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Distribution and larval habitats of Anophelinae (Diptera, Culicidae) in aquatic systems of the Valencian Autonomous Region (Spain)

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ABSTRACT

Distribution and larval habitats of Anophelinae (Diptera, Culicidae) in aquatic systems of the Valencian Autonomous Region (Spain).

The current and increasing problem of imported cases of Culicidae-transmitted diseases in Europe, together with the increasingly recurrent appearance of autochthonous cases of arboviruses in temperate latitudes and the history of areas previously endemic for them, have led to a great recovery in the research on these dipterans. The present study focuses on anopheline mosquitoes from Eastern Spain, particularly from the Valencian Autonomous Region, which is a formerly endemic area of malaria. The geographical distribution as well as tolerance ranges to abiotic factors such as water temperature, salinity, pH and the altitude of the aquatic habitats occupied by their preimaginal stages are presented for the seven identified mosquito species of the genus *Anopheles* and subgenus *Anopheles*: *Anopheles algeriensis* Theobald, 1903; *Anopheles atroparvus* Van Thiel, 1927; *Anopheles claviger* (Meigen, 1804); *Anopheles maculipennis s.s.* Meigen, 1818; *Anopheles marteri* Senevet & Prunnelle, 1927; *Anopheles petragnani* Del Vecchio 1939 and *Anopheles plumbeus* Stephens, 1828. In this way, we have updated the knowledge on these species, which are considered potential vectors of malaria and other pathogens that cause disease at a local level in the studied area. Therefore, new insights of great consequence for the public health, epidemiology, entomology, ecology and pest control sectors are provided, which should be taken into consideration in surveillance and control programmes.

KEY WORDS: Anopheline mosquitoes, abiotic factors, aquatic habitats, species diversity, geographical distribution, malariogenic potential, Eastern Spain.

RESUMEN

Distribución y hábitats larvarios de Anophelinae (Diptera, Culicidae) en sistemas acuáticos de la Comunidad Valenciana (España).

La actual y creciente problemática de casos importados de enfermedades transmitidas por Culicidae en Europa, junto con la aparición cada vez más recurrente de casos autóctonos de arbovirosis en latitudes templadas y los antecedentes de zonas anteriormente endémicas para las mismas, han llevado a una gran recuperación en la investigación de estos dípteros. El presente estudio se centra en los mosquitos anofelinos en el este de España, concretamente en la Comunidad Valenciana, la cual fue un área de malaria endémica en el pasado. Se presentan los datos referentes a la distribución geográfica, así como los rangos de tolerancia a factores abióticos tales como la temperatura del agua, la salinidad de la misma, su pH y la altitud a la que se encuentran los hábitats acuáticos ocupados por los estados preimaginales *de las siete especies identificadas de mosquitos del género* Anopheles *y subgénero* Anopheles*:* Anopheles algeriensis *Theobald, 1903;* Anopheles atroparvus *Van Thiel, 1927;* Anopheles claviger *(Meigen, 1804);* Anopheles maculipennis s.s. *Meigen, 1818;* Anopheles marteri *Senevet & Prunnelle, 1927;* Anopheles petragnani *Del Vecchio 1939 y* Anopheles plumbeus *Stephens, 1828. De esta manera nosotros hemos actualizado el conocimiento sobre estas especies, las cuales son consideradas potencialmente vectoras de malaria y de otros patógenos causantes de enfermedad a nivel local en la zona estudiada. Por tanto, se proporcionan nuevos datos de gran repercusión para los sectores de la salud pública, la epidemiología, la entomología, la ecología y el control de plagas a tener en cuenta en programas de vigilancia y control..*

PALABRAS CLAVE: *Mosquitos anofelinos, factores abióticos, hábitats acuáticos, diversidad de especies, distribución geográfica, potencial malariogénico, Este de España.*

INTRODUCTION

The study of anopheline mosquitoes (Diptera: Culicidae) in Spain has been addressed deeply and thoroughly, mainly due to the historical existence of endemic zones of malaria in the country (Bueno Marí & Jiménez Peydró, 2008), as occurred in the Valencian Autonomous Region during the 18th and 20th centuries (Bueno Marí & Jiménez Peydró, 2010a). It is critically important to highlight that certain species of the *Anopheles* genus can act as carrier agents for disease-causing agents of an endoparasitic nature, such as protists and nematodes, and even those of a viral type.

Malaria is a disease caused by several apicomplexan protist species of the genus *Plasmodium*, which belongs to the family Plasmodiidae, and is transmitted to humans through the bite of female mosquitoes of the genus *Anopheles*. In Spain, the main transmitter of malaria is the species *Anopheles atroparvus* Van Thiel, 1927 (Eritja et al., 2000, Bueno-Marí et al., 2012). In addition, the species *Anopheles maculipennis s.s.* Meigen, 1818 can act as a secondary vector in certain areas and under specific conditions; likewise, *Anopheles claviger* (Meigen, 1804) also has the potential to act as a secondary vector of this parasitic disease (Jiménez Peydró et al., 2023). Besides this, Taheri et al. (2024), modelling the spatial risk of malaria transmission in Spain, have identified the Mediterranean basin, and particularly some parts of the Valencian Autonomous Region, as potential high-risk areas of malaria transmission.

