Rediscovery of *Anopheles algeriensis* Theob. (Diptera: Culicidae) in Germany after half a century

Andreas Krüger[1*], Egbert Tannich[2]

[1] *Department of Tropical Medicine, Bundeswehr Hospital Hamburg, Hamburg, Germany*
[2] *Department of Parasitology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
**corresponding author: krueger@bnitm.de*

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Abstract: *Anopheles algeriensis*, a mosquito species primarily found in the Mediterranean region and susceptible to *Plasmodium* spp., is one of seven *Anopheles* species previously reported from Germany. However, the last record dates back to 1958. Whether it was really absent since then or whether this is just a sign of neglected mosquito studies over several decades is unclear. The present study is part of a nationwide mosquito mapping project launched in 2011. We describe larval dip collections of *An. algeriensis* in 2013 from the inland salt meadow "Brenner Moor", located in Schleswig-Holstein, northern Germany, which represents the most northerly of all German *An. algeriensis* collection sites known so far. Despite several mosquito mapping projects carried out in Germany over recent years, *An. algeriensis* has not been detected within the last 55 years. Therefore this species appears to be extremely rare or at least rurally localised. Hence, it remains a very low risk vector in terms of autochthonous malaria transmission in Germany. ***Journal of the European Mosquito Control Association* 31: 14-16, 2013**

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Introduction

Blood-feeding mosquitoes can transmit some of the most devastating diseases in the tropics, but are not only a threat in tropical climates. In the case of malaria, it is nowadays widely neglected that the Tertian form of the disease, i.e. *Plasmodium vivax*, persisted as far north as England and most North Sea coastal areas of continental Europe for several hundred years until the mid-twentieth century (Bruce-Chwatt & Zulueta, 1980). The main vectors here were members of the *Anopheles maculipennis* complex. In Germany, the complex comprises four species: *An. maculipennis* Meigen, *An. messeae* Falleroni, *An. daciae* Nicolescu and *An. atroparvus* Van Thiel (Mohrig, 1969; Kronefeld *et al*., 2012; Weitzel *et al*., 2012), the last of which was responsible for most of the historic transmissions. In addition, the species *An. claviger* (Meigen), *An. plumbeus* Dyar & Knab and *An. algeriensis* Theobald have been previously recorded (Mohrig, 1969). *Anopheles algeriensis* is considered to be the rarest one, only being reported from four distant places in Germany. The last finding dates back to 1956-58 when Scherpner (1960) found larvae and one overwintering female in Neu-Isenburg south of Frankfurt/Main. The first record was from Hinsbeck located in western Germany between the town of Krefeld and the Dutch border (Martini, 1931).

Anopheles algeriensis is a Mediterranean species with only a few extensions north- and eastwards, e.g. Estonia, Near and Middle East (Ramsdale & Snow, 2000). In Western Europe, it was found in Britain and more recently in Ireland as well as in the Netherlands (Ashe *et al*., 1991; Snow, 1998; Reusken *et al*., 2010).

Regarding its vector importance, *An. algeriensis* is susceptible to *Plasmodium* species (Weyer, 1939; Russell *et al*., 1943; Becker *et al*., 2010), but is only considered a potential or secondary malaria vector in endemic regions without proof of natural transmission. Due to its scarcity in Europe and its mammalo- and exophilic behaviour, the species does not presently, and did not historically pose a risk (Peus, 1942; Russell *et al*., 1943; Becker *et al*., 2010).

Here we report on a focus of *An. algeriensis*, which was recently detected in northern Germany during a nationwide mosquito mapping survey, running since 2011.

Materials and Methods

The study area is located in Schleswig-Holstein in the nature reserve "Brenner Moor" near the town Bad Oldesloe located about 20 km northeast of Hamburg (N53°49'10" E10°21'7", altitude 2 m). The reserve protects the largest inland salt meadow in the Federal State of Schleswig-Holstein. The salinity may reach that of the North Sea. The area is mainly covered by dense *Phragmites* reed, interrupted by small ponds and ditches. On April 21st and May 20th 2013 larval mosquitoes (L3-4) were collected by dipping with plastic bowls. The water temperature was 12-14°C, and air temperature 16-17°C. Other water parameters were as follows: pH 7.34; electrical conductivity 2460 µS/cm; total dissolved solids 1230 ppm (measured with a multi-parameter pocket tester HI98129, Hanna Instruments Germany, three days after collecting, held at 4°C). The larvae were taken into the laboratory for further development and adult emergence, using a mixture of original and distilled water. Emerged adults were identified morphologically (Mohrig, 1969; Becker *et al*., 2010) under a stereomicroscope, digitally photographed and preserved dry-pinned or frozen. The male genitalia were prepared as a permanent-mounted slide using Euparal, and the abdomen and one leg of the female were preserved in absolute ethanol as a DNA voucher. For comparison of the general morphological aspects, seven male *An. algeriensis* from the first German collection site in Hinsbeck (Martini, 1931) were available.

Results

The first larval collection consisted of nine *Anopheles* larvae, of which six were successfully reared to adults within 14 days. One female and three males belonged to *An. claviger*, whereas another female and male differed from *An. claviger* by having a uniformly brownish scutum, brownish erect scales on the vertex and brownish scales on the anterior acrostichal area

(Figure 1), as typical for *An. algeriensis*. In contrast, in *An. claviger* the scutum has a median grey stripe and the scales on the vertex and in the anterior acrostichal area are cream-coloured. Both species lack any dark spots or pale coloration on the wing veins. In addition, the male was definitely identified as *An. algeriensis* by its genitalia (Figure 2), which have only one strong parabasal seta at the base of the gonocoxite.

