

# Biodiversity and Larval Habitat Heterogeneity of Mosquitoes (Diptera: Culicidae) in Northern Spain

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**Abstract** A comprehensive mosquito larval study was conducted in order to improve the knowledge of the culicid mosquito diversity in the Autonomous Region of La Rioja, northern Spain. Between May and October 2011, a total of 1381 mosquitoes belonging to 13 species were collected and identified. Among these species, some significant malaria vectors such as *Anopheles atroparvus* or *Anopheles claviger* were detected. The most important invasive mosquito in Europe, the Asian tiger mosquito (*Aedes albopictus*), was not recorded. We used the information of our samplings to identify the influence of anthropisation (basing on the difference between low, medium and highly anthropised environments) and altitude of biotopes (under or below 800 meters) on mosquito diversity. The biodiversity indices have shown that the medium anthropisation affects positively the mosquito species richness since the typology of potential larval sites is increased, mainly due to the man-made breeding sites that can be colonised. Furthermore, in similarly anthropised environments, the species composition resulted in a high equitability. With regard to altitude, the biotopes situated at altitudes below 800 meters show a higher diversity and lower dominance of mosquitoes, probably due to reasons related to their temperature tolerance and feeding preferences.

**Keywords:** Culicids, diversity, disease vectors, medical entomology, anthropisation, La Rioja, Spain

## Introduction

Mosquitoes (Diptera: Culicidae) are considered as the most important organisms in wetlands and rainforests worldwide, not only for ecological reasons but also for issues related to human and animal health. Currently, around 3500 species of mosquitoes are recognised all over the world and some of them are potential vectors of devastating diseases as malaria, dengue, filariasis, yellow fever and West Nile fever. In recent years, the climate change together with several processes linked to the human activities, such as deforestation, habitat modification and globalisation, have allowed the appearance and establishment of some selvatic species of mosquitoes, traditionally restricted to tropical areas, in urban environments of temperate territories. One of the examples of this worrying tendency is the case of the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), which has colonised most of the Mediterranean countries

of Europe in last decades and nowadays begins to transmit tropical arboviruses, such as agents of dengue or chikungunya on the Old Continent.

Spain is one of the European countries where mosquitoes were more thoroughly studied since the beginning of the 20<sup>th</sup> century due to the endemicity of malaria. The investigations carried out by some excellent entomologists and malariologists from the first half of the 1900s, such as Gil Collado, Clavero, de Buen and Torres Cañamares, allowed extending the knowledge on the Spanish mosquito fauna and the understanding of epidemiology of mosquito-borne diseases. However, since the eradication of malaria in Spain in 1961, the comprehensive studies of mosquitoes have been scanty and mainly restricted to particular areas where malaria morbidity was historically very high (ENCINAS GRANDES 1982, LÓPEZ SÁNCHEZ 1982, BUENO MARÍ 2010). A total of

64 mosquito species have been recorded in Spain, including some species currently considered eradicated from the country, such as *Anopheles labranchiae* Falleroni, 1926 and *Aedes aegypti* (Linnaeus, 1762), as well as some recently introduced species as *Aedes albopictus* (BUENO MARI *et al.* 2012a).

The aim of this study is to extend the knowledge of culicids of the Autonomous Region of La Rioja, an area with no previous comprehensive data on the diversity of mosquitoes. In addition, we analyse how mosquitoes' biodiversity is modified across varying altitudes (above or below 800 meters, which is the average altitude of the territory) and land-use categories. The results of these investigations can be very important to enlarge the information about the ecology (type of habitat preference, adaptability to different biotopes and main host preferences) of some species, as well as to establish predictive models of mosquitoes' populations that can be used for determining areas of epidemiological risk.

