Geographical limits of the Southeastern distribution of *Aedes aegypti* (Diptera, Culicidae) in Argentina

Leonardo M. Díaz-Nieto[1], Arnaldo Maciá[2], M. Alejandra Perotti[3], Corina M. Berón[1*]

1 Centro de Estudios de Biodiversidad y Biotecnología (CEBB-CIB-FIBA-Mar del Plata) – CONICET, Mar del Plata, Argentina, **2** División Entomología, Facultad de Ciencias Naturales y Museo de La Plata (UNLP), La Plata, Argentina, **3** School of Biological Sciences, University of Reading, Reading, UK

***** E-mail: cberon@fiba.org.ar

Background

Aedes aegypti (Linnaeus) is a human-biting mosquito and the primary vector of human dengue and yellow fever viruses; it is also considered the principal vector of Chikungunya virus in Asia [1,2]. In particular, dengue and dengue hemorrhagic fever constitute an important burden to humankind in terms of morbidity and mortality. About 3.6 billion people in the tropics, mainly in Asia, the Western Pacific region, the Caribbean, as well as Central and South America, live under risk of infection with one or more of the four dengue virus serotypes (DEN-1 to DEN-4), and recent reports estimate over 230 million infections, over 2 million cases of the severe form of the disease and 21,000 deaths [3].

It is believed that *A. aegypti* originally migrated from West Africa to the New World beginning with the 15th century aboard slave ships, and after that yellow fever appeared in the new world. Presumably the yellow fever virus was introduced by travellers on these ships, especially African slaves. The adaptation of this insect to survive in human environments was crucial for colonization and development in water storage containers in the holds of sailing ships [4]. At present, *A. aegypti* lives in close proximity to people, in urban areas, breeding in all types of domestic and peridomestic collections of fresh water, including flower vases, water drums, tins, broken coconut shells, old tyres and gutters. A major range of expansion of *Aedes* mosquitoes into these urban areas is also attributable to the adaptation of the genera *Aedes* to breed in water-holding automobile tyres [5].

A. aegypti is a tropical and subtropical species spanning a geographical distribution from 35°N to 35°S. Its lower thermal threshold corresponds to 10°C isotherms during the winter, and although it has been found up to 45°N, its presence in colder regions is due to its ability to colonise new areas during the warm season [6]. In South America, the historic direction of dispersal of *Aedes* mosquitoes has been towards higher latitudes and from tropical to sub-tropical areas, in particular in the Southern Cone. We propose, that the south eastern movement of *A. aegypti* might be related to human migrations from rural areas to towns lacking in a proper housing policy and essential services like water, and sewage disposal systems (http://www.migraciones.gov.ar/pdf_varios/estadisticas/Patria_Grande.pdf) [7].

Between the 1950s, 1960s and most of the 1970s, in Central and South America epidemic dengue was rare because *A. aegypti* had been eliminated from most of the countries. The eradication program organized by the Pan American Health Organization (PAHO) was discontinued in the early 1970s, and consequently the mosquito was reintroduced in countries from which it had been eradicated [6,8]. In Argentina, the earliest records of *A. aegypti* go back to the 1900s and are concurrent with the dengue-like epidemic of 1916, which affected the coastal areas of the Uruguay River (31°44’S, 60°31’W) [9]. However, in 1986 re-infestation took place in the northern border with Paraguay, deriving in its spread over wide areas of the country. Nowadays, the current geographical distribution of *A. aegypti* in Argentina is wider than during its eradication in 1967 [10,11]. Recently it has been demonstrated that the three *A. aegypti* main haplogroups identified in Argentina would represent different colonization events, probably from neighboring countries: Bolivia, Paraguay, and Brazil (Fig. 1A and B) [7]. Particularly, in Buenos Aires province, the most densely populated area of the country, the records of high abundances of well-established populations of *A. aegypti* were taken in La Plata (capital of the province) and in Buenos Aires (capital city of the country), both located on the East coast, and the southernmost findings in Chascomús, from 132 Km from Buenos Aires city (35°33’S, 58°00’W, Fig 1) [10-15].

