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Assessment of water bodies as larval habitat and oviposition activity in ovitraps of mosquitos in a subtropical city of Salta province, Argentina

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Assessment of water bodies as larval habitat and oviposition activity in ovitraps of mosquitos in a subtropical city of Salta province, Argentina

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Abstract. Many mosquito species are vectors of significant medical and veterinary importance. This study aimed to analyze and describe the oviposition activity of *Ae. aegypti* in ovitraps, determine whether other mosquito species also oviposit in these traps, and characterize the mosquito species present in urban water bodies, such as streams, irrigation channels and puddles, in Hipólito Yrigoyen city, Salta, Argentina. Thirty-four houses were sampled using ovitraps in April 2017, and 21 water body sites were sampled for immature mosquito stages using dippers in October 2018 and December 2019. A total of 348 *Ae. aegypti* eggs were collected from ovitrap substrates, with 63% of the ovitraps testing positive. Larvae of *Limatus durhamii* (50) and *Ae. aegypti* (29) were found in ovitrap water. By contrast, 196 larvae were collected from natural or artificial water bodies, primarily *Culex* (74.47%), *Anopheles* (24.99%), and *Ochlerotatus* (0.51%) genus. Of these, 114 specimens were identified at species level: *Cx. coronator* (30.61%), *Cx. quinquefasciatus* (23.97%), *An. argentinus* (3.06%) and *Oc. patersoni* (0.51%). *Aedes aegypti* was found to be widely distributed across the city. Notably, the species detected in ovitraps were not found in natural or artificial bodies, and vice versa. It is important to highlight the presence of *Anopheles* species in drainage channels within the urban area. Most of the species identified have known medical relevance, underscoring the need for comprehensive vector control strategies that go beyond targeting only *Ae. aegypti*.

Keywords: Culicidae; urban larval habitat; water body; ovitrap; urban diversity

Introduction

Culicids, commonly known as mosquitoes, are arthropods belonging to the class Insecta, order Diptera, and family Culicidae. To date, more than 3,729 culicids species have been identified worldwide (Harbach, 2013), many of which are capable of transmitting pathogens -such as viruses, parasites and bacteria- of both medical and veterinary relevance. These insects are

distributed from sea level up to approximately 3,000 meters above sea level, occupying a broad diversity of ecosystems, including deserts, forests and tropical ecoregions, circumpolar zones, and urban environments (Forattini, 1996; Campos and Maciá, 1998).

Most mosquito species are concentrated in tropical and subtropical regions, where favorable climatic conditions (e.g., rain, temperature, humidity), combined with local topography and availability of aquatic habitats for immature stages, promote their development and persistence (Franklinos et al. 2019). In Argentina 252 mosquito species have been reported, with the Northwest region (NOA) hosting the second-highest diversity in the country. Within this region Salta Province stands out as the most diverse, with 102 documented species (Rossi, 2015; Stein et al. 2016; Stein et al. 2018).

Understanding the mosquito species inhabiting urban environments is particularly important due to their close association with human populations. Urban ecosystems provide numerous natural and artificial larval habitats, as well as resources and shelter for adult mosquitoes. Consequently, cities -especially those in tropical and subtropical areas- can support a certain diversity of Culicidae (Lee et al. 2018; Lee et al. 2020).

Human activities have significantly altered natural environments, often leading to habitat loss and facilitating the spread and mixing of previously isolated biota (Smart et al. 2006). This environmental transformation has been linked to increased prevalence of pest species and pathogen vectors (Bonnefoy et al. 2008). In this context, while *Aedes aegypti* (Linnaeus) is the most well-known and extensively studied urban mosquito, due to its role as a vector of arboviruses and its strong preference for human hosts, the overall diversity of mosquitoes in many urban settings remains poorly understood. It is likely that multiple species are already adapted, or are currently adapting, to urban conditions.

Mosquito species show preferences for various breeding sites: some favor freshwater environments, others brackish or polluted waters. Larval habitats can be natural, such as phytotelmata (plant-held water) (Kitching 2000, Stein et al. 2018; Flores et al. 2025), tree

hollows (Mangudo et al. 2011) or water bodies like streams and puddles, or artificial as drainage channels, including a variety of man-made containers (tires, water cans, bottles, among others). Species also differ in their preference for temporary versus others breeding sites occurring usually in permanent ones (Pires & Gleiser 2010; Ramirez et al. 2016; Rosero-García et al. 2018).