Regarding nematodial agents, filariasis stands out. In fact, in the coastal marsh areas of the Valencian region, the species *An. atroparvus* and *An. claviger*, due to their marked anthropophilia, can play a secondary role in the transmission of heartworms, keeping it enzootic among the natural reservoirs of the disease and rapidly transmitting it to humans (Jiménez Peydró et al., 2023).

On the other hand, among the mosquito-borne diseases of viral origin, the Batai, Tahyna and West Nile viruses are notable. The first of them can be transmitted to humans mainly by the species *An. maculipennis s.s.*, and to a lesser extent by *An. claviger*. In any case, this host is rarely part of the transmission cycle (Traavik et al., 1985, Francy et al., 1989). The second can be transmitted to humans through the species *An. maculipennis* (Lundström, 1999). Specifically, in the interior mountain ranges of the Valencian natural parks, *An. maculipennis s.s.* maintains the Tahyna virus as enzootic, while, due to its anthropophilic tendency, *An. atroparvus* has the capacity to act as a possible bridge of transmission between humans and natural reservoirs (Jiménez Peydró et al., 2023). Finally, West Nile virus, a zoonotic virus with birds as its primary host (Eldridge et al., 2000), can be accidentally transmitted to humans by *An. atroparvus* due to its abovementioned anthropophilia, as well as to various species of equids, both behaving as secondary or accidental hosts (Mouchet et al., 1970). Consequently, this mosquito species can act as a bridge for the transmission of this virus between birds and humans (Bueno Marí & Jiménez Peydró, 2010b). Therefore, and as already indicated by Jiménez Peydró et al. (2023), West Nile virus may constitute one of the arboviruses with the greatest potential for the initiation and maintenance of transmission cycles in the inland mountains of Valencia, where

An. atroparvus can act as a secondary vector.

The inland mountain ranges of the Valencian Autonomous Region thus represent the most likely areas for possible epidemic outbreaks of the aforementioned diseases transmitted by these mosquito species, specifically during times in which their population levels are high (Bernués Bañeres, 2013).

Thus, the main aim of the Laboratory of Entomology and Pest Control's (LEPC) has been to update and increase the knowledge on the *Anopheles* species present throughout the three provinces of the Valencian Autonomous Region (Castellón, Valencia and Alicante), as well as to study their ecology. The focus has always been on understanding the existing connections between the immature mosquito habitats with the local environmental parameters that determine their occurrence.

METHODOLOGY

Area of study: orography and climatology (precipitation, atmospheric humidity, and temperature)

The orography of the Valencian Autonomous Region is mainly characterised by inland mountainous areas and extensive plains close to the littoral zone. Due to this geographical configuration, the region presents a complex network of wetlands along the entire coastline. This geography influences the climatology of this Spanish region, which is distinguished by the presence of irregular rainfall, with maximums in spring and autumn that occasionally occur with a torrential nature during the months of September and October; mild and dry winters; and a harsh and long-lasting summer drought (GVA). This high degree of aridity is reflected in the variation in the rainfall range, which fluctuates between 1000 mm in the rainiest mountain areas to just 250 mm of annual precipitation in the territory of the province of Alicante (GVA). In line with the rainfall, humidity plays an important role in this area, with a humid Mediterranean regime present in the northern regions of the province of Castellón, the central and southern regions of the province of Valencia, as well as the northeastern parts of the province of Alicante, while a dry Mediterranean regime occurs in the rest of the Valencian region (GVA). Furthermore, the temperature also contributes to this situation, ranging between 11 and 17 ºC on an annual average; however, this increases progressively from the inland areas towards the coastal zones, reaching averages between 17 and 19 ºC (GVA). Nonetheless, the recorded thermal amplitude reveals minimums of -2 ºC in the mountainous areas of the interior and between 0 and 15 ºC with increasing proximity to the coast, as well as maximums of 28 ºC in these areas and lower temperatures in the interior of the territory (GVA).

