BioInvasions Records (2019) Volume 8, Issue 3: 551–557

Rapid Communication

First record of *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in Botswana

Mmabaledi Buxton*, Kebaneilwe Lebani, Casper Nyamukondiwa and Ryan J. Wasserman

Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology (BIUST), Palapye, Botswana

Author e-mails: *lodybuxton@hotmail.co.uk (MB), lebanik@biust.ac.bw (KL), nyamukondiwac@biust.ac.bw (CN), ryanwas21@gmail.com (RJW)*

**Corresponding author*

Citation: Buxton M, Lebani K, Nyamukondiwa C, Wasserman RJ (2019) First record of *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in Botswana. *BioInvasions Records* 8(3): 551–557, https://doi.org/10.3391/bir.2019.8.3.10

Received: 27 February 2019

Accepted: 3 June 2019

Published: 14 August 2019

Handling editor: Angeliki F. Martinou

Thematic editor: Stelios Katsanevakis

Abstract

Aedes aegypti is an important vector mosquito for arboviruses globally. It is believed to originate from North Africa and was introduced to other parts of the world through trade systems and transportation. Despite its now widespread distribution, there are still many regions in which the species is likely to occur, but is yet to be reported, hindering our understanding of invasion debt dynamics. Here, we record *Ae. aegypti*, a semi-aquatic invasive species, for the first time in Botswana. The species was sampled from an urban location in Palapye, a village in the arid central district of the country. The larvae were collected from plastic containers with rain water, reared to adults and subsequently identified using morphological and molecular techniques.

Key words: vector, invasive species, arboviruses, arid tropics, southern Africa

Introduction

Aedes aegypti is a vector of some of the most significant arboviral infections, such as Zika, chikungunya, yellow fever and dengue, with significant economic burdens and direct impacts on health risks globally (Bhatt et al. 2013; Weetman et al. 2018). These vectors are adapted to inhabit human settlements, where they breed in artificial systems, e.g. disposed containers (Cheong 1967). The record of vector-arboviral diversity and distribution in Africa has been recently reviewed (see Braack et al. 2018; Powell et al. 2018). Improved transportation systems, urbanization, climate change and vectorial invasive behavior are potentially facilitating increased spread and successful transmission of arbovirus infections (Gould et al. 2017). Beyond its native range, *Ae. aegypti* is considered an invasive species mainly across tropics and sub tropics of the world, exploiting human habitation for its success (Powell and Tabachnick 2013). *Aedes aegypti* has an aquatic component comprising larval and pupal stages which utilise small, often artificial containers holding water (Getachew et al. 2015). The eggs can be readily transported and survive for many months as they can tolerate desiccation with prolonged embryonic viability (Oliva et al. 2018). *Aedes*-borne arboviruses are facilitated by human activities to

expand ranges as they may then be received by vectors in novel environments. Recently, it was progressively determined that arboviral transmission is not only horizontal (between infected *Aedes* species and humans) but can also circulate naturally from infected mosquitoes to its offspring (Ferreira-de-Lima and Lima-Camara 2018). As such, vertical transmission may effectively proliferate arboviral infections within the vector itself before being extended to humans.

Ongoing climate change is facilitating global range expansion of medically important *Aedes* species, particularly through changes to temperature regimes and habitats (reviewed in Reinhold et al. 2018). Similarly, temperature is recognised as a driving factor of the transmission and establishment of *Aedes*-borne infections globally (Tesla et al. 2018). In sub-Saharan Africa, *Ae. aegypti* has been recorded in some countries whilst others have no records (Weetman et al. 2018). The lack of records could be due to a lack of mosquito research, or diverted attention to other vector species such as the Anopheline mosquitoes that transmit malaria. Given that globally, the expansion of arboviruses has followed the spread of their vectors, recording of the presence of vector species in new areas is important in mitigating risks associated with human infection (Bhatt et al. 2013).

In Botswana, the only published information on *Aedes* is a record for *Aedes mcintoshi* from the north of the country (Cornel et al. 2018). Here, we report on the first detection of *Ae. aegypti* from an urbanized location in an arid sub-tropical region of Botswana. This record is important for our understanding of re-distribution dynamics of medically important vectors, with implications for emerging infections, early warning systems and the adequate incorporation of arid ecosystems into invasion debt models.