## Material and Methods

### Study area

The study was carried out in the Autonomous Region of La Rioja (N 42°28'00"/ W 02°27'00"), which is a territory situated in northern Spain and occupying 5.045 km<sup>2</sup> (Fig. 1). La Rioja is a very heterogeneous region, with high-mountainous environments (with some peaks of above 2000 meters in altitude) in the south and vast plain areas used for cultivation, mainly vine-growing, in the north. Along the northern part of the region, the Ebro River runs, which is the largest river of Spain and has 7 tributaries in La Rioja, namely (from east to west) Alhama, Cidacos, Leza, Iregua, Najerilla, Oja and Tirón. Therefore, La Rioja is frequently and commonly called the "region of 7 valleys". Due to the influence of the Ebro Valley, the climate of the northern area may be defined as typical Mediterranean with continental influence, with the average annual temperature being approximately 13.5°C and an annual rainfall of 400 mm. On the other hand, the southern part is characterised by continental climate with an average annual temperature close to 9°C and about 600 mm of annual rainfall.

The study area can be divided in 3 subregions according to different structure of natural landscapes and human influence: low, medium and highly anthropised areas. The areas of low anthropisation (LA) are composed of natural biotopes located far from human activities, with a great variety of potential animal hosts that could be bitten by mosquito females. The sampling effort in this areas was mainly focused in two clearly heterogeneous nature re-

serves strictly protected by local authorities, i.e. the Natural Park of Sierra Cebollera (characterised by mountainous environments, even above 2000 meters of altitude, with high richness of small rivers, ponds and sources) and the Nature Reserve of Los Sotos de Alfaro (including different mosquito breeding sites associated with the riparian area of the Ebro River, such as temporary puddles and isolated meanders). The areas of medium anthropisation (MA) are represented by territories where certain human influence of weak intensity (farming and livestock activities, or small villages in neighbouring areas) is present, as well as wild territories, in which man-made sites of larval mosquitoes such as containers or artificial ditches and ponds exist. Finally, the highly anthropised areas (HA) are related to potential mosquito breeding sites located in urban environments (ornamental fountains, scuppers or sewer systems) or even at intra-domiciliary level (small containers, pools or different domestic utensils capable of containing water for several days). These breeding sites are frequently characterised by highly polluted water and a low diversity of potential animal hosts that could be bitten.

### Faunistic and statistical analyses

In order to improve the information on mosquitoes, between May and October 2011 we carried out intensive larval sampling at all identifiable breeding sites in the three types of areas (LA, MA, HA), by using the standard dipping method (SERVICE 1993). Sampling of small larval habitats, such as tree-holes and small containers, was done directly by emptying or pipetting the contents for immature stages. The sampling effort was fixed at ten minutes, which included the active search for larvae in each biotope visited (BUENO MARI, JIMÉNEZ PEYDRÓ 2011). Finally, the collected mosquitoes were identified to a species



Fig. 1. Location of the studied area over the Iberian Peninsula

**Table 1.** Values of alphadiversity indices according to diverse land-use categories (low anthropisation – LA, medium anthropisation – MA and high anthropisation – HA) and different altitudes (> 800 m and < 800 m)

Indices	LA	MA	HA	<800 m	>800 m
Species Richness (S)	9	13	10	13	10
Abundance (N)	392	445	544	897	484
Shannon-Wiener (H')	1.514	2.322	1.667	1.909	1.321
Menhinick (Mn)	0.455	0.616	0.429	0.434	0.455
Margalef (Mg)	1.340	1.968	1.429	1.765	1.456
Equitability (J')	0.689	0.905	0.724	0.744	0.574
Berger-Parker (d)	0.429	0.144	0.465	0.438	0.645
Dominance (D)	0.300	0.109	0.276	0.234	0.438
Simpson (1-D)	0.701	0.891	0.724	0.766	0.562

level according to the criteria in the taxonomic keys of ENCINAS GRANDES (1982), SCHAFFNER *et al.* (2001) and BUENO MARÍ (2010). Data were analysed statistically using the PAST Program (HAMMER *et al.* 2001). We calculated several biodiversity indices for each environment as well as the ecological distance between those environments through the development of a principal components analysis (PCA) based on the Jaccard distance.