In the neighbouring pools and ditches, the typical halophilic species *Ochlerotatus detritus* (Haliday) and *Oc. dorsalis* (Meigen) as well as further *An. claviger* specimens were found in great numbers in the first collection, whereas larvae of *Oc. caspius* and *An. maculipennis* complex were found four weeks later.

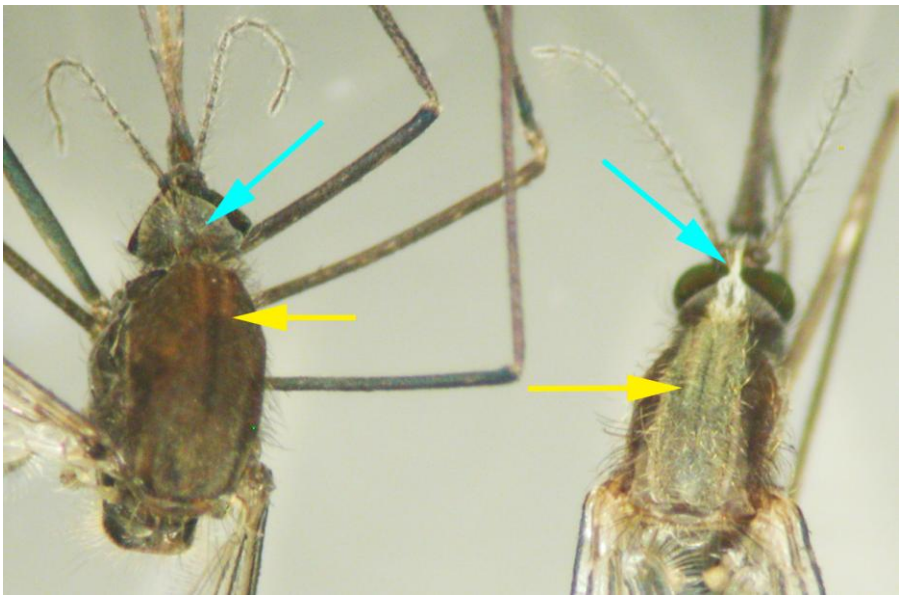

Figure 1: Morphological aspects of *An. algeriensis* vs. *An. claviger*. Shown are two adult females reared from larvae collected in "Brenner Moor". Left: *An. algeriensis*, with highlighted uniformly brownish scutum and only brownish erect scales on the vertex and in the anterior acrostichal area. Right: *An. claviger* with median grey stripe on the scutum and cream-coloured scales on the vertex and the anterior acrostichal area.

Figure 2: Male genitalia of *An. algeriensis*. The asterisks highlight the single strong parabasal seta at the base of each gonocoxite.

Discussion

The last mosquito sampling reported for the "Brenner Moor" took place in the late 1960s, when Zielke (1970) described finding 12 mosquito species, including *An. claviger* and the halophilic species *An. atroparvus*, *Oc. detritus* and *Oc. dorsalis* in the saltwater ditches of a site, which he called "Alt Freesenburg". Whether *An. algeriensis* was just overlooked or indeed absent at that time cannot be determined retrospectively. In the remains of Zielke's collection, *An. claviger* but no *An. algeriensis* were found. On the other hand, some of the previously known collection sites of *An. algeriensis* in Germany were published with insufficient geographic details, so that it is almost impossible to exactly identify the old locations. Weyer (1939) mentioned Müritz in Mecklenburg and Spreewald, both representing large areas of more than 300 km^2, without giving any reference, whereas Baer (1960) cited Peus (1929), who in fact gave no account on *An. algeriensis* at all. The exception is the site of Hinsbeck (Martini, 1931), which was further localised by mentioning "Haus Bey". Since this is nowadays a golf course, it seems doubtful that *An. algeriensis* would still find appropriate larval environments in this area. Similarly, Scherpner (1960) reported the demise of the site in Frankfurt through habitat sanitation.

The new record reported here does not only represent a rediscovery of *An. algeriensis* in Germany after 55 years, it is also the most northerly record in this country. Europe-wide, only the record from Estonia (Ramsdale & Snow, 2000) is clearly further north, whereas the sites in Britain and Ireland are roughly at the same latitude as the new German site.

This new site is characterised by slight brackish salinity, whilst other ponds in the reserve show true saltwater quality. So far, *An. algeriensis* was not considered a typical halophilic species, at least not in Europe. The only account on reproducing in brackish water was given for a population in northern Africa (Weyer, 1939; Becker *et al*., 2010). Otherwise, the habitat preferences were described as swamps, silting zones of lakes, ponds and other freshwater habitats, but usually with shady vegetation such as reeds (Peus, 1942).

During recent decades, ample mosquito mapping projects, using more than 100 CO_2-baited adult traps, were carried out in southwestern Germany (Becker *et al*., 2011) and several others were performed in various parts of Germany within the last three years (Kronefeld *et al*., 2012; Werner *et al*., 2012). However, *An. algeriensis* was not detected. Noticeably, in the Netherlands *An. algeriensis* was only recorded in CO_2-baited adult traps during a short period in 2009. Thus, it is concluded that *An. algeriensis* remains a very rare or at least rurally localised species in Germany and apparently elsewhere in Europe (Ramsdale & Snow 2000). Hence, it is considered a very low risk vector in terms of autochthonous malaria transmission.

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