Materials and methods

Larval collection, rearing and morphological identification

Larval specimens were collected in January 2019 in Palapye village, Botswana (22°32.976S; 027°11.504E) (Figure 1A), from three open 20 L paint containers (300 mm diameter) that collected ~ 10 L rain water adjacent to buildings (Figure 1B). A representation of 4th instar larvae were collected using a 1000 µm mesh sieve, placed in two small plastic containers (300 ml) filled with rain water and housed in a rearing cage (Bugdorm-BD43030F, 240 mm^3, Megaview Science Co., Ltd, Taiwan). Fish food (Lopis: Tropical fish food flakes) was used to feed the larvae *ad libitum*, reared at 27 ± 2 °C and 75 ± 10% RH, under a 12:12 light:dark photocycle in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) housed in the laboratory. After two days a number of adults had emerged and were identified using gross morphology under a stereo microscope (Model: Leica M205 C: TL 5000 Ergo Transmitted Light Base, Leica Microsystems Ltd. Singapore), following Rueda (2004) (Figure 1C). *Aedes aegypti* is generally a small, dark mosquito,

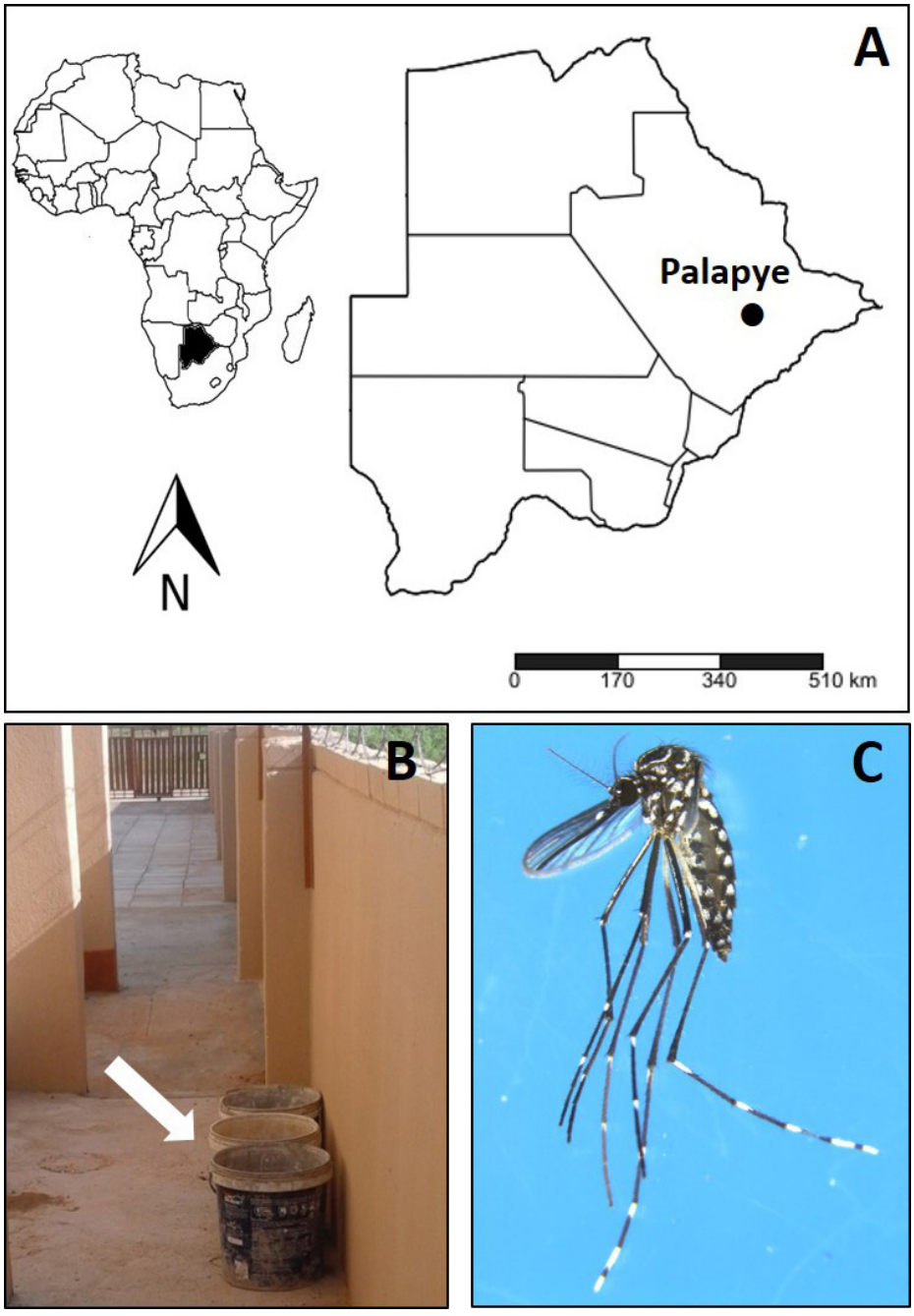

Figure 1. Collection site, habitat and picture of whole specimen. A: A map of the collection locality. B: Habitat from which larvae were collected. C: The entire body of an adult female, emerged from a collected larval specimen. Photographs by MB and RJW.

with white banded striped legs, white femoral knee-spot, clypeus with two white scale patches, white lyre-shaped markings and two white parallel stripes on the scutum (Rueda 2004).

