Short Report: Historical Analysis of a Near Disaster: *Anopheles gambiae* in Brazil

Aristeidis Parmakelis,* Michael A. Russello, Adalgisa Caccone, Carlos Brisola Marcondes, Jane Costa, Oswaldo P. Forattini, Maria Anice Mureb Sallum, Richard C. Wilkerson, and Jeffrey R. Powell

Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut; Department of Microbiology and Parasitology, Center of Biological Sciences, Universidade Federal de Santa Catarina, Trindade, Florianópolis, Brazil; Laboratório de Biodiversidade Entomológica, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brazil; Division of Entomology Walter Reed Army Institute of Research, Silver Spring, Maryland; Department of Entomology, Smithsonian Institution, National Museum of Natural History, Washington, DC

Abstract. Attributed to human-mediated dispersal, a species of the *Anopheles gambiae* complex invaded northeastern Brazil in 1930. This event is considered unique among the intercontinental introductions of disease vectors and the most serious one: “Few threats to the future health of the Americas have equaled that inherent in the invasion of Brazil, in 1930, by *Anopheles gambiae*.” Because it was only in the 1960s that *An. gambiae* was recognized as a species complex now including seven species, the precise species identity of the Brazilian invader remains a mystery. Here we used historical DNA analysis of museum specimens, collected at the time of invasion from Brazil, and aimed at the identification of the Brazilian invader. Our results identify the arid-adapted *Anopheles arabiensis* as being the actual invading species. Establishing the identity of the species, in addition to being of intrinsic historical interest, can inform future threats of this sort especially in a changing environment. Furthermore, these results highlight the potential danger of human-mediated range expansions of insect disease vectors and the importance of museum collections in retrieving historical information.

The *Anopheles gambiae* species complex contains the most important vectors of the deadliest form of malaria in sub-Saharan Africa, where globally, ~80% of malaria mortality and morbidity occurs. Incidents of *Anopheles* introduction have been reported worldwide but have been restricted. However, introductions coupled with suitable environmental conditions for *Anopheles* establishment are the most threatening. The Brazil invasion was one of these cases. On March 23, 1930, R. C. Shannon collected larvae of *An. gambiae* s.l., near Natal, Brazil (State of Rio Grande do Norte; Figure 1). The species expanded its range, and a serious increase in human malaria cases over 9 years followed with a 20–25% death rate in a largely immune-naïve population. The efforts of a Rockefeller Foundation–supported team led to the eradication of the invading species by 1940, averting a potential public health catastrophe.

Because of the level of shipping traffic between Brazil and Senegal in the 1920s/1930s, it has been assumed that the invader came from this African region, which is inhabited by *An. gambiae* s.s. (with its two molecular forms, M and S), *Anopheles arabiensis*, and *Anopheles melas*. Of these three taxa, *An. melas* is an unlikely candidate given its larval ecology (brackish water); both *An. gambiae* s.s. and *An. arabiensis* have been speculated to be the invaders. In this paper, we used museum specimens (Table 1), collected at the time of invasion from various localities in Brazil, and through DNA analysis, we aimed to identify the Brazilian invader. To avoid potential contaminations, DNA was extracted following strict ancient DNA (aDNA) protocols in an aDNA facility. DNA extractions (from entire mosquito or a few legs) were carried out using the EasyDNA kit (Invitrogen, Carlsbad, CA), using mussel glycogen and protein degrader according to the manufacturer’s protocol. For polymerase chain reaction (PCR) amplification, we targeted short regions (124–419 bp) of the ribosomal DNA (rDNA) intergenic spacer (IGS). This region contains one diagnostic site separating *An. gambiae* (both M and S forms) from *An. arabiensis* and one diagnostic site that allows the separation between the M and S *An. gambiae* molecular forms and between *An. arabiensis* and *An. gambiae* M molecular form. The legitimacy of the diagnostic sites is robust, having been assessed on a large database of IGS DNA sequences from *An. gambiae* s.l. samples collected from all over the African continent. The primers initially used in the PCR amplifications were IGS441 and IGS783, whereas additional primers targeting smaller and larger fragments were also designed and used when the initial IGS primers were not effective. The primers designed for this study were IGS565-forward, IGS581-forward, IGS659-forward, and IGS839-reverse (located in positions 565–584, 581–600, 659–679, and 839–858 of the *An. gambiae* IGS sequence with accession number AF470116, respectively). Primers were used either directly on the DNA extracts, or whenever that was feasible, in a nested PCR protocol. PCR products were visualized in ethidium bromide-

