appeared in CDC traps, although this species comprises one of the most common mosquitoes resting inside animal shelters (Almeida *et al.*, 2008).

*Ochlerotatus detritus* s.l. was found in the districts of Setúbal and Faro, where collections were made in estuarine regions. This is in agreement with previous studies which have shown that this mosquito is common in wetlands and coastal habitats (Ribeiro *et al.*, 1988).

*Anopheles algeriensis* was previously reported only once, when a few larva and adults were collected in western Algarve in 1982 (Ramos *et al.*, 1982). Our data not only confirm the presence of this species in Algarve, but also show that its distribution includes the more northerly districts of Beja, Setúbal and Portalegre.

*Uranotaenia unguiculata* was considered to be a very rare mosquito species in Portugal. A male reared from a pupa, was recorded for the first time in the southern district of Beja (Ramos *et al.*, 1977-78) and two additional male specimens were recorded in 1991, also in the southern districts of Beja and Faro (Ramos *et al.*, 1992). The first record of a female was in 2004, from Almancil, also in Faro district (Almeida *et al.*, 2005). In this study 90 specimens of *Ur. unguiculata* were captured in the district of Faro and in the more northerly district of Setúbal, which shows that *Ur.*

*anguiculata* is not as rare as hitherto reported and is widespread throughout the south of mainland Portugal.

Smaller numbers of adult *Cs. annulata*, *Cq. richiardii*, *An. claviger*, *An. plumbeus* and *Cx. modestus* were collected (<0.1%), the last two, each with just one identified specimen. Larval collections detected two additional species, *Cx. hortensis* and *Cx. laticinctus*, neither of which were found in CDC adult traps.

*Stegomyia albopicta* and *St. aegypti* were not detected. These are considered the most widespread and invasive mosquito species in Europe, and both are known to transmit several arboviruses, including yellow fever, dengue, West Nile (Flaviviridae), and Chikungunya (Togaviridae). The recent establishment of these species in distinct and wider geographic regions makes the development of appropriate mosquito surveillance programmes imperative (Gratz, 2004, Eritja *et al.*, 2005).

In summary *Oc. caspius*, *Cx. pipiens* s.l. and *Cx. theileri* were the most abundant mosquitoes found. These species are recognized as vectors of arboviruses, such as West Nile virus (Lundström, 1999) and may constitute a public health threat. Furthermore, the proximity of migratory bird sanctuaries increases the risk of involvement in arbovirus transmission cycles. Both *Cx. pipiens* s.l. and *Cx. perexiguus*, have already been found positive for West Nile virus in the southern district of Faro (Parreira *et al.*, 2007). Due to its abundance and distribution *Cx. pipiens* s.l. and also other mosquitoes with catholic host preferences may contribute to the establishment of local arbovirus circulation in Portugal.

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