Since some species, particularly *Ae. aegypti* prefer artificial containers as larval habitats, ovitraps are used as monitoring networks and represent a useful tool for tracking the spatial and temporal variation of *Ae. aegypti* (Abán Moreyra et al. 2022). In the context of studying mosquito diversity, we also found it important to assess whether other mosquito species present in the area may lay eggs on such devices.

The objective of this study was to characterize the mosquito fauna present in different types of water habitats, to describe the oviposition activity of *Ae. aegypti* in ovitraps, and to evaluate whether other mosquito species also use these devices for oviposition in the city of Hipólito Yrigoyen, Salta, Argentina. This work contributes valuable data on the culicid diversity in Hipólito Yrigoyen, a subtropical city located in the Yungas rainforests, a biodiversity hotspot, and highlights its potential implications for the epidemiology of mosquito-borne diseases. The finding aims to support local health authorities in optimizing vector control and disease prevention.

Materials and methods

Study area

The study was carried out in the city of Hipólito Yrigoyen (23° 14' 39" S, 64° 16' 38" W; fig. 1), located in Oran Department, Salta Province, in northern Argentina, approximately 73 km from the Bolivian border. Hipolito Yrigoyen is a small city with an estimated population of 12832 inhabitants (INDEC, 2022; Figure 1A and B). It lies within the Yungas rainforest ecoregion, characterized by a humid subtropical climate influenced by its proximity to the Tropic of Capricorn. Annual rainfall ranges from 900 to 1000 mm, with most precipitation occurring

during the summer (Lomáscolo et al. 2017). The northeastern edge of the city is bordered by remnant patches of native Yungas Forest, while the surrounding landscape is predominantly agricultural (Grau & Brown, 2000)

Oviposition activity of *Aedes aegypti*

To describe the spatial oviposition activity of *Ae. aegypti*, in April 2017 ovitraps were placed as evenly as possible across the city, covering different urban areas. A total of 34 ovitraps (one ovitrap per premise) were installed in shaded areas within the gardens of private residences (Figure 1C). Sampling was conducted during a month with moderate *Ae. aegypti* activity, selected based on the operational availability of the research team. This timing is ecologically relevant, as *Ae. aegypti* is expected to concentrate in stable source habitats rather than being broadly distributed across both source and sink sites, as typically observed during peak season (Aban Moreyra et al. 2022, Chanampa et al. 2024). Other mosquito species are also expected to remain active during this period, allowing for the detection of larval habitats and species of sanitary relevance.

The selected houses were located approximately 300 meters apart. Each ovitrap consisted of a 300 ml plastic container painted black on the outside. A wooden paddle was placed vertically inside each container to serve as an oviposition substrate. The containers were filled to 2/3 of their volume with dechlorinated water. After seven days, the ovitraps were removed and wooden paddles taken to the laboratory, and were left to dry in the shade prior to egg counting under a stereoscopic microscope. To detect the presence of immature mosquito stages, the water from each ovitrap was also collected in plastic cups and transferred to the laboratory.

Entomological sampling of water bodies

To further characterize the mosquito fauna in Hipólito Yrigoyen, different areas of the city were surveyed to identify potential larval habitats, which were subsequently sampled for mosquitoes. Different types of stagnant or lentic ground-level water bodies -such as puddles, streams, irrigation channels and drainage ditches- were examined throughout the city. A total of 21

siteswere sampled in October 2018 and December 2019, as the temperatures during these months (spring and early summer) are favorable for mosquito proliferation in this region. Larvae and pupae were collected using 300 ml dippers, with sampling carried out for approximately 15 minutes at each site (Figure 1D).

Laboratory processing

In the laboratory, specimens collected from the water in the ovitraps and those collected in the water bodies were transferred to white plastic trays to facilitate visualization. Early instar larvae and pupae were individually reared in containers filled with dechlorinated water and fed grounded fish food, until they reached the IV larval instar or adult stage, respectively. Rearing was conducted under controlled laboratory conditions: 60–80% relative humidity, a mean temperature of 27°C, and a 12:12 hour light: dark photoperiod.

For morphological identification of the specimens (fourth instar larvae or adult), dichotomous keys (Darsie 1985) and specific descriptions when necessary were used (Gorham et al. 1967; Faran, 1980; Faran and Linthicum, 1981; Wilkerson and Strickman, 1990; Forattini, 2002).

