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Bamboo, clay and plastic artisanal ovitraps for mosquito surveillance across an urban–forest gradient in Colombia, with a video record of maternal care in *Trichoprosopon digitatum*

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Abstract

Low-cost ovitraps are the backbone of *Aedes* surveillance in cities, but their value for monitoring the wider mosquito community, including sylvatic species, is poorly known. We evaluated artisanal ovitraps ("OviCol") made of plastic, fired clay and bamboo (*Guadua* sp) , baited with canary seed (*Phalaris canariensis*) or sugarcane molasses with dry yeast (*Saccharomyces cerevisiae*), in three phases in Santa Marta, northern Colombia. In Phase 1, six plastic ovitraps placed in tropical humid forest in the foothills of the Sierra Nevada de Santa Marta (SNSM) collected 20 eggs and 63 larvae of *Trichoprosopon digitatum* and 19 larvae of *Limatus durhamii*. They also yielded what is, to our knowledge, the first video record of egg brooding in this species: a female guarded her egg raft for at least 7 h and resumed brooding after the raft was removed and returned. In Phase 2, 83 ovitraps on an urban university campus collected 43,764 *Aedes* eggs in 28 days. Positivity did not differ among materials, but plastic and bamboo traps accumulated about three times more eggs than clay traps (rate ratios 3.09 and 3.61), whereas the attractant had no detectable effect. Egg counts showed field evidence of skip oviposition, and 19 persistent traps held 62% of all eggs. An artificial-intelligence counter (Col-Ovo) agreed with manual counts (CCC = 0.79, rising to 0.95). Larvae reared from positive traps included *Ae. aegypti, Culex quinquefasciatus, Cx. (Carrollia) cf. bihaicola, Cx. (Phenacomyia) corniger* and *Ae. albopictus*, the first evidence of this invasive vector in Santa Marta. In Phase 3, ovitraps in the SNSM forest yielded a Sabethini-dominated assemblage (*Tr. digitatum, Li. durhamii, Wyeomyia spp.)* together with *Orthopodomyia cf. fascipes, Toxorhynchites (Lynchiella) sp.* and *Cx. nigripalpus,* with no *Aedes* detected. Simple, inexpensive traps therefore detected invasive urban vectors, sylvatic potential vectors and natural predators, and documented a rarely observed behavior. Clay traps are adequate for presence–absence surveillance, plastic or bamboo traps are preferable when egg density or egg removal is the goal, and clay and bamboo offer biodegradable alternatives to plastic.

1. **Introduction**

Mosquito-borne arboviruses are expanding in incidence and geographic range. In 2024, the Americas recorded more than 13 million dengue cases, the highest number ever reported(PAHO, 2025b), and yellow fever re-emerged in several South American countries in 2024–2025 with a case-fatality rate close to 40% (PAHO, 2025a). *Aedes aegypti* and *Aedes albopictus*, the main urban vectors of dengue, Zika and chikungunya viruses, are now present in approximately 170 countries on every continent except Antarctica(Kraemer et al., 2015; Powell et al., 2018; Wilkerson et al., 2021), and the presence of competent *Aedes* populations in cities close to sylvatic yellow fever foci keeps the risk of urban re-establishment alive (PAHO, 2024). The global economic cost of invasive *Aedes* and the diseases they transmit exceeded USD 94 billion between 1975 and 2020, with the Americas bearing almost half of it (Roiz et al., 2024). Yet the control measures most used by vector programs, chiefly insecticide spraying, have shown small and unsustained effects on vector populations: of 848 intervention studies reviewed, only 18 reported scientifically valid impact indicators, and chemical control reduced vector density by about 1%(Alvarado-Castro et al., 2017; Bouzid et al., 2016; Low et al., 2025). This has renewed calls for new continental strategies against synanthropic *Aedes* (The Lancet Regional Health - Americas, 2023).

