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Anopheles maculipennis complex in Switzerland: reassessing taxonomic status and malaria potential

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Anopheles larvae were collected during summer 2000 at 40 sites throughout Switzerland with altitudes between 190m and 720m above sea level. The larvae were raised in the laboratory until fourth instar; then they were screened for *A. maculipennis* sensu lato and *A. claviger*. By means of a PCR method with species-specific primers, members of the *A. maculipennis* complex could be tested. In all collections we detected only *A. maculipennis* sensu stricto and *A. messeae* as sibling species besides *A. claviger*. Earlier reports on *A. melanoon* and *A. atroparvus* could not be substantiated, and we assume misidentifications because the morphological criteria for sibling species are unreliable. With respect to malaria, the vectors are present, but the risk for re-establishing *Anopheles-Plasmodium* associations, i.e. human malaria, is discussed with respect to tourism, and considered to be minimal. Despite an increased tourism and imported malaria together with a trend for global warming, there is no reason for public fears in Switzerland. The vector populations are far too low in abundance to allow imported malaria to become stable.

INTRODUCTION

One hundred years ago, the interest in the distribution and occurrence of Culicidae in Switzerland has been initiated by the pioneering results on the role of mosquitos in the transmission of malaria by ROSS (1897, 1898) in India and GRASSI (1898) and GRASSI et al. (1899) in Italy. It was the latter who recognised the role of European *Anopheles maculipennis* MEIGEN, 1818 as vector for *Plasmodium*. GALLI-VALERIO (1901) was the first to investigate the mosquito fauna in Switzerland, particularly of *A. maculipennis* in the western part of our country. Malaria has been known to occur in this country since a long time at various lowland areas, such as many river plains (e.g. Rhine, Linth, Aare, Ticino) which experienced frequent floodings. GUHL (1944) published maps comparing the existence of historical malarious areas with the distribution of *Anopheles*. GEIGY (1945) summarised and discussed the issue of malaria in Switzerland. Various observations on the occurrence of *Anopheles* and other mosquitos in Switzerland were compiled, together with new collections by BRIEGEL (1973).

A. maculipennis sensu lato as the principal vector of Plasmodia in Switzerland was early recognised to represent a group of species (VAN THIEL 1933, 1939), although in an inconclusive manner. Keys were provided to differentiate among *A. messeae* FALLERONI, 1926, *A. melanoon* HACKETT, 1934, *A. atroparvus* VAN THIEL, 1927, *A. subalpinus* HACKETT & LEWIS, 1935, and *A. labranchiae* FALLERONI, 1926, based on the color patterns of the eggs by HACKETT & MISSIROLI (1935), together

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with some ecological and behavioural parameters (WEYER 1942). In earlier publications there was a confusion with the terminology: GRASSI et al. (1899) referred to *A. claviger* (MEIGEN, 1804) (= zanzarone in Italian), GALLI-VALERIO (1917) found *A. maculipennis* together with *A. claviger*, but the latter was also called *A. bifurcatus* MEIGEN, 1818 (e.g. BÜTTIKER 1948), which is an invalid synonym. According to the current knowledge (KETTLE 1995), the species complex of *maculipennis* consists of nine sibling species in the palearctic region, together with 5 nearctic species in the sense of MAYR (1974). Several crossing experiments among members have been reported by KITZMILLER et al. (1967). For geographic reasons, only three are potential candidates for our country: *A. maculipennis* sensu stricto and *A. messeae*, the two most common ones in Europe (WHITE 1978), and *A. atroparvus*. *A. melanoon* and *A. labranchiae* are confined to Southern Europe, while *A. beklemishevi* STEGNII & KABANOVA, 1976 occurs in Scandinavia and eastwards.

With the development of modern techniques, the polymerase chain reaction (PCR), it is now possible to accurately analyse species complexes (COLLINS & PASKEWITZ 1996), such as *A. gambiae* GILES, 1902 (SCOTT et al. 1993), *A. quadrimaculatus* SAY, 1824 (CORNEL et al. 1996), or *A. maculipennis* (PROFT et al. 1999). In this report we have collected fresh *Anopheles* material throughout Switzerland and subjected it to the PCR assay to clarify the presence and distribution of the *maculipennis* complex in our country. *A. claviger* also belongs to a species complex (KETTLE 1995), but its other sibling species *A. petragnani* DEL VECCHIO, 1939 belongs to the Mediterranean area and therefore was out of consideration for our present purposes. The results will be related and discussed with the present malaria situation abroad and in Switzerland.

