

## Update on the distribution of *Aedes albopictus* (Diptera: Culicidae) in Misiones, Argentina

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### ABSTRACT

The Asian tiger mosquito (*Aedes albopictus*, Diptera: Culicidae) has spread rapidly in the last 30 years from its native region in Southeast Asia. In Argentina, studies on its potential distribution suggest that this species could be found in temperate zones of the province of Buenos Aires. However, since its initial detection in 1998 *Ae. albopictus* is bounded to the subtropical province of Misiones. To evaluate the presence and abundance of *Ae. albopictus* in the Northeast of Argentina, we preliminarily evaluated the presence of this vector by analyzing its presence in tires of 20 cities belonging to the province of Misiones and four cities in Northern Corrientes, and then performed an evaluation of the vector in the towns where the vector was detected. *Aedes albopictus* was present only in two cities of Misiones: Eldorado and Colonia Aurora. *Aedes aegypti* and *Ae. albopictus* accounted for 86% of the individuals collected in the domiciles of both towns. In Colonia Aurora both species were in similar abundances suggesting a co-dominance. The present study extends the austral distribution of *Ae. albopictus* in Argentina to the city of Colonia Aurora where the highest abundance recorded in Argentina was detected. Nevertheless, the reasons of its bounded distribution in the region are not known.

**KEYWORDS:** Mosquitoes. Tires. Vector. *Aedes aegypti*. *Ae. albopictus*. Asian tiger mosquito. Diptera: Culicidae. Distribution. Arboviruses.

### INTRODUCTION

In the world, the Asian tiger mosquito *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) is a competent vector for at least 22 arboviruses. These include chikungunya and the four serotypes of dengue virus, mainly transmitted by *Ae. aegypti*<sup>1</sup>.

This species has spread rapidly in the last 30 years from its native region in Southeast Asia. It is an invasive mosquito whose dry-resistant eggs have facilitated its transport to other continents through used tires and other artificial containers<sup>1,2</sup>. At present, this mosquito is well established in different parts of North and South America, Southern Europe, and islands of Oceania and part of Africa<sup>3</sup>.

*Aedes albopictus* was discovered almost simultaneously in the United States and Brazil in the mid 1980s<sup>2</sup>. This species had previously been detected in tire shipments in North American ports and in a Tennessee cemetery. The Northern limit of this species corresponds roughly to the -5 °C isotherm, as predicted by cold-resistance studies<sup>3</sup>. The species entered Florida in 1986, from the temperate North and had already colonized all Florida counties by 1994, coinciding with a decline in *Ae. aegypti*'s population<sup>2</sup>.

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In Central and South America, this species was reported in 16 countries or islands<sup>4,5</sup>. In Brazil, 24 out of 27 States are already colonized<sup>6</sup>, but in Argentina, *Ae. albopictus* is bounded to the province of Misiones since its discovery in 1998<sup>7</sup>. Adult, pupae and larvae were recorded at Iguazu National Park and in the cities of San Antonio, Eldorado, Puerto Iguazu and Comandante Andresito<sup>7-9</sup> (Figure 1). In 1998, the only ecological study to date reported a relative low abundance when compared to *Ae. aegypti* ( $\approx 1: 10$ ) in the municipality of Eldorado, and described that larvae and pupae of both species were collected in the same artificial breeding sites<sup>8</sup>.

To date, studies have described that *Ae. albopictus* predominates in rural areas and *Ae. aegypti* in highly urbanized habitats, while both are present in suburban areas<sup>10</sup>. This pattern was proposed as a promoter of the

