Rediscovery of *Aedes cretinus* (Edwards, 1921) (Diptera; Culicidae) in Cyprus, 66 years after the first and unique report.

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Abstract: Mosquitoes are important vectors of parasitic helminths, protozoan and viral pathogens causing human disease. There has been a growing interest in the establishment and spread of invasive aedine mosquito species but also in understanding native mosquitoes. Following concerns regarding black and white-striped mosquitoes biting aggressively during the day, survey visits were organised by the Joint Services Health Unit on the Kryos river at the village of St. Mavra, Limassol district, Cyprus. Adult mosquitoes collected either during human landing catches in June 2015 or adults that emerged in the laboratory from water collected from tree holes in January 2016, were identified as *Aedes* (*Stegomyia*) *cretinus.* During the same period (June 2015), *Ae. cretinus* adults were also collected by the Public Health authorities of the Republic of Cyprus from a local residence at Avdellero village, Larnaca district. *Aedes cretinus* is a species with limited distribution and close resemblance to the Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus.* Information on mosquito species and their respective geographic distribution is fundamental for the goal of reducing the impact of emerging vector-borne viral zoonoses in Europe and targeting control for vector and nuisance species. Our study highlights the need for educational and raising awareness programmes regarding insects of medical importance as well as the importance of collaboration and networking between the relevant authorities. ***Journal of the European Mosquito Control Association* 34: 10-13, 2016**

Keywords: Mosquito, *Aedes, Stegomyia,* Mediterranean basin, distribution, Cyprus.

Introduction

Worldwide, mosquitoes (Diptera: Culicidae) are vectors for a large variety of pathogens. The blood-feeding females transmit the protozoa causing malaria, the filarial worms causing lymphatic elephantiasis, the viruses causing dengue, yellow fever, chikungunya, Zika, Rift Valley fever and West Nile fever as well as additional viruses causing encephalitis and polyarthritis. In addition to their major importance as vectors of human pathogens, mosquitoes can bite humans and other mammals in very large numbers causing nuisance of a magnitude that negatively affects individuals and society, and may have large economic consequences (Mullen & Durden, 2000; Lündstrom *et al*., 2013). In Europe there has been a growing interest in recent years in the establishment and spread of invasive aedine mosquito species (Medlock *et al*., 2012; Medlock *et al*., 2015) but also in understanding the heterogeneity of native mosquito diversity (Medlock and Vaux, 2015a).

Understanding the distribution, abundance and life strategies of native mosquitoes at the local scale can inform surveillance and lead to targeted control programmes (Medlock & Vaux, 2015b). Data collection on the presence of vector or nuisance mosquito species is the first essential step for any risk assessment to be possible (Schaffner *et al*., 2013).

Aedes (*Stegomyia*) *cretinus* Edwards 1921 is a rarely collected and poorly documented mosquito species across Europe (Gaunt *et al*., 2004). Its distribution is limited to the Mediterranean and due to its low population densities it has not been extensively researched. It is mostly due to its close resemblance to the Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1895) that a certain interest has been raised regarding its distribution and bionomics in European countries (Samanidou, 1998). Understanding *Aedes cretinus* current distribution in relation to *Aedes albopictus* (Giatropoulos *et al.* 2012) as well as any interactions with other container breeding species, native or invasive, could be of importance especially since it belongs to the subgenus *Stegomyia* which contains many vector species such as *Ae. albopictus* and *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762). Little is known about the biology of *Ae. cretinus* (Lane, 1982; Becker *et al.* 2010) and its vector potential is unknown (Schaffner & Mathis, 2014). The species is an aggressive anthropophilic daytime biter (Samanidou-Voyadjoglou and Koliopoulos, 1998; Darsie, 1999) and can cause a great amount of nuisance at the locations where it is present.

