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Mosquito vectors of yellow fever virus in areas of epidemiological risk in northeastern Argentina

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Abstract. Due to several epizootic events between 2020 and 2021 in the south of Brazil near the border with Argentina, we performed mosquito surveys in areas of epidemiological risk in northeast Argentina to evaluate abundance and distributions of mosquito vectors from the yellow fever virus (YFV) (Diptera: Culicidae). For the most abundant species, the number of individuals captured per collector was evaluated based on the capturing time range. With a total sampling effort of 191 collector-hours, we captured 676 mosquitoes belonging to 6 genera and 16 species. The most abundant species were *Aedes* (*Ochlerotatus*) *scapularis* (Rondani) (33.58%), *Sabethes* (*Sabethes*) *albiprivus* Theobald (20.27%), *Aedes* (*Stegomyia*) *albopictus* (Skuse) (17.75%) and *Haemagogus* (*Conopostegus*) *leucocelaenus* (Dyar and Shannon) (15.86%). *Sabethes albiprivus* and *Ae. albopictus* showed activity peaks at noon and morning, respectively, while other species showed no important time differences between 10:00AM and 15:00PM. Our results show that the most abundant mosquito species in those environments with epidemiological risk in northern Corrientes and southern Misiones are categorized as important in the transmission of the YFV. Moreover, we report an expansion of *Ae. albopictus* distribution and the first record of *Aedes* (*Ochlerotatus*) *patersoni* Shannon and Del Ponte in Corrientes and Misiones provinces.

[Keywords: *Aedes albopictus*, *Haemagogus leucocelaenus*, *Sabethes albiprivus*, arbovirus]

Resumen. Vectores del virus de la fiebre amarilla en zonas de riesgo epidemiológico del noreste argentino. Debido a varios eventos epizoóticos entre 2020 y 2021 en el sur de Brasil, cerca de la frontera argentina, realizamos muestreos con el fin de evaluar la abundancia y distribución de los mosquitos (Diptera: Culicidae) vectores del virus de la fiebre amarilla en áreas de riesgo epidemiológico del noreste de Argentina. Además, para las especies más abundantes, se evaluó el número de individuos capturados por colector en función del tiempo. Con un esfuerzo de muestreo total de 191 horas-colector se capturaron 676 mosquitos pertenecientes a 6 géneros y 16 especies. Las especies más abundantes fueron *Aedes* (*Ochlerotatus*) *scapularis* (Rondani) (33.58%), *Sabethes* (*Sabethes*) *albiprivus* Theobald (20.27%), *Aedes* (*Stegomyia*) *albopictus* (Skuse) (17.75%) y *Haemagogus* (*Conopostegus*) *leucocelaenus* (Dyar y Shannon) (15.86%). *Sabethes albiprivus* y *Ae. albopictus* mostraron picos de actividad, mientras que el resto de las especies presentaron actividad sin diferencias entre las 10:00AM y las 15:00PM. Nuestros resultados evidencian que las especies de mosquitos más abundantes en ambientes con riesgo epidemiológico se encuentran incriminadas en la transmisión del virus de la fiebre amarilla. Por otro lado, informamos sobre una expansión de la distribución de *Ae. albopictus* en el país y el primer registro de *Aedes* (*Ochlerotatus*) *patersoni* Shannon y Del Ponte en las provincias de Corrientes y Misiones.

[Palabras clave: *Aedes albopictus*, *Haemagogus leucocelaenus*, *Sabethes albiprivus*, arbovirus]

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Introduction

Viral diseases increased their importance in recent decades due to changes in viruses and vector distribution and the reappearance of neglected arboviruses that have the potential to become a serious health problem in the short term (Yu and Cheng 2022). Yellow fever is considered a risk disease for South America since outbreaks have frequently occurred in countries such as Brazil, Paraguay and Bolivia during the last decades (MSN 2018). Between 2007 and 2009, an epizootic was recorded; it affected the howler monkeys *Alouatta caraya* (Humboldt) and *Alouatta guariba* (Humboldt) in the provinces of Corrientes and Misiones (Argentina), impacting both primate populations (Holzmann et al. 2010). During this outbreak, nine human cases were recorded (five of them fatal) and viral isolation was achieved in samples from dead monkeys, patients and mosquitoes of the species *Sabethes* (*Sabethes*) *albiprivus* Theobald (Holzmann et al. 2010; Goenaga et al. 2012). Furthermore, Argentina declared an epidemiological alert in 2019 and recommended epidemiological surveillance for Misiones and Corrientes provinces due to the risk of epizootics reported in Brazilian bordering areas (Santa Catarina, Paraná and Rio Grande do Sul). Viral circulation has been registered since 2017 in this country (Mares-Guia et al 2020). As a result, seven imported human cases of yellow fever were recorded in Argentina, three of which died (MSN 2018).