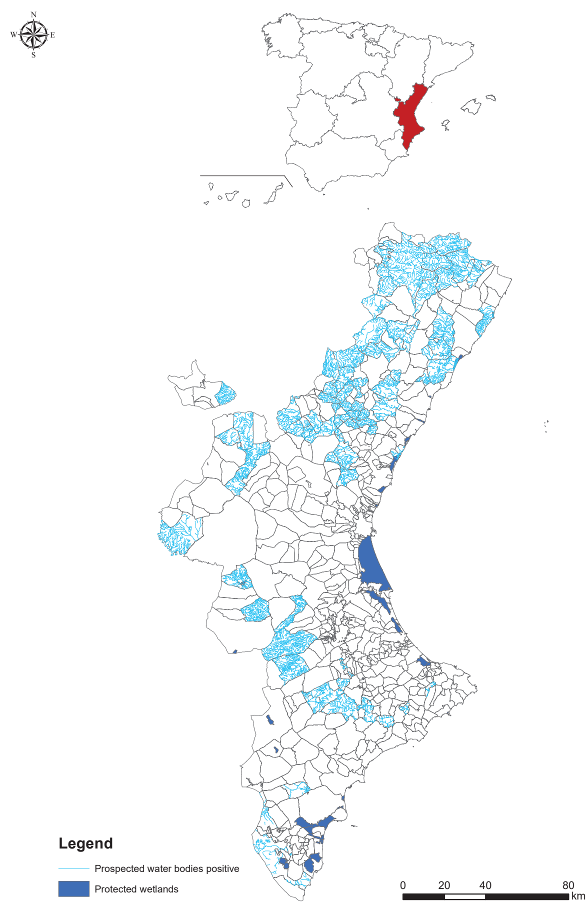

Figure 1. Geographical location of the Valencian Autonomous Region in the Spanish territory (upper side). The areas coloured in blue indicate the position of the prospected water bodies positive for the presence of anopheline mosquito species (bottom side). *Situación geográfica de la Comunidad Valenciana en el territorio español (lado superior). Las áreas coloreadas en azul indican la posición de los cuerpos de agua prospectados positivos para la presencia de especies de mosquitos anofelinos (lado inferior).*

Sample collection techniques, sampling areas and specimen identification

In the abovementioned region of Spain, the LEPC has been carrying out mosquito sampling for twelve years in urban, peri-urban and wild areas, always focusing on the detection and study of immature stages of development in their breeding habitats using both the 'dipping' technique (Service, 1993) and through collection of water samples with field pipettes, conditioned by the characteristics of the breeding sites. Therefore, an area of 23 254 km^2 (IGN) – or 23 271 km^2 if considering the regional government (GVA), which is divided into 5823 km^2 in Alicante province, 10 813 km^2 in Valencia province and 6635 km^2 in Castellón province (GVA) – was studied by prospecting each water space susceptible to harbouring species of the genus *Anopheles* (Fig. 1). Each sampling effort took approximately 10 minutes, enabling standardisation of the sampling methodology.

Thus, and as the larval developmental sites of the anopheline species comprise a wide variety of biotopes, mainly characterised by clean and fresh stagnant and/or lotic waters, aquatic developmental breeding sites such as inland and seaside wetlands, marshes, coastal lagoons, puddles, ponds, pools of temporary rivers, rivulets, streams, river margins, tree holes (dendrotelmas), irrigation channels and artificial water containers were studied (Fig. 2).

The random sampling was conducted from February to November in two different periods: from 2005 to 2011 and from 2017 to 2021. Researchers travelled to each of the 542 municipalities that make up the provinces of Alicante (141), Castellón (135) and Valencia (266) in an all-terrain vehicle, surveying all those places capable of hosting favourable characteristics for settlement and life-cycle development of *Anopheles* mosquitoes, followed by an active dipping search of the water bodies to be prospected. Once the presence of anophelines was detected, the specimens were collected, stored in Duchess-type plastic jars and placed inside refrigerated coolers until their arrival at the laboratory facilities. At the same time, the physical-chemical variables, such as the water temperature (ºC), water pH (potential of Hydrogen) and water salinity (g/L), were measured in situ with a portable multi-parameter probe (Consort-C535). Likewise, the elevation (m a.s.l.) and the Universal Transverse Mercator (UTM) coordinates were obtained using a portable Global Positioning System (GPS) device (Garmin 12/datum WGS 84). This and other relevant information for each sampling station, like the name of the municipality, the province and the date of collection, were recorded in the field notebook (Table S1, supplementary information, available at https://www.limnetica.net/en/limnetica). Consequently, each preimaginal biotope was registered in order to elucidate nuances and differences in the species levels of tolerance for the abiotic factors.

Once in the laboratory, the mature larvae were selected and treated for conservation. The live

Figure 2. Types of aquatic habitats prospected. *Tipos de hábitats acuáticos prospectados.*

immature larvae were kept in laboratory conditions until reaching the mature stage for processing, then preserved in 80% ethanol and identified by means of morphological-based identification keys (Encinas Grandes, 1982, Schaffner et al., 2001, Becker et al., 2010) under a stereomicroscope (Leica MZ APO) with a cool light (Leica CLS100X, Germany). After the identification of the larvae, the samples were stored in the entomological collection of the Institut Cavanilles de Biodiversitat i Biologia Evolutiva (ICBiBE) at the Universitat de València (Estudi General).

Figure creation

The geographic distribution maps of each of the identified species in the area of study, as well as the figure of the municipalities with detected presence of species belonging to the genus *Anopheles*, were generated using the Geographic Information System (GIS) software ArcMapTM 10.5 in ESRI's ArcGIS® (Redlands, CA, USA).