Ovitraps, containers that offer gravid females an attractive oviposition site, have been used for *Aedes* surveillance since the 1960s (Fay & Eliason DA, 1966) because they are cheap, sensitive at low vector densities, and easy to operate. Simple, handmade ovitraps can detect synanthropic *Aedes* as well as or better than commercial traps (Codeço et al., 2015; Mosquera et al., 2026). Ovitraps have also moved from surveillance to control: lethal ovitraps kill visiting females with an insecticide-treated oviposition strip (impregnated with deltamethrin)(Perich et al., 2003), and larvicides such as *Bacillus thuringiensis israelensis* added to the water prevent the traps from becoming breeding sites (Regis et al., 2008).

We recently showed that handmade ovitraps (“OviCol”) costing less than USD 0.2 per unit and baited with homemade attractants such as canary seed reach positivity indices above 78% and collect more than 380 eggs per trap per week in Colombian cities (Arrieta-Ángel et al., 2025). The eggs laid on the paper substrate can be inactivated by adding diflubenzuron to the trap or by immersing the substrate in hot water, which killed 100% of the eggs. Every 100 eggs removed prevent the emergence of about 70 adults, 45% of them females (Arrieta-Ángel et al., 2025). The feasibility of incorporating OviCol ovitraps and hot-water egg inactivation into routine vector surveillance and control programs has since been evaluated in several endemic regions of Colombia, with promising results compared with AGO and BG-Sentinel traps (Mosquera et al., 2026). To remove the bottleneck of manual egg counting, we also developed Col-Ovo, an artificial intelligence tool that counts *Aedes* eggs from smartphone photographs of ovitrap substrates taken under field conditions. Its counts agree closely with those of trained technicians (Lin’s concordance coefficient 0.89) and it reduces processing time from about 15min to less than 3s per substrate (Almanza & Montenegro, 2026).

All of this work, like most ovitrap research, has been carried out in cities, with plastic traps, and with *Ae. aegypti* as the target. The performance of ovitraps in sylvatic environments remains poorly documented (Alencar et al., 2016; Chaverri et al., 2018). Forests neighbourhoods a far richer culicid community, including species of the tribe Sabethini that develop in phytotelmata and tree holes. Some of these are naturally infected with arboviruses such as Saint Louis encephalitis, Ilhéus and Wyeomyia viruses (Uribe-Soto & Suaza-Vasco, 2021), yet they are rarely monitored because conventional adult traps are costly and logistically demanding (Wilke et al., 2022). A low-cost

ovitrap that also works in forest would extend community-based surveillance to the urban–sylvatic interface, where invasive *Aedes,* principally *Ae. albopictus,* may come into contact with sylvatic arboviruses.

Moreover, the original OviCol is made of plastic. Deploying hundreds of traps in cities or forests adds plastic waste, and traps that are lost or abandoned can themselves become breeding sites. Fired clay and bamboo (*Guadua*) are inexpensive, locally available in Colombia, and biodegradable, and they resemble the natural containers used by many container-breeding mosquitoes. Whether traps made of these materials perform as well as plastic ones is, however, unknown, and this must be established before a fully biodegradable OviCol can be proposed.

Here, we evaluated the effectiveness of OviCol in sylvatic environments and compared the original plastic trap with clay and bamboo versions, each baited with canary seed (*Ph. canariensis*) or molasses with yeast (*S. cerevisiae*). Our objectives were (**i**) to compare *Aedes* egg collection and trap positivity among materials and bio-attractants, (**ii**) to describe the culicid assemblages collected in urban and forest settings, and (**iii**) to document behavioral observations made possible by the traps, including egg brooding by *Tr. digitatum*, a behavior first noted in 1968(Aitken et al., 1968) but, to our knowledge, never documented on video.

2. Materials and Methods

2.1. Ovitrap designs and attractants

Three artisanal ovitrap types were used (**Fig. 1**): (i) black plastic containers; (ii) fired clay (unglazed terracotta) pots; and (iii) bamboo (*Guadua angustifolia*). In Phases 2 and 3, each trap received approximately 250 mL of water and a 20.5 × 20.5 cm absorbent paper towel lining the inner wall as oviposition substrate, plus one of two attractants: canary seed (*P. canariensis*, ~1 g of whole seeds) or sugarcane molasses (5 mL of a 1:10 dilution in water) with dry baker's yeast (*S. cerevisiae*, < 0.2 g). Construction details for the plastic model are given in (Arrieta-Ángel et al., 2025).