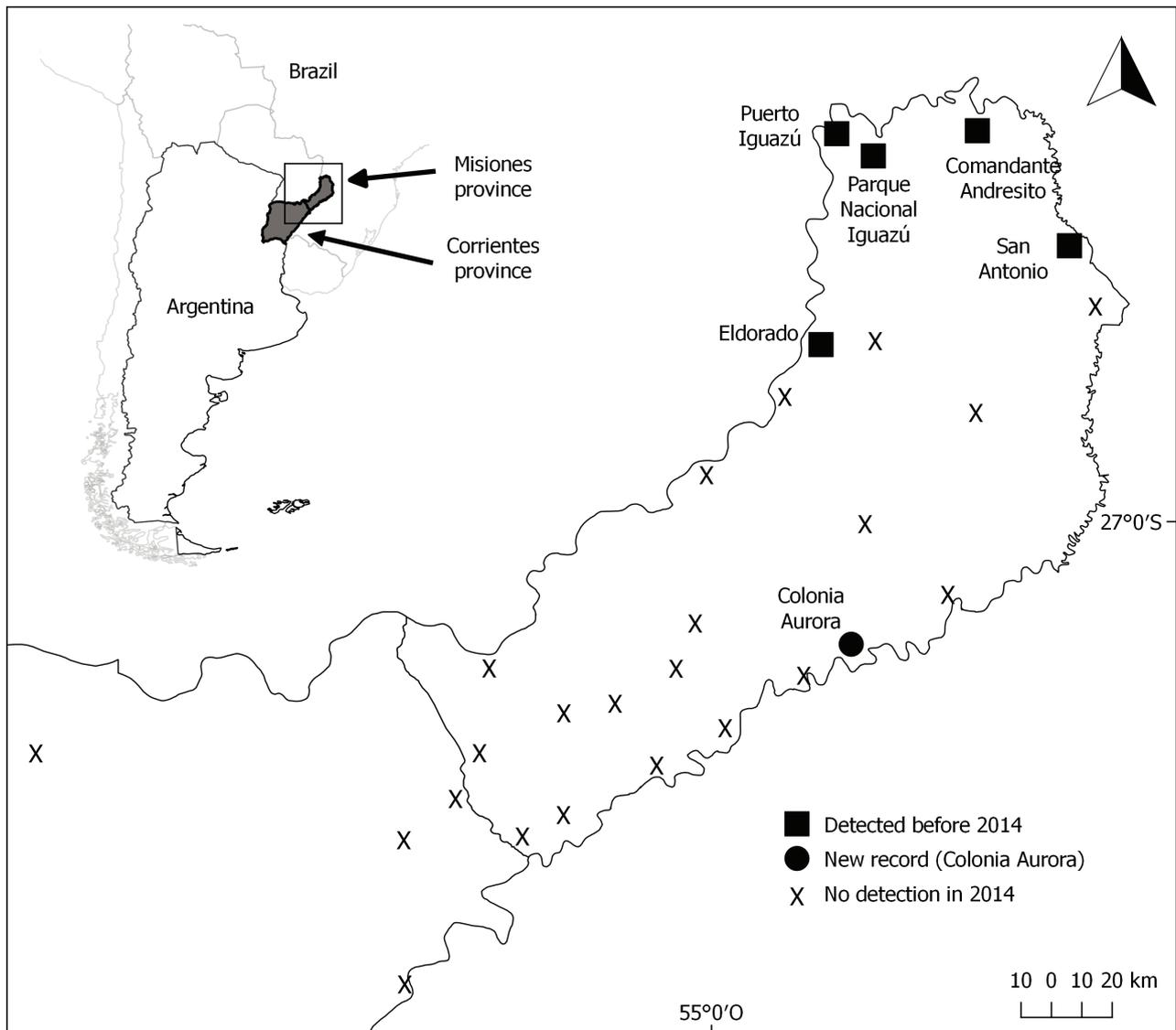
coexistence between species, avoiding direct competition.

Since its dispersion across the world occurred passively through transport and commercial exchange of used tires with presence of preimaginal stages and eggs, the present work has preliminarily evaluated the presence of this vector in tires of different districts of the province of Misiones and Northern of Corrientes; then evaluated the vector in the houses of the cities where its presence was detected.

## MATERIALS AND METHODS

### Study area

We sampled 20 cities of the province of Misiones and four in the North of the province of Corrientes (Figure 1, Table 1). The study area has a subtropical climate, with



**Figure 1** - Location of the province of Misiones and Corrientes in Argentina (upper left). Cities evaluated in 2014 and records with presence of *Ae. albopictus* in the province of Misiones.