MATERIAL AND METHODS

Anopheles larvae were collected from June to September 2000 at 40 localities from all over Switzerland, brought to the laboratory and fed our standard food mixture (TIMMERMANN & BRIEGEL 1993) until the fourth and final instar. *A. maculipennis* sensu lato was identified and distinguished from *A. claviger* in each sample by the morphology of the outer clypeal hairs on the anterior margin (WEYER 1942). The majority of the larvae of each collection site was fixed in 70% ethanol and stored individually until analysis. Coexisting larvae of other mosquito taxa were discarded. For analysis single larvae were quickly dried and the ethanol was evaporated to prevent possible disturbancies during the later procedures. Of each larva,

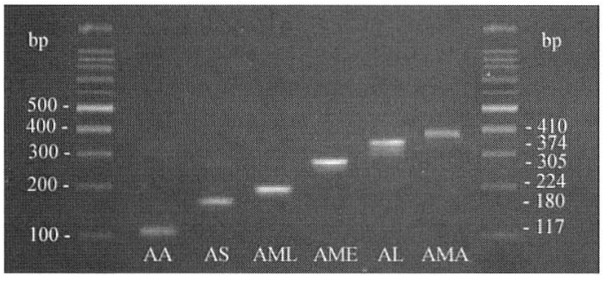

Fig. 1. Agarose-gel of species-specific PCR products for six sibling species of *Anopheles maculipennis* s. l. Markers to the left are base-pairs of specific lengths of the DNA-ladder, to the right the actual lengths of the fragments from the species. AA = *A. atroparvus*; AS = *A. sacharovi*; AML = *A. melanoon*; AME = *A. messeae*; AL = *A. labranchiae*; AMA = *A. maculipennis* s. s.

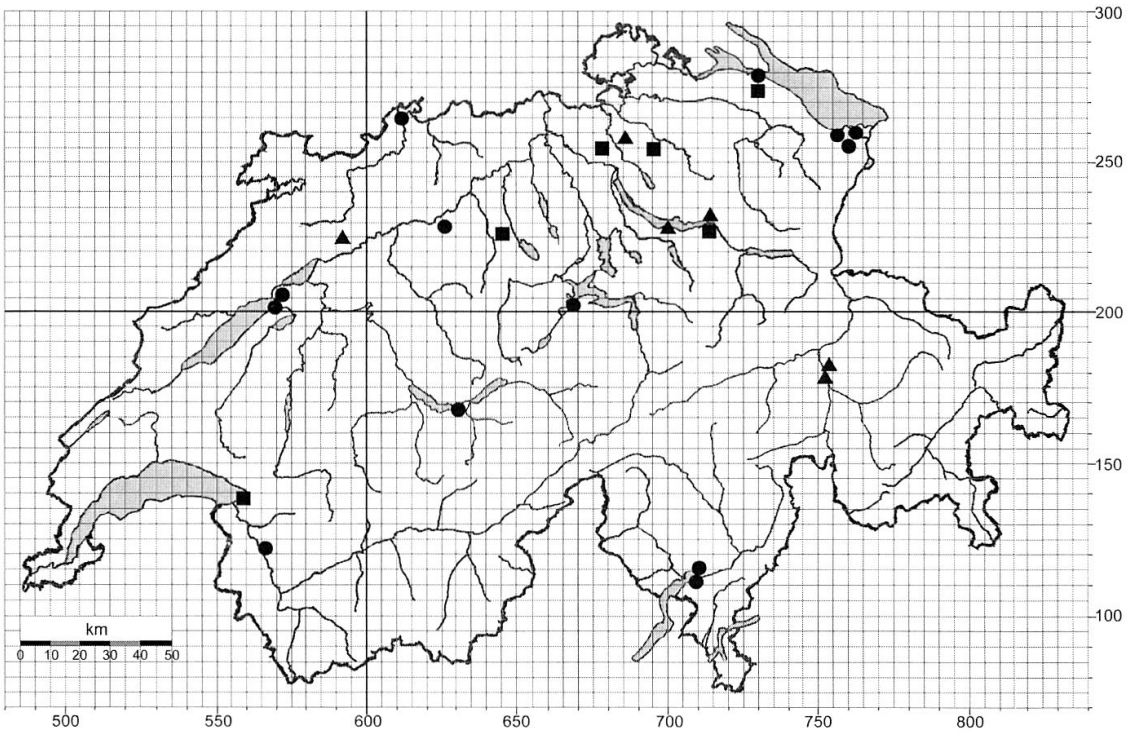

Fig. 2. Collection sites of *Anopheles maculipennis* s. s. and *A. messeae* in Switzerland during summer 2000. ▲ = *A. maculipennis* s.s. alone; ■ = *A. messeae* alone; ● = *A. maculipennis* s.s. sympatric with *A. messeae*.

the last two terminal segments were cut-off and transferred into SARSTEDT tubes (72.737.002; 50 µl).