## Results

A total of 1381 mosquitoes belonging to 13 species were collected and identified (Appendix I). These were: *Anopheles atroparvus* Van Thiel, 1927, *Anopheles claviger* (Meigen, 1804), *Anopheles maculipennis* Meigen, 1818, *Anopheles petragrani* Del Vecchio, 1939, *Culex hortensis* Ficalbi, 1889, *Culex impudicus* Ficalbi, 1890, *Culex mimeticus* Noè, 1899, *Culex modestus* Ficalbi, 1889, *Culex pipiens* Linnaeus, 1758, *Culex territans* Walker, 1856, *Culiseta annulata* (Schrank, 1776), *Culiseta longiareolata* (Macquart, 1838), and *Ochlerotatus caspius* (Pallas, 1771). The most abundant species in the study were *Culex pipiens* (n=434) and *C. hortensis* (n=331). Specimens of *Anopheles albopictus*, which is currently the most important invasive mosquito in Europe, have not been detected in the studied area.

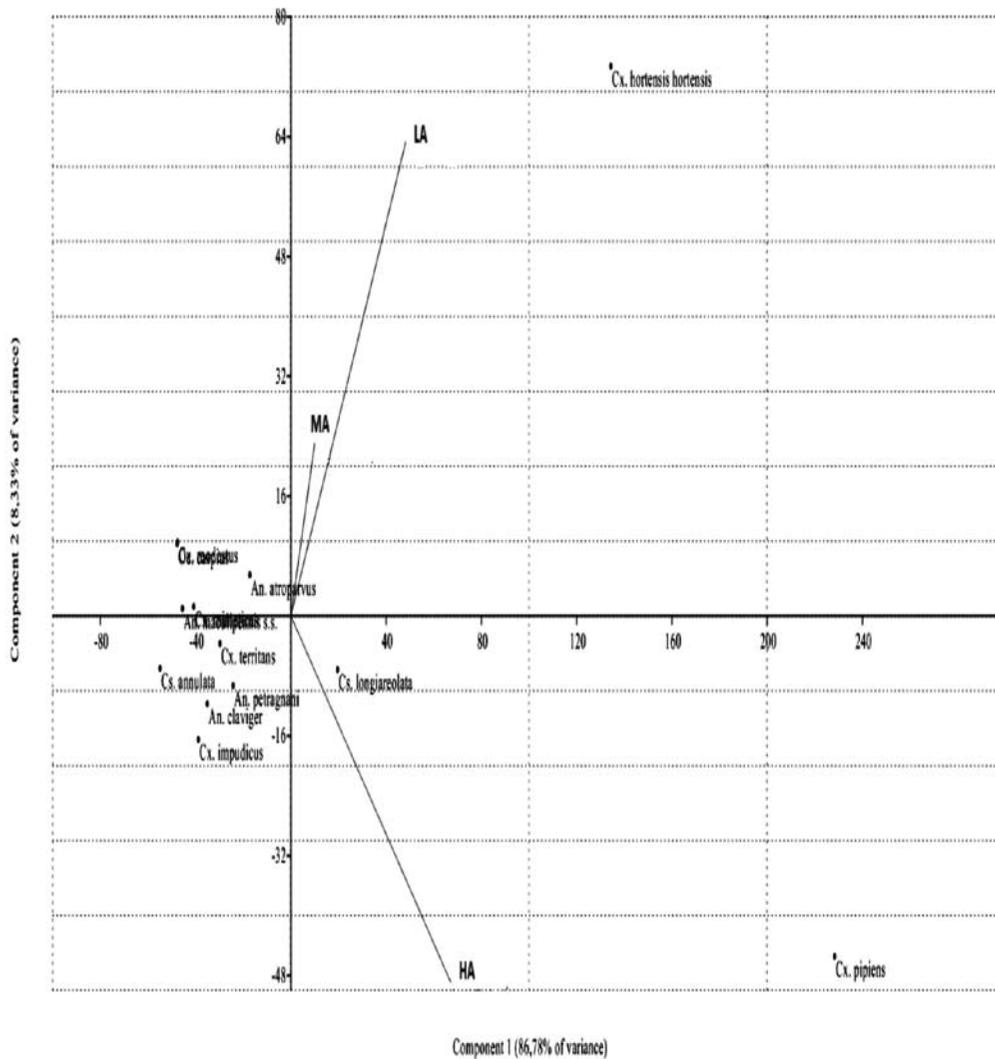
According to the results from the calculated values of the biodiversity indices (Table 1), we can confirm that MA environments show the highest diversity of mosquitoes from the point of view of the anthropisation level of landscapes. Moreover, the larval sites located at altitudes lower than 800 meters exhibit higher diversity of mosquito species than those situated at higher altitudes.