Data analysis

To explore the spatial variation by species, thematic maps were made using QGIS software (https://www.qgis.org/es/site/). To compare proportions, the Chi-Square test was applied, while differences in means were assessed using Student's t-test. These statistical analyses were performed using InfoStat Professional software, version 1.0 (http://www.infostat.com.ar). Differences were considered statistically significant at $p < 0.05$.

Results

A total of 275 immature Culicidae specimens (larvae and pupae) were collected in all from water bodies and ovitraps water. These specimens belonged to two subfamilies (Anophelinae and Culicinae), five genera, and six species. The following genera were identified: *Aedes*

Meigen, 1818; *Limatus* Theobald, 1901; *Culex* Linnaeus; *Ochlerotatus* Lynch Arribálzaga; and *Anopheles* Meigen.

Of the 34 ovitraps deployed, 33 wooden paddles were recovered, as one ovitrap was lost. Twenty-one ovitraps (64%), placed across various sectors of the city, tested positive for at least one *Ae*. *aegypti* egg (Figure 2). A total of 348 *Ae. aegypti* eggs were detected, with an average of 10.54 eggs per wooden paddle, ranging from 1 to 72 eggs. Additionally, the number of eggs showed a positive correlation with the number of *Ae. aegypti* larvae in the same ovitraps (Spearman coefficient = 0.55; $p < 0.05$; Supplementary Information 1).

A total of 79 larvae were collected from the water in the ovitraps, with 29 (37%) belonging to *Ae. aegypti* and 50 (63%) to *Li. durhamii*. *Aedes aegypti* was the only mosquito species found in 31.57% ovitraps, while 39.39% were positive only for *Li. durhamii*. There were no statistically significant differences between these proportions ($p > 0.05$). The mean abundance of *Li. durhamii* per ovitrap was 1.52, ranging from 1 to 12, whereas for *Ae. aegypti*, it was 0.88, ranging from 1 to 10. No statistically differences were observed between these values ($p > 0.05$). Larvae of both species were found together in 5 (26.31%) ovitraps. Additionally, when evaluating the correlation between the number of *Ae. aegypti* and *Li. durhamii* larvae in the ovitraps, no significant association was detected ($p = 0.98$). No correlation was observed between the number of *Li. durhamii* larvae and the number of *Ae. aegypti* eggs per ovitrap ($p > 0.05$; Figure 3; Supplementary Information 1).

In water bodies, a total of 196 immature specimens were collected, of which 114 were identified to species level, corresponding to four species across three genera. In descending order of abundance, the identified species were: *Culex coronator* Dyar and Knab, 1906 (30.6% of the specimens collected); *Cx. quinquefasciatus* Say, 1823 (24.0%); Anopheles (Anopheles) argentinus Brèthes, 1912 (3.1%) and *Ochlerotatus patersoni* (Shannon and Del Ponte, 1928) (0.5%). The immatures specimens that could not be identified to species level belonged to the

177 genera *Anopheles* and *Culex.* The distribution of Culicidae collected according to water body
178 type is presented in Table 1 (Supplementary Information 2).

179 *Culex coronator* was found in all surveyed water bodies and was the most abundant species
180 overall, with particularly high numbers in drainage channels. *Culex quinquefasciatus,* the
181 second most abundant species, was present in all the water bodies except the puddle and showed
182 its highest abundance in the irrigation channel. Immature stages of *An. argentinus* -the least
183 abundant among the identified species- were found exclusively in the stream, while only a
184 single *Ochlerotatus patersoni* larva represented an isolated finding from the drainage channel.
185 Among the specimens not identified to species level, *Culex* sp. were found only in the stream,
186 whereas *Anopheles* spp. larvae were collected mainly from the stream, but also from both the
187 irrigation and drainage channels (Table 1).

188 In 2018, immature stages of *Culex* and *Anopheles* were present in all surveyed water bodies,
189 except for the puddle, where *Anopheles* was absent. In both the irrigation and drainage channels,
190 *Culex* immatures were more abundant than *Anopheles*; *Anopheles* was more abundant and more
191 frequently observed in the stream. Both genera were found with equally frequency in the
192 irrigation channel, while *Culex* was more frequently collected in the drainage channel. In 2019,
193 culicids were collected in only two water bodies: the stream, where both genera were present,
194 and the drainage channel, where only *Culex* was found (Table 2; Figure 4).