On the other hand, cases of dengue have increased in the last few years in Argentina. From January to June 2012, 2,043 patients with symptoms were reported, and 194 were confirmed with serotypes DEN-1, DEN-2 or DEN-3 (http://www.msal.gov.ar/dengue/images/stories/partes_dengue/parte74.pdf). In 2011 PAHO emitted an epidemiological alert due to the introduction of DEN-4 serotype in the Americas (http://new.paho.org), being Brazil, Paraguay and Bolivia countries of high risk of dengue infection, with 57,267 possible cases and 5 deaths (Brazil); 10,827 suspected cases, 30 victims (Paraguay) and 3,233 notified cases with 28 deaths (Bolivia) (Fig 1B) (http://www.msal.gov.ar/dengue/images/stories/partes_dengue/parte74.pdf).

In the USA, the dispersal of *Aedes albopictus* Skuse offered an opportunity to understand the synanthropic behavior of *Aedes* mosquitoes. The mosquito was introduced in 1985 in the continental territory through shipments of used tyres containing eggs originated in Asia [16]. In subsequent years, the pattern of spread of this container-dwelling species followed the main interstate highways [17], quickly reaching and colonizing several new areas of the USA in a few years. We wondered whether *A. aegypti* would present a similar behavior, and is making use of human transportation [18]. For this, we investigated the occurrence of the mosquito in major roads connecting densely populated cities with the Southeast of Argentina (Table 1).

One of the most important highways in Argentina is the Provincial Route Nº2, connecting Buenos Aires and La Plata cities with Mar del Plata city and the most visited beaches of the country, principally in summer time, representing about two million people commuting between those places (Fig 1C and Table 1) (http://www.indec.mecon.ar). Route Nº2 crosses the most prominent wetland areas of the Pampas, and its construction has definitely reshaped the landscape, making available new manmade wetlands, which offer shelter to an increasing diversity of flora and fauna, including mosquitoes [19]. On this artery there are some small towns that offer several travel services such as tyre-repair stations or "gomerías", which store used automobile and truck tyres for long periods of time, thus these tyres accumulate rainwater (Fig 2 and 3A). Moreover, along this highway a lot of vehicles transport goods from the north of the country to the coastal area without any sanitary control to prevent insects exchange from one region to the other. The latest scientific southernmost record of *A. aegypti* carried on in Buenos Aires province, was obtained in Chascomús a town located on Route Nº2 [11]. Route Nº2 takes the bulk of the traffic and people in south-eastern direction. On the other hand, Route Nº11, connecting Buenos Aires and La Plata cities with the Atlantic coast, is a short motorway parallel to the coastline and Route Nº226 runs south-west and is mostly used by freight transport (Fig 1C).

Present distribution of *A. aegypti* in the most populated areas of the Buenos Aires province

In order to understand the status of the southern distribution of ***A. aegypti***, we sampled mosquito larvae and pupae during the rain period, in January and March 2011, and only in March 2012 because rainfall levels were very low in January (Fig. 2). The sampling stations were located in towns situated along Route Nº2 and the other two major arteries that connect Buenos Aires with the South. The sampling stations were cemeteries, that are far from the towns and are shortly visited and "gomerías" located in densely populated areas of each town, both at the edge of the roads (Fig. 3 and Table 2). Larval specimens were collected and reared until fourth instar or adult stage to facilitate identification using specific keys [13,20]. Voucher specimens, prepared from all localities, were submitted to the local museum, Museo de Ciencias Naturales "Lorenzo Scaglia" (Mar del Plata, Argentina).

Larvae of *A. aegypti* were found in March 2011 and 2012 in Chascomús, agreeing with and confirming previous records [11,13]. Here we report the finding of *A. aegypti* in the towns of Lezama, Castelli and Dolores for the first time. A population of mosquitoes was found in Lezama in March 2011, 39.2 Km southeast of Chascomús, being both localities separated by farmland and uniquely connected by Route Nº2. As a high number of larvae of all stages and pupae were found in multiple containers in this locality, we feel confident that Lezama holds a natural, well-established population. In March 2012, we found a higher number of larvae of all stages and pupae in the same type of containers for a second time in Lezama; and for the first time in Castelli (27.7 Km south from Lezama) and Dolores (59.5 Km south from Lezama), stating Dolores the southernmost limit of the species' range within Argentina, now 98.7 Km south from

Chascomús (Fig 1C). In Routes N°11 and 226 *A. aegypti* was not found in any of the water containers examined.