**Table 1** - Mosquitoes captured by species in the cities surveyed in 2014.

City	Human population	# Tire deposits	Tires with larval stages	AEG	QUI	FLU	BIG	ALB	Total
ED	63,931	4	18	146	64	49	4	2	287
ES	22,898	4	8	5	179	3	5		196
LA	31,051	2	15	93	19	59	10		185
SL	1,950	1	4	5	2	8	1		17
BI	13,768	3	7	12	5	38	6		71
SP	31,051	3	16	110	7	20	2		143
SV	44,999	3	6	16	29	2	1		48
VI	32,983	1	12	16	39	126			182
AZ	4,113	2	3	3	1	2			22
CN	14,181	1	6	8	3	68			80
AP	7,098	1	2	6	42	1			49
JA	13,030	2	4	23	1	42			66
LO	3,012	1	11	20	36	1			57
CV	10,078	3	9	40	19	4			63
MO	24,338	1	5	35	13				56
CL	3,900	1	2	4	1				5
JO	7,095	2	2	15	8				23
ST	25,824	2	3	4	3				14
PR	19,500	1	2	10		6			20
CS	7,988	1	2	4		12			16
CZ	5,854	1	1	14					14
CA	7,744	5	18		41	29	8	37	138
PA	5,928	1	2		5	4	2		11
OB	66,112	1	4			8	1		9
Total		47	162	589	517	482	40	39	1,772

From Misiones: Alba Posse (AP), Azara (AZ), Bernardo de Irigoyen (BI), Candelaria (CN), Cerro Azul (CZ), Colonia Aurora (CA), Concepcion de la Sierra (CS), Campo Viera (CV), Eldorado (ED), El Soberbio (ES), San Javier (JA), San Jose (JO), Leandro N. Alem (LA), Montecarlo (MO), Obera (OB), Panambi (PA), Puerto Rico (PR), Santiago de Liniers (SL), San Pedro (SP), San Vicente (SV). From Corrientes: Colonia Liebig (CL), Loreto (LO), Santo Tome (ST), Gobernador Ingeniero Valentin Virasoro (VI). Human population according to Ministerio del Interior<sup>22</sup>; number of specimens of the most abundant species: *Ae. aegypti* (AEG), *Ae. fluviatilis* (FLU), *Cx. quinquefasciatus* (QUI), *Lt. bigoti* (BIG), *Ae. albopictus* (ALB).

precipitations throughout the year (from 1564 mm to 2012 mm, annually). The average annual temperature varies between 20 and 21 °C, with mild winters (phytogeographical region: Parana Province, Amazonian Domain)<sup>11</sup>.

### Collection and analysis of data

In January 2014, we collected preimaginal stages present in the study area (Figure 1, Table 1). We evaluated 5-30 tires per tire deposit in each city (1 to 5 deposits/ city). Up to a total of five deposits were examined in each city, providing that no larvae were found at the first deposit (in some of the cities all deposits available were sampled)<sup>12</sup>.

Pre-imaginal stages were collected and fixed *in situ* with alcohol 70%.

In March 2015, samples were collected from 50 houses with breeding sites in the towns of Colonia Aurora (20) and Eldorado (30) (Misiones, Figure 1). In Colonia Aurora, sampling was carried out throughout the village. As Eldorado has a greater extension (Table 1), the city was stratified into two areas: one urban and one peri-urban. In each zone, samples were taken from 13 and 17 houses, respectively. All the houses were selected according to the criteria of accessibility and the informed consent of all the owners of the houses was obtained with the approved consent of the Ethics Commission.

Only artificial containers with water in the peridomicile of the houses were examined. Each artificial breeding site was classified according to the type of container. Mosquito larvae were collected by filtration and fixation in alcohol 70%. All specimens collected were transported to the laboratory, examined under a stereoscopic microscope and specimens were identified at the species level<sup>13</sup>.

To evaluate the existence of independence between towns, a Pearson's Chi-squared test was performed using *Infostat* with the relative abundances of *Ae. aegypti* (Linnaeus, 1762), *Ae. albopictus* and the rest of the collected species.

### Comparison with Eldorado in 1998

In order to compare this study with the situation of *Ae. albopictus* in Eldorado observed in 1998<sup>8</sup>, we compared the number of positive breeding sites for *Ae. albopictus*, the number of larvae of *Ae. albopictus* per artificial breeding site, the percentage of breeding sites for *Ae. albopictus* with *Ae. aegypti*, the ratio between *Ae. albopictus* and *Ae. aegypti* and the occurrence of other species sharing the breeding site with *Ae. albopictus*.