Aedes cretinus is a dendrolimnic species, found in tree holes (Gutsevich *et al*., 1974) but it could be encountered in containers such as tyres (Schaffner, 2003). There are *Ae. cretinus*

records from Cyprus (Lane, 1982), the island of Crete and several locations on the Greek mainland (Edwards, 1921; Samanidou, 1998; Giatropoulos *et al.*, 2012), Georgia and Antalya in Turkey (Lane, 1982; Alten *et al.*, 2000). In Cyprus, it was first recorded in 1949 in Nicosia (Lane, 1982) but there are no other available records since and it is not included in the list of the mosquitoes of Cyprus published by Violaris *et al.* (2009).

Following concerns expressed by an environmental officer from the Republic of Cyprus in June 2015 who reported the following: *"Many black and white-striped mosquitoes looking like the tiger mosquito bite people aggressively at the yards of the restaurants at St. Mavra"* subsequent surveys were arranged by the Joint Services Health Unit (JSHU) at the village of St. Mavra at Koilani (Limassol district). During the same period (June 2015) they were also complaints from a local resident at Avdellero village (Larnaca district) who reported to the Public Health authorities of the Republic of Cyprus the presence of tiger-looking mosquitoes at his residence.

Materials and Methods

Sites of survey visits

Agia Mavri or St. Mavra (34.84657N, 032.86916E), Koilani Limassol was visited twice in 2015, on the 15th and 26th June 2015. Two more survey visits were arranged in 2016 on the 27th of January and the 13th of March. St. Mavra is an idyllic location in a valley crossed by the Kryos river. The St. Mavra church is built at the west side of the river and apart from the church there are also two restaurants in close proximity. The area is dominated by plane trees with large tree trunks; the St. Mavra plane (*Platanus orientalis*) is 1200 years old and 35 metres high. The Kryos river flows next to the church. The area receives many visitors (tourists, pilgrims, school children) during spring and summer especially on the 3rd of May each year, which is an important celebration date for the church of St. Mavra. Local traditionalists want the wider area of Koilani to which St. Mavra belongs to have been named after Kyllini in Peloponnesus, Greece, by its first residents who were from Arcadia, Greece. There are many artefacts from the Roman period and there has been a lot of agricultural and commercial activity due to the wineries in this region since ancient times, whether mosquitoes were introduced to St. Mavra during the Roman period through commerce can only be speculated. Mosquitoes were also collected in Larnaca by the Public Health authorities of the Republic of Cyprus from a residence at Avdellero village. The village of Avdellero is 3-4 km from the sea front of Larnaca. The local residence was a new house with a garden without many trees and without a lot of vegetation.

Sampling methodology

St Mavra village, Koilani Limassol District

Mosquitoes were sampled as larvae (larval dipping), and as adults (Human Landing Catch [HLC], and adult traps [Heavy Duty EVS CO_2-baited Mosquito Trap, Bioquip USA]). St. Mavra was visited over a period of hours from 09.00 on 15th June and again on the 26th June 2015. Local restaurant owners and their families confirmed the severe mosquito nuisance in the area despite the treatment conducted by local authorities of the Kryos river with larvicides. River Kryos is a small, rocky creek shaded by old plane trees with big trunks with tree-holes. Two surveyors walked for approximately 300 metres upstream, one person at each side of the stream. Each person stopped at 30 points dipping with a standard dipper (50 ml) three times at each point. Dip samples were taken from pools formed by the rocks but also from flowing water. A HLC was then performed. Two surveyors exposed their bare arms for thirty minutes. A CO_2 trap was also left overnight hanging from a tree branch seven metres above the river during the first visit and at three metres above the river during the second visit. Two more survey visits took place in 2016, January 27th and March 13th. During these subsequent surveys all trees along the valley were surveyed for tree holes, with water and humus collected from a number of the tree holes.

Avdellero village, Larnaca District

Four mosquitoes had been collected by the resident around 18th of June, 2015, and given to the Public Health specialists. Upon the collection of these specimens BG-Sentinel™ (Biogents AG, Regensburg, Germany) traps with BG-Lure™ were set at the local residence for a whole year, from 18th June 2015 to 30th June 2016.