## Discussion

La Rioja shows a wide variety of culicid species, all of which being well known as an important part

of the Spanish mosquito fauna (BUENO MARÍ *et al.* 2012a). From a public health perspective, particular importance should be given to the detection of major malaria vectors, such as *Anopheles atroparvus* and *A. claviger*. The former is currently considered the most important malaria vector in Europe (SCHAFFNER *et al.* 2001) and this species is suspected to be responsible for the transmission of an autochthonous malaria case occurred in 2010 in Northern Spain (SANTA OLALLA *et al.* 2010, BUENO MARÍ *et al.* 2012b). It should be noted that the episode of *Plasmodium vivax* (GRASSI, FELLETTI 1890) indigenous transmission was the first documented in Spain since 1961. Although more studies about malaria receptivity are needed to have clear conclusions, in this study we must note the presence of larval sites of *A. atroparvus* very close to human settlements (HA environments) as a very important epidemiological issue. With regard to *A. claviger*, the species was related to several malaria outbreaks, which occurred in some Eastern Mediterranean countries (GRAMICCIA 1956, COLUZZI *et al.* 1964). Though we also detected *A. claviger* in surrounding urban areas, the species is considered as a minor malaria vector due to its strong zoophilic tendency (BECKER *et al.* 2010).

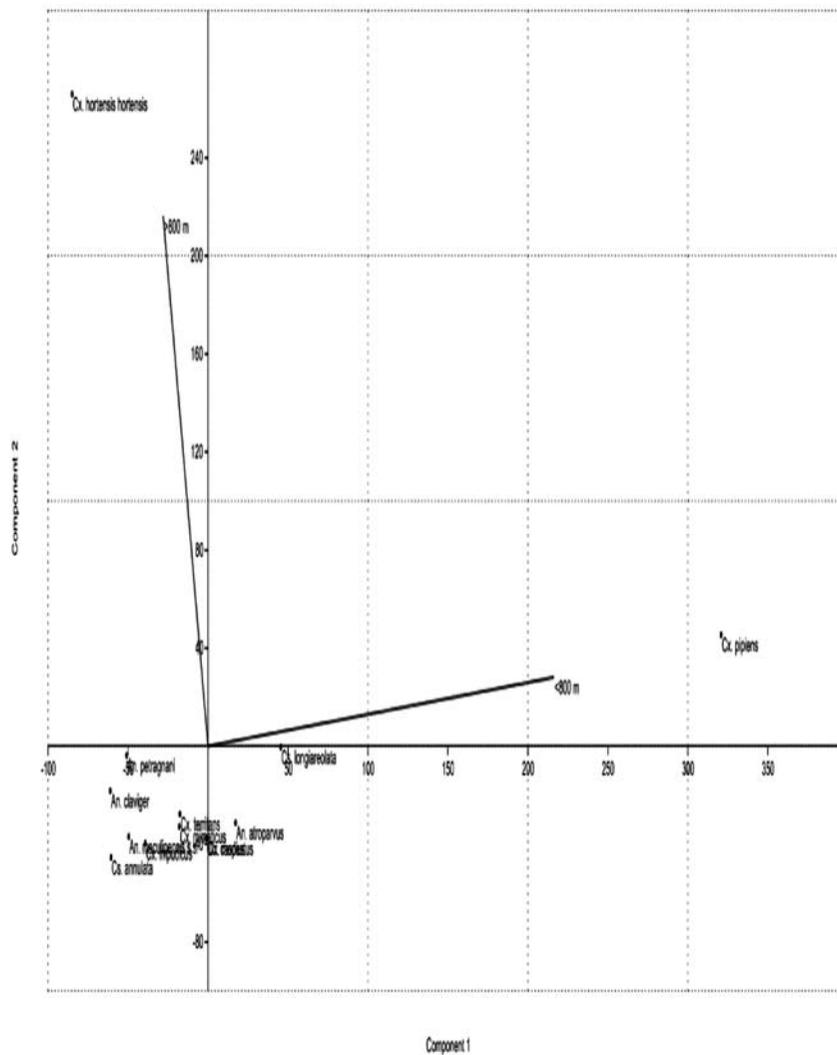
Regarding the analysis of the mosquito biodiversity depending on different degrees of landscape anthropisation, the results of the employed indices show that MA environments are those with the highest diversity and lowest dominancy. All the classical diversity indices used (Shannon-Wiener – H', Menhinick – Mn, and Margalef – Mg) reveal the highest values for MA environments, while the diversity indices related to the community structure (Equitability – J', Berger-Parker – d, Dominance –D, and Simpson – 1-D) clearly show that MA environments are those with the lowest abundance of dominant species. These results may be interpreted in the sense that the MA environments allow the availability of a great variety of different larval biotopes (ovopositing sites related to certain human influence, such as man-made con-