RESULTS

Species documented, and their distribution

Seven Anophelinae species were identified from the Valencian territory: *Anopheles algeriensis* Theobald, 1903; *Anopheles atroparvus*; *Anopheles claviger*; *Anopheles maculipennis s.s.*; *Anopheles marteri* Senevet & Prunnelle, 1927; *Anopheles petragnani* Del Vecchio 1939 and *Anopheles plumbeus* Stephens, 1828. As a result, new data about their geographic distribution and their location from sea level to high altitudes are reported (Fig. 3 and Table S2 (supplementary information, available at https://www.limnetica.net/en/limnetica)). The first species, *An. algeriensis*, was found in 5 municipalities (3 of Valencia and 2 of Alicante provinces), *An. atroparvus* in 14 municipalities (11 of Castellón province and 3 of Valencia province), *An. claviger* in 11 municipalities (6 of Castellón province and 5 of Valencia province), *An. maculipennis s.s.* in 32 municipalities (29 of Castellón province and 3 of Valencia province), *An. marteri* in 3 municipalities (2 of Castellón province and 1 of Valencia province), *An. petragnani* in 56 municipalities (36 of Castellón province, 12 of Valencia province and 8 of Alicante province) and *An. plumbeus* in 2 municipalities (1 of Castellón province and 1 of Valencia province), the last two being the species with the widest and narrowest distributions, respectively. It is worth emphasising that only *An. petragnani* is distributed throughout the three provinces that make up the Valencian region, whilst the other six species were each detected in two provinces. Apart from this, the results revealed that the province with the highest presence of anopheline species was Castellón, with samples collected from 61 municipalities, followed by Valencia province, with samples from 16 municipalities, and finally Alicante province, with samples from 10 municipalities. Moreover, the insights obtained indicate that most of the anopheline discoveries took place in the inland part of the study area, followed by the pre-littoral zone, while the littoral zone had the lowest presence of mosquitoes of this genus (Fig. 3).

Species relative abundance

Over the twelve years of our study, the LEPC visited a large number of municipalities throughout the Valencian Autonomous Region, which led to a number of records that varied from one species to another (Table S3, supplementary information, available at https://www.limnetica.net/en/limnetica). The species that was found most frequently is *An. petragnani*, with 127 records in 56 municipalities spread throughout the study area, and with a medium average population density. This is undoubtedly the most common species in the region, as it has twice as many records as *An. maculipennis s.s.*, the species with the second-highest number; 55 records in 32 municipalities. On the other hand, the species with the lowest number of records is *An. plumbeus*, with 2 records and a medium average population density, followed by *An. algeriensis* and *An. marteri*, both with 6 records and with a low average population densities. It should be noted that *An. atroparvus* is the species with the lowest average population density, which is important due to its malariogenic potential, while the species with the highest average population densities are *An. petragnani* and *An. plumbeus*.