Among yellow fever vectors, the Asian tiger mosquito —*Aedes* (*Stegomyia*) *albopictus* (Skuse)— is a recent spread species in the province of Corrientes (Goenaga et al. 2020). This invasive mosquito was first detected in the country in 1985 in San Antonio, in the province of Misiones (Rossi 1999). Later on it was found in other localities in Misiones, such as Eldorado (Schweigmann et al. 2004), Puerto Iguazú, Comandante Andresito (Rossi 2006) and Colonia Aurora (Lizuain et al. 2019). Recently, Goenaga et al. (2020) reported *Ae. albopictus* in the province of Corrientes for the first time, one adult individual in Gobernador Virasoro and three in Garruchos. Although the presence of other yellow fever virus (YFV) vectors such as *Haemagogus* (*Conopostegus*) *leucocelaenus* (Dyar and Shannon) (primary vector) and *Sa. albiprivus* (Cano et al. 2021) is known in the northeast of Corrientes, their abundances and ecological characteristics have not been thoroughly studied so far.

Due to the limited knowledge about YFV vectors in Northeastern Argentina and several epizootic events in the south of Brazil between 2020 and 2021, we conducted a mosquito survey to evaluate abundance and distributions of YFV vectors in several locations with the presence of primates in the Northeast of Corrientes province and the southeast of the province of Misiones.

Materials and Methods

Surveys were carried out in seven localities during March and April 2021 in the northeast of Corrientes and the southeast of Misiones, Argentina (Figure 1A,B): Santo Tomé (Taji Poty Natural Municipal Reserve) (28°34′16.66′′ S - 56°0′42.87′′ W); Gobernador Virasoro (Las Marías establishment) (28°6′39.05′′ S - 56°3′16.58′′ W); San Carlos (27°44′31′′ S - 55°53′41.18′′ W); Colonia Liebig (27°53′35.32′′ S - 55°52′58.62′′ W); Garabí (Paraje Las Chispas) (28°12′37.88′′ S - 55°49′11.84′′ W); Garruchos (28°10′37.04′′ S - 55°38′31.13′′ W), and Puerto Azara, next to El Chimiray stream (Misiones province) (28°7′39.37′′ S - 55°38′44.95′′ W). Sampling points correspond to the phytogeographic region called Campos y Malezales, that extends along the southern part of Misiones and east of Corrientes, a landscape mainly covered by grasslands, small patches of forests and gallery forest along the edge of streams (Burkart et al. 1999). As many regions in Argentina, cattle ranching and forest plantations with exotic trees (*Pinus* spp. and *Eucalyptus* spp.) have increasingly replaced native vegetation (Nanni et al. 2020). Sampling sites were selected based on the known presence of social groups of black-and-gold howler monkeys, which are considered yellow fever sentinels due to their high susceptibility to YFV (Holzmann et al. 2010; Kowalewski et al. 2011).

Three to eight collectors stayed at fixed points within shaded areas of gallery forests at each location, collecting adult mosquitoes approaching for blood meals with nets and manual vacuum devices between 10:00AM and 15:00PM (local midday: 12:50PM). This time period was selected in order to capture YFV vectors that have diurnal activity with the highest probability to capture *Sabethes* species around midday (Pinto et al. 2009). Despite not using repellent, collectors' bodies were fully covered. The captured were stored in aspirator collection tubes and transported to the laboratory. Each site was

