Anopheles plumbeus Stephens, 1828: a neglected malaria vector in Europe

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Abstract

Traditionally field and laboratory research about malaria vectors in Europe have been mainly focused on the species of the *Anopheles maculipennis* complex. However, although malaria is essentially a rural disease, potential urban vectors merit attention. Because only a few European *Anopheles* species can breed in urban environments, improving knowledge about their bioecology is necessary to implement effective control measures. Among these opportunistic species, *Anopheles plumbeus* has a distinctive dendrolimnic behavior, being able to complete its larval development in small containers. The aim of this paper is to provide a thorough review of the limited studies on An. plumbeus with the aim of providing useful epidemiological information.

Introduction

It is well known that malaria is the most important parasitic disease in the world. According to the World Health Organization (WHO), malaria annually affects 250 million people and threatens directly or indirectly 50% of the world population.[1] The disease is endemic in much of Africa and several countries of Asia, Central America, and South America. In Europe, malaria was also an endemic disease until after the end of World War II and was historically eliminated by a mixture of vector control, disease treatment, habitat modification, and improvements in general living standards. The morbidity and mortality from parasitosis was particularly high in Southern Europe, although seasonal epidemics or outbreaks occurred as far north as Scandinavia, even reaching as far as 68°N latitude during the 19th century.

Flooding practices, mainly rice cultivation, were clearly associated with malaria endemicity in Mediterranean countries until the beginning of the 20th century. In this area, different multivoltine species of the *Anopheles maculipennis* complex were considered the main disease vectors.[2] Due to climatic conditions, these anophelines showed an extended period of activity and consequently malaria episodes were continuous in Southern Europe. However, there are several reasons for which it has not been possible to define for certain which mosquito species was the most important for malaria transmission in Northern Europe. The major reason is related to the fact that temperature conditions of Scandinavian countries should have meant that malaria transmission has mainly occurred under indoor conditions due to transmission of sporozoites throughout the winter by semiactive hibernating mosquitoes,[3] since it is well known that in warm conditions the overwintering females of *Anopheles* can take several blood meals.[4-5] Therefore, the best malaria vectors in Northern Europe were those anthropophilic and endophagic anophelines, which present hibernating females with semiactive winter habits but not a complete diapause. In conclusion, northern malaria existed in such a cold climate by means of the summer dormancy of *Plasmodium vivax* (Grassi & Feletti, 1890) hypnozoites, in addition to the indoor feeding activity of overwintering *Anopheles* females mentioned previously.

Currently, malaria provides an excellent example of a disease for which the occurrence of several global changes may influence its re-emergence in Europe.[6] Global change can be defined as the impact of human activity on the fundamental mechanisms of biosphere functioning. Therefore, global change includes not only climate change, but also globalization, habitat transformation, water cycle modification, biodiversity loss, synanthropic incursion of alien species into new territories, or the introduction of new chemicals in nature.

Given these complex interacting factors, the cycles of malaria transmission in Europe are nowadays relatively common again in Georgia, Azerbaijan, Kyrgyzstan, Tajikistan, Uzbekistan and Turkey.[7] Moreover the recent autochthonous cases diagnosed in Spain, France, Greece or Italy[8-11] have shown the need to better understand the potential influence of this tropical disease in Southern Europe, where the warmer climate obviously favors the continued development of the disease.

The risk of the re-establishment of malaria in Europe is believed to be low.[12-14] This low risk is attributed, among others, to the temperature requirements for completing the Plasmodium cycle, to the efficient health system in Europe, which allows for the early detection of malaria patients, and to the low density of *Anopheles* vectors in mainly rural areas limiting the contact between vectors and infected persons. The aim of this paper is to discuss the potential increase of malaria risk in urban settings due to the presence and abundance of suitable disease vectors such as *Anopheles plumbeus* Stephens, 1828.