Figure 1. Artisanal ovitraps ("OviCol") evaluated and their unit cost, in Colombia **(A)** Original plastic model, shown with its components: a black plastic container (0.9 L), a paper towel lining the inner wall as oviposition substrate (USD $0.2). **(B)** Bamboo (*G. angustifolia*) internode cut below a node to form a closed cup (USD

$3.12). **(C)** Fired clay pot with a perforated lid (USD $9.38). All traps were ≤ 25 cm high, had a capacity of 800–1,000 mL, and received approximately 250 mL of water, the paper substrate, and canary seed or molasses with yeast.

2.2. Phase 1: pilot in tropical humid forest

Phase 1 was carried out from 18 November to 21 December 2024 at Caracolí farm (10.7926 N, −74.0882 W; 200 m a.s.l. [**Fig. 2**]), on the western foothills of the SNSM, a UNESCO Biosphere Reserve that rises from sea level to 5,700 m and is under increasing pressure from cattle ranching and monocultures (Duran-Izquierdo & Olivero-Verbel, 2021). Of the 16-ha farm, 3 ha are cultivated with cacao and plantain and the remainder is riparian tropical humid forest. Six black plastic ovitraps, each with 300 mL of site water and 1g of canary seed, were placed at least 10 m apart over approximately 300 m², either on the ground next to trees or up to 1.5 m high on cacao trunks. Traps were inspected three times at 8–15-day intervals, and subsample larvae were collected at each inspection.

On the last inspection, a *Tr. digitatum* female was found resting on an egg raft in one of the traps. This trap was observed from 07:30 h, and the female was video-recorded with a smartphone (iPhone X, Apple) while she remained on the raft. During the observation, the trap was moved 30 m and the raft was removed with a plastic pipette and returned to the water surface. The female left the raft at 14:50 h. The eggs were then collected and was preserved in 70% ethanol. Larvae were reared in the laboratory on ground dog food until adult emergence.