Polymerase Chain Reaction (PCR) and DNA Sequencing

Six of the morphologically identified specimens were used for molecular verification of identity. DNA extraction from whole specimens was performed using Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, USA) according to manufacturer's instructions. DNA was eluted using 60 µL

of nuclease free water (VWR International LLC, USA). Amplification of a 710 bp region of cytochrome *c* oxidase subunit I (COI) by PCR was performed using universal primers LCO 1490 and HCO 2198 for species identification. The 50 µl PCR reaction consisted of 8 µl of extracted DNA, 25 µL of 2x Phusion U Multiplex PCR Master Mix (Thermo Fisher Scientific, USA), 9 µL of nuclease free water (VWR International LLC, USA), and 0.8 µM of each primer. PCR reaction conditions were as follows: initial denaturation at 98 °C for 30 seconds, followed by 35 cycles of denaturation at 98 °C for 10 seconds, annealing at 48 °C for 30 seconds and extension at 72 °C for 30 seconds. This was followed by a final extension at 72 °C for 7 minutes.

Amplicons were visualised on 1% agarose gels stained with ethidium bromide. 40 µL of the PCR product was purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA), according to manufacturer's recommendations. Elution was performed using 20 µL of nuclease free water (VWR International LLC, USA). Sequencing was performed at Inqaba Biotechnical Industries (Pretoria, South Africa) according to recommended protocols for BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA).

Sequence Assembly and Sequence Queries

To obtain consensus sequences, raw sequences were trimmed and contigs were assembled using CodonCode Aligner 8.0.2 software package. The consensus sequences were then subjected to a nucleotide blast (NCBI) and protein translation (http://insilico.ehu.es/translate/ - invertebrate mitochondrial genetic code). The translated amino acid sequence was subjected to a protein blast (NCBI). The nucleotide sequence was deposited in Genbank under accession number MK571449.

Results and discussion

The mosquitos were identified as *Ae. (Stegomyia) aegypti* (Linnaeus, 1762) by diagnostic morphological features on the head, thorax and legs (Figure 2). Four distinct diagnostic features were used, namely: (A) the mesepimeron with two well-separated white scale patches; (B) white lyre-shaped markings on a black scutum and a pair of submedian-longitudinal white stripes; (C) the anterior portion of midfemur having a longitudinal white stripe, and; (D) clypeus on the head with two white separate scale patches.

All six specimens had identical nucleotide sequences. A single consensus sequence was formed. Results confirmed that the specimens were *Ae. aegypti*. The nucleotide sequence had 99.64% homology to a partial cytochrome oxidase subunit I (COI) gene from an isolate of *Ae. aegypti* (GenBank, Accession number: MK300221.1; Appendix 1). When the nucleotide sequence was translated to a protein sequence using a compensatory invertebrate mitochondrial genetic code, the translated sequence had 100% homology to the cytochrome oxidase subunit I protein of *Ae. aegypti*.

