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Haemagogus leucocelaenus (Diptera: Culicidae), el potencial vector silvestre de la fiebre amarilla en zona de borde del norte de Misiones, Argentina

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Abstract: *Haemagogus leucocelaenus* (Diptera: Culicidae) is considered the primary vector of yellow fever virus (Flaviviridae) in wild environments in South America. Previous research has defined *Hg. leucocelaenus* as a wild species with phytotelmata-type breeding sites. The objective of this study was to report the temporal and space occurrence of *Hg. leucocelaenus* at the microscale in the wild-periurban fringe through a systematic and spatially stratified sampling using 81 ovitraps between April 2019 and February 2020 in the locality of Puerto Iguazú, Argentina. Of the total ovitraps, eight were positive for *Hg. leucocelaenus*, six in the wild environment and two in the periurban environment. Regarding the time distribution, 98.5% of the occurrence was concentrated in November and December 2019 towards the beginning of the rainy season. The results confirm the habitat plasticity of *Hg. leucocelaenus* and establish the aptitude of artificial containers as a methodology in monitoring studies, since they illustrate the potential of wild populations to deposit fertile eggs in them at least up to 300 m from the wild-periurban fringe. This work is a contribution to determine the temporal and space risk of yellow fever virus transmission in the region, based on the distribution patterns of this species as a function to the ecotone associated with forest borders and climatic variables.

Keywords: Infectious disease, Mosquitoes, Public health.

Resumen: *Haemagogus leucocelaenus* (Diptera: Culicidae) se considera el principal vector del virus de la fiebre amarilla (Flaviviridae) en entornos silvestres de Sudamérica. Investigaciones anteriores han definido a *Hg. leucocelaenus* como una especie silvestre con sitios de reproducción natural en fitotelmata. El objetivo de este estudio fue reportar

la ocurrencia espacio-temporal de *Hg. leucocelaenus* a microescala en la franja silvestre-periurbana a través de un muestreo sistemático y espacialmente estratificado utilizando 81 ovitrampas realizado entre abril del 2019 y febrero del 2020 en la localidad de Puerto Iguazú, Argentina. Del total de las ovitrampas colocadas, ocho fueron positivas para *Hg. leucocelaenus*, seis en el ambiente selvático y dos en el periurbano. En cuanto a la distribución temporal, el 98,5% de las apariciones se concentraron en noviembre y diciembre de 2019, hacia el inicio de la temporada lluviosa. Estos resultados confirman la plasticidad del hábitat de *Hg. leucocelaenus* y establecen la aptitud de los contenedores artificiales como metodología en los estudios de seguimiento, ya que ilustran el potencial de las poblaciones silvestres para depositar huevos fértiles en ellos, al menos hasta 300 m de la franja silvestre-periurbana. Este trabajo es una contribución al riesgo espacio-temporal de transmisión del virus de la fiebre amarilla en la región, a partir de los patrones de distribución de esta especie en función del ecotono asociado a los bordes silvestres y variables climáticas.

Palabras clave: Enfermedades infecciosas, Mosquitos, Salud pública.

Since the appearance of the outbreak of yellow fever virus (Flaviviridae) (YFV) by *Haemagogus* spp. and forest related mosquitos in the Midwest region of Brazil in 2016 (Abreu et al., 2019; de Olivera et al., 2020), considered the most important sylvatic outbreak in the last eight decades, the risk of expansion from the sylvatic cycle by sylvan species of mosquitos to the urban cycle by *Aedes aegypti* (L) and *Aedes albopictus* (Sukse) (Diptera: Culicidae) was predicted many times as the epidemic wave spread south out of the Amazon basin and approached densely populated cities (Marques et al., 1998; Massad et al., 2018; Rezende et al., 2018; Childs et al., 2019; de Olivera et al., 2020). However, no infected females of *Ae. aegypti* were near recent YFV foci (Abreu et al., 2020). On the other hand, the risk of YFV transmission to humans by the sylvan mosquitoes *Haemagogus leucocelaenus* (Dyar & Shannon) (Culicidae) and *Aedes scapularis* (Rondani) was associated with forest-rural-urban interfaces and green patches within the cities that harbor nonhuman primates (NHP) (Cunha et al., 2020; Sacchetto, 2020). The spatial scales used to characterize this interface-related risk range from national trends to 2 km buffers, which are useful scales for strategic programmatic planning; however, the landscape's structural patterns and mosquito behavior research in sylvan-urban ecotones at the microscale could be used to design better preventive and operational interventions at the foci level (de Thoisy et al., 2020; Wilk-da-Silva et al., 2020). Cardoso (2010) isolated YFV from *Hg. leucocelaenus* and *Aedes serratus* (Theobald) in southeastern Brazil, almost at the border with Misiones, Argentina. Abreu et al. (2019) confirmed that *Hg. leucocelaenus* was the main vector in central Brazil in the 2016-2018 outbreak along with *Haemagogus janthinomys* (Dyar). The last YF sylvatic outbreak in Argentina occurred between 2007 and 2008, with epizootics in monkeys and confirmed human cases. One of these epizootics occurred in a private reserve in Puerto Iguazú in 2007, which borders Iguazú National Park. Subsequently, five human cases and one death were confirmed in the departments of Guaraní, San Pedro and Eldorado, Misiones province (Ministerio de Salud, 2008). Although YFV was isolated in