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Malaria vectors in Europe

Compiling data on the occurrence and distribution of former and present malaria vectors in southern Europe is a difficult task. Firstly, there are several *Anopheles* species belonging to species complexes, such as the sibling species of the *Anopheles maculipennis* and the *Anopheles claviger* complexes, which are very difficult to distinguish morphologically. Therefore, there is much discussion over existing data as they depend on a correct identification of these anophelines. Correct identification is essential because closely related sister species can vary widely in their ability to transmit malaria. Secondly, most of the information about *Anopheles* collections is from periods when malaria was endemic. This is important to note because, in the past, malariologists sometimes had limited knowledge of the systematics of the genus. Additionally, malaria eradication programs in southern Europe usually included intense mosquito control not only through the use of insecticides, but also through environmental modifications, resulting in changes in the *Anopheles* fauna.[15]

The disappearance of *Anopheles labranchiae* (Falleroni, 1926) from Spain[16] or *Anopheles sacharovi* (Favre, 1903) from Malta and Romania[17] are examples of such a faunistic change. Thirdly, there is a worrying decrease in the number of specialists in medical entomology in numerous countries and this may lead to a decline in the number of field studies on mosquitoes. Consequently, given the potential limitations in existing publications, the literature on malaria vectors, especially in southern Europe, must be evaluated with caution.

Twenty *Anopheles* species are currently listed as occurring in Europe (Table 1). From an epidemiological point of view, the species of the *An. maculipennis* complex are the most important malaria vectors. Although *Anopheles maculipennis* s.s. (Meigen, 1818), *Anopheles melanoon* (Hackett, 1934) and *Anopheles messeae* (Falleroni, 1926) have been implicated as local or secondary vectors across their distribution range,[25,26] the most competent vectors in the group are undoubtedly *Anopheles atroparvus* (Van Thiel, 1927),[27,28] *An. labranchiae*[29,30] and *An. sacharovi*.[31,32]

Anopheles plumbeus and its possible interest in malaria outbreaks

Anopheles plumbeus (synonyms: *Anopheles corsicanus* Edwards, 1928; *Anopheles intermedius* Shingarev, 1928; *Anopheles nigripes* Staeger, 1839) is a Palearctic species, widely distributed around Europe (Figure 1), but detailed studies about its malariogenic potential are scarse. It can be found in forest areas from sea level to altitudes of 2,000 meters[33,34] and is the only tree hole breeding (Figure 2) species of the genus *Anopheles* in Europe. The eggs are laid on the side of tree holes, not directly on the water surface. Thus, egg hatch-

Figure 1. Larvae of *An. plumbeus*.

Table 1. *Anopheles* species with endemic presence in Europe and indication of historical data about its vectorial role.[18,19,20-24] Countries with anopheline records considered as doubtful or sporadic were not included. If it is thought that the species has been eradicated, the country is also not listed. Abbreviations are: Brit (Britain), Ire (Ireland), Nor (Norway), Swe (Sweden), Den (Denmark), Fra (France), Cors (Corsica), Spain, Bala (Balearic Islands), Port (Portugal), Belg (Belgium), Neth (Netherlands), Lux (Luxemburg), Ger (Germany), Aust (Austria), Czech (Czech Republic), Slovk (Slovakia), Pol (Poland), Switz (Switzerland), Ital (Italy), Sard (Sardinia), Sic (Sicily), Malt (Malta), Ser-Mon (Serbia-Montenegro), Croa (Croatia), Bosn (Bosnia), Slovn (Slovenia), Mace (Macedonia), Alb (Albania), Gree (Greece), Turk (Turkey), Cypr (Cyprus), Hung (Hungary), Rom (Romania), Bulg (Bulgaria), Moldv (Moldavia), Ukr (Ukraine), Bela (Belarus), EurRus (Eropean Russia), Lith (Lithuania), Latv (Latvia), Est (Estonia).

