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Confirmation of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in Greece

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Abstract

The presence of *Aedes albopictus* (Skuse) was recently confirmed for the first time in Greece. During the last two years, a number of mosquito specimens were sent to our laboratory for identification, with the note that they were abundant and a nuisance in the northwest part of the country. The specimens were seriously damaged and problems were encountered during the process of morphological differentiation between *Ae. albopictus* and *Aedes cretinus* Edwards, two related species of the subgenus *Stegomyia*. *Aedes cretinus* is endemic in many parts of Greece.

Of the morphological characters that differentiate *Ae. albopictus* from *Ae. cretinus*, the only one visible in the specimens was the simple claws of the mid- and foretarsomeres, characterising *Ae. albopictus*. Molecular methods of DNA amplification for the nuclear internal transcribed spacer gene (ITS2) were applied to the specimens under investigation. The consequent DNA sequence confirmed that these specimens were *Ae. albopictus*. The findings of the present study are of particular interest due to the importance of this mosquito species for public health.

Introduction

Aedes albopictus (Skuse), an East Asian mosquito species, was first recorded outside of its indigenous area in the USA. Before the discovery of a substantial population in Houston, Texas (Sprenger & Wuithiranyagool, 1986), sporadic records were reported by Eads (1972) and Reiter & Darsie (1984). Since then, *Ae. albopictus* has been established in a considerable part of the USA, and it is also now present in South America, Australia, New Zealand, Africa and parts of the Middle East. The first European record of *Ae. albopictus* was from Albania in 1979 (Adami & Murati, 1987) followed by Italy in 1990 (Sabatini *et al.*, 1990; Dalla Pozza & Majori, 2002). *Aedes albopictus* has also been reported from other European countries: France (Schaffner *et al.*, 2001), Belgium (Schaffner, personal communication), Montenegro (Petric *et al.*, 2001) and Spain (Aranda *et al.*, 2004). Close to Europe, it has been reported from Israel (Pener *et al.*, 2003). There was no evidence for the occurrence of *Ae. albopictus* in Greece until now although several times in the past misidentifications of the morphologically similar *Ae. cretinus* caused concern. *Aedes cretinus* is the only species of the subgenus *Stegomyia* endemic to Europe with records from Greece (several parts), Cyprus (Nicosia), Turkey (Antalya) and Georgia (Sokhomi) (Lane, 1982; Becker *et al.*, 2003). Because of its limited distribution, there are few publications dealing with *Ae. cretinus* and many aspects of its bionomics are still unknown. *Aedes cretinus* was described by Edwards (1921), who named it after the island of Crete, where the type specimen came from. Because morphological differences are slight, Edwards considered *Ae. cretinus* to be the "Mediterranean representative" of *Ae. albopictus* (Edwards, 1921).

Materials and methods

The material used in this study comprised 14 adult mosquitoes collected during routine sampling by the Departments of Public Health of the Prefectures of Thesprotia and Corfu, in the northwestern part of Greece, and sent to the National School of Public Health for identification.

Due to inexperience in mosquito handling, the mosquitoes were crushed at capture and important characters were missing. Careful examination of the remaining characters differentiated them from *Ae. cretinus*, which is endemic in many parts of the Country, and shares many morphological characters with *Ae. albopictus*.

Molecular techniques were applied to confirm the results. DNA was extracted from the samples following the phenol-chloroform extraction protocol described by Linton *et al.* (2001). PCR amplification of the second internal transcribed spacer 2 (ITS2) region of rDNA was performed using 5.8SF and 28SR primers (Collins & Paskewitz, 1996). The PCR product is 568 bp for *Ae. albopictus* and 435 bp for *Ae. cretinus*. PCR was carried out in a 50 µl volume containing 1 x PCR buffer, 2mM $MgCl_2$, 0.2 mM of each dNTP (Invitrogen Paisley, UK), 1mM of each primer (MWG Biotech, High Point, NC), 2U of Taq DNA polymerase (Invitrogen), and 2µl of template DNA. The thermocycler parameters described in Linton *et al.* (2001) were used. Products were visualised on a 1.5% agarose gel containing ethidium bromide and cleaned using a commercially available PCR purification kit (QIAgen Hilden, Germany), according to the manufacturers instructions. Products were sequenced in both directions, using the ABI 310 automated sequencer at Hygeia Hospital and the chromatograms read by the Chromas programme. Similarity with sequences available in GenBank was assessed using BLAST version 2.2.9 (Basic Local Alignment Tool) (Altschul *et al.*, 1997).