195 **Discussion**

196 There remains a significant gap in the knowledge regarding the diversity and geographic
197 distribution of culicid species in northwestern Argentina. Given that mosquitoes are among the
198 most relevant insect groups in Public Health, due to their role as vectors of numerous diseases
199 affecting humans and other animals, it is essential to conduct comprehensive surveys of both
200 confirmed and potential vectors of different etiological agents. In this context, the present study
201 contributes valuable information on mosquito diversity in urban settings within the Yungas
202 rainforest region, a subtropical area of high ecological importance.

Regarding the oviposition pattern of *Ae. aegypti* in the study area, the species was detected at most sampling sites. Previous research by Abán Moreyra et al. (2022), conducted in San Ramón de la Nueva Oran (SRNO) -a city approximately 15 km from the study site with similar environmental conditions- reported a reduction in the abundance and spatial distribution of *Ae. aegypti* during April, compared to its broader presence during the summer months. However, this contraction pattern was not observed in the present study in Hipólito Yrigoyen, where the mosquito remained widely distributed in April.

Furthermore, when comparing the proportion of positive ovitraps between this study and the one conducted in SRNO (16.33%), a markedly higher positivity was observed in Hipólito Yrigoyen (63.64%; $p < 0.0001$), despite both surveys being conducted in April (albeit in different years) (Abán Moreyra et al. 2022). Additionally, the average number of eggs per ovitrap was notably higher in Hipólito Yrigoyen (10.55) compared to SRNO (2.89). When extrapolating to 100 ovitraps, this translates to approximately 1054 eggs in Hipólito Yrigoyen versus 307 eggs in SRNO, further highlighting the greater abundance and oviposition activity of *Ae. aegypti* in the former locality.

The differences observed in the abundance and distribution of *Ae. aegypti* between these localities may be largely attributed to varying intensity of vector control intervention. In SRNO, during 2017-2018 period, the municipality developed more than 150 socio-environmental agents (SEAs) to conduct interventions, which included the identification of risk areas through ovitraps surveys and the implementation of large-scale larval source reduction campaign in January (Abán Moreyra et al. 2022; Gil et al. 2016). In contrast, Hipólito Yrigoyen has significantly fewer SEAs, limiting the scope and effectiveness of anti-vector actions.

The water collected from the ovitraps revealed co-occurrence of oviposition by *Ae. aegypti* and *Li. durhamii*. However, since *Li. durhamii* lays its eggs directly in the water and not on the oviposition substrate (in this case, a wooden paddle), its presence does not compromise the specificity of the ovitrampas for detecting *Ae. aegypti*. *Limatus durhamii* is a native neotropical

229 species that was first reported in the Oran department of the Salta province by Dantur Juri in
230 2012. Furthermore, in the same region, this species has been observed developing in small water
231 accumulations such as snail shells (Mangudo et al. 2016). Studies investigating the potential
232 ecological interaction between *Li. durhamii* (native) on *Ae. aegypti* (invasive) suggest that the
233 former is a strong competitor against *Ae. aegypti* (Talaga et al. 2018). *Limatus durhamii* is a
234 facultative predatory species, and under certain laboratory conditions, its presence has been
235 associated with reduced *Ae. aegypti* abundance (Lopes, 1999; Talaga et al. 2018).

236 The results from the sampling of channels, streams, and puddles in Hipólito Yrigoyen highlight
237 the epidemiological relevance of understanding the ecology, diversity, and distribution of
238 culicid species in northern Argentina. The most abundant species recorded was *Cx. coronator*,
239 followed by *Cx. quinquefasciatus*, both of which are associated with the transmission of key
240 arboviruses, including Venezuelan equine encephalitis virus (VEEV), San Luis encephalitis
241 virus (SLEV) and West Nile virus (WNV) (Diéguez-Fernández et al. 2020). These cosmopolitan
242 species have a wide geographical distribution; and both their immature and adult forms have
243 been found in a broad range of habitats (Flores et al. 2025; Diaz et al. 2013; Grech et al. 2013;
244 Rossi and Vezzani, 2011; Visintin et al. 2010; Morais et al. 2010; Almirón et al. 1995; Almirón
245 and Brewer, 1996).

246 *Anopheles argentinus* is primarily a wild mosquito species that requires clean water bodies for
247 the development of its immature stages. Its presence has been previously documented in streams
248 and rural irrigation canals located in peri-urban or rural areas. However, in this study, *An.*
249 *argentinus* was detected in an urban drainage channel that traverses a significant portion of the
250 city, an atypical habitat for this species. Given that historically *An. argentinus* was the primary
251 vector of malaria in Argentina prior to the elimination of autochthonous transmission, its
252 presence in Hipólito Yrigoyen raises epidemiological concern, particularly given the city's
253 proximity to the Bolivian border where malaria remains endemic (Dantur Juri, 2009).