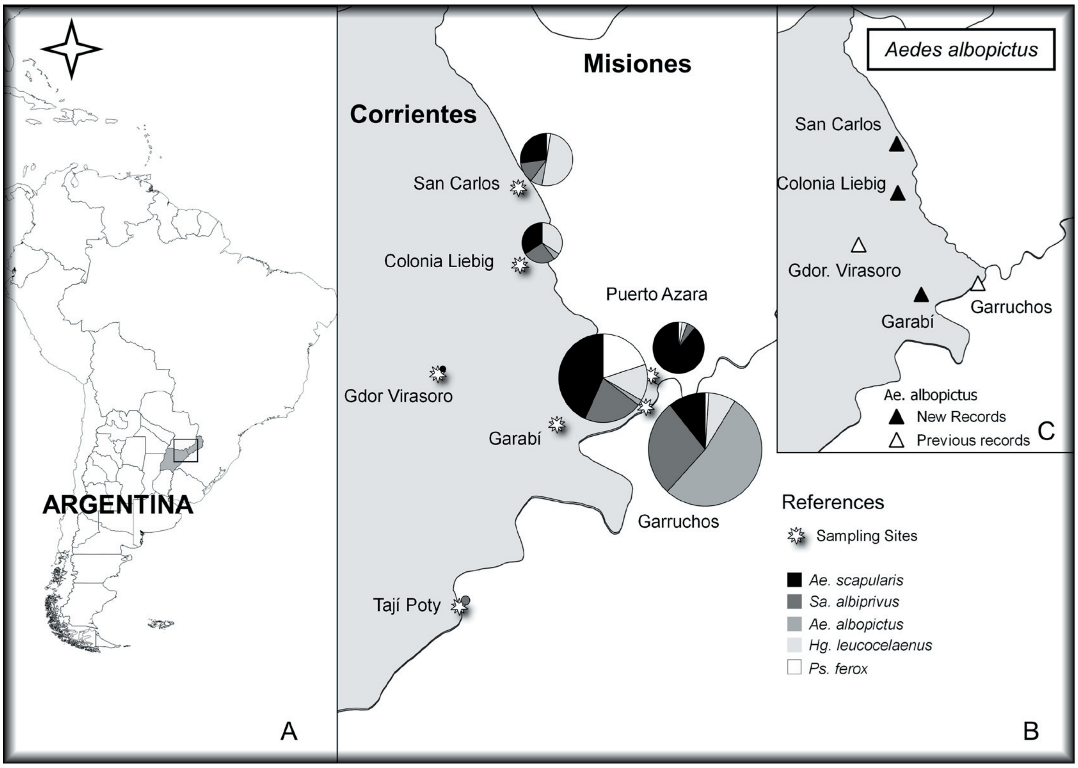

Figure 1. A) Location of Argentina in South America and studied area in the north of Corrientes province and south of Misiones province (box). B) Total number of specimens captured at each site and relative abundance of the five most abundant mosquito species (*Aedes albopictus*, *Aedes scapularis*, *Haemagogus leucocelaenus*, *Psorophora ferox* and *Sabethes albiprivus*). The size of the pie indicates the number of specimens captured at each site (from 10 in Taji Poti to 206 in Garruchos). C) New and previous records of *Ae. albopictus* in Corrientes province.

Figura 1. A) Ubicación de la Argentina en América del Sur y área de estudio en el norte de la provincia de Corrientes y el sur de la provincia de Misiones (recuadro). B) Número total de ejemplares capturados en cada sitio y la abundancia relativa de las cinco especies más abundantes (*Aedes albopictus*, *Aedes scapularis*, *Haemagogus leucocelaenus*, *Psorophora ferox* y *Sabethes albiprivus*). El tamaño del círculo indica el número de ejemplares capturados en cada sitio, siendo mínimo en Taji Poty (n=10) y máximo en Garruchos (n=206). C) Registros nuevos y previos de *Ae. albopictus* en la provincia de Corrientes.

visited twice in a row, with the exception of Puerto Azara, which was visited only one day. In the lab, adults were frozen until deceased, pin-mounted and identified using general dichotomous keys (Lane 1953; Darsie 1985), with original descriptions, redescriptions and revisions also used when necessary (Shannon and Delponte 1928; Guedes and De Souza 1964; Arnell 1976).

The number of captured mosquitoes of the most abundant species per hour and day was modeled with generalized linear and mixed models (GLMM), considering time as the fixed factor of interest and the sampling site as a random factor due to the lack of independence between observations from the same location. To control for the different number of people who collected mosquitoes between sites, the capturing effort was incorporated as an offset as the logarithm of the number of collectors. For models of *Sa. albiprivus*, *Hg. leucocelaenus* and *Ae. albopictus*, we assumed Poisson error distributions (log link); but for *Ae.* (*Ochlerotatus*) *scapularis* (Rondani), a negative binomial error distribution was assumed, and for *Ps.* (*Janthinosoma*) *ferox* (von Humboldt), a Conway-Maxwell Poisson distribution (because of diagnostics of error over-dispersion and under-dispersion, respectively, compared to those expected under Poisson distributions). Deviance tests were conducted to evaluate differences for the fixed factor (assuming χ^2 distributions for the difference between their deviations), and —when appropriate— post hoc Tukey tests were performed to compare between time periods. All models were fitted with the lme4 package (Bates et al. 2015) within the R environment (R Core Team 2023), except for the model for *Ps. ferox*, which was fitted with functions in the glmmTMB package (Brooks et al. 2017).