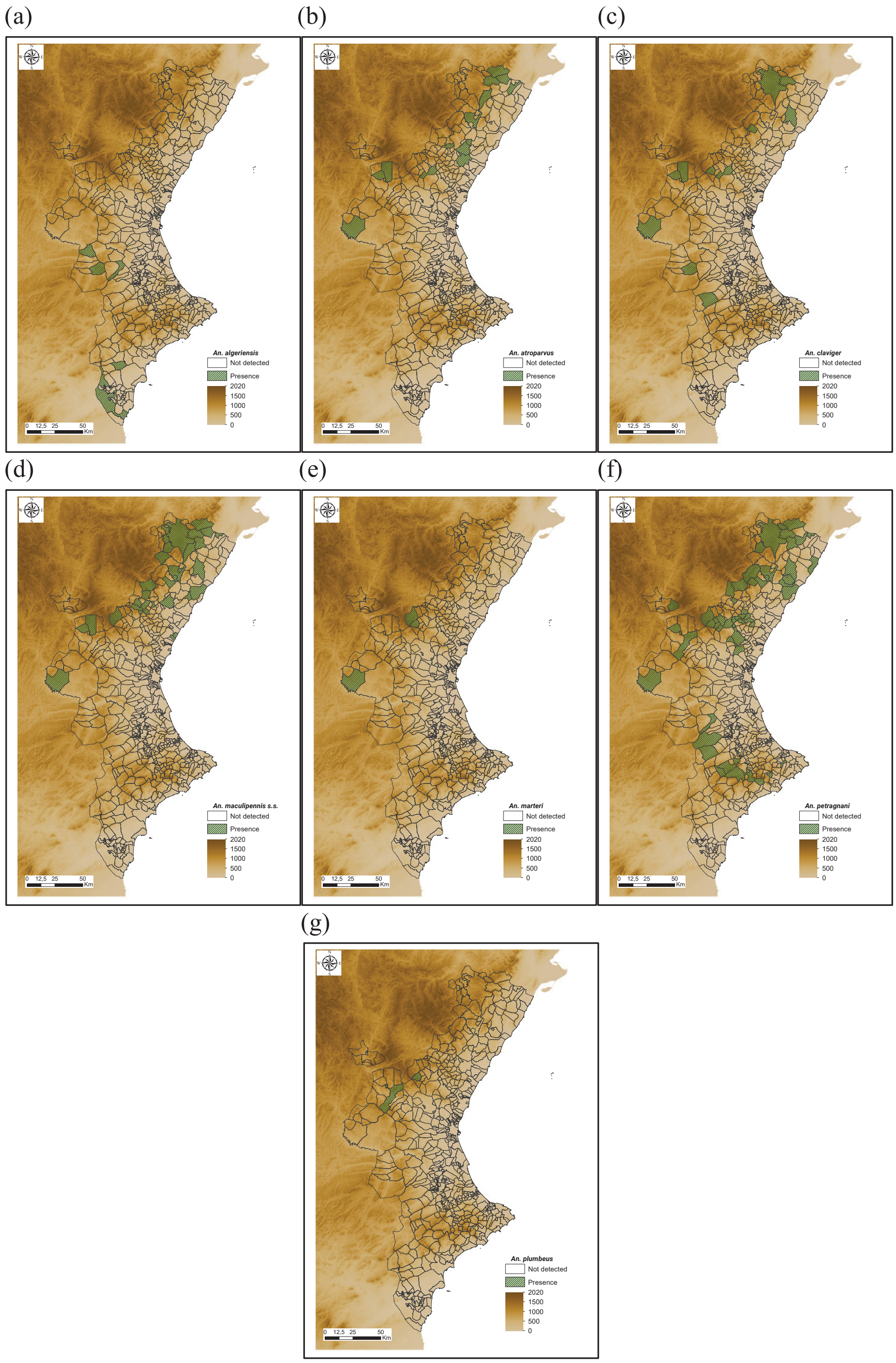

Figure 3. Municipal distribution maps of the 7 species recorded from the area of study: (a) *An. algeriensis*, (b) *An. atroparvus*, (c) *An. claviger*, (d) *An. maculipennis s.s.*, (e) *An. marteri*, (f) *An. petragnani*, (g) *An. plumbeus*. The altitudinal profile of the territory, in brown colour scale, is also shown. *Mapas de distribución provincial de las 7 especies registradas en el área de estudio: (a)* An. algeriensis*, (b)* An. atroparvus*, (c)* An. claviger*, (d)* An. maculipennis s.s.*, (e)* An. marteri*, (f)* An. petragnani*, (g)* An. plumbeus*. También se representa el perfil altitudinal del territorio, en escala de color marrón.*

Elevation and water temperature, salinity and pH ranges observed for the species recorded for the Valencian Autonomous Region

Elevation is a factor that is known to influence many living organisms, as is the case in mosquitoes, whose biodiversity it affects. In fact, the increase of this abiotic factor usually results in a reduction of species diversity due to the extreme weather conditions and, in the particular case of mosquitoes, the reduced abundance and diversity of their main hosts. Therefore, with respect to the elevation (Fig. 3), the species *An. petragnani* – reported in the provinces of Castellón, Valencia and Alicante – shows the widest range, between 122 m and 1472 m, while the species *An. marteri* – recorded for the provinces of Castellón and Valencia – has the narrowest, ranging from 673 m to 883 m. The rest of the species revealed varied elevation ranges; for instance, the species *An. algeriensis*, described for the provinces of Valencia and Alicante, was collected from 80 to 458 m, which represents the second-narrowest elevation range. The species *An. atroparvus*, reported for the provinces of Castellón and Valencia, was recorded between 150 and 1067 m. Similarly, *An. claviger*, recorded for the provinces of Castellón and Valencia, has an elevation range that varies between 170 and 1117 m. The species *An. maculipennis s.s.* – reported for the provinces of Castellón and Valencia – was found from 0 to 1195 m, which is the second-widest elevation range; it was also the species discovered at the lowest altitude in the present study. Finally, the species *An. plumbeus*, described for the provinces of Castellón and Valencia, has an elevation range between 405 and 794 m.

Regarding water temperature, the species *An. petragnani*, *An. maculipennis s.s.* and *An. atroparvus* have the widest water temperature ranges. Moreover, *An. atroparvus* was found to be the species capable of occupying aquatic habitats with the highest water temperature, this being higher than 30 ºC. In contrast, *An. petragnani* showed an ability to inhabit waters below 10 ºC. Furthermore, *An. marteri*, *An. algeriensis* and *An. plumbeus* were found to have narrow water temperature ranges, with *An. plumbeus* having the narrowest (Fig. 4).