Anopheles species	European distribution	Malaria outbreaks
An. algeriensis	Brit, Ire, Fra, Cors, Spain, Bala, Port, Ger, Aust, Ital, Sard, Sic, Croa, Alb, Gree, Turk, Hung, Bulg, Moldv, Ukr, EurRus, Est	Argelia (not demonstrated to be a vector in Europe)
An. atroparvus	Brit, Ire[a], Swe, Den, Fra, Spain, Port, Belg, Neth, Ger, Aust, Czech, Slovk, Pol, Switz,[a] Ital, Ser-Mon, Croa, Bosn, Slovn, Mace, Hung, Rom, Bulg, Moldv, Ukr, Bela, EurRus, Lith, Latv	Northern Europe, Central Europe, Eastern Europe, Mediterranean Europe
An. beklemishevi	Swe, Fin, EurRus	-
An. cinereus	Spain, Port	-
An. claviger	Brit, Ire, Nor, Swe, Den, Fra, Cors, Spain, Port, Belg, Neth, Lux, Ger, Aust, Czech, Slovk, Pol, Switz, Ital, Sic, Ser-Mon, Croa, Bosn, Slovn, Mace, Alb, Gree, Turk, Cypr, Hung, Rom, Bulg, Moldv, Ukr, Bela, EurRus, Lith, Latv, Est	Eastern Mediterranean countries, Central Asia
An. daciae[b]	Brit, Rom	-
An. hyrcanus	Fra, Cors, Spain, Ital, Sard, Sic, Ser-Mon, Croa, Mace, Alb, Gree, Turk, Hung, Rom, Bulg, Moldv, Ukr, EurRus	Asia (as An. hyrcanus s.l.)
An. labranchiae	Cors, Ital, Sard, Sic, Croa	France (Corsica), Italy (Italian peninsular, Sardinia and Sicily), Southeastern Spain (disappeared since 1973)
An. maculipennis s.s.	Nor, Swe, Den, Fra, Cors, Spain, Port, Belg, Neth, Lux*, Ger, Aust, Czech, Slovk, Pol, Switza, Ital, Sic, Ser-Mon, Croa, Bosn, Slovn, Mace, Alb, Gree, Turk, Hung, Rom, Bulg, Moldv, Ukr, Bela, EurRus, Lith, Latv, Est	Coastal areas in the Balkans, Asia Minor, Northern Iran
An. marteri	Cors, Spain, Port, Ital, Sard, Sic, Alb, Gree, Turk, Bulg	-
An. melanoon[c]	Fra, Cors, Spain, Ital, Rom, EurRus	-
An. messeae	Brit, Irea, Nor, Swe, Den, Fra, Cors, Belg, Neth, Ger, Aust, Czech, Slovk, Pol, Switza, Ital, Ser-Mon, Croa, Bosn, Slovn, Mace, Alb, Gree, Hung, Rom, Bulg, Moldv, Ukr, Bela, EurRus, Lith, Latv, Est	Eastern Europe
An. multicolor	Spain	-
An. petragnani	Fra, Cors, Spain, Port, Ital, Sard, Sic	-
An. plumbeus	Brit, Ire, Swe, Den, Fra, Cors, Spain, Port, Belg, Neth, Lux, Ger, Aust, Czech, Slovk, Pol, Switz, Ital, Sic, Ser-Mon, Croa, Bosn, Slovn, Mace, Alb, Gree, Turk, Hung, Rom, Bulg, Ukr, Bela, EurRus, Lith, Est	England, Germany, Caucasus
An. pulcherrimus[d]	Turk	Middle East
An. sacharovi	Cors, Ser-Mon, Croa, Mace, Alb, Gree, Turk, Bulg, EurRus	Near East
An. subalpinus[c]	Fra, Cors, Port, Ser-Mon, Croa, Mace, Alb, Gree, Turk, Bulg, EurRus	Albania, Greece
An. sergentii	Sic	Mediterranean Africa
An. superpictus	Cors, Ital, Sic, Ser-Mon, Croa, Mace, Alb, Gree, Turk, Bulg, EurRus	Middle East

[a]Records referred to *Anopheles maculipennis* s.l.; [b]species recently described by molecular and morphological techniques; [c]there is confusion between these two species; [d]present in Asiatic Turkey.

ing is closely related to the intensity and number of flooding events. Consequently rainfall patterns are key factors for estimating the number of generations per year. Although it is a strictly dendrolimnic species, during dry periods, females can also lay the eggs in small domestic and peridomestic containers, as well as other artificial breeding sites underground, such as catch basins and septic tanks with water contaminated with organic waste.[18] There are several reports in Europe about the presence of larvae in a different biotope to the tree cavity.[35-40] Moreover, considerable populations may also be found in urban settings, where larvae develop in tree holes in gardens and parks. This is especially the case in Central Europe where *An. plumbeus* has increased in numbers over the last decades and can be a major nuisance.[19] Several studies have revealed that recent outbreaks of *An. plumbeus* in Belgium can be explained by a habitat shift toward human-created habitats. The expansion of *An. plumbeus* is in particular related to a larval habitat shift of this species from tree-holes in forests to large manure collecting pits of abandoned and uncleaned pig