A single specimen of *Ochlerotatus patersoni* was recorded during the study. Although the presence of this species in Salta Province has been previously documented, available information on its biology and ecology remains scarce. Furthermore, the extensive literature reviewed did not yield data regarding its potential epidemiological relevance, highlighting the need for further studies to better understand the role of this species within local mosquito communities and its possible implications for public health.

It is important to emphasize that the mosquito species found in drainage channels were not detected in the ovitraps, and conversely, those recorded in ovitraps did not appear in the channel samples. This distinction is highly relevant for the strategic planning of vector control measures, particularly for *Ae. aegypti*, as it underscores the importance of targeting its preferred breeding sites, such as discarded tires, flower vases, water jars, and other artificial containers. It is also well established that certain *Culex* species, including *Cx. quinquefasciatus* and *Cx. coronator*, may utilize these same containers for oviposition. Therefore, interventions aimed at controlling *Ae. aegypti* may also have indirect effects on reducing populations of these *Culex* species, which are important vectors of other arboviruses.

However, focusing exclusively on the control of *Ae. aegypti* risks overlooking the presence of other important vector species such as *Anopheles* spp. and *Culex* spp. which breed in natural and artificial water bodies and may be involved in the transmission of arboviruses and parasites of the genus *Plasmodium*. It is essential to adopt a broader perspective on vector control that also considers these genera and the associated public health risks. Additionally, although sandflies (family Psichodidae) -the vectors of leishmaniasis- do not belong to the Culicidae family, they should be included in any integrated vector management strategy. Previous studies have reported the presence of these insects both on the periphery and the urban center of Hipólito Yrigoyen, in association with recurrent cases of cutaneous leishmaniasis at the area (Hoyos et al. 2016 Chanampa et al. 2018). This scenario highlights the need for comprehensive vector control strategies that extend beyond the exclusive management of *Ae. aegypti*, and instead address the full spectrum of medical arthropods present in the locality of Hipólito Yrigoyen.

Finally, it is important to acknowledge certain limitations of this study, particularly the limited number of surveys conducted and the restricted sampling coverage for some types of water bodies (for example puddles). A considerable proportion of specimens could not be identified at the species level, mainly due to the development stage at which they were collected (very early stages, and could not reach the fourth larval instar or adult stage in the laboratory). Given that Hipólito Yrigoyen is located within the Yungas ecoregion, a higher species richness was expected. Indeed, among the specimens identified only to the genus level, such as *Culex* and *Anopheles*, it is likely that multiple distinct species are represented. Another plausible factor contributing to the observed species richness is the influence of human activities in the area, which may reduce overall biodiversity while favoring the persistence of species of public health importance, as was observed in SRNO in urban compared to Yungas Forest tree hole mosquito fauna (Mangudo et al. 2018).

Nevertheless, these findings show the importance of sustained and targeted intervention strategies. They may also serve as a baseline for future evaluations of vector control programs, particularly if complemented by longitudinal monitoring in nearby non -intervened areas to serve as external control.

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Authors' contributions: PMC, JFG contributed to the study design, mosquitoes collection, data analysis, laboratory experiments, literature search and writing of the manuscript. DNAM, CM, CA contributed to literature search and writing of the manuscript. GNC and MDF contributed to samples collection and writing of the manuscript. MJDJ, GR contributed to samples taxonomic identification and writing of the manuscript. RMG contributed to the data analysis, literature search and writing of the manuscript. All authors read and approved the final manuscript version.

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Fig. 1 Location of the study area. **A-** Salta Province, Argentina. **B-** Hipólito Yrigoyen locality, Salta Province, Argentina. **C-** Sampling sites with ovitraps. **D-** Sampling sites in different types of water bodies: puddles (dark red), streams (white), irrigation channels (pink) and drainage ditches (yellow).

Fig. 2 Distribution of *Ae. aegypti* eggs recorded with ovitraps in Hipólito Yrigoyen locality. Graduated symbols represent the number of eggs, with larger and darker red circles indicating higher abundances.

Fig. 3 Distribution of *Ae. aegypti* and *Li. durhamii* larvae found in the water of ovitraps used in the town of Hipólito Yrigoyen.

