

**New anopheline records from the Valencian Autonomous Region of Eastern Spain
(Diptera: Culicidae: Anophelinae)**

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Abstract

Updated information on the distribution of *Anopheles* in the Valencian Autonomous Region of Eastern Spain is presented. Larval surveys over a four year period (2005-2008) detected seven species, all in the subgenus *Anopheles*, namely *Anopheles algeriensis*, *An. atroparvus*, *An. claviger*, *An. maculipennis*, *An. marteri*, *An. petragrani* and *An. plumbeus*. Distribution, biology and ecology of each are discussed and vectorial potential is considered against the current growing incidence of imported malaria in Spain.

Key words: *Anopheles*, malaria, mosquitoes, distribution, Spain.

Introduction

Malaria was highly endemic in Spain until about the middle of the twentieth century (Pletsch, 1965). Since then, faunistic studies of mosquitoes have been scanty and limited to certain, mainly central, inland provinces (García Calder-Smith, 1965; Encinas Grandes, 1982; Sánchez Covisa, 1985; Báez, 1987; López Sánchez, 1989; Melero-Alcibar 2006a, 2006b; Bueno Marí *et al.*, 2008). The aim of this work was to update knowledge of the *Anopheles* present in the Valencian Autonomous Region (comprising the Provinces of Castellón, Valencia, and Alicante) by examining the distribution and types of aquatic developmental site exploited by each species. This information is necessary for assessment of the potential for renewed malaria transmission in the context of the current increasing incidence of imported malaria in Spain (Dirección General de Salud Pública, 2010).

Materials and Methods

Our area of study was the Valencian Autonomous Region of Eastern Spain (Figure 1). Parts of this area were formerly highly endemic for malaria, but existing data referring to its anopheline fauna are few and old (Table 1). Using simple random sampling during a four year period (2005-2008), we collected larvae using the dipping method of Service (1993). Data were collected from a wide variety of aquatic sites throughout the 23,260 km² study area. Mosquitoes were identified according to the keys of Encinas Grandes (1982), Darsie & Saminadou Voyadjoglou (1997) and Schaffner *et al.* (2001).

Results

A total of seven anopheline species were identified:

***Anopheles algeriensis* Theobald, 1903:** larvae of this species were common in the south of the study area (Figure 2). The tolerance of this species to brackish water (Becker *et al.*, 2003) was corroborated by the finding of larvae in coastal marshes (9.4 ‰ salinity) in coexistence with other halophilic species including *Ochlerotatus caspius* (Pallas, 1771). *Anopheles algeriensis* larvae were also found in inland freshwater sites up to 667 meters altitude. Despite not being regarded as a primary malaria vector, it is important to note that Horsfall (1955) found *An. algeriensis* females with *Plasmodium* oocysts in Algeria. Furthermore, the species can transmit *Plasmodium falciparum* under laboratory conditions (Becker *et al.*, 2003). Though it has been recorded in Britain, France and Germany (Becker *et al.*, 2003), our catches represent the northernmost records of the species in the Iberian Peninsula.

***Anopheles atroparvus* Van Thiel, 1927:** this species was the main malaria vector in Spain. In our collections it was frequently found in small lagoons, temporary puddles, irrigation canals and river margins (Figure 2). Although we did not find *An. atroparvus* in rice fields, this species is common in the rice fields of Southern Spain (López Sánchez, 1989; Ruiz & Cáceres, 2004). It has been shown that European populations of this species are capable of transmitting *Plasmodium vivax* but are refractory to tropical Asian and African strains of *P. falciparum* (James *et al.*, 1932; Shute, 1940; Shute & Maryon, 1974; Ramsdale & Coluzzi, 1975; de Zulueta *et al.*, 1975; Daskova & Rasnitsyn 1982; Ribeiro *et al.*, 1989).

It is important to note that *An. atroparvus* is suspected of being the vector of an autochthonous case of *Plasmodium ovale* which occurred in Central Spain, although airport malaria cannot be discarded due to the proximity of the patient's residence to two international airports (Cuadros *et al.*, 2002). We should keep in mind the possible future emergence of strains of exotic plasmodia capable of developing in Spanish mosquitoes (Bueno Marí & Jiménez Peydró, 2010).