As for the salinity, *An. plumbeus* displayed the widest range and revealed the highest tolerance to this factor, this being around 5 g/L. The spe-

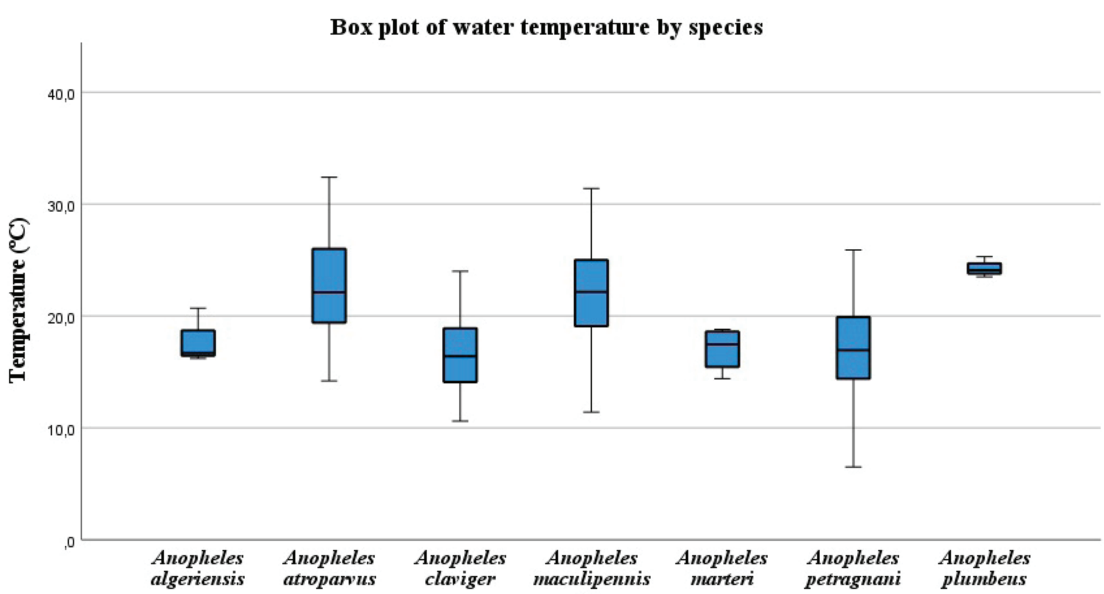

Figure 4. Box plot showing correlations between the water temperature of the aquatic habitats prospected with the seven species of anophelines identified in each of them. *Diagrama de caja y bigotes que muestra las correlaciones entre la temperatura del agua de los hábitats acuáticos prospectados con las siete especies de anofelinos identificadas en cada uno de ellos.*

cies with the second-widest range of salinity was found to be *An. algeriensis*, while the rest of the species showed much lower tolerances to salinity, which in no case exceeded 2 g/L. *An. atroparvus* was highlighted as the species with the narrowest salinity range, immediately followed by *An. maculipennis s.s.*, *An. petragnani*, *An. claviger* and *An. marteri* (Fig. 5).

For the water pH, all the identified species showed an average value of around eight. However, there were some differences and nuances that are worth noting. For instance, *An. atroparvus* has the widest water pH range, which varies between slightly acidic (about six) and basic (close to eleven). Furthermore, the larvae of this species were collected from waters bodies with the most alkaline pH. On the other hand, the species with the narrowest pH range is *An. algerienses*, whose larvae were mostly found in waters with a pH value of eight. Finally, the species that was found to be capable of inhabiting waters with the most acidic pH values is *An. claviger*, whose larvae were sometimes found in waters with a pH of around six (Fig. 6).

Species with potential as vectors of disease-causing agents

Only three out of the seven species of Anophelinae recorded in the region could have an important role in the transmission of malaria due to the female's need for blood meals. These species are *An. atroparvus*, *An. claviger* and *An. maculipennis s.s.* However, nowadays the malariogenic potential is low at the level of this autonomous region due to the epidemiological parameters such as the high receptivity, the infectivity and low vulnerability. Despite this, it is important to remember that *An. atroparvus* was suspected of being the autochthonous malaria vector of the 2010 case reported in Huesca province (Autonomous Region of Aragón) (Santa Olalla et al., 2010); an unsurprising suspicion, considering that this species is considered the most important malaria vector on the European continent (Schaffner et al., 2001).

In addition to this, it is important to point out that the latest reported cases of malaria in the Valencian Autonomous Region were recorded as having been imported from other countries, and no autochthonous cases have been recorded so far.