Results

The scutum, where the main differential characters of *Ae. albopictus* and *Ae. cretinus* are located, was seriously damaged in all the specimens. No remnants of white scales were found, which could suggest the presence of the typical white line that occurs laterally on the scutum of *Ae. cretinus*. Simple claws were observed on the mid- and forelegs. This character is found in *Ae. albopictus* only, compared to the toothed claws of *Ae. cretinus*.

Nuclear ITS2 rDNA sequences were generated for all 14 specimens. Amplified products were 568 bp long. The ITS2 fragments of *Ae. albopictus* and *Ae. cretinus* would be visually different by gel electrophoresis. However, DNA sequencing of the PCR products was performed to confirm the results. The ITS2 fragment amplified for the *Ae. albopictus* specimens in our study corresponded to GenBank entry L22060 (Kjer *et al.*, 1994).

Discussion

Due to the increased concern regarding mosquito-borne diseases, local health authorities in northwest Greece captured a number of insects and sent them to our laboratory for identification. The majority of these insects were not mosquitoes, but a few were typical *Ae. cretinus*. Among these specimens were some seemingly different mosquitoes from Corfu and the city of Igoumenitsa in Thesprotia, which proved to be *Ae. albopictus*

The presence of *Ae. albopictus* in the northwestern part of the country (Corfu and Igoumenitsa) was not unexpected. Corfu is an island in the northern Ionian Sea (39^0 49'-39^0 22'N, 19^0 38'-19^0 57' E), with a total surface area of 592.1 km^2. The climate is Mediterranean with high annual precipitation and average relative humidity above 70%. It faces the mainland city of Igoumenitsa (39^0 31'N, 20^0 14'E), from where there is regular transport by ferryboat. In the summer there is also frequent connection with the Albanian city of Himara, where *Ae. albopictus* is well established (Velo & Bino, 2002). Igoumenitsa, with ferry links to Italy, is the capital of the maritime prefecture of Thesprotia, which borders Albania in the north and the prefectures of Ioannina and Preveza in the east and south.

The establishment of *Ae. albopictus* in Greece constitutes a new threat to public health. It is known that this species is a vector of Dengue fever and a potential vector of other arboviruses, among them the causal agents of Yellow fever, West Nile fever and Rift Valley fever (Mitchell, 1995). It is also known that Greece experienced one of the worst recorded epidemics of Dengue fever in 1927-1928, with over a million cases resulting in more than 1500 deaths (Papaevangelou & Halstead, 1977). This fact, combined with the severe nuisance created by *Ae. albopictus*, should be of great concern to public health services.

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Risk of airport malaria in the UK

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Abstract

The inexorable increase in air traffic has fuelled concern that the risk of malaria may rise if vector mosquitoes are imported into non-endemic countries onboard aircraft from malarious zones. We assessed this risk by searching for mosquitoes in 52 aircraft that had flown from Africa and arrived at Gatwick airport, one of the UK's busiest airports. After 43 hours of searching by experts only three exotic mosquitoes were found. All of these were *Culex quinquefasciatus*, a species incapable of transmitting human malaria. The low numbers of mosquitoes recovered indicates that aircraft disinfection is being implemented successfully and the risk of imported malaria vectors by aircraft into the UK is extremely low.

Introduction

There is growing concern about the risk of malaria in and around European airports, resulting from the importation of infective mosquitoes by aircraft, particularly as a result of increased travel to malaria-endemic countries. Airport malaria has been reported from several European countries in the last 30 years including Belgium, France, Germany, Italy, Netherlands, Spain, Switzerland and UK (Danis *et al.,* 1996). The UK was recently listed third in Europe with 14 cases of actual or suspected cases of airport malaria between 1969 and 1999 (Gratz *et al.,* 2000). Despite these cases and the fact that there are over 685,000 flights into the UK each year (Civil Aviation Authority, 2000), there have been no published surveys of aircraft searched for disease vectors in this country for the past 18 years.