In the south of Argentina *A. aegypti* is very likely to be moving by passive dispersal using the major highway connecting the North with the Southeast of the country. It is noteworthy that this same behavior has been studied and documented in a closely related species, *A. albopictus* in the USA. Previous observations on this mosquito in North America are consistent with the hypothesis of mosquito migration facilitated by anthropic action, presumably by transportation of scrapped tyres through the interstate highway system [17]. In *A. aegypti*, egg resistance in absence of water, a feature shared with *A. albopictus*, can lead to a similar way of transferring to new places in order to breed. Therefore, passive dispersal of *Aedes* species using frequented freeways should be considered at the time of designing new monitoring programs.

According to Shepherd *et al.* [21] dengue virus transmission follows two general patterns: epidemic dengue and hyperendemic dengue. Epidemic dengue transmission occurs when dengue virus is introduced into a region as an isolated event that involves a single viral strain. If the number of vectors and susceptible hosts are sufficient, explosive transmission can occur with an infection incidence of 25-50%. Hyperendemic dengue transmission is characterized by the circulation of multiple viral serotypes in an area with susceptible hosts and competent vector (with or without seasonal variation) and appears to be a major risk for dengue hemorrhagic fever. Travelers to these areas are more likely to be infected than travelers going to areas that experience only epidemic transmission.

In South America, particularly in Buenos Aires Province, it is known that the provincial Health Ministry has a program of surveillance of *A. aegypti*, which involves mosquito larvae and eggs monitoring and their control. However, this surveillance does not follow a regular pattern, being erratic in terms of time and each council or municipality decides to carry it on or not. In addition, to obtain official data from concrete actions is sometimes unlikely to find.

The new biogeographical record of Central and Southern Argentina, reported in this article, is an important fact of the constant expansion of *A. aegypti* into new southernmost areas. Together with the presence of the different dengue serotypes indicate that the situation is far more dangerous than previously thought. Urgent and responsible actions must be taken to control the Dengue vector and its further expansion into new areas.

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Legends to figures

Figure 1. *Aedes aegypti* and dengue fever in South America. (A) Historical distribution of *A. aegypti* in Argentina, indicating: 1916, firth dengue-like epidemic; 1986 re-infestation places and biogeographical records between 1991 and 1999, **(B)** Current geographic distribution of *A. aegypti* and regions with risk of transmission of dengue in South America, **(C)** Studied area, showing highways between Buenos Aires and Mar del Plata cities, sampling points and distances between them. (A and B) adapted from Curto *et al*., Vezzani and Carbajo, [10,15] and http://www.healthmap.org/dengue/index.php.

Figure 2. Weather conditions of the studied area, from July 2010 to June 2012. On the left mean temperature in ºC (T), on the right % of relative humidity (RH) and total precipitation in mm (PP). http://www.tutiempo.net/clima. Arrows indicate sampling times.

Figure 3. Sampling places in Buenos Aires province: (A) Tyre-repair stations showing tyres with accumulated rainwater, **(B)** flowerpots at cemeteries.

Table 1. Information of cities connected by Route Nº 2 in Buenos Aires Province (http://www.censo2010.indec.gov.ar/).