Figure 2. Typical breeding site of *An. plumbeus.*

pens.[41] The continuous development of this species in urban environments could considerably increase the possible interaction between malaria vectors and humans.[42]

Despite the fact that the number of generations per year depends on the hydrological situation, most authors suggest a bivoltinism of the species.[43] Females are aggressive and persistent biters, feeding principally on mammalian blood,[44] but also on birds and reptiles. However, some populations show a strong anthropophilic preference.[45] Even though *An. plumbeus* is mainly active during the crepuscular periods, occasionally females of the species have been observed to attack humans during the day; this is always in shaded areas on the edges of forest.[19]

The species usually overwinter in the larval stage, but in adverse conditions (extreme drought) eggs can also withstand the winter and hatch after spring rains. Overwintering larvae (mainly second and third instars) can even survive when the water surface is frozen, spending most of the time at the bottom of the hole and obtaining oxygen through a high percentage of cuticular respiration. Given this winter biology, larval control of *An. plumbeus* in urban gardens and parks during winter months could be a potential prophylactic measure. Several infectivity tests have revealed the ability of *An. plumbeus* to transmit *Plasmodium falciparum* (Welch, 1897), with percentages of positive generation of oocysts varying from 60 to 90% (Table 2).[46,47] These results confirm the competence of European populations of *An. plumbeus* to act as a bridge vector of tropical *P. falciparum* strains *versus* negative results obtained in similar tests with European *An. atroparvus* strains.[48-53] However, more recent studies have shown the ability of *An. atroparvus* to generate oocysts of *P. falciparum* 46, but not to complete sporogony. Futhermore, *An. plumbeus* can also produce sporozoites of Eurasiatic strains of *P. vivax*.[54,55] Some authors even suggest that *An. plumbeus* is capable of transmitting all four Plasmodium species.[56] *Anopheles plumbeus* has been suspected to be responsible for two recorded cases of locally transmitted malaria in London, United Kingdom[57,58] and another two cases in Duisburg, Germany.[59] The medical interest in the species is not only restricted to malaria, because it probably has a considerable role in the maintenance of arboviruses such as West Nile or even dirofilariasis.[18,60,61]

Table 2. Results of several infectivity tests of *Plasmodium falciparum* conducted on *Anopheles plumbeus* females.[46,47]

Mosquito n. (origin)	N. of oocysts	Sporozoites in salivary glands
1,2,3 (Epping, UK)[a]	3.02[c,d]	-
4 (Epping, UK)[a]	0	-
5 (Epping, UK)[a]	0	-
6 (London, UK)[b]	40	Yes
7 (London, UK)[b]	12	No
8 (London, UK)[b]	8	Yes
9 (London, UK)[b]	1	No
10 (London, UK)[b]	7	Yes
11 (London, UK)[b]	0	No
12 (London, UK)[b]	31	Yes
13 (London, UK)[b]	7	No
14 (London, UK)[b]	2	Yes
15 (London, UK)[b]	46	Yes

[a]Dissection at 7 days after feeding; [b]Dissection at 18-21 days after feeding; [c]Mean number of oocysts per mosquito according to the formula of Williams' mean. $W= [\text{antilog}(\sum\log(x+1)\sqrt{n})]-1$; [d]Maximum number of oocysts in one individual was 24.

Conclusions

The present situation in most of Europe can be described as what malariologists of the first half of the last century would have called *anophelism without malaria*. Although malaria is essentially a rural disease, we must pay some attention to urban vectors of the parasitosis. Because only a few European *Anopheles* species can breed in urban environments, we need to improve our bioecological knowledge of potential malaria vectors to be able to implement effective control measures. Among these opportunistic species, *An. plumbeus* is especially noteworthy given its distinctive arboreal behavior and its ability to complete its larval development in small domestic and peridomestic containers. In conclusion, gardens and parks, as well as other potential breeding sites such as catch basins or septic tanks, should be monitored continuously, especially those placed near hospitals where malaria patients could be a source of infection for mosquitoes.

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