Fig. 4 Distribution of sampling sites that harboured culicid larvae and/or pupae (coloured figures) on four types of water bodies (puddle, stream, irrigation and drainage channels) in the city of Hipolito Yrigoyen, Salta Province, Argentina. The image shows the sampling sites where *Culex* (green figures) and *Anopheles* (grey figures)were found as the only genus, and sampling sites where both genera were found together (red figures). White figures indicate sampling sites without culicids.

Figures

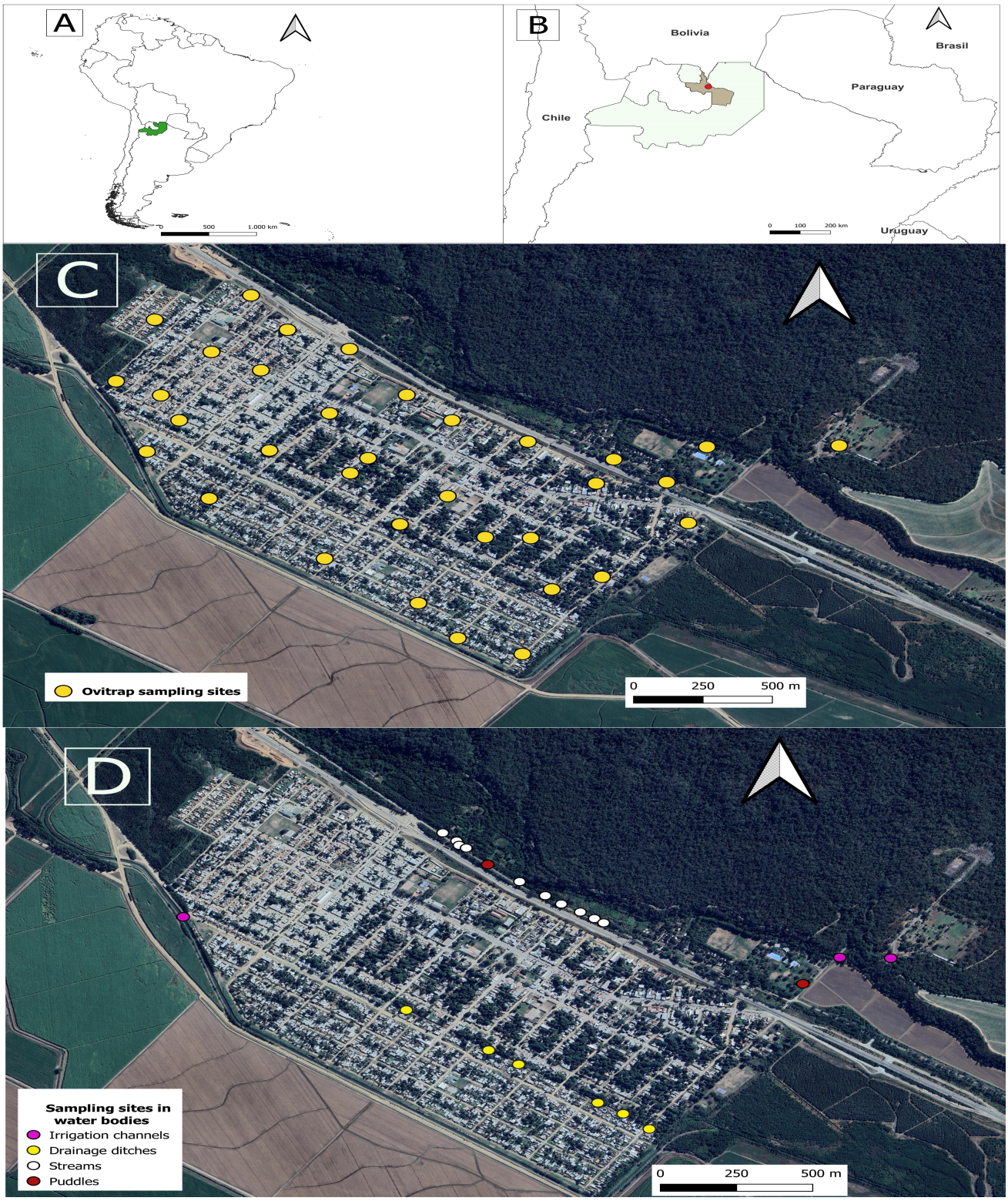

Figure 1

Location of the study area. **A-** Salta Province, Argentina. **B-**Hipólito Yrigoyen locality, Salta Province, Argentina. **C-** Sampling sites with ovitraps. **D-** Sampling sites in different types of water bodies: puddles (dark red), streams (white), irrigation channels (pink) and drainage ditches (yellow).