Sabethes alviprivus (Theobald) (Culicidae) in 2009 in southern Misiones (Goenaga et al., 2012), the ecoepidemiology of this disease in Argentina is unknown as well as the role of *Hg. leucocelaenus* and the other species considered transmitters. However, its role as a vector of YFV was reported in southern Brazil, including states bordering the province of Misiones (Cardoso et al., 2010; Abreu et al., 2019). The present study aims to report the space-time occurrence of *Hg. leucocelaenus* at the microscale in the wild-periurban fringe of Puerto Iguazú in Argentina, using environmentally stratified sampling.

Entomological monitoring was carried out in Puerto Iguazú (25° 36' 39" S; 54° 34' 49" W), Misiones Province (Fig. 1), in the three-country border area of Argentina, Brazil and Paraguay. This area is included in the ecoregion of the Paranaense Forest where NHP troops can move between countries and where YFV epizootics approach from the Brazilian border (SESP, 2020). The climate is subtropical with an annual average temperature of 21°C (15°C-25°C) and rainfall between 1,000 and 2,200 mm / year (Oyarzabal et al., 2018).

Monthly samples were taken from April 2019 to February 2020. The study area was classified into three types of environments as follows, based on visual analysis of satellite images and socio-demographic data (INDEC, 2010): urban densely populated areas, periurban areas with subsistence agricultural family farms, and wild environments in the public use and trail areas of Iguazú National Park (INP) (Fig. 1). Based on data collected by Silver (2008) we modified the methodology using type oviposition traps which consist of black plastic containers (1L) with the interior lined with corrugated cardboard as egg supports. The corrugated cardboard was tested in a preliminary experiment to ensure that withstands rainwater and collects eggs successfully. These traps were filled with clean water covering 50% of their capacity. The ovitraps were distributed randomly in each of the defined environmental strata, placed between 0.5 and 1.5 meters from the ground (Alencar et al., 2010). The areas from selected environments were not homogeneous in shape and size, so they were divided randomly into quadrants of 400 m² and the quadrants amount to cover the 10% necessary of the surface of each environment was selected. Then, three or four ovitraps were randomly distributed within each of the selected quadrants. A total of 35 ovitraps were placed in the urban environment with a distance to the edge from 0 m to 1,174.62 m, 30 ovitraps in the periurban environment with a distance to the edge from 45 m to 1,346 m and 16 in the wild environment with a distance from 13.55 m to 248.29 m from the edge, each remaining active for seven days *per* sampling. The egg supports were transferred to the laboratory dried and preserved in an incubator (19 ± 2 °C) until immersion. Larvae were fed with a standard laboratory diet (Puggioli et al., 2013) in 250 ml plastic containers until the pupal stage. Pupae were placed in smaller containers (20 ml) in emergence cages. Fourth-instar larvae that did not reach the adult stage were placed in 80% ethanol for later identification. The emerged adults were sacrificed with acetone after 48 hs and mounted as described by Rossi et al. (2006)

Species identification was performed using keys of Forattini (2002) and Zavortink (1972). Preserved specimens were incorporated into the entomological collection of the National Institute of Tropical Medicine (INMeT).

Of the 81 total ovitraps, eight were positive for *Hg. leucocelaenus*: six in the wild environment and two in the periurban environment (Fig. 1, Table I). Regarding the time distribution, 98.5% of the occurrence was concentrated in November and December towards the beginning of the rainy season, with accumulated rainfall for these months of 110.4 mm and 220.5 mm, respectively, and average temperatures of 30.5 °C in November and 26.8 °C in December. Also, 1.5% of the captures were in May at the end of the rainy season, with accumulated rainfall of 174 mm and an average temperature of 21.4°C. *Haemagogus leucocelaenus* was not found in the urban environment.