Mosquito identification

Mosquito specimens were identified by morphology at the JSHU laboratory with the aid of a key by Darsie and Samanidou-Voyadjoglou (1999) and photographic material provided by Giatropoulos (personal communication; Benaki Phytopathological Institute, Greece), and confirmed by entomologists at Public Health England (PHE). The specimens collected from Avdellero village in Larnaca were first identified by morphology based on an electronic identification key (Schaffner *et al.*, 2001). Afterwards, they were submitted to genetic analysis. DNA isolation and amplification of part (460 bp) of the mitochondrial cytochrome oxidase subunit I gene (mt COI) with primers C1-J-1718_mod (5-GWGGRTTTGGWAAYTGAYTAG-3) and C1-N-2191_mod (5-GTAAAATTAAAATATAAACTTCTGG-3) were done as described (Schönenberger *et al.*, 2016). The rDNA ITS2 region was amplified according to Collins and Paskewitz (1996). Sequencing of the purified amplicons (minelute PCR kit, Qiagen, Hildesheim, Germany) was done by a private company (Synergene, Switzerland).

Results

During the visits at St. Mavra in June 2015, seven female *Ae. cretinus* mosquitoes were collected by HLC, exact landing times were not recorded but individuals were collected between 09:30-11:00 hours. No *Ae. cretinus* were collected from the CO_2 traps on either survey dates. No mosquitoes were found during larval dipping during the first 2015 visit. Dipping on the 26th June 2015 resulted in no collection of immature *Ae. cretinus* but two *Anopheles claviger* sensu lato (Meigen, 1804) immatures were collected from one of the pools formed by the rocky substrate. The anophelines were reared to the adult stage in the laboratory and sent to PHE for identification. They were identified as *An. claviger* based on morphological characters and known species distribution (Becker *et al.* 2010). Tree-holes were inspected where they occurred, but were dry on these dates.

During both visits at St. Mavra in 2016 there were no signs of adult mosquitoes flying. Dip sampling was performed but did not result in any larval collections. Some tree holes were full of water during the January survey so water and humus from dry tree holes were collected and placed in a mosquito chamber in the JSHU entomology laboratory. Three male and two female *Ae. cretinus* emerged from the organic matter collected from the tree holes in the first week of March. During the visit in March 2016, larval dipping and HLC did not

generate any catches, tree holes were inspected but were found dry. All containers noticed in the surroundings of the stream were also inspected and water and debris were collected and brought to the laboratory but no mosquitoes were found.

The four female specimens caught at Avdellero, Larnaca were damaged but their morphology suggested they belong to the *Aedes (Stegomyia)* Scutellaris Group, and two of them could be identified as *Ae. cretinus*, with the presence of a scale patch on metameron being diagnostic to *Ae. albopictus*. Two specimens, one identified as *Ae. cretinus* (specimen 1) and one as Scutellaris Group member (specimen 2), were submitted to molecular analysis. COI sequences (>465 base pairs[bp]) of both specimens were 97.65-98% identical to *Ae. cretinus* GenBank and BOLD entries, just like ITS2 sequences (346 bp) for specimen 1 was 99% identical to GenBank entries. No more *Ae. cretinus* were collected in Larnaca from the BG-Sentinel traps that were set at the residence. Contemporary and historical records of *Ae. cretinus* can be viewed in figure 1.

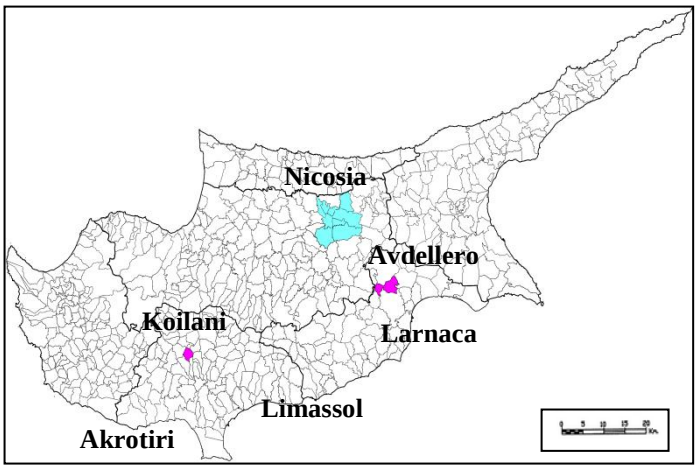

Figure 1: Location of collection sites for *Aedes cretinus* in Cyprus. Blue: historical record (1949); Purple: contemporary records (2015).