DISCUSSION

Previous studies reported useful and relevant information regarding the bioecology of some of the anopheline species recorded in the present research. For instance, as in the present research, the wettest areas of the inland sections of the Valencia and Castellón provinces were revealed to be the ones with the highest diversity of anopheline mosquito species, while the driest places, such as those located in Alicante province, showed the lowest richness of anopheline species (Bueno Marí & Jiménez-Peydró, 2011). Another example is that of the species *An. atroparvus*, which was previously found in river margins, irrigation channels, temporary puddles, small costal lagoons and rice fields (Bueno-Marí & Jiménez-Peydró, 2010c, 2012, 2013). We did not find this species in rice-field breeding sites located in the three provinces that make up the Valencian region. However, as this species is capable of colonising this kind of habitats such as rice fields (Romeo Viamonte, 1950, Pérez Moreda, 1982, Mateu, 1987, López Sánchez, 1989, Ruiz & Cáceres, 2004, Bueno-Marí & Jiménez-Peydró, 2013), and the imago stage has the ability to fly around 12 km when seeking its host to obtain a blood meal (Kaufmann & Briegel, 2004), its surveillance is instrumental to anticipating a likely local and autochthonous outbreak of malaria, which is also considered a Notifiable Disease in Spain. In addition, the multivoltine life-cycle dynamics of this species (Encinas Grandes, 1982), its possible endophagic habits and its semiactive behaviour during the winter season (Bueno-Marí & Jiménez-Peydró, 2013) could lead to indoor malaria transmission events, as was already described by Huldén et al. (2005) in northern Europe. These events are more likely if vector populations are present in high densities, as occurred in the rural areas of the interior of this autonomous region (Bueno-Marí & Jiménez-Peydró, 2012), and which was seen in low populations sizes (average level of 1.35) in the present study.

In like manner, the preimaginal development stages of the zoophilic species *An. claviger* and

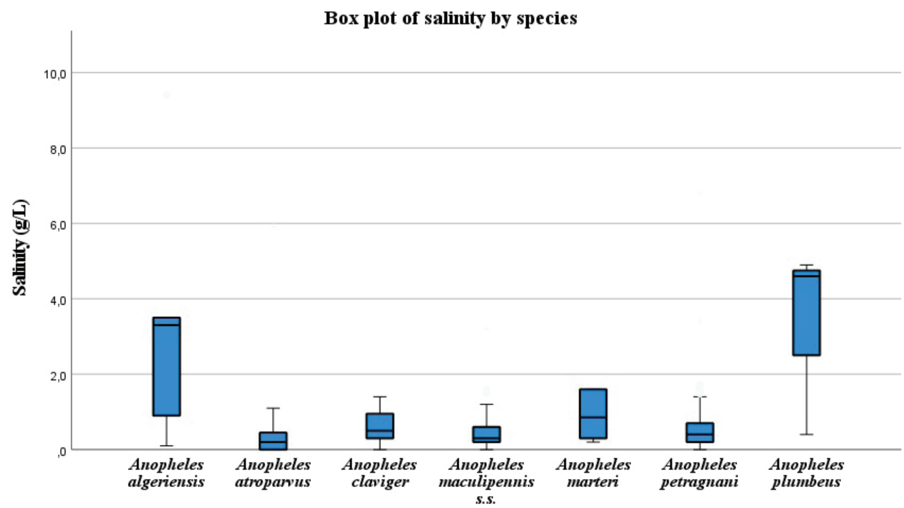

Figure 5. Box plot showing correlations between the water salinity of the aquatic habitats prospected with the seven species of anophelines identified in each of them. *Diagrama de caja y bigotes que muestra las correlaciones entre la salinidad del agua de los hábitats acuáticos prospectados con las siete especies de anofelinos identificadas en cada uno de ellos.*

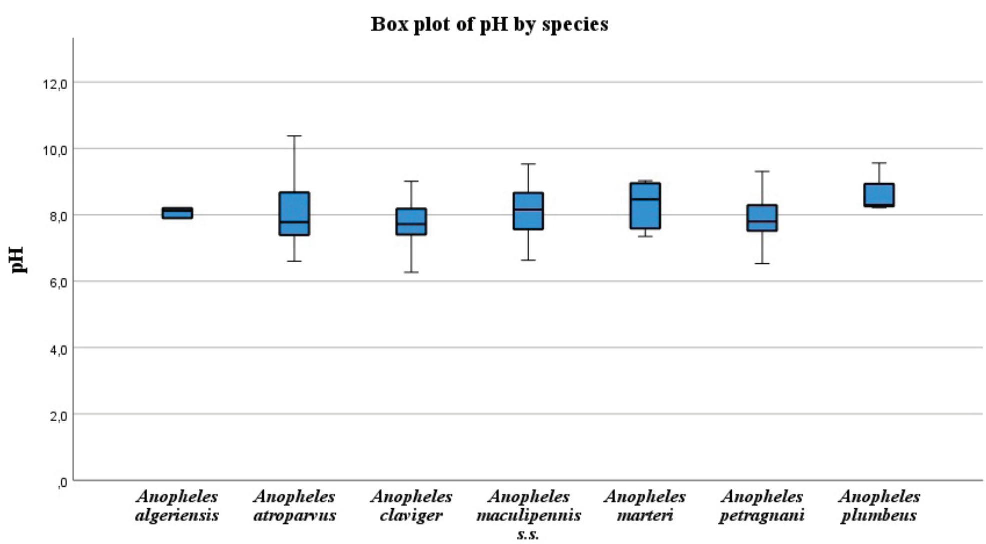

Figure 6. Box plot showing correlations between the water pH of the aquatic habitats prospected with the seven species of anophelines identified in each of them. *Diagrama de caja y bigotes que muestra las correlaciones entre el pH del agua de los hábitats acuáticos prospectados con las siete especies de anofelinos identificadas en cada uno de ellos.*