***Anopheles claviger* (Meigen, 1804):** larval sites of this species were found only at altitudes between 455-849 meters (Figure 2), which agrees with an orophilic tendency described from other places within the distribution of this species (Schaffner *et al.*, 2001; Bueno Marí *et al.*, 2009a). Autogeny, eurigamy, exophagy and zoophily have all been reported in this species (Markovic, 1941; Coluzzi, 1962; Encinas Grandes, 1982). It will readily feed on man, and has been shown to transmit malaria in some Eastern Mediterranean towns, where it is an urban mosquito breeding in the cool water stored in urban reservoirs (Gramiccia, 1956; Russel *et al.*, 1963; Coluzzi *et al.*, 1964; Muir & Keilany, 1972).

***Anopheles maculipennis* Meigen 1818:** the aquatic stages were always found in fresh or slightly brackish water in inland mountainous regions away from anthropised environments (Figure 2). These aspects indicate a non-vector role, as in much of continental Europe (Bueno Marí & Jiménez Peydró, 2008). However, where it occurs in coastal areas in the Balkans, Asia Minor and Northern Iran, it is a well known malaria vector (Postiglione *et al.*, 1973; Zaim, 1987; Manouchehri *et al.*, 1992; Schaffner *et al.*, 2001).

***Anopheles marteri* Sévenet & Prunelle, 1927:** this is the first report of *An. marteri* in the study area. The scanty data regarding the distribution of the species in Spain refer to a few catches in several mountainous regions of the southern half of the country (Torres Cañamares, 1945; Romeo Viamonte, 1950). We collected several larvae near the source of the river Palancia at 812 meters altitude (Figure 2). This zoophilic species usually breeds in wild areas of low anthropisation (Aitken, 1954). Consequently is not considered an important malaria vector.

***Anopheles petragani* Del Vecchio, 1939:** this was the most abundant anopheline in our study area (Figure 2). Larvae were found in a great diversity of environments (e.g. rivers, temporary puddles, natural fountains, reservoirs) at altitudes between 127-1155 meters. We also collected larvae during the month of February with a water temperature of 5.5°C. This finding confirms that this species overwinters in the larval stages as reported by Schaffner *et al.* (2001). *Anopheles petragani* is autogenous, stenogamous, exophagic and usually zoophilic (Coluzzi, 1962; Lachmajer, 1971), although man may be bitten in the surroundings of their larval biotopes (Encinas Grandes, 1982). This is the first documented report of the species in the Valencian Autonomous Region.

***Anopheles plumbeus* Stephens, 1828:** is the only strictly dendrolimnic species of the genus *Anopheles* in Europe. We usually found *An. plumbeus* on white poplar (*Populus alba* L.) in cohabitation with other tree hole species such as *Ochlerotatus echinus* Edwards, 1920, *Oc. geniculatus* (Olivier, 1791), *Oc. gillcolladoi* (Sánchez Covisa, Rodríguez & Guillén, 1985), *Oc. berlandi* (Séguy, 1921), *Oc. pulcritarsis* (Rondani, 1872) and *Orthopodomyia pulcripalpis* (Rondani, 1872). The most striking larval site was a small plastic bag containing water, and near several tree hole breeding sites in a wooded area (Figure 2). This is the first time the presence of larvae in a biotope different from the tree cavity is reported in Spain, although there are several reports in foreign literature (Aitken, 1954; Senevet *et al.*, 1955; Rioux, 1958; Tovornik, 1978). The females feed at any time of the day, even in daylight, biting humans with persistence and aggressiveness in urban as well as forested areas (Shute, 1954). The species also feeds on domestic ungulates and birds (Service, 1971). Due to its restricted distribution it is a sporadic malaria vector. Nevertheless it is suspected of responsibility for several episodes of malaria in England (Blacklock, 1921; Shute, 1954) and Germany (Krillger *et al.*, 2001) and laboratory studies have shown it to be capable of transmitting *P. falciparum* (Marchant *et al.*, 1998).

Discussion

Our data indicate a relatively low malariogenic potential for the Valencian Autonomous Region, thus supporting the theses of other authors for the whole country (López Vélez & Molina Moreno, 2005). Though current socio-economic conditions in Spain reduce possibilities of re-emergence of malaria transmission (Bueno Marí *et al.*, 2009b), vigilance must be maintained. With the exception of the markedly zoophilic *An. marteri* and *An. petragani*, the anophelines caught in this study are capable of initiating greater or lesser degrees of malaria transmission. However, though *An. atroparvus* is considered the most dangerous vector species, the fact that all its detected larval sites were in areas remote from centers of human population limits their significance. As indicated before, *An. atroparvus* was not found in rice fields of the study area, where it is well known that the species was common in the past (Romeo Viamonte, 1950; Pérez Moreda, 1982; Mateu, 1987). It is suggested that the high eutrophication (due to massive employment of fertilizer with

nitrogen compounds) and the presence of large residual amounts of various insecticides in Valencian rice fields (Mendoza, 2002; Tarazona *et al.*, 2003) are two possible drawbacks to the larval development of *An. atroparvus*. Moreover, potential biotopes of *An. atroparvus* surrounding rice fields were also drastically modified in the last 50 years. Most of the irrigation channels have been destroyed by the strong urban development around these crops and the scanty waters that are currently present are deeply colonized by eastern mosquito fish - *Gambusia holbrooki* (Girard, 1859), which was introduced for the fight against malaria in 1921.

There are several discrepancies with regard to species found in the past and in our sampling. The most important western Mediterranean malaria vector, *Anopheles labranchiae* Falleroni, 1926 was found to be abundant in a restricted area of the contiguous Alicante and Murcia Provinces in 1946 (Clavero & Romeo Viamonte, 1948), but had disappeared by 1973 (Blázquez & de Zulueta, 1980) due to abandonment of rice cultivation in this area (Eritja *et al.*, 2000). This was the only area where this species has been able to establish itself in the Iberian Peninsula (Blázquez & de Zulueta, 1980). Though abundant along the African coastline between Ceuta and Tangiers, *An. labranchiae* has been unable to obtain a toe-hold in 15 km distant coastal plains of southern Spain, where rice fields support large populations of *An. atroparvus* (Ramsdale & Snow, 2000).

The absence from our collections of *Anopheles hyrcanus* (Pallas, 1771), an important potential vector of malaria in rice growing areas of southern France (Ponçon *et al.*, 2007), is not surprising, since our only record dates from 1911, when Professor Pittaluga captured several specimens in the rice fields near L'Albufera de Valencia (Gil Collado, 1930). The lack of records of since then, together with the limited knowledge of the systematics of the genus at that time, leads us to regard the presence of *An. hyrcanus* in the study area as highly dubious.

There are old records of *Anopheles cinereus* Theobald, 1901 and *Anopheles melanoon* Hackett, 1934, but neither was detected in our surveys. Though both may be regarded as secondary vectors the finding of infective specimens is exceptional (Schaffner *et al.*, 2001). Old records of *An. cinereus* were related to irrigation canals in the south of the study area. *An. melanoon* was recorded in rice fields and marshes scattered throughout the coastal territory. These larval biotopes are precisely those which have suffered higher degrees of modification, so that human pressure on these coastal wetlands may have caused the limitation or disappearance of both species.

We may conclude that a combination of incidental human activities, active intervention by the National Epidemiological Surveillance Network and awareness of the human population all contribute to minimizing risks of a return of vector-borne disease and the present situation can be described as what malariologists of the first half of the last century would have called "anophelism without malaria".

The presence, distribution, behaviour and abundance of *Anopheles* species must be further analyzed and researched in order to deepen knowledge of the vector potential of our anopheline fauna, and allow stratification of the province according to epidemiological risk.

Acknowledgments

We are also grateful to the Regional Ministry of the Generalitat Valenciana (*Conselleria de Medi Ambient, Aigua, Urbanisme i Habitatge*) for granting permission to collect insects in the protected enclaves. We wish to acknowledge that work reported in this article was partially funded by the Research Project CGL 2009-11364 (BOS), supported by the Ministry of Science and Innovation of Spain (*Ministerio de Ciencia e Innovación del Gobierno de España*).

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Table 1. Species of *Anopheles* reported in Valencian Autonomous Region until 2005 with indication of province and locality (Gil Collado, 1930; Clavero, 1946; Romeo Viamonte, 1950; Encinas Grandes, 1982). ¹ Reported as *An. labranchiae atroparvus*. ² Reports of *An. melanoon subalpinus* included.

Species	Localities reported of Castellón province	Localities reported of Valencia province	Localities reported of Alicante province
<i>An. algeriensis</i>	-	-	Alicante
<i>An. atroparvus</i> ¹	Unspecified localities	Unspecified localities	Elche
<i>An. cinereus hispaniola</i>	-	-	Alicante, Cox, Orihuela
<i>An. claviger</i>	El Grao de Castellón	-	-
<i>An. hyrcanus</i>	-	Valencia (La Albufera)	-
<i>An. labranchiae</i>	-	-	Cox, Elche, Guardamar, San Felipe Neri, San Fulgencio, Orihuela
<i>An. maculipennis s.l.</i>	Almenara, El Grao de Castellón, Las Llosas	Alfajar, Carcagente, Catarroja, Cullera, El Grao, El Palmar, Gandía, Perelló, Silla, Sollana, Sueca, Tabernes de la Valldigna	San Fulgencio
<i>An. melanoon</i> ²	Castellón, Peñíscola	Oliva, Saler, Villanueva de Castellón	Orba, Pego
<i>An. plumbeus</i>	-	Utiel	-

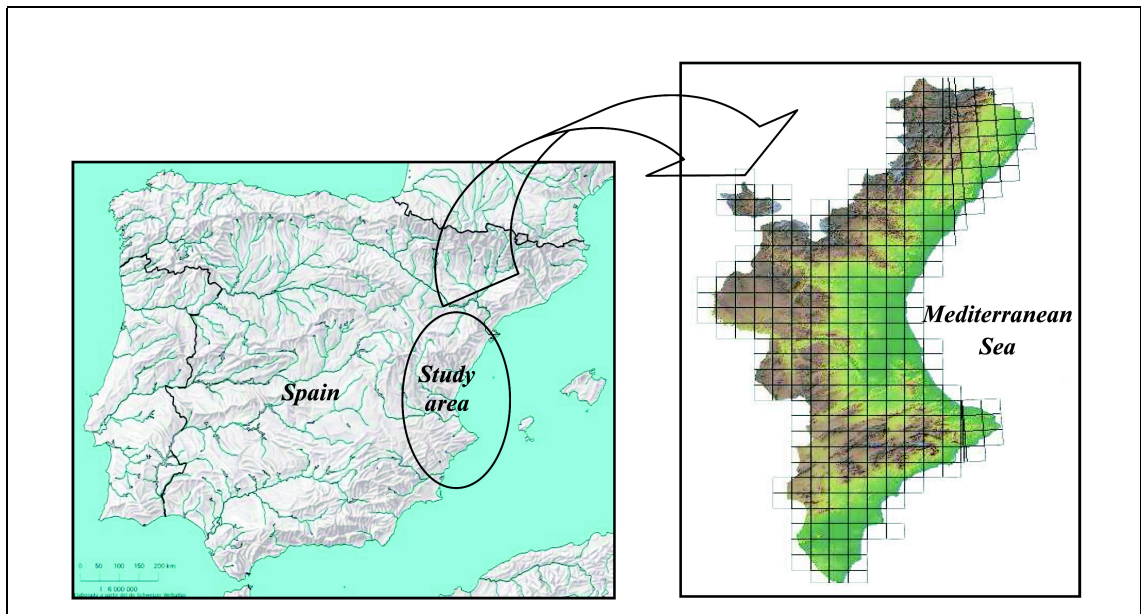


Figure 1. Situation of the study area.

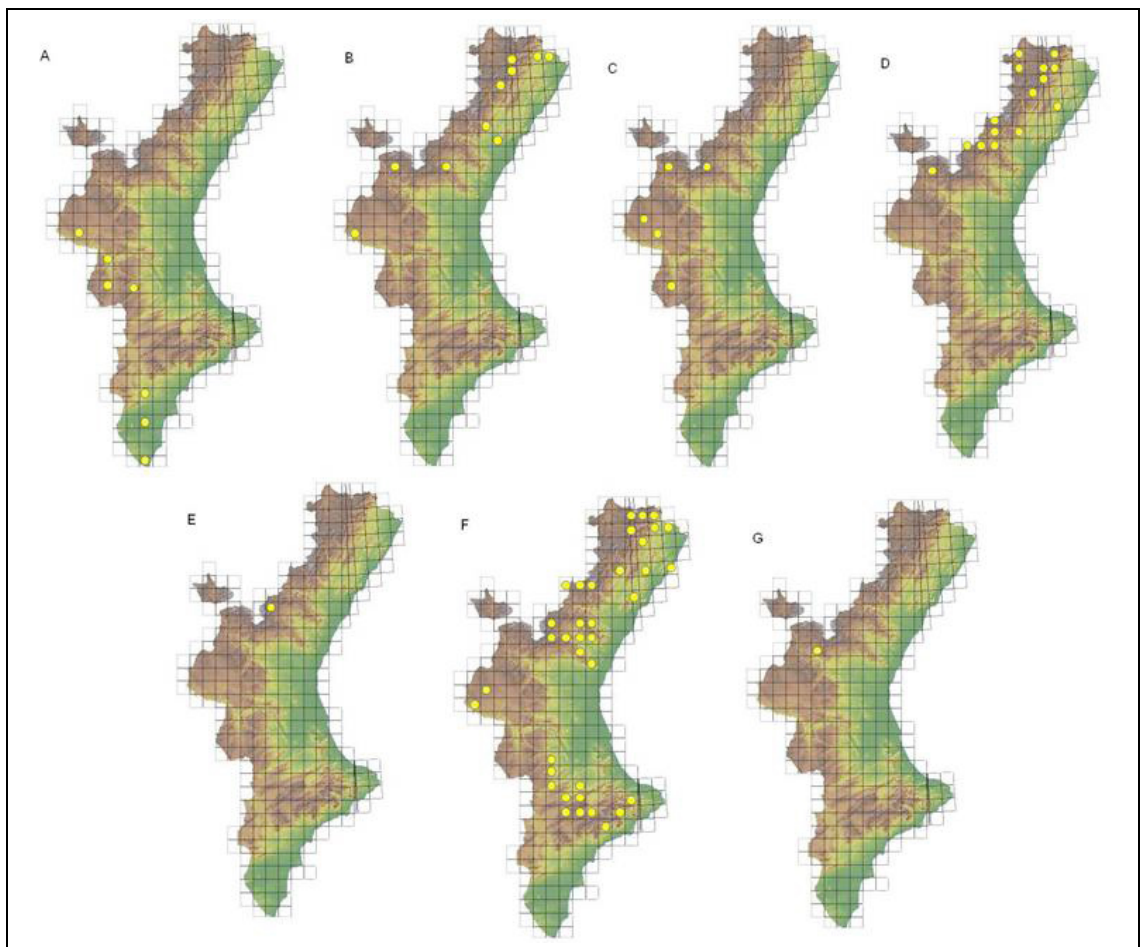


Figure 2. Distribution of the anophelines caught. (A): *An. algeriensis*, (B): *An. atroparvus*, (C): *An. claviger*, (D): *An. maculipennis*, (E): *An. marteri*, (F): *An. petragrani*, (G): *An. plumbeus*.