For the PCR we followed exactly the procedure elaborated and described by PROFT et al. (1999) which was based on the analysis of the *gambiae* complex (SCOTT et al. 1993). A negative control, without DNA, and a positive control with DNA from identified species of members of the *maculipennis* complex were routinely included in every PCR assay. Purification of DNA before amplification, as suggested by COLLINS et al. (1987) was not required because a single abdominal segment gave identical results as after DNA extraction (unpublished result). Identification was carried out in the ITS2 region of the ribosomal DNA (COLLINS & PASKEWITZ 1996) with six species-specific primers (complementary to the DNA– strand) and one universal primer (complementary to the DNA+ strand), the latter being identical for all species. The primers were established at the Institute for Medical Parasitology at the University of Bonn (PROFT et al. 1999).

Finally, the DNA bands were visualized in short-wave UV light and documented with a Kodak scanner (digital science TM Image Station 440 CF), and the documents were treated with a graphic program (PhotoShop 5.5) to insert numbers and names. In Fig. 1 we present a gel with the reaction products of six species out of the *A. maculipennis*-complex.

RESULTS

Out of 40 new collection sites during summer 2000 in Switzerland, 36 were positive for *Anopheles* larvae, as shown in the map (Fig. 2). The PCR method revealed the existence of *A. maculipennis* s. s. at 6 localities, *A. messeae* at 6 different

localitites, *A. claviger* at 11 different spots; in 13 additional pools *A. maculipennis* s. s. coexisted with *A. messeae*. When plotted on separate maps (not shown), we could not recognise a distribution pattern and *A. melanoon* was never encountered. Based on our material, the allopatric or sympatric occurrence of the two sibling species in Switzerland appears randomly spread, within an altitudinal range of 196–720 m above sea level. However, it was interesting, that *A. claviger* was never found together with a member out of the *maculipennis* complex. All the larva-positive pools usually were characterised by dense vegetation, but sometimes also with bare edges, but we were unable to assign a particular species to any type of pools or ponds. In contrast to other mosquito taxa however, the larval biotopes were always sunlit, standing water accumulations with floating vegetation.

DISCUSSION

Two members of the *A. maculipennis* complex have been firmly established by PCR for Switzerland: *A. maculipennis* s. s. and *A. messeae*, both being nearly impossible to differentiate by morphological means. *A. melanoon* which has been mentioned in earlier reports (e.g. BÜTTIKER 1948; BRIEGEL 1973) was absent from our samples, even from southern Ticino, and it is very unlikely to form part of the Swiss mosquito fauna, because it rather belongs to the Mediterranean area. Therefore, in the earlier reports we probably are dealing with misidentifications. The distribution maps for *A. maculipennis* s. l. published earlier (GUHL 1944; GEIGY 1945; BÜTTIKER 1948; BRIEGEL 1973) are now assumed to be a conglomeration of these two sibling species.

An interesting result of this report is the absence of *A. claviger* larvae from biotopes harbouring members of the *maculipennis* complex, or vice versa, contrasting reports by RABOUD (1980). We do not know of any obvious reasons for this behaviour, but we admit that the analysis and characterisation of the nature of the breeding sites and their water qualities have not been considered as relevant for this project. Based on our experience with other Culicidae and their breeding sites we believe that the quantitative parameters of a given pool or puddle are less important than its mere existence. Since our country and its lowlands are largely overpopulated and overfarmed, there is not much choice of breeding sites for females seeking oviposition sites. Nevertheless, it would be a project by itself to investigate possible differences in the breeding requirements for *A. maculipennis* versus *A. claviger*. Amongst members of the *maculipennis* complex no overt ecological or behavioural differences exist. Their sympatric occurrence in our samples does refute the claim by WEYER (1940) for *A. messeae* as a representative of the lowlands, and *A. maculipennis* s. s. as one of the more elevated regions.

Furthermore, the occurrence of *A. melanoon* and *A. atroparvus* mentioned by BORRANI (1937), GASCHEN (1940), BÜTTIKER (1948), FOUQUE et al. (1991), JETTEN & TAKKEN (1994), and RAMSDALE & SNOW (2000) is doubtful. *A. atroparvus* with its preference for brackish water cannot be expected in this country because its range is within 100 km from the coastline of the sea (WEYER 1940). The occurrence of *A. melanoon* cannot be excluded completely; it might be encountered one day in the Piano di Magadino, one of the most southern tips of the country with regular floodings, but a positive proof would then be required.