Discussion

Information on mosquito species and their respective geographic distribution is fundamental for the goal of reducing the impact of emerging vector-borne viral zoonoses in Europe and targeting control for vector and nuisance species (Ahmed *et al.* 2009). This study highlights the importance of mosquito recording and surveillance schemes. *Aedes cretinus* is not included in the list of the mosquitoes of Cyprus published by Violaris *et al.* (2009) and there is only one published record regarding the species, from 1949 (Lane, 1982). However, interviews with the locals at St. Mavra and also with some of the residents of the wider Koilani area report the presence of black and white-striped mosquitoes around the area causing great nuisance. Further work should be undertaken at St. Mavra and the wider Koilani area, to establish the extent of the distribution and abundance of *Ae. cretinus* or/and any other *Aedes* spp.. Since *Ae. cretinus* belongs to the subgenus *Stegomyia*, more information should be gathered regarding its biology and ecology as well as its vector potential with screening for arboviruses, and vector competence studies. Although the *Ae. cretinus* records generated from our current study are limited they show that tree holes are breeding habitats for *Ae. cretinus* in Cyprus, however despite the fact that the species was not collected from small rock pools by the Kryos river we would not like to exclude the possibility that the species could also breed in other habitats. The observed adult mosquito activity while tree holes were dry could be an indication that adults breed in other habitats as well. Our current findings clearly show the importance of surveys and the need for mosquito identification. Mosquito life habits are important to know in order to run a successful management programme. Suitable methods for treating or managing tree holes should be considered for the control of *Ae. cretinus* at the St. Mavra location. Application of biological larvicides such as *Bacillus thuringiensis israelensis* within each tree hole is an option but it could be challenging and not cost-effective. It is perhaps also unlikely to be adopted by public health workers due to the difficulties in accessing the St. Mavra site on a regular basis to apply the larvicide. The effectiveness of creating physical barriers for mosquito breeding *e.g.*, by netting or placing styrofoam or sand in the tree holes could be explored during a pilot study and the most suitable approach could be adopted. The role of tree holes as breeding habitats for *Ae. cretinus* and other organisms *e.g.*, mosquito predators should be further investigated in Cyprus. Surveillance programmes for *Ae. cretinus* should not exclude other possible breeding habitats, according to the literature the species has been recovered from forests and open areas, from potholes, and small hollows in forests as well as tree holes (Alten *et al.*, 2000) and containers such as tyres (Schaffner, 2003). Although our data is limited we think that *Ae. cretinus* in Cyprus is well adapted to the particular forested/wild/creek habitat and it is not likely to spread to more domesticated environments however monitoring the sites and wider area where *Ae. cretinus* occurs is essential especially since in Greece, it has also been shown that if invasive *Ae. albopictus* is introduced, it has the ability to colonize habitats where *Ae. cretinus* occurs (Giatropoulos *et al.*, 2012).

The lack of finding *Ae. cretinus* in the adult traps is surprising given that this species has been found in Turkey in CO_2 traps (Alten *et al.*, 2000). It would be useful to test a range of traps in the field for a longer period to ascertain the usefulness of traps for *Ae. cretinus* surveys, e.g., the Gravid *Aedes* Traps (GAT™, Biogents), BG-Sentinel with chemical BG-Lure and with or without CO_2, and Mosquito Magnet (Mosquito Magnet®) with octanol lure.

Anopheles claviger s.l. also collected during this study is considered to have been a malaria vector in Cyprus and neighbouring countries such as Azerbaijan, Italy (south), Lebanon, Mesopotamia, and Palestine (Muir & Keilany, 1972). There were cases of malaria on the island of Cyprus for many years until 1946 when an extensive *Anopheles* eradication campaign began. The *Anopheles* Eradication Service operated until 1949 and an official announcement as to the success of the campaign was made on the 10th January, 1950 (Constantinou, 1998). Nowadays, Cyprus is considered malaria-free, however various factors including topography, climate, and the presence of anopheline species are associated with a risk that malaria could be re-introduced. In addition to malaria, there is a risk for a number of arboviruses that infect birds and other species such as West Nile virus, chikungunya, Usutu, Sindbis and Tahyna to become introduced.