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*Address correspondence to Aristeidis Parmakelis, 21 Sachem St., New Haven, CT 06520. E-mail: parmakel@nhmc.uoc.gr*
stained agarose gels. The PCR products were either purified using the QIAquick PCR purification kit (Qiagen, Germantown, MD) or excised from the gel and purified using the Freeze N’ Squeeze kit (Bio-Rad, Hercules, CA). Cycle sequencing of the purified PCR products was performed using Big Dye chemistry, and sequences were determined on an ABI 3730 automated sequencer.

IGS DNA sequences were successfully obtained (125–204 bp) from 11 of the 13 specimens. All 11 were *An. arabiensis*.

(Figure 2). This result is consistent with the prediction of *An. arabiensis* in Brazil based primarily on its ecology. *An. arabiensis* is the most arid-adapted member of the complex, and the area invaded is arid (Figure 1). Because *An. arabiensis* is arid-adapted, the humid rainforest surrounding the invaded area could well have been crucial in precluding its further spread in 1930s. With the increasing destruction of tropical forests in South America, more territory is converted into an ideal habitat for the previous invader and its equally dangerous sibling species, *An. gambiae* s.s.

Insect vectors of disease can spread to new regions, and it is critical to have surveillance capable of detecting new introductions early. The eradication of this fatal vector in Brazil in the 1930s was a success only because mosquito workers were in the region to combat yellow fever and, by chance, detected *An. gambiae* s.l. Our results point out the importance of museum collections in allowing retrieval of historical information as new understanding (e.g., taxonomy) and technology advances to take advantage of collections.

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**Authors’ addresses:** Aristeidis Parmakelis, Michael A. Russello, Adalgisa Caccone, and Jeffrey R. Powell, Department of Ecology and Evolutionary Biology, Yale University, 21 Sachem St., 06520, New Haven, CT, Telephone: 203-432-3866, Fax: 203-432-7394, E-mails: parmakel@nhmc.uoc.gr, adalgisa.caccone@yale.edu, jeffrey.powell@yale.edu. Aristides Parmakelis, present address: Department of Biology, University of Crete, Knossou Avenue, Irakleio, Crete GR-71409, Greece, Telephone: 2810-392182, E-mail: parmakel@nhmc.uoc.gr, Michael A. Russello, present address: Unit of Biology and Physical Geography, University of British Columbia Okanagan, 3333 University Way, SCI381, Kelowna, British Columbia V1V 1V7, Canada, Telephone: 250-807-8762, Fax: 250-807-8005, E-mail: michael.russello@ubc.ca. Carlos Brito Marcondes, Department of Microbiology and Parasitology, Center of Biological Sciences, Universidade Federal de Santa Catarina, Trindade, 88040-900 Florianópolis, SC, Brazil, Telephone: 55-48-3721-5208, Fax: 55-48-3721-5208, E-mail: cbritosla@umb1.ufsc.br. Jane Costa, Laboratório de Biodiversidade Entomológica, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil, Telephone: 55-21-2598-4401, Fax: 55-21-2573-7276, E-mail: jcosta@ioc.fiocruz.br. Maria Anice Mureb Sallum and Oswaldo P. Forattini, Departamento de Epidemiologia, Faculdade de
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