Since the goal of this study was of taxonomic and faunistic nature, frequencies and densities of the *Anopheles* populations were not statistically treated. But in comparison to *Aedes*- or *Culex*-species, the anopheline fauna in general is extremely small and low.

Malaria in Switzerland

Malaria has been a common disease in Switzerland, caused mainly by *Plasmodium vivax* as in all Central and Northern European countries, while *P. falciparum* was frequent in the southern countries (HACKETT & MISSIROLI 1931; BRUCE-CHWATT & ZULUETA 1980; REITER 2001). In Switzerland, the vector of *P. vivax* was *A. maculipennis* (GEIGY 1945), but this species and *A. messeae* are less anthropophilic as long as domestic animals abound in large numbers (KETTLE 1995). The vector potentials of *A. claviger* and *A. plumbeus* STEPHENS, 1828, which both are also present (BRIEGEL 1973), are less pronounced or even unknown as vectors, respectively. If *P. falciparum* infections occur, they must have been imported, either by a tourist, or by a foreign vector, in most cases through air traffic, because laboratory infections of *A. messeae* with *P. falciparum* failed (MARCHANT et al. 1998).

Towards the end of the 19th century a clear decline of malaria was observed in Europe (REITER 2001) and also in Switzerland (GALLI-VALERIO 1901, 1917). For Switzerland, canalisation of rivers, large-scale reclamation of marsh land, and increased cattle farming were some of the most prevalent reasons. Vectors not only declined, but supposedly they also turned to increased zoophily.

Despite Switzerland was declared free of malaria in 1966 by WHO, we are currently confronted with increased cases, as all European countries (REITER 2001). There was quite a similar situation in Switzerland after World War I, when federal health authorities were concerned about possible returns of malaria (GASCHEN 1940). The reason in those days was the arrival of many refugees from malarious regions (GEIGY 1945), and today it is the remarkable tourism to tropical countries. Again, the question arises of whether malaria could re-establish, particularly in connection with global warming.

A few points need to be mentioned to counter public fears. We do not share the view of pessimistic scientists (e.g. MAC KENZIE 1999; PEARCE 1992, 1998) for the following reasons. From a recent review by REITER (2001) it is evident that *Anopheles-Plasmodium* associations, i.e. human malaria, increased and decreased in historical times and in temperate regions independent of cold (Little Ice Age in the 17th and 18th century) or warm temperatures (end of 19th century). As a matter of fact, it even decreased towards the end of the 19th century, parallel to a general temperature increase. In temperate regions it is far more a matter of politics, economics, and human activities that render the spreading or the import of mosquito-borne diseases such as malaria, than climate changes. In the tropical areas however, the interplay of climate and vector biology are far more complex and sensitive, thus far less predictable. In Central Europe tourism and human migrations from south to north undoubtedly carry certain risks, but this can be kept under control by our medical community. And as indicated above, the density for larval breeding sites and the possibilities of *Anopheles* population growth are far too low for an efficient vector system. *A. plumbeus*, for which a vectorial potential for human Plasmodia has been assumed but not substantiated (N. BECKER, pers. comm.), even can be regarded as an endangered species in Switzerland. As shown by this report, it took us four warm summer months of intense collecting to find 36 sites with small numbers of *Anopheles* larvae.

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ZUSAMMENFASSUNG

In früheren Arbeiten war die Situation von *Anopheles maculipennis* sensu lato, einem Artenkomplex von 9 kryptischen Arten, in der Schweiz stets unklar. Deshalb sammelten wir im Sommer 2000 an 40 Orten *Anopheles*-Larven, welche im Labor bis zum 4. Stadium aufgezogen, dann grob bestimmt und in Ethanol fixiert wurden. Mittels PCR und artspezifischen Primern wurden die Schwesterarten aufgeschlüsselt. Im gesamten Material waren lediglich *A. maculipennis* sensu stricto und *A. messeae* präsent, abgesehen von *A. claviger*, die ausserhalb des *maculipennis* Komplexes steht. Frühere Berichte über *A. melanoon* und *A. atroparvus* scheinen auf Fehlbestimmungen zu beruhen. Da die beiden nachgewiesenen Schwesterarten Vektoren von *Plasmodium* darstellen, wird die Frage nach einer Rückkehr menschlicher Malaria diskutiert. Trotz sich häufender Importe von Malaria durch Tourismus und Völkerbewegungen, erscheint das Risiko einer erneuten Etablierung von Malaria in der Schweiz minimal. Auch eine globale Erwärmung vermag die bei uns viel zu geringe Vektorpopulation nicht mehr zu steigern, wohl endgültig mangels günstiger Brutbiotope.

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