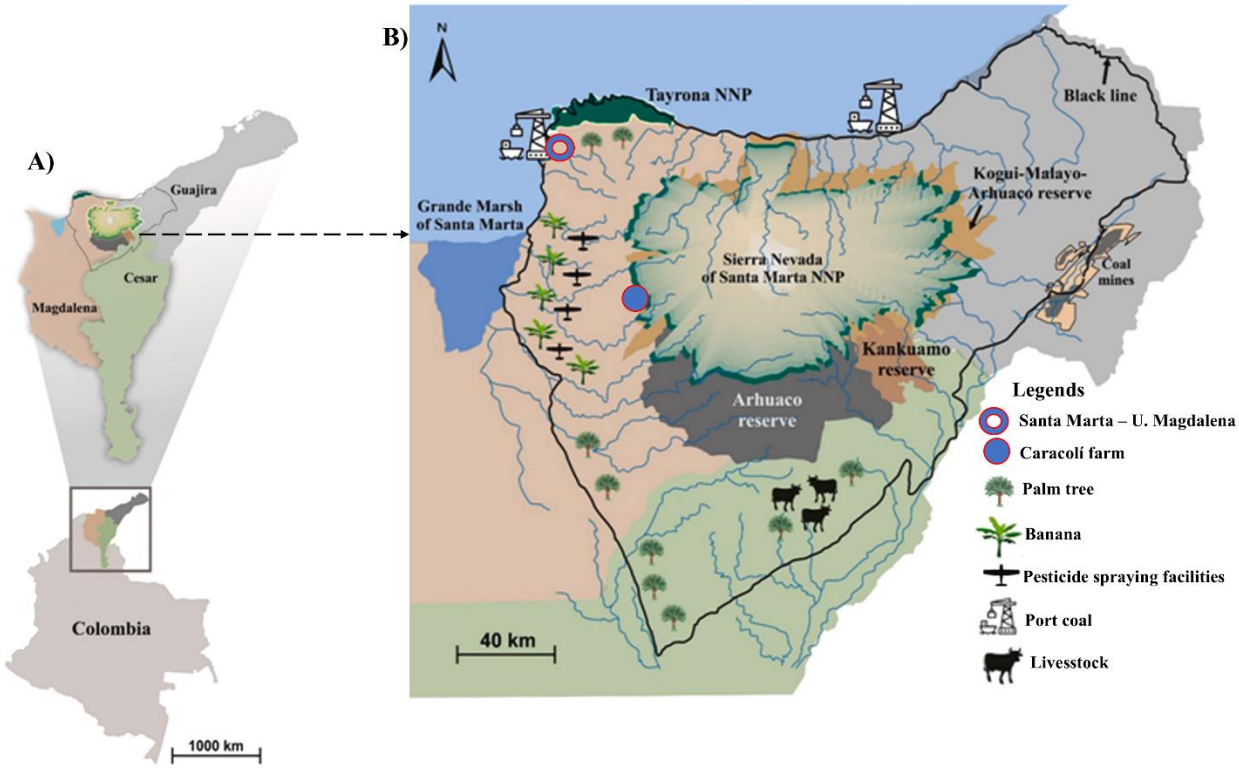


Figure 2. Study area. (**A**) Location of the departments of Magdalena, Cesar and La Guajira in northern Colombia. (**B**) The Sierra Nevada de Santa Marta (SNSM), showing the two sampling sites: the Universidad del Magdalena campus in Santa Marta (Phase 2) and Caracolí farm in the SNSM foothills (Phases 1 and 3).

Protected areas, indigenous reserves, the Black Line and the main land uses of the region are also shown. **Adapted from** (Duran-Izquierdo & Olivero-Verbel, 2021).

2.3. Phase 2: factorial comparison on an urban campus

Phase 2 took place on the campus of the Universidad del Magdalena (UNIMAG), Santa Marta (11.2247 N, −74.1842 W; ~10 m a.s.l.; **Fig. 2**), a densely frequented urban area with gardens, buildings and drainage structures. On 11 June 2025, 83 ovitraps were installed over an area of approximately 450 × 480 m: 23 clay (11 with canary seed and 12 with molasses–yeast), 30 plastic (15 + 15) and 30 bamboo (15 + 15). Traps of the three materials were interspersed across the campus, and all traps were geo-referenced. The median distance to the nearest trap was 13 m.

Oviposition substrates were inspected on day 1 (12 June); eggs on positive substrates were counted and the substrates were returned to the traps. On day 2 (13 June), all substrates were removed, eggs were counted manually with the naked eye, positive substrates were photographed with a smartphone (iPhone 11, Apple), and new substrates were installed. Traps were considered positive at 0–48 h if eggs were found on day 1 or day 2. The procedure was repeated on days 7, 14, 21 and 28 (18 and 25 June, 2 and 9 July). Because the day-2 substrate had remained in the trap since installation, the day-2 count was taken as the cumulative count for 0–48 h. Eggs were recorded as *Aedes* spp. Field data were recorded with OviLab https://reportes.chilloa.org/, a virtual form designed by our group for ovitrap surveillance. At installation, the form recorded each trap's code, geographic coordinates and a description of its location; at each inspection, it recorded the number of eggs, the presence of larvae and pupae, and other observations.

To evaluate automated counting under field conditions, photographs of the positive substrates collected on day 28 were analyzed with Col-Ovo (Almanza & Montenegro, 2026), and these counts were compared with the naked-eye counts.

After the egg experiment, traps remained in place and were inspected for immature stages until September 2025. Positive traps were selected at random and their larvae were reared in the laboratory to the fourth instar for identification. These subsamples were used only to describe species composition, not to estimate relative abundance. Each subsample was linked to its trap code to obtain its location, material and attractant.