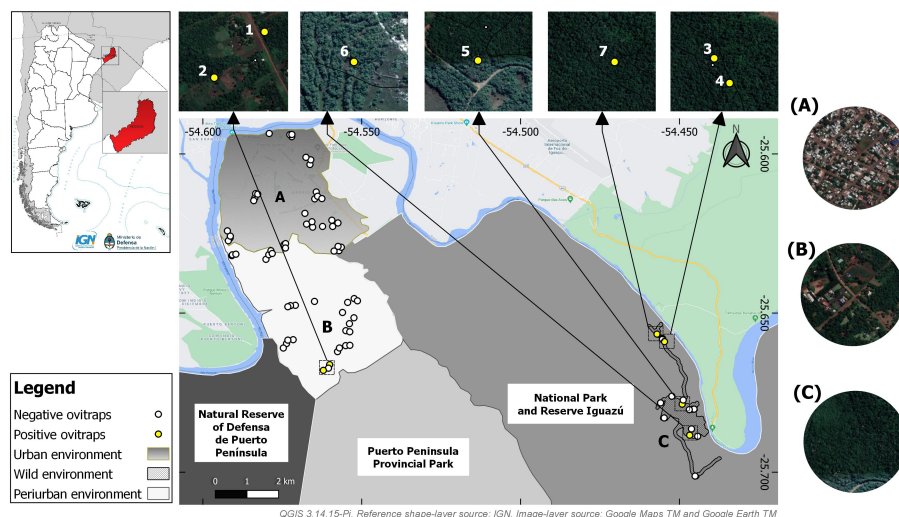


Fig. 1. Detail of the study area and the distribution of ovitraps. (A) urban environment; (B) periurban environment; (C) wild environment. Occurrence of *Haemagogus leucocelaenus* in periurban and wild environments is indicated in yellow. Numbers 1 to 7 represent the IDs of the positive ovitraps.

ID	Latitude	Longitude	Environment	Distance to the forested edge	Month	Stage reached (n)	Sex
1	25°39'58.49"S	54°33'36.07"W	P	336,76 m	May	A (1)	1 M
2	25°40'5.01"S	54°33'43.14"W	P	508,06 m	November	A (7) L (5)	4 F/3 M
3	25°39'28.86"S	54°27'19.07"W	W	In the forest	November	A (32) L (1)	25 M / 7 F
4	25°39'32.45"S	54°27'16.92"W	W				
5	25°40'43.09"S	54°26'56.87"W	W				
6	25°41'18.17"S	54°26'48.36"W	W	In the forest	December	A (3) L (7)	1 M / 2 F
5	25°40'43.09"S	54°26'56.87"W	W				
7	25°39'23.82"S	54°27'25.40"W	W				
Total						A (43) L (25)	31 M / 12 F

Table I. Spatial and temporal distribution of positive ovitraps of *Haemagogus leucocelaenus* in Puerto Iguazú city and Iguazú National Park, from April 2019 to February 2020.

A = Adult; L = larva; M = Male; F = Female; ID = ovitrap label; P = periurban environment; W = wild environment. In April, June, July, August, September, October 2019 and January, and February 2020 samplings, the vector at ground level was not found.

Haemagogus leucocelaenus has been recorded in Puerto Iguazú in the area characterized here as wild environments since the 1940s (Duret,

1950), even in the last report, which included one specimen (25°35'28" S; 54°33'38" W) (D'Oria et al., 2010).

Our study of the occurrence of *Hg. leucocelaenus* confirms the habitat plasticity of this species, and also shows the potential of the females in their wild source populations to deposit fertile eggs in periurban artificial containers at least up to 300 m from the wild-periurban fringe. Abreu et al. (2019) proposed intensified human vaccination against YFV in a buffer of 12 km from the forest-urban ecotone due to the colonization capability of secondary forest patches by *Haemagogus* species and its flight range and endophagic behavior. However, from a metapopulation dynamics perspective, our results at the microscale suggest that intensive monitoring of wild vectors of YFV should be performed at least in a 500 m buffer from the sylvan edge. The presence of ovitraps in the periurban-wild or wild-urban edge or in its proximity without oviposition of *Hg. leucocelaenus* could be due to a patch colonization of the species corresponding to source and sink population dynamics (Hanski et al., 1997; Hanski, 1999). According to Alencar et al. (2013), *Hg. leucocelaenus* has an eclectic behavior in its feeding habits. This could be observed directly related to its mobility between the canopy and the ground in search of food or breeding sites to deposit its eggs. In the study area, the presence of *Ae. albopictus* (Lizuin et al., 2019) has been proposed as a bridge between the sylvatic and domestic YFV cycles (Pereira Dos Santos et al., 2018). On the other hand, vertical transmission of YFV has been observed in *Hg. janthinomys* by natural infection and in *Haemagogus equinus* (Theobald) by experimental infection (Mondet et al., 2002), but this transmission has not been observed in field-collected *Haemagogus* spp. in recent outbreaks of the region (Abreu et al., 2019). Oviposition by *Hg. leucocelaenus* in periurban environments in our study mainly occurred at the beginning of the rainy season. The best predictor for the positivity of ovitraps and the number of eggs of *Hg. leucocelaenus* in Rio de Janeiro State, Brazil, was the mean temperature above 27 °C and accumulated precipitation above 100 mm, with a time lag of four weeks between the climatic record and the ovitrap collection (Couto-Lima et al., 2020). In conclusion, our study indicates the appropriateness of the surveillance of sylvan synanthropic mosquitoes, such as *Hg. leucocelaenus*, with artificial containers in wild-periurban fringes and around urban green patches large enough to harbor NHP. Also, this work provides insight into the temporal and spatial risk scenario of YFV transmission in the region, based on distribution patterns as a function of the urban-periurban-wild ecotone associated with the forest border and climatic variables such as rainfall, which would affect the presence of *Hg. leucocelaenus*. Nonetheless, to provide better operational protocols for monitoring, additional studies at the microscale are still required, taking into account the limitations of ovitraps (Alencar et al., 2016).

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