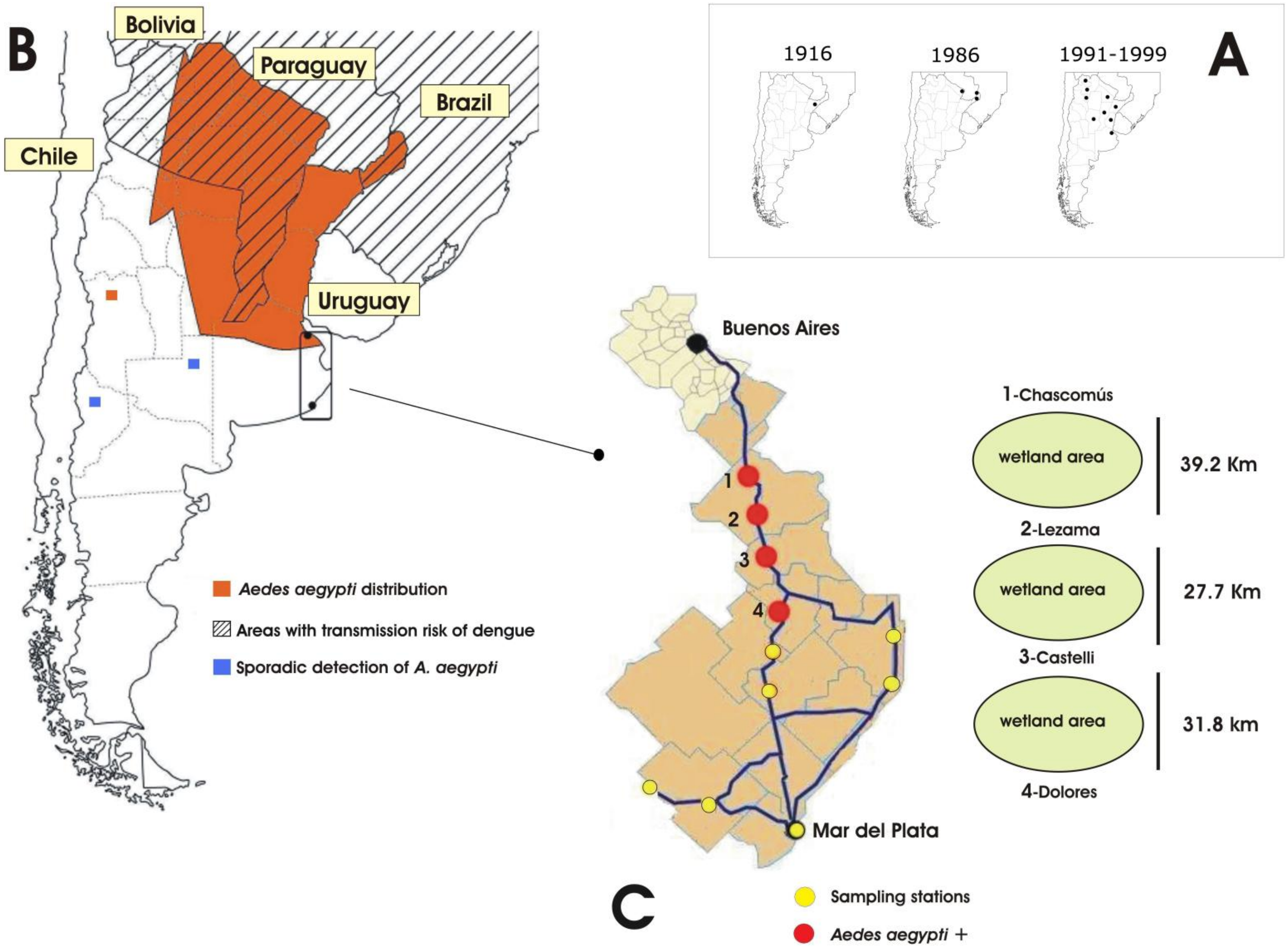
City	Area km^2	Population size	Number of households
Buenos Aires	2,681	12,801,365	3,147,638
Chascomús	3,452	38,477	18,277
Lezama	1,102	4,111	nd[c]
Castelli	2,063	8,206	3,448
Dolores	1,973	26,601	10,687
General Guido	2,814	2,814	1,508
Maipú	2,641	10,172	4,375
Mar del Plata	1,461	618,989	308,570
MdP, Summer time[a]	1,461	2,000,000	nd[c]