**Fig. 2.** Mosquito species distribution according to the anthropisation degree of landscapes (Principal Components Analysis based on the Jaccard distance)

tainers or other artificial structures, but also other sites completely apart from human influence), which might be potentially colonised by multiple species with different breeding behaviour. Therefore, this high diversity of different breeding sites may be the reason for the high levels of mosquito diversity. Moreover, the MA environments are characterised by a high equitability and correspondingly by a low abundance of dominant species. On the other hand, the LA and HA environments show a high dominance of its mosquito species composition, with *Culex hortensis* and respectively *C. pipiens* being the dominant species in those two environments, as can be seen from the Principal Components Analysis (Fig. 2). In conclusion, certain human influence in some environments can positively affect the diversity and structure composition of mosquitoes. Our observations coincide with those carried out recently in other parts of Spain (BUENO MARI, JIMÉNEZ PEYDRÓ 2011).

Referring to the influence of altitude on the mosquito diversity, the calculated indices show that the sampled breeding sites at altitudes above 800 m had lower diversity and higher dominance (Table 2). Although it is well known that an increase in altitude can affect the mosquito diversity negatively (e.g. among others, through extreme cold conditions in winter months, low diversity and abundance of potential hosts or less presence of different typology of the larval sites), we must point out that these results could also be influenced by anthropisation. This is because there is a strong decrease in the number of the mosquito breeding sites characterised by high levels of anthropisation at altitudes above 800 meters, as can be seen in Appendix I. Naturally, such a possibility must be considered before making any inference. In any case, the high level of dominance in environments at high altitude is clearly due again to *C. hortensis*, while *C. pipiens* can be de-



**Fig. 3.** Mosquito species distribution according to the altitude of breeding sites (Principal Components Analysis based on the Jaccard distance)

fined as the species best associated to landscapes of altitudes below 800 meters (Fig. 3). The orophilic tendency of *C. hortensis* has been profoundly documented (GIL COLLADO 1930, RIOUX 1958), even at altitudes oscillating between 3000-3500 meters in the Himalayan Mountains (PROVONSHA 2009) and the species collections in the Iberian Peninsula seem to be more significant above 600 m (BUENO MARÍ 2010). This species feed mainly on reptiles and batrachians which share the same aquatic habitats with *C. hortensis* (SCHAFFNER *et al.* 2001). This is one of the reasons, which can explain the abundance of *C. hortensis* in territories with high altitude, low temperature and non-polluted waters, at larger distances from urban areas. On the other hand, *C. pipiens* is well known as the most important mosquito in urban areas worldwide. At this point, the difference between *C. pipiens* subsp. *pipiens* and *Cx. pipiens* subsp. *molestus* should be noted, as they have quite