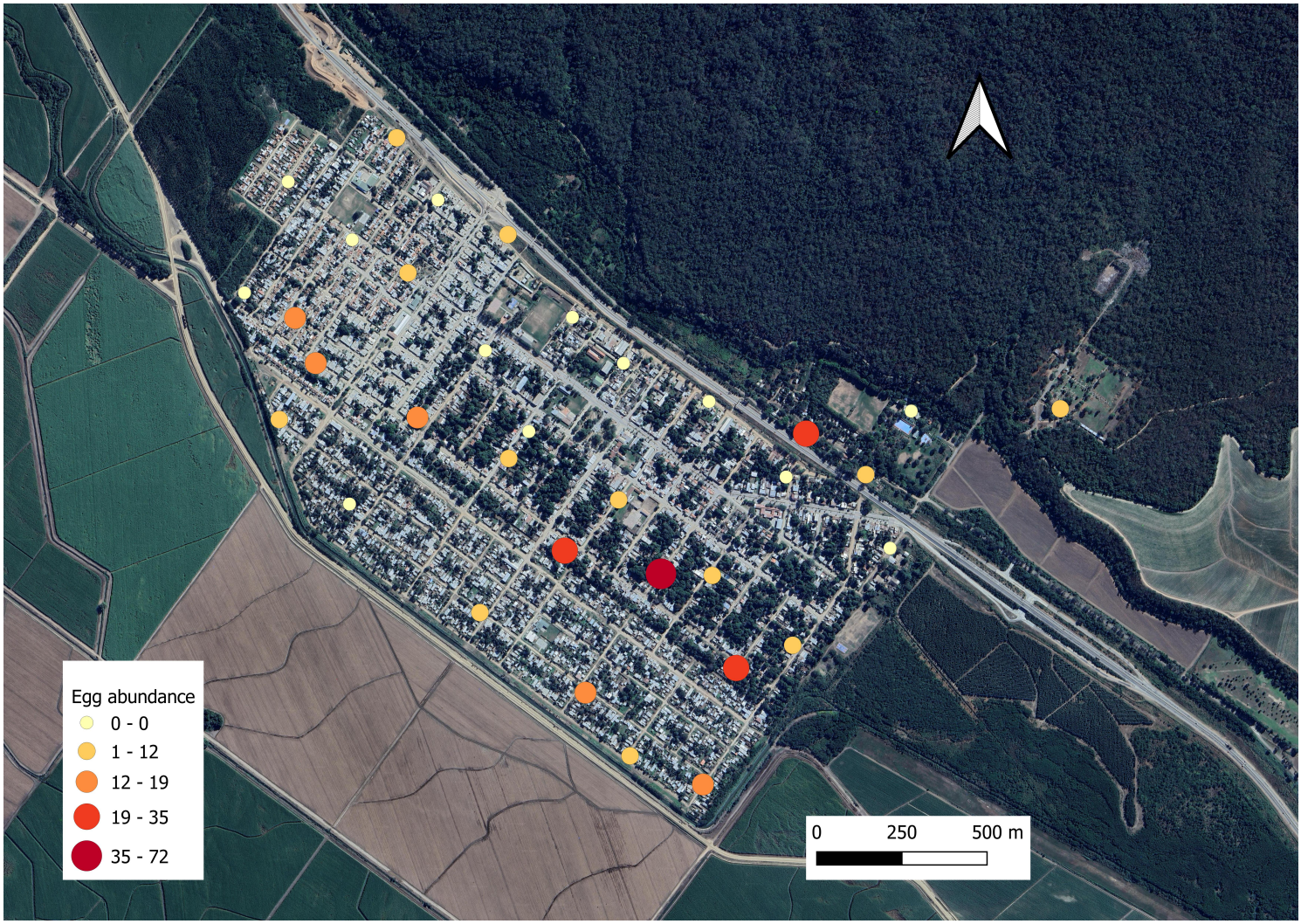

Figure 2

Distribution of *Ae. aegypti* eggs recorded with ovitraps in Hipólito Yrigoyen locality. Graduated symbols represent the number of eggs, with larger and darker red circles indicating higher abundances.