Aedes aegypti, the vector of Zika, dengue, chikungunya, and yellow fever viruses has also been historically recorded in Cyprus. It was last observed in the 1930s (Aziz, 1934) but it was not recorded during a survey that took place between 1996 and 2007 (Violaris *et al.*, 2009) and is reported as extinct in an earlier list by Hadjivassilis (2000). There are concerns for other

invasive species not yet recorded in Cyprus but present in the Mediterranean basin such as *Ae. albopictus*, therefore surveillance schemes are essential and so is the immediate response to citizens concerned about the presence of native or invasive species, particularly those similar in appearance to the Asian tiger mosquito. Other mosquitoes such as the saltmarsh mosquito *Aedes* (*Ochlerotatus*) *detritus* (Haliday) can cause massive nuisance problems in tourist areas of Larnaca and Akrotiri, especially around the important wetland sites.

The current findings of *Ae. cretinus* by the investigations of nuisance complaints linked to 'black and white-striped mosquitoes' underpin the importance of public engagement in the detection of invasive species and timely and efficient communication between different environmental and public health units across Cyprus. It is necessary to inform and share information regarding the presence of insect pests and vectors at the local, regional and European level. These were two cases of a well-informed officer and a well-informed local resident about the Asian tiger mosquito who contacted the relevant authorities, but this is not always the case. Educational material targeting the general public to raise awareness regarding arthropods of medical importance, particularly invasive mosquitoes, is crucial and we encourage a network of environmental health officers, local authorities, public health specialists, and entomologists to develop this further.

References

Ahmed, J., Bouloy, M., Ergonul, O., Fooks, A.R., Paweska, J., Chevalier, V., Drosten, C., Moormann, R., Tordo, N., Vatansever, Z., Calistri, P., Estrada-Peña, A., Mirazimi, A., Unger, H., Yin, H. & Seitzer, U. (2009). International network for capacity building for the control of emerging viral vector-borne zoonotic diseases: ARBO-ZOONET. *Eurosurveillance*, 14(12), 1-4. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19160

Alten, B., Bellini, R., Caglar, S.S., Simsek, F.M. & Kaynas, S. (2000). Species composition and seasonal dynamics of mosquitoes in the Belek region of Turkey. *Journal of Vector Ecology*, 25, 145-164.

Aziz, M. (1934) The anopheline mosquitoes of Cyprus. Cyprus Health Department. Paper No. 4, 32 pp.

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M., Dahl, C. & Kaiser, A. (2010). Mosquitoes and their control. 2nd Edition. Springer Verlag Berlin Heidelberg, 577 pp.

Collins, F.H. and S.M. Paskewitz (1996). A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic Anopheles species. *Insect Molecular Biology*, 5, 1-9.

Constantinou, K. (1998). *Anopheles* (malaria) eradication in Cyprus. *Parassitologia.* 40(1-2), 131-135.

Darsie, R.F.Jr. (1999). Description of the pupa of *Aedes cretinus* Edwards, a key to the pupae of the *Albopictus* subgroup, subgenus *Stegomyia* Theobald, genus *Aedes* Meigen, and characters to separate the European *Stegomyia* species (Diptera: Culicidae). *Proceedings of the Entomological Society of Washington*, 101, 614-618.

Darsie, R.F. & Samanidou-Voyadjoglou, A. (1997). Keys for the indentification of the mosquitoes of Greece. *Journal of the American Mosquito Control Association*, 13, 247-254.

Edwards, F.W. (1921). A revision of the mosquitoes of the Palaearctic region. *Bulletin of Entomological Research*, 12, 263-351.