Results

With a total sampling effort of 191 collector-hours, we captured 676 mosquitoes belonging to 16 species from genera *Aedes*, *Haemagogus*, *Limatus*, *Mansonia*, *Psorophora* and *Sabethes* (Table 1). The most abundant species were *Ae. scapularis* (33.58%), *Sa. albiprivus* (20.27%), *Ae. albopictus* (17.75%), *Hg. leucocelaenus* (15.86%) and *Ps. ferox* (5.32%). *Aedes scapularis* was the most abundant in Garabi, Gobernador Virasoro and Puerto Azara; *Hg. leucocelaenus*, in San Carlos and Colonia Liebig; *Sa. albiprivus*, in Taji Poty, and *Ae. albopictus*, in Garruchos (Figure 1B). Furthermore, *Ae. albopictus* was also present in Garabí, San Carlos and Colonia Liebig (Figure 1C). Other identified species were collected in low proportion (<2%): *Ae.* (*Ste.*) *aegypti* (Linnaeus), *Ae.* (*Och.*) *crinifer* (Theobald), *Ae.* (*Georgecraigius*) *fluviatilis* (Lutz), *Ae.* (*Och.*) *patersoni* Shannon and Del Ponte, *Ae.* (*Och.*) *serratus* (Theobald), *Li. durhamii* Theobald, *Ma.* (*Mansonia*) *indubitans* Dyar and Shannon, *Ma.* (*Man.*) *titillans* (Walker), *Ps.* (*Jan.*) *albipes* (Theobald), *Ps.* (*Jan.*) *discrucians* (Walker) and *Sa.* (*Peytonulus*) *identicus* Dyar and Knab (Table 1). Specifically, two specimens of *Ae. patersoni* were captured in San Carlos (Corrientes) and Puerto Azara (Misiones), respectively. They were the first records for this species in both provinces and were deposited in the Diptera collection of the Museo de La Plata (vouchers MLPDipC10000, 10001).

Regarding host-seeking activity, both *Sa. albiprivus* (χ^2=24.61; df=4; P<0.001) and *Ae. albopictus* (χ^2=15.48; df=4; P=0.004) showed significant differences in the number of individuals captured per hour and collector. *Sabethes albiprivus* presented the highest value (0.78±0.30 individuals.collector^{-1}.h^{-1}) at 13:00PM-14:00PM, while *Ae. albopictus* had a maximum value of 0.10±0.10 individuals.collector^{-1}.h^{-1} at 10:00AM-11:00AM (Figure 2). The other three species did not show significant hourly differences between 10:00AM and 15:00PM: *Aedes scapularis* presented a mean capture rate of 0.69±0.39 individuals.collector^{-1}.h^{-1} (χ^2=1.83; df=4; P=0.76), *Hg. leucocelaenus* 0.21±0.15 individuals.collector^{-1}.h^{-1} (χ^2=1.43; df=4; P=0.84) and *Ps. ferox* 0.11±0.11 individuals.collector^{-1}.h^{-1} (χ^2=1.28; df=4; P=0.86) (Figure 2).

Discussion

According to the vector incrimination criteria (Cano et al. 2022), 13 of the 16 species recorded in northeastern environments of Argentina might be important species in YFV transmission, including the five most abundant species recorded in the studied area. In Argentina, *Sa. albiprivus* is the most important species, incriminated as a vector due to its association with primates, the detection of the virus in natural infected specimens and its association with YFV outbreaks (Goenaga et al. 2012). Although there is still no local

Table 1. Number of adult mosquitoes per species captured in each location and total number of individuals per species in each location: Colonia Liebig (CL), Garabí (Gb), Garruchos (Ga), Las Marias (LM), Puerto Azara (PA), San Carlos (SC) and Taji Poty (TP).

Tabla 1. Número de mosquitos adultos capturados por especie en cada ubicación y número total de individuos por ubicación y especie: Colonia Liebig (CL), Garabí (Gb), Garruchos (Ga), Gobernador Virasoro (GV), Puerto Azara (PA), San Carlos (SC) y Taji Poty (TP).