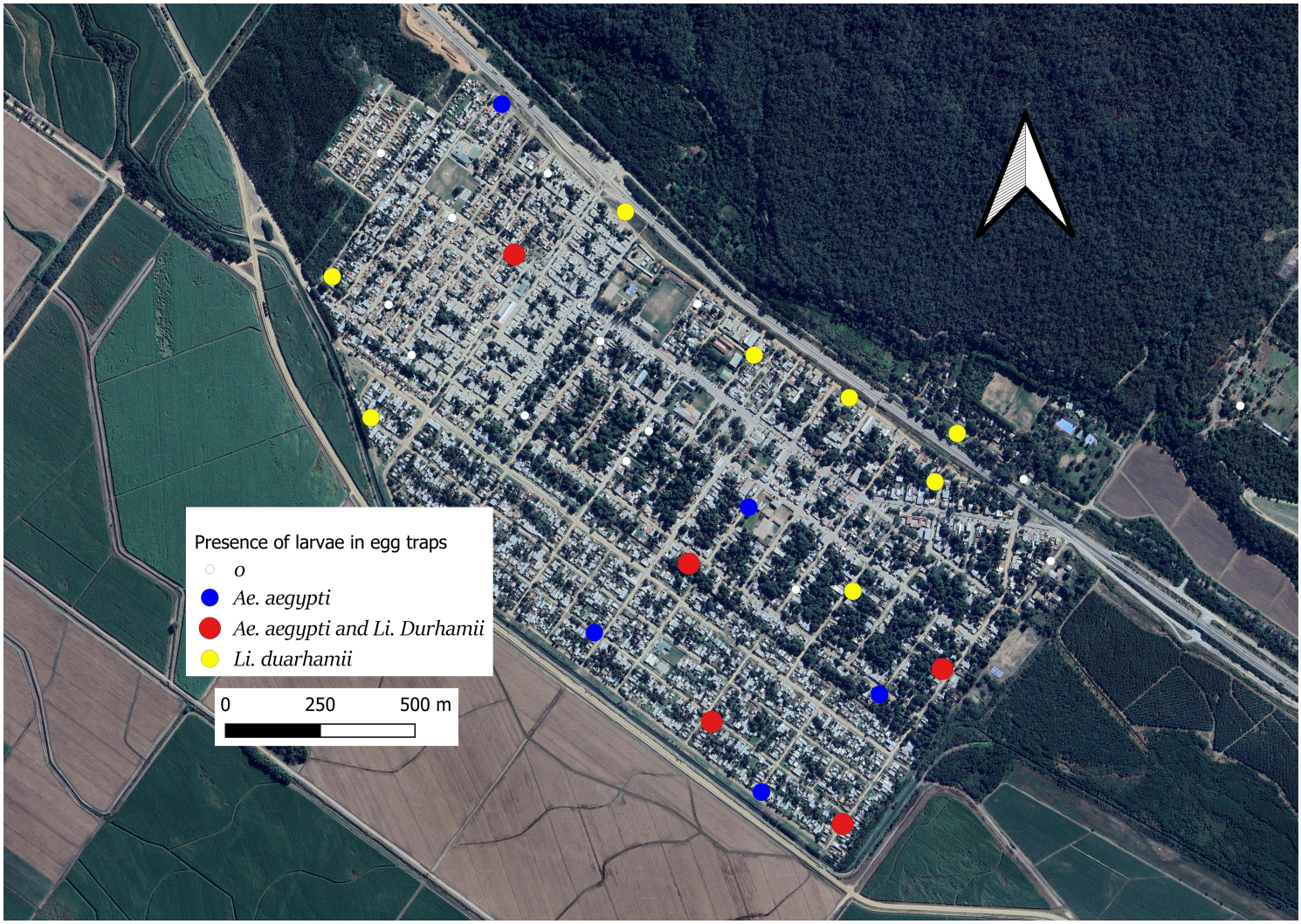

Figure 3

Distribution of *Ae. aegypti* and *Li. durhamii*larvae found in the water of ovitraps used in the town of Hipólito Yrigoyen.



Figure 4

Distribution of sampling sites that harboured culicid larvae and/or pupae (coloured figures) on four types of water bodies (puddle, stream, irrigation and drainage channels) in the city of Hipolito Yrigoyen, Salta Province, Argentina. The image shows the sampling sites where *Culex* (green figures) and *Anopheles* (grey figures)were found as the only genus, and sampling sites where both genera were found together (red figures). White figures indicate sampling sites without culicids.

Supplementary Files

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