2.4. Phase 3: deployment in SNSM forest

Phase 3 was carried out between August and September 2025 at the same Caracolí farm used in Phase 1 (16 ha, comprising 3 ha of cacao and plantain crops and riparian tropical humid forest; **Fig. 2**). Its aim was to evaluate whether the three trap materials could detect container-breeding mosquitoes in a sylvatic environment, not to compare their productivity. Ten clay traps were placed at ground level at randomly selected points at least 5 m apart, five baited with canary seed and five with molasses–yeast. Six plastic and six bamboo traps were suspended with ropes at two heights: 5 m (two traps per material) and 10 m or higher (four traps per material, at 10 and 15 m). Within each height stratum, half of the traps of each material were baited with canary seed and half with molasses–yeast, so that materials and attractants were compared at the same height. Thus, each

combination of material and attractant was represented by one trap at 5 m and by two traps at ≥ 10 m. Clay traps were placed on the ground, as their weight (~2 kg) prevented suspension.

Traps were first inspected 7 days after installation and every 15 days thereafter; all positive traps were sub-sampled for larvae, which were fixed in 70% ethanol or reared and identified as in Phase 2. Because the number of traps per combination was small, Phase 3 data are reported descriptively as species presence and number of larvae, without statistical tests.

2.5. Taxonomic identification

From day 14 onward, whenever larvae were found in a trap, a subsample was collected and fixed in 70% ethanol in a vial labeled with the trap code. The largest larvae in each subsample were selected for taxonomic identification under a stereomicroscope and photographed with a calibrated scale (SteREO Discovery V20, ZEISS, Jena, Germany). Genera and species were identified with the keys (Becker et al., 2020; Clark-Gil & Darsie, 1983; Cova García, 1964; En et al., 2016; González & Darsie Jr, 1996; Harburguer & Gonzalez, 2018; Rossi & Almirón, 2004; Stojanovich & Pratt, 1966). Nomenclature follows the Mosquito Taxonomic Inventory(Harbach, 2013).

2.6. Statistical analysis

Positivity and egg density. A trap was considered positive when at least one egg was found on its substrate. The ovitrap positivity index (OPI) was calculated as the percentage of positive traps, and the egg density index (EDI) as the mean number of eggs per positive trap, for each collection and for the whole study. Both indices are reported descriptively; positivity at least once and at 0–48 h was compared among materials with χ^2 or Fisher's exact tests, and weekly positivity (days 7–28) with a logistic regression including material, attractant and collection day, with standard errors clustered by trap; the effect of material was tested with a Wald χ^2 test.

Egg collection by material and attractant. The total number of eggs per trap over 28 days was compared among materials and attractants with a negative binomial generalized linear model, and effects are reported as rate ratios (RR) with 95% confidence intervals (CI). The same model was fitted to the eggs of each weekly collection (days 7–28), with collection day and its interaction with material as additional terms and standard errors clustered by trap (Liang & Zeger, 1986). To separate the effect of material on egg numbers from its effect on positivity, the weekly model was also fitted to positive trap-collections only.

Classification of egg counts. To map how intensely females used each trap, the number of eggs per trap at each weekly collection was assigned to four classes based on the oviposition behavior of *Ae. aegypti*, which lays a mean of about 73 eggs per gonotrophic cycle distributed among four to six sites(de Abreu et al., 2015): 0; 1–30 eggs, about one female visit; 31–74, up to one complete clutch; and ≥ 75, more than one female. A five-class scheme that split the 1–30 class (1–15 and 16–30) was also tested but was less informative, because these classes contained very few traps (**Table S1**).

Spatial distribution. For each weekly collection (days 7, 14, 21 and 28), traps were mapped by material and egg class over an OpenStreetMap basemap. Areas of concentrated oviposition were visualised with a Gaussian kernel density surface (50-m bandwidth) of the total eggs per trap.

Automated counting. Col-Ovo counts were compared with manual counts from the last collection (day 28) only, after the observers had gained experience in naked-eye counting during the previous collections. The 50 positive substrates (manual counts 10–1,340 eggs) were analyzed; negative substrates were not processed, so the comparison assesses counting accuracy rather than detection. Agreement was evaluated with Spearman's correlation, Lin's concordance correlation coefficient (CCC)(Lin, 1989) , the mean absolute error and the mean difference (bias, Col-Ovo minus manual). Bland–Altman limits of agreement were calculated as the bias ± 1.96 standard deviations of the differences. Differences were tested with the Wilcoxon signed-rank test, overall and by trