New anopheline records from the Valencian Autonomous Region of Eastern Spain
(Diptera: Culicidae: Anophelinae)
Rubén Bueno-Mari and Ricardo Jiménez-Peydró
Entomology and Pest Control Laboratory, Cavanilles Institute of Biodiversity and
Evolutionary Biology, University of Valencia (Spain).
E-mail: ruben.bueno@uv.es

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
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; Sanchez Covisa, 1985; Báez, 1987; López Sánchez, 1989; Melero-
Alcibar 2006a, 2006b; Bueno Mari 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
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 atroparvs 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. atroparvs 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 & Rasmicyn 1982; Ribeiro et al., 1989).

It is important to note that An. atroparvs 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 Mari & 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 Mari 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 Mari & 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 referring to the distribution of the species in Spain refer to a few catches in several mountainous regions of the southern half of the country (Torres Cañamases, 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 petragnani** 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 petragnani* 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. gilcolladoi* (Sánchez Covisa, Rodriguez & 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 (Krüger 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 malarigenic 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 Mari et al., 2009b), vigilance must be maintained. With the exception of the markedly zoophilic *An. marteri* and *An. petragnani*, 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).

References


### 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Localities reported of Castellón province</th>
<th>Localities reported of Valencia province</th>
<th>Localities reported of Alicante province</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. algeriensis</em></td>
<td>-</td>
<td>-</td>
<td>Alicante</td>
</tr>
<tr>
<td><em>An. atroparvus</em> 1</td>
<td>Unspecified localities</td>
<td>Unspecified localities</td>
<td>Elche</td>
</tr>
<tr>
<td><em>An. cinereus hispaniola</em></td>
<td>-</td>
<td>-</td>
<td>Alicante, Cox, Orihuela</td>
</tr>
<tr>
<td><em>An. claviger</em></td>
<td>El Grao de Castellón</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>An. hyrcanus</em></td>
<td>-</td>
<td>Valencia (La Albufera)</td>
<td></td>
</tr>
<tr>
<td><em>An. labranchiae</em></td>
<td>-</td>
<td>-</td>
<td>Cox, Elche, Guardamar, San Felipe Neri, San Fulgencio, Orihuela</td>
</tr>
<tr>
<td><em>An. maculipennis</em> s.l.</td>
<td>Almenara, El Grao de Castellón, Las Llosas</td>
<td>Alfafar, Carcagente, Catarroja, Cullera, El Grao, El Palmar, Gandia, Perelló, Silla, Sollana, Sueca, Tabernes de la Valldigna</td>
<td>San Fulgencio</td>
</tr>
<tr>
<td><em>An. melanoon</em> 2</td>
<td>Castellón, Peñíscola</td>
<td>Oliva, Saler, Villanueva de Castellón</td>
<td>Orba, Pego</td>
</tr>
<tr>
<td><em>An. plumbeus</em></td>
<td>-</td>
<td>-</td>
<td>Utiel</td>
</tr>
</tbody>
</table>
Figure 1. Situation of the study area.