An. maculipennis s.s. have been reported from aquatic habitats characterised by fresh or slightly brackish waters in mountainous areas isolated from human settlements (Bueno-Marí & Jiménez-Peydró, 2010c, 2012, 2013). In our case, and congruent with López Sánchez (1989), the salinity where these species were sampled also stands out, ranging from fresh waters (0.00 - 0.49 g/L) to oligobrackish waters (0.50 - 4.99 g/L), revealing the species *An. claviger* as the most salinity-tolerant of these two species.

In the same way, the exclusively zoophilic species *An. petragnani* has been reported in small mountainous habitats in scarcely anthropised places, circumstances that lead to it being contemplated as a less-important malaria vector (Bueno Marí & Jiménez-Peydró, 2011, 2012) and considered this species as a bioindicator of wild environments (Bueno Marí & Jiménez-Peydró, 2011). Regarding its abundance, this species revealed the highest preimaginal populations among the mosquito species studied by Bueno-Marí & Jiménez-Peydró (2012), as in this study, where it also showed the highest distribution. Likewise, this species has previously been found in various habitats, including rivers, temporary puddles, reservoirs and natural fountains (Bueno-Marí & Jiménez-Peydró, 2010c). It was also found in these habitats in the present study, as well as in places such as troughs, irrigation channels and old public laundries.

On the other hand, *An. plumbeus*, an exclusively dendrolimnic species that tends to occupy tree cavities of black and white poplars (Bueno-Marí & Jiménez-Peydró, 2010c, 2012, 2013), was also collected at tree holes in the present study.

With respect to the altitude, larvae of *An. algeriensis* were previously reported from inland freshwaters located up to an elevation of 667 m (Bueno-Marí & Jiménez-Peydró, 2010c), while in our case this species was found from 80 to 458 m. The larval biotopes where *An. claviger* was previously reported ranged from 455 to 849 m (Bueno-Marí & Jiménez-Peydró, 2010c); in our study, this species was collected from 170 to 1117 m. The species *An. petragnani* was previously reported between 127 and 1155 m (Bueno-Marí & Jiménez-Peydró, 2010c), compared to between 122 and 1472 m in this study. In addition, *An. marteri* was collected for the first time at 812 m, in the surroundings of the source of the Palancia river (Bueno-Marí & Jiménez-Peydró, 2010c); in the present study, it was found between 673 and 888 m.

Climate change may play a significant role in the distribution and transmission of malaria cases in this Spanish region, as it directly affects the rainfall that occurs. Increasingly severe fluctuations in rainfall are limiting the possible and potential breeding sites of the aforementioned anopheline species, as well as the multiplication of the pathogen with the increase in average temperature (Arcos et al., 2011). In fact, species with the potential to be transmitted by these anophelines such as *Plasmodium falciparum* Welch, 1897 and *Plasmodium vivax* (Grassi & Feletti, 1890), require a minimum temperature of 19 ºC and 16 ºC, respectively (Castro-Díez et al., 2007), average temperatures that have been frequently reached in recent years and that could enable both the infection of these anopheline species and the completion of the sporogonic cycle of *Plasmodium* species in these vectors.

CONCLUSION

The present study has reported on seven haematophagous mosquito species of the family Anophelinae – important from both a human health and a veterinarian point of view – and characterised their breeding habitat types, geographical distribution, elevation ranges and water temperature, salinity and pH, and highlighting the study's importance within the framework of 'One Health' (environmental, human and animal health). All the new insights provided have contributed to increasing the knowledge on anophelines in eastern Spain. This research has also been crucial to shedding light on the pressing need to develop an active and long-lasting surveillance programme in this particular region of Spain. Such surveillance is essential due to the continuous flow of tourists and goods from all over the world, as well as the imported cases of illnesses whose causative agents might be locally transmitted to human populations by these anopheline vectors of public health importance, potentially which causing the re-emergence of different anthroponoses in this temperate latitude. Last but not least, we would like to remind travellers about the importance of adopting the available prophylactic measures during their trips to endemic areas of any vector-borne disease mediated by arthropods.

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AUTHOR CONTRIBUTIONS

D.L.P.: Sample collection, Processing and identification of samples, Data processing, Prepa-

ration of the original draft, Figures and Tables design, Review, Editing, and Validity; Á.L.C.: Sample collection, Processing and identification of samples, Figures creation, Review, and Validity A.G.A.: Sample collection, Processing and identification of samples, Data processing, Contribution to some sections of the original draft, Figures and Tables creation, Review, and Validity; J.V.F.G.: Fund acquisition, Project conceptualization and supervision, Review, Editing, and Validity.

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