[a]MdP, Mar del Plata.
[c]No data

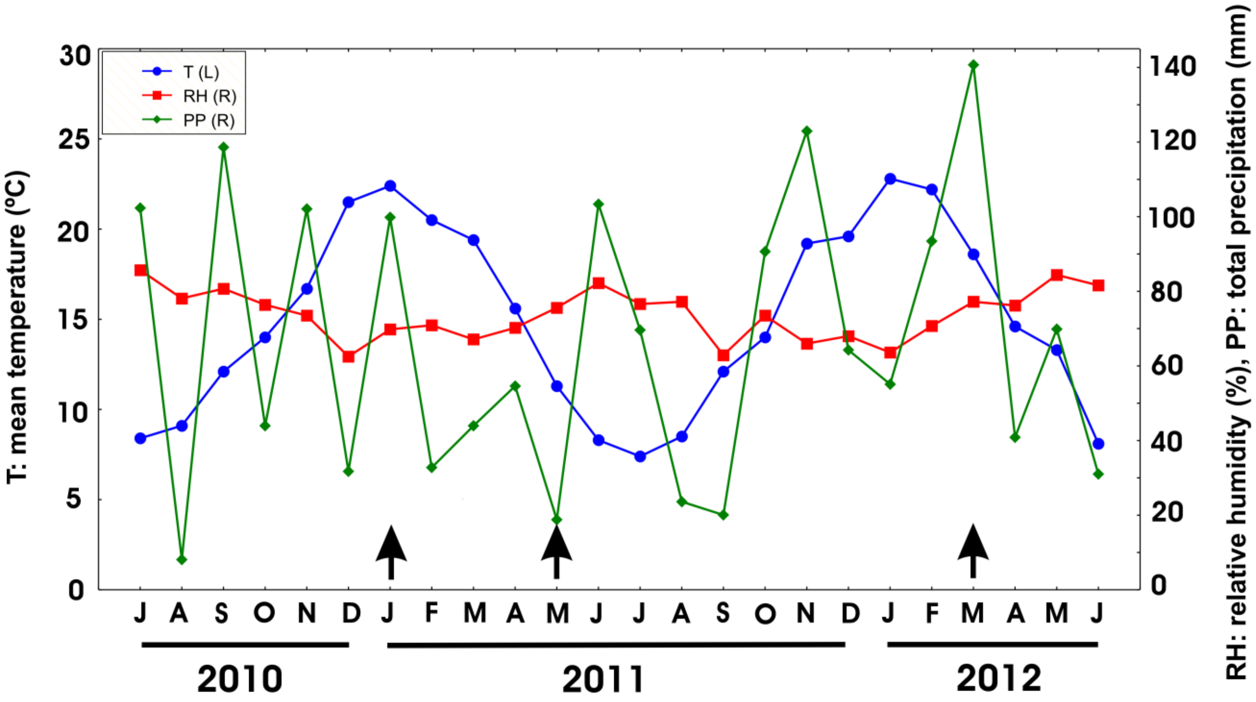
Table 2. Sampling stations and species collected in cities along Route Nº 2, in the Southeast of Argentina.

	2011						**2012**					
City	**Flowerpots**[a]	***Culex* sp.**	***A. aegypti***	**Tyre-repair stations**[b]	***Culex* sp.**	***A. aegypti***	**Flowerpots**[a]	***Culex* sp.**	***A. aegypti***	**Tyre-repair stations**[b]	***Culex* sp.**	***A. aegypti***
Chascomús	239 (12/0)	+	-	1 (1/1)	+	+	300 (8/2)	+	+	2 (2/2)	+	+
Lezama	200 (0/0)	-	-	3 (3/1)	+	+	200 (5/0)	+	-	3 (3/3)	+	+
Castelli	480 (0/0)	-	-	3 (3/0)	+	-	200 (3/2)	+	+	3 (3/3)	+	+
Dolores	730 (29/0)	+	-	2 (2/0)	+	-	400 (12/1)	+	+	2 (2/2)	+	+
Gral. Guido	280 (1/0)	+	-	2 (2/0)	+	-	300 (7/0)	+	-	3 (3/0)	+	-
Maipú	440 (5/0)	+	-	2 (2/0)	+	-	nd[c]	nd[c]	nd[c]	2 (1/0)	+	-
Mar del Plata	3,600 (~45/0)	+	-	10 (8/0)	+	-	3,600 (~45/0)	+	-	10 (8/0)	+	-

[a] Number of flowerpots sampled, in brackets positive ones for *Culex sp* and for *A. aegypti* respectively.
[b] The number of *A. aegypti* was 500 larvae or more in each tyre-repair station, in brackets positive ones for *Culex sp* and for *A. aegypti* respectively.
[c] No data



A
1916
1986
1991-1999
B
Bolivia
Paraguay
Brazil
Chile
Uruguay
Aedes aegypti distribution
Areas with transmission risk of dengue
Sporadic detection of A. aegypti
C
Buenos Aires
1
2
3
4
Mar del Plata
1-Chascomús
wetland area
39.2 Km
2-Lezama
wetland area
27.7 Km
3-Castelli
wetland area
31.8 km
4-Dolores
Sampling stations
Aedes aegypti +



T (L)
RH (R)
PP (R)
T: mean temperature (ºC)
RH: relative humidity (%), PP: total precipitation (mm)
30
25
20
15
10
5
0
140
120
100
80
60
40
20
0
J A S O N D J F M A M J J A S O N D J F M A M J
2010
2011
2012


GOMERIA
CUBIERTAS DE OCASION
A
B