## RESULTS

In January 2014, *Aedes albopictus* was present only in two cities out of the 24 sampled (Figure 1, Table 1). Taking the total number of culicine collected in tire deposits (1772), *Ae. aegypti*, *Cx. quinquefasciatus* (Say, 1823) and *Ae. fluviatilis* (Lutz, 1904) were the most abundant species, corresponding to 89.6% of the sample (Table 1),

so *Ae. albopictus* represented 5.6% of the total sample. The rest of the species were: *Lutzia bigoti* (Bellardi, 1862), *Toxorhynchites* sp., *Cx. eduardoi* (Casal & Garcia, 1968), *Cx. coronator* (Dyar & Knab 1906), *Cx. tatoi* (Casal & Garcia, 1971), *Cx. maxi* (Dyar, 1928) and *Cx. apicinus* (Philippi, 1865).

In March 2015, we collected, in the houses, a total of 2,945 specimens (1,842 in Eldorado and 1,103 in Colonia Aurora) belonging to 11 species of the genera *Aedes*, *Culex*, *Limatus* and *Toxorhynchites*. *Aedes aegypti* and *Ae. albopictus* accounted for 86% of total individuals. A total of 149 containers were inspected, of which 71 (47.6%) were positive. The relative abundances of these species depended on the town (Pearson's Chi-square = 932.63,  $gl = 2$ ;  $p < 0.0001$ ). While in Eldorado *Ae. aegypti* was the dominant species with a relative abundance of 88.4%, *Ae. albopictus* accounted for 6.4% of the individuals caught. On the contrary, in Colonia Aurora both species were dominant, representing 71.4% of the specimens collected, being *Ae. albopictus* slightly more abundant (37.1% for *Ae. albopictus* and 34.3% for *Ae. aegypti*).

Regarding the comparison between the 1998 study and ours, in 2015, one can observe that although in the first study the number of inspected containers with water was of 2,237 in comparison with 90 in 2015; in the latter the percentage of containers with *Ae. albopictus* and the number of individuals of this species was higher (Table 2).

## DISCUSSION

The present study extends the austral distribution of *Ae. albopictus* for Argentina to the municipality of Colonia

**Table 2** - Comparison of *Ae. albopictus* status in Eldorado in 1998 (according to Schweigmann *et al.*<sup>8</sup>) with the status in Eldorado and Colonia Aurora in 2015.

Variables/ year	Eldorado		Colonia Aurora
	1998	2015	2015
Inspected containers	2,237	90	59
Breeding sites with <i>Ae. albopictus</i> (%)	33 (1.47)	13 (14.44)	15 (25.42)
Breeding sites with <i>Ae. albopictus</i> / houses with <i>Ae. albopictus</i>	1.08	1.3	1.36
Number of <i>Ae. albopictus</i> larvae/ breeding sites of <i>Ae. albopictus</i>	1.43	9	27.27
Percentage of breeding sites of <i>Ae. albopictus</i> with <i>Ae. aegypti</i> (%)	85	100	93
Proportion ( <i>Ae. albopictus</i> : <i>Ae. aegypti</i> )	≈(1:10)	≈(1:14)	≈(1:1)
Other species that shared the breeding site with <i>Ae. albopictus</i>	<i>Li. durhamii</i>	<i>Li. durhamii</i>	<i>Li. durhamii</i>
	<i>Ae. fluviatilis</i>	<i>Ae. fluviatilis</i>	<i>Ae. fluviatilis</i>
	<i>Toxorhynchites</i> sp.		<i>Cx. quinquefasciatus</i>
	<i>Culex</i> sp.		<i>Cx. bidens</i>

Aurora (27°28' S; 54°31' W), 120 km of the Southernmost record in the city of Eldorado<sup>8</sup> (26°24' S; 54°38' W). To date, most of the studies on this vector have only indicated its occurrence in the country<sup>7,9</sup>. In the only ecological report, Schweigmann *et al.*<sup>8</sup> found low abundances in Eldorado, homogeneously distributed.