Gaunt, C.M. , Mutebi, J.P. & Munstermann, L.E. (2004). Biochemical taxonomy and enzyme electrophoretic profiles during development, for three morphologically similar *Aedes* species (Diptera: Culicidae) of the subgenus *Stegomyia*. *Journal of Medical Entomology*, 41(1), 23-32.

Giatropoulos, A.K., Michaelakis, A.N., Koliopoulos, G.Th. & Pontikakos, C.M. (2012). Records of *Aedes albopictus* and *Aedes cretinus* (Diptera: Culicidae) in Greece from 2009 to 2011. *Hellenic Plant Protection Journal*, 5, 49-56.

Gutsevich, A.V., Monchadskii, A.S. & Shtakelberg, A.A. (1974). Fauna of the USSR Diptera volume 3(4), Mosquitoes, Family Culicidae, 408 pp.

Hadjivassilis, A. (2000). Mosquitoes (Diptera, Culicidae) of Cyprus. *European Mosquito Bulletin*, 7, 38.

Lane J (1982). *Aedes* (*Stegomyia*) *cretinus* Edwards 1921 (Diptera: Culicidae). *Mosquito Systematics* 14(2), 81-85.

Lundström, J.O., Schäfer, M.L., Hesson, J., Blomgren, E., Lindström, A., Wahlqvist, P., Halling, A., Hagelin, A., Ahlm, C., Evander, M., Broman, T., Forsman, M. & Persson Vinnersten, T.Z. (2013). The geographic distribution of mosquito species in Sweden. *Journal of the European Mosquito Control Association*, 31, 21-35.

Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H. & Van Bortel, W. (2012). A review of the invasive mosquitoes in Europe: Ecology, Public Health Risks and Control Options *Vector-borne and Zoonotic Diseases*, 12(6), 435-447.

Medlock, J.M. & Vaux, A.G.C. (2015a). Seasonal dynamics and habitat specificity of mosquitoes in an English wetland: implications for UK wetland management and restoration. *Journal of Vector Ecology*, 40(1), 90-105.

Medlock, J.M. & Vaux, A.G.C. (2015b). Impact of the creation, expansion and management of English wetlands on mosquito presence and abundance – developing strategies for future disease mitigation. *Parasites & Vectors*, 8. 142.

Muir, D.A. & Keilany, M. (1972). *Anopheles claviger* Meigen as a malaria vector in Syria WHO/MAL/72.757.

Mullen, G.R. & Durden, L.A. eds (2000). Medical and Veterinary Entomology, second edition. Academic Press, Burlington, MA, USA, 637 pp.

Samanidou, A. (1998). *Aedes cretinus*: Is it a threat to the Mediterranean Countries? *European Mosquito Bulletin*, 1, 8.

Samanidou-Voyadjoglou, A. & Koliopoulos, G. (1998). Some notes on *Aedes* (*Stegomyia*) *cretinus* Edwards (Culicidae) in northern Athens, Attiki, Greece, pp. 194-195. In *Fourth International Congress of Dipterology*, 6-13 September, Oxford, United Kingdom.

Schaffner, F. (2003). Mosquitoes in used tyres in Europe: species list and larval key. *European Mosquito Bulletin*, 16, 7-12.

Schaffner, F., Bellini, R., Petrić, D., Scholte, E.-J., Zeller, H. & Marrama Rakotoarivony L. (2013) Development of guidelines for the surveillance of invasive mosquitoes in Europe. *Parasites & Vectors*, 6, 209.

Schaffner, F. & Mathis, A. (2014) Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. *Lancet Infectious Diseases*, 14(12). 1271-1280.

Schönenberger, A.C., Wagner, S.. Tuten, H.C., Schaffner, F. Torgerson, P., Furrer, S., Mathis, A. & Silaghi C. (2016). Host preferences of host-seeking and blood-fed mosquitoes in Switzerland. *Medical and Veterinary Entomology*, 30, 39-52.

Violaris, M., Vasquez, M.I., Samanidou, A., Wirth, M.C. & Hadjivassilis, A. (2009). The mosquito fauna of the Republic of Cyprus: A revised list. *Journal of the American Mosquito Control Association*, 25(2), 199-202.