	CL	Gb	Ga	GV	PA	SC	TP	Total
Aedes aegypti		1		1		11		13
Aedes albopictus	4	3	109			6		122
Aedes crinifer	1	8						9
Aedes fluviatilis						1		1
Aedes patersoni					1	1		2
Aedes serratus		3			1	1		5
Aedes scapularis	25	65	22	10	82	22		226
Aedes sp.					1			1
Haemagogus leucocelaenus	25	20	16		3	43		107
Limatus durhamii	1		1					2
Mansonia indubitans							2	2
Mansonia titillans		1					8	9
Psorophora albipes			1		1			2
Psorophora discrucians					1			1
Psorophora ferox		30	2		2	2		36
Sabethes albiprivus	19	32	57		5	11	13	137
Sabethes identicus							1	1
Total	75	163	208	11	97	98	24	676

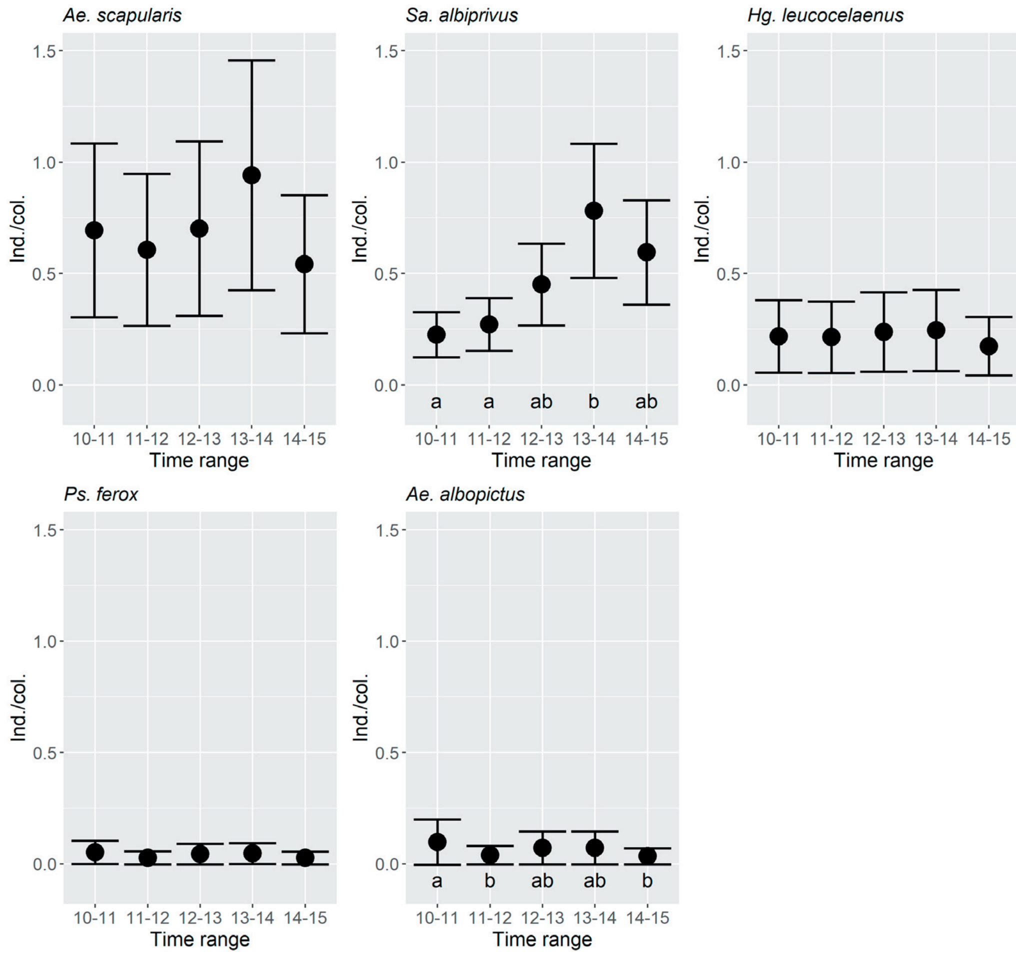

Figure 2. Mean (±standard error) number of adult mosquitoes per collector (individuals-collector) captured at all samplings points per time range (10:00-11:00AM, 11:00-12:00PM, 12:00-13:00PM, 13:00-14:00PM and 14:00-15:00PM) for *Aedes scapularis*, *Sabethes albiprivus*, *Hemagogus leucocelaenus*, *Psorophora ferox* and *Aedes albopictus*. For species that showed temporal variations in their activity (*Sa. albiprivus* and *Ae. albopictus*; deviance tests: $P<0.05$), same letters show time ranges that did not differ according to the Tukey test ($P<0.05$).