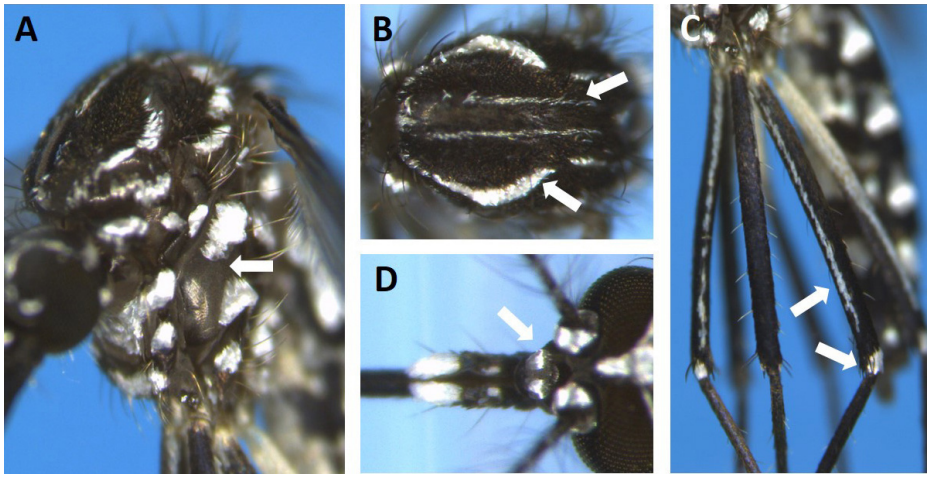

Figure 2. Key morphological characteristics of a collected mosquito: A: Mesepimeron, showing two well-separated white scale patches (arrow). B: Thorax, showing scutum with a pair of submedian-longitudinal white stripes (top arrow), and white lyre-shaped markings (bottom arrow). C: Midfemur, showing white knee-spot (bottom arrow) and anterior portion with a longitudinal white stripe (top arrow). D: Clypeus, showing white scale patches (top arrow). Photographs by MB and RJW.

The distribution of *Ae. aegypti* has been studied in Africa and globally (Kraemer et al. 2015; Ngoagouni et al. 2017). Modeling of vector species and associated arboviral infections demonstrated the capacity for geographic range expansion through correlation with environmental niches and current global change (Tjaden et al. 2017). Recently, southern Africa produced new reports of *Ae. aegypti* in Namibia, Zambia, Angola and Mozambique (Weetman et al. 2018). In particular, Namibia, Angola and South Africa, have close proximity to the neighboring Botswana with potential risks of cross-border invasion. However, the origins of *Ae. aegypti* in Botswana are not yet known. South Africa as the hub for regional and international trade and travel links in southern Africa, has the highest risk of *Aedes* arboviral infections, though currently there are legal constraints to pharmacists issuing preventive travel medicines and vaccines to migrants (Baker 2018). However, cross-border screening of *Aedes*- borne arboviral infections and biological samples thereof, are currently not effectively carried out, thus perpetuating the invasion risk between countries due to travel and trade systems.

The presence of *Ae. aegypti* in Botswana and its formal documentation in the primary literature, contributes to its current global re-distribution and mapping under climate change. Most mosquito studies in Botswana have focused on *Anopheles* as vector of malaria, while a few have reported on *Culex* species (Tawe et al. 2017; Cornel et al. 2018). Very few mosquito surveys have, however, been conducted at a national level and considered the full spectrum of culicid diversity, with no published information available to our knowledge. In the country, the Ministry of Health and Wellness through vector control unit, carries out surveillance on vectors of medical importance annually during the transmission season but the data

on distribution, diversity, breeding hotspots, insecticide resistance status and others, remains unpublished. Thus, further local research on spatial and temporal distribution of mosquito vectors and their invasive potential is required. Further, the prevalence of associated pathogenic infections should be investigated to strengthen existing strategic campaigns on mosquito related diseases and prevention thereof. Botswana, as a tourism destination, attracts an influx of tourists from across the globe. As a result, the arrival of arboviral strains that can potentially be received by the locally reported *Aedes* vectors is a potential risk. While vaccines exist for e.g. dengue (Hadinegoro et al. 2015), there are reported risks associated with its deployment (Ferguson et al. 2016). Furthermore, no vaccines have yet been developed for Zika. Therefore, risks associated with these diseases can only be managed through control of the vector populations, and monitoring of potential arbovirus infections in humans. Given results from this and other regional studies, local research on monitoring relevant *Aedes* species distribution, screening their associated pathogens and control measures, must be prioritized (Baldacchino et al. 2015).

Acknowledgements

We thank BIUST for providing funding to MB, KL, CN and RW, and the Department of Chemical and Forensic Sciences (BIUST) for use of their equipment. We further acknowledge the Ministry of Environment and Wildlife Resource Management for issuing a permit (REF: ENT 8/36/4 XXXXII [14]) for the research. In addition, we sincerely acknowledge the contributions made by two anonymous reviewers to a previous version of the manuscript.

References

Baker L (2018) The role of pharmacists in travel medicine in South Africa. *Pharmacy* 6: 68, https://doi.org/10.3390/pharmacy6030068

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Myers MF (2013) The global distribution and burden of dengue. *Nature* 496: 504, https://doi.org/10.1038/nature12060

Baldacchino F, Caputo B, Chandre F, Drago A, della Torre A, Montarsi F, Rizzoli A (2015) Control methods against invasive *Aedes* mosquitoes in Europe: a review. *Pest Management Science* 71: 1471–1485, https://doi.org/10.1002/ps.4044

Braack L, de Almeida APG, Cornel AJ, Swanepoel R, De Jager C (2018) Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites and Vectors* 11: 29, https://doi.org/10.1186/s13071-017-2559-9

Cheong WH (1967) Preferred *Aedes aegypti* larval habitats in urban areas. *Bulletin of the World Health Organization* 36: 586