different behaviour. The subsp. *molestus* is greatly related to urban areas and has a marked anthropophilic feeding behaviour, while the subsp. *pipiens* is vastly present in non-urbanised areas and has an ornithophilic feeding behaviour (BECKER *et al.*, 2010). However, in our study no differentiation between the subspecies was carried out, mainly due to the lack of clear taxonomic characters that can be used to separate the specimens at larval stage.

In conclusion, such kind of research must be promoted in order not only to extend the knowledge of these insects, which may potentially provoke devastating pests, but also to have the possibility for elaborating predictive maps of epidemiological interest. The surveillance of these disease vectors of major public health significance is imperative to the prevention and control of current emerging and re-emerging anthroponoses or zoonoses in Europe such as malaria, dengue or West Nile.

Table 2. Data of larval sites detected

Sampling site	Coordinates	Altitude	Habitat type	<i>A. atroparvus</i>	<i>A. claviger</i>	<i>A. maculipennis</i>	<i>A. petragrani</i>	<i>C. annulata</i>	<i>C. longiareolata</i>	<i>C. hortensis</i>	<i>C. impudicus</i>	<i>C. mimeticus</i>	<i>C. modestus</i>	<i>C. pipiens</i>	<i>C. territans</i>	<i>O. caspius</i>
1	N42°03'33,1" W2°01'00,6"	580	LA	19		7									5	
2	N42°02'58,2" W1°55'36,5"	463	MA	12	4	4						17				
3	N42°12'52,6" W2°00'36,1"	425	MA	2							2			17	5	
4	N42°17'03,0" W1°58'36,1"	354	HA	1												
5	N42°17'52,8" W1°58'21,2"	325	HA	2							5				1	
6	N42°11'16,3" W2°17'37,4"	774	MA	8								5		1	6	
7	N42°19'23,2" W2°30'52,2"	569	HA	4												
8	N42°04'30,6" W2°36'16,4"	1236	LA	4												
9	N42°16'24,2" W2°17'29,8"	720	MA	8		3						8		4	7	
10	N42°17'13,6" W2°25'37,9"	717	MA	5			3									
11	N42°26'51,2" W2°18'33,6"	362	HA	4												
12	N42°26'59,9" W2°18'11,3"	344	HA	10								3				
13	N42°11'45,6" W1°44'38,8"	274	MA	3							4		5		4	
14	N42°13'26,5" W2°15'11,0"	937	MA		3		4			9					2	
15	N42°13'23,1" W2°15'12,3"	944	HA							7		3		3		
16	N42°03'41,3" W2°35'42,8"	1229	LA		2	2										
17	N42°04'41,1" W2°40'39,3"	1131	LA		5		2			5				2	3	
18	N42°08'47,6" W2°57'59,7"	978	HA		3	3										
19	N42°14'02,0" W3°02'34,8"	967	HA		14		12			27	3				5	
20	N42°29'24,3" W2°57'48,1"	577	HA		2		5							61		
21	N42°11'16,1" W2°17'37,7"	775	MA			1						11				
22	N42°04'35,9" W2°40'38,7"	1131	LA			2				28					2	
23	N42°00'28,8" W1°56'50,8"	533	MA				2			3	2			7	6	
24	N42°08'50,6" W2°16'12,5"	765	HA				2					3			3	
25	N42°07'19,8" W2°12'20,5"	810	MA				10		1	27						
26	N42°04'48,7" W2°40'40,1"	1413	LA				2					4		2		
27	N42°04'23,4" W2°42'26,4"	1424	LA				1		2	26						
28	N42°04'16,0" W2°41'88,3"	1225	LA				7									
29	N42°24'39,4" W2°44'02,8"	489	HA				6									