Figura 2. Número de individuos por colector (individuos-colector) capturados en todos los puntos de muestreo y por rango de tiempo (10:00-11:00AM, 11:00-12:00PM, 12:00-13:00PM, 13:00-14:00PM y 14:00-15:00PM) (media±error estándar) para *Aedes scapularis*, *Sabethes albiprivus*, *Hemagogus leucocelaenus*, *Psorophora ferox* y *Aedes albopictus*. Letras iguales informan que no se detectaron diferencias significativas entre los rangos horarios según la prueba de Tukey ($P<0.05$).

evidence of the vectorial role of *Ae. albopictus*, *Ae. scapularis*, *Hg. leucocelaenus* and *Ps. ferox*, *Hg. leucocelaenus* is a primary vector in Brazil (de Abreu et al. 2019) and *Ae. albopictus*, *Ae. scapularis* and *Ps. ferox* were found infected with YFV in the same country (Cunha et al. 2019, 2020; de Abreu et al. 2019; Moreno et al. 2011).

While the main objective of this study was to assess the abundance and distribution of YFV mosquito vectors, it still seems relevant to highlight variations in host activity because it directly influences the virus transmission dynamics of the virus (Kramer and Ciota 2015). Although there are some data available on this behavior in *Sabethes* species for other sites in America (Chadee 1990; Pinto et al. 2009), no information on *Sa. albiprivus* was available for Argentina. The populations of this mosquito species from our studied sites showed its peak of host-seeking activity between 13:00PM and 14:00PM. Similar results were found in Brazil, where Pinto et al. (2009) showed that other *Sabethes* species (*Sa. chloropterus*, *Sa. cyaneus*, *Sa. glaucodaemon* and *Sa. belisarioi*) had similar peaks of activity around midday, and a second peak at around 15:00PM. Surprisingly, a recent work in the Cerrado, a semiarid region of Brazil, found that the activity peak of two captured *Sabethes* species (*Sa. chloropterus* and *Sa. albiprivus*) was later, between 16:30PM and 17:30PM (Oliveira et al. 2023). Given that we did not include those time periods in our study, future studies should explore these specific time periods. We also identified a peak in capture rates between 10:00AM and 11:00AM for the invasive *Ae. albopictus*. However, our observation period did not span the entire day, preventing us from testing for differences in its behavior compared to Asian countries, where it shows bimodal activity, with prominent peaks at dawn and dusk (Yin et al. 2019). Differences among study results could also be explained by environmental and climatic factors. For example, another study on *Sa. chloropterus* showed differences in host-seeking activity between dry and wet periods (Chadee 1990).

We did not detect differences in the activity of *Ae. scapularis* and *Hg. leucocelaenus* within the time periods we sampled. Forattini and Gomes (1988) observed high activity for *Ae. scapularis* at dusk (between 17:00PM and 20:00PM) and a basal activity (but not null) in the morning, noon and early afternoon. A peak of *Hg. leucocelaenus* activity has been observed at noon in other studies (Forattini and Gomes 1988; Pinto et al. 2009), which is compatible with the higher number of individuals we captured between 12:00PM and 14:00PM.

Our results extend the distribution of the Asian tiger mosquito in Argentina, with new records in Garabi, Colonia Liebig and San Carlos (Corrientes). Also, we detected higher abundances than the previous record in Garruchos (Goenaga et al. 2020), suggesting that this species is established and dispersing in natural environments of Corrientes. Otherwise, there are still no records in cities of Corrientes, and in Misiones, it is documented only in two cities (Leporace et al. 2019; Lizuain et al. 2019), despite its successful establishment in cities elsewhere (e.g., Lounibos [2002] in Europe or the United States). Possibly, there are other factors that limit its establishment in Argentinean cities such as the absence of diapause (Lounibos et al. 2003), the survival of eggs and adults (Sota and Mogi 1992; Mogi et al. 1996) and a poor competitive performance among larvae for food (Lizuain et al. 2022).

In a world affected by climate change, the geographical expansion of potential vectors and the emergence of new viruses increase the chance of extending endemic diseases and amplifying outbreaks. Our findings highlight the importance of sustaining long-term ecological, virological and entomological studies to understand the population dynamics of sylvatic yellow fever vectors.

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