After 20 years, *Ae. aegypti* remains the most abundant species in this city and occurs simultaneously with the Asian tiger mosquito in all breeding sites. *Aedes albopictus* continues to be found in a smaller proportion, but in Colonia Aurora both species are the most abundant and they present similar abundances. Regarding the species that share breeding sites with *Ae. albopictus*, *Ae. aegypti* remains the species with which it coexists mostly, and to a lesser extent with *Li. durhamii*, *Ae. fluviatilis* and *Culex* species.

Based on the present study and other reports that have also monitored the same study area<sup>9</sup>, the bounded distribution of *Ae. albopictus* in the province of Misiones is striking, considering that the vector occurs at higher latitudes in Uruguay<sup>4</sup> and is widely distributed in Southern Brazil, more precisely in Rio Grande do Sul State<sup>14</sup> that borders the provinces of Corrientes and Misiones. In this context, it is possible that a greater sampling effort on tires is required to detect *Ae. albopictus* in the cities of Misiones. Brazilian studies indicate that it is the most frequent and dominant species in this type of breeding site<sup>15,16</sup> so that searching for *Ae. albopictus* in tires is expected to retrieve positive results.

Despite this, the reported distribution of *Ae. albopictus* differs from other studies that predicted that this species could be potentially found to the South of the province of Buenos Aires ( $\approx 38^\circ$  S Latitude)<sup>5,17</sup>. According to Kreamer *et al.*<sup>5</sup>, one of the most important predictors is the temperature that limits the gonadotrophic cycles of the vector. These studies were based on bibliographic data that investigated the vector survival, information that is unknown for local populations.

Due to the lack of diapause in the South American populations, several studies indicate that the local population of *Ae. albopictus* could have a tropical origin<sup>18</sup>. It is possible that this attribute influences the distribution of this mosquito. Since warm area insects exhibit a high minimum development threshold temperature<sup>19</sup>, an evaluation of larval development and survival rates of Latin American populations at low temperatures would be necessary to indicate whether temperate zones can affect survival and development. However, the lack of diapause was not a limitation for the expansion of *Ae. aegypti*, species that also lacks diapause and whose range reaches temperate regions such as the province of Buenos Aires<sup>12</sup>. In this context, it would also be interesting to evaluate other

factors that could affect the distribution of the vector in the region, such as eggs mortality, a situation observed in the United States, where eggs of *Ae. albopictus* have a higher mortality than the ones of *Ae. aegypti*<sup>20</sup>.

According to the Provincial Institute of Statistics and Census, Province of Misiones<sup>21</sup>, Eldorado is characterized as an urban area, while Colonia Aurora is rural. However, the results of this study differ in part from those observed in the American continent where spatial segregation of both vectors is observed<sup>10</sup>. In our study, in Colonia Aurora, both species were in similar abundances characterizing a co-dominance. According to Braks *et al.*<sup>10</sup>, *Ae. aegypti* generally predominates in highly urbanized habitats and *Ae. albopictus* in rural areas. In that study, they attributed the high abundances of this species in rural environments to a greater competitive advantage compared to *Ae. aegypti*.

After 20 years since its discovery in the province of Misiones, the reasons for the limited distribution of *Ae. albopictus* in Argentina remain unknown, considering that it has a great dispersive capacity in other regions of the world<sup>2</sup>. This study demonstrated that *Ae. albopictus* has not increased its distribution with the speed that was registered in others regions of the world. However, the high abundances in Colonia Aurora and the fact that this species went South in neighboring countries, is warning that this vector has still the potential to expand its geographic niche, posing a risk to the public health in Argentina. For this reason, evaluating the population attributes and interaction with other species and parasites in the region will contribute to understand what factors are affecting the presence and abundance of the vector in the country and to determine the control measures of this vector.

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## AUTHORS' CONTRIBUTIONS

Arturo A. Lizuain participated on the conception, planning, sampling, analysis, interpretation and writing of the work; Marina Leporace participated on the planning, sampling, interpretation and writing of the work; Maria Soledad Santini and María E. Utgés participated on the planning, interpretation and writing of the work; and Nicolás Schweigmann participated on the planning, sampling, analysis, interpretation and writing of the work. All authors approve the final submitted version.

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