Cornel AJ, Lee Y, Almeida APG, Johnson T, Mouatcho J, Venter M, Braack L (2018) Mosquito community composition in South Africa and some neighboring countries. *Parasites and Vectors* 11: 331, https://doi.org/10.1186/s13071-018-2824-6

Ferguson NM, Rodríguez-Barraquer I, Dorigatti I, Mier-y-Teran-Romero L, Laydon DJ, Cummings DA (2016) Benefits and risks of the Sanofi-Pasteur dengue vaccine: Modeling optimal deployment. *Science* 353: 1033–1036, https://doi.org/10.1126/science.aaf9590

Ferreira-de-Lima VH, Lima-Camara TN (2018) Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus*: a systematic review. *Parasites and Vectors* 11: 77, https://doi.org/10.1186/s13071-018-2643-9

Getachew D, Tekie H, Gebre-Michael T, Balkew M, Mesfin A (2015) Breeding sites of *Aedes aegypti*: potential dengue vectors in Dire Dawa, East Ethiopia. *Interdisciplinary Perspectives on Infectious Diseases* 2015: 706276, https://doi.org/10.1155/2015/706276

Gould E, Pettersson J, Higg S, Charre R, de Lamballerie X (2017) Emerging arboviruses: why today? *One Health* 4: 1–13, https://doi.org/10.1016/j.onehlt.2017.06.001

Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, Tran HN (2015) Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *New England Journal of Medicine* 373: 1195–1206, https://doi.org/10.1056/NEJMoa1506223

Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Hendrickx G (2015) The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife* 4: e08347, https://doi.org/10.7554/eLife.08347

Ngoagouni C, Kamgang B, Kazanji M, Paupy C, Nakouné E (2017) Potential of *Aedes aegypti* and *Aedes albopictus* populations in the Central African Republic to transmit enzootic chikungunya virus strains. *Parasites and Vectors* 10: 164, https://doi.org/10.1186/s13071-017-2101-0

Oliva L, La Corte R, Santana M, Albuquerque C (2018) Quiescence in *Aedes aegypti*: Interpopulation Differences Contribute to Population Dynamics and Vectorial Capacity. *Insects* 9: 111, https://doi.org/10.3390/insects9030111

Powell JR, Tabachnick WJ (2013) History of domestication and spread of *Aedes aegypti* - A Review. *Memorias do Instituto Oswaldo Cruz* 108: 11–17, https://doi.org/10.1590/0074-0276130395

Powell JR, Gloria-Soria A, Kotsakiozi P (2018) Recent history of *Aedes aegypti*: vector genomics and epidemiology records. *BioScience* 68: 854–860, https://doi.org/10.1093/biosci/biy119

Reinhold J, Lazzari C, Lahondère C (2018) Effects of the Environmental Temperature on *Aedes aegypti* and *Aedes albopictus* Mosquitoes: A Review. *Insects* 9: 158, https://doi.org/10.3390/insects9040158

Rueda LM (2004) Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Walter Reed Army Institute Of Research Washington Dc, Department Of Entomology, USA, https://doi.org/10.11646/zootaxa.589.1.1

Tawe L, Ramatlho P, Waniwa K, Muthoga CW, Makate N, Ntebela DS, Paganotti GM (2017) Preliminary survey on *Anopheles* species distribution in Botswana shows the presence of *Anopheles gambiae* and *Anopheles funestus* complexes. *Malaria Journal* 16: 106, https://doi.org/10.1186/s12936-017-1756-5

Tesla B, Demakovsky LR, Mordecai EA, Ryan SJ, Bonds MH, Ngonghala CN, Murdock CC (2018) Temperature drives Zika virus transmission: evidence from empirical and mathematical models. *Proceedings of the Royal Society B. Biological Sciences* 285: 20180795, https://doi.org/10.1098/rspb.2018.0795

Tjaden NB, Suk JE, Fischer D, Thomas SM, Beierkuhnlein C, Semenza JC (2017) Modelling the effects of global climate change on Chikungunya transmission in the 21st century. *Scientific Reports* 7: 3813, https://doi.org/10.1038/s41598-017-03566-3

Weetman D, Kamgang B, Badolo A, Moyes C, Shearer F, Coulibaly M, McCall P (2018) *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: Current and future threats. International Journal of Environmental Research and Public Health 15: 220, https://doi.org/10.3390/ijerph15020220

Supplementary material

The following supplementary material is available for this article:

Appendix 1. Polymerase Chain Reaction (PCR) and DNA Sequencing data outputs.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Buxton_etal_SupplementaryMaterial.pdf