Table 2. Continued

Sampling site	Coordinates	Altitude	Habitat type	<i>A. atroparvus</i>	<i>A. claviger</i>	<i>A. maculipennis</i>	<i>A. petragrani</i>	<i>C. annulata</i>	<i>C. longiareolata</i>	<i>C. hortensis</i>	<i>C. impudicus</i>	<i>C. mimeticus</i>	<i>C. modestus</i>	<i>C. pipiens</i>	<i>C. territans</i>	<i>O. caspius</i>
30	N42°14'37,9" W3°02'18,1"	930	HA				2			5						
31	N42°27'09,4" W2°51'44,9"	638	MA					3	9				59	14		63
32	N41°58'26,5" W1°54'08,3"	552	LA						29					117		
33	N42°12'56,6" W2°15'10,9"	860	MA						12	2						
34	N42°16'51,6" W1°58'52,0"	353	HA						13					3		
35	N42°11'39,2" W2°41'47,2"	1080	HA						16					8		
36	N42°12'43,4" W1°46'05,3"	280	MA						3					7		
37	N42°06'28,6" W1°59'55,9"	732	MA						11							
38	N42°34'48,3" W2°50'48,1"	455	HA						8					15		
39	N42°27'04,0" W2°52'03,1"	646	MA						15							
40	N42°28'10,9" W2°26'53,4"	378	HA						17					9		
41	N42°20'10,9" W2°03'57,7"	376	HA						9					14		
42	N42°01'25,6" W2°40'26,0"	1611	LA							26						
43	N42°03'48,7" W2°42'54,3"	1677	LA							2						
44	N42°03'55,6" W2°42'52,8"	1676	LA							13						
45	N42°04'13,5" W2°43'11,2"	1645	LA							8				1		
46	N42°01'16,5" W2°40'16,1"	1631	LA							10				2		
47	N42°02'03,3" W2°41'35,6"	1593	LA							13				2		
48	N42°03'36,4" W2°42'50,2"	1543	LA							4						
49	N42°01'43,3" W2°41'01,1"	1451	LA							7						
50	N42°02'07,8" W2°40'23,2"	1581	LA							3						
51	N42°05'39,7" W2°40'33,0"	1086	LA							10						
52	N42°05'28,6" W2°40'31,9"	1090	LA							13						
53	N42°05'44,4" W2°50'07,9"	1194	HA							9						
54	N42°15'43,9" W2°58'26,2"	1537	HA							40						
55	N42°17'10,1" W2°57'58,5"	1408	HA							18				13		
56	N42°10'41,2" W1°45'35,8"	294	MA							16						
57	N42°30'14,4" W2°40'54,1"	424	HA								11				4	
58	N42°10'41,2" W1°45'35,8"	294	MA								1					

Table 2. Continued

Sampling site	Coordinates	Altitude	Habitat type	<i>A. atroparvus</i>	<i>A. claviger</i>	<i>A. maculipennis</i>	<i>A. petragrani</i>	<i>C. annulata</i>	<i>C. longiareolata</i>	<i>C. hortensis</i>	<i>C. impudicus</i>	<i>C. mimeticus</i>	<i>C. modestus</i>	<i>C. pipiens</i>	<i>C. territans</i>	<i>O. caspius</i>
59	N42°17'02,4" W1°58'35,1"	346	MA								9					
60	N42°30'11,1" W2°40'47,4"	420	HA											35	2	
61	N42°16'04,5" W2°58'16,4"	1492	HA											8		
62	N42°33'18,2" W2°47'15,8"	445	HA											65		
63	N42°26'36,6" W2°30'36,4"	434	HA											6		
64	N42°28'16,4" W2°27'08,7"	376	HA											13		
65	N42°26'35,1" W2°57'37,8"	636	HA											5		
	TOTAL			82	33	22	58	3	145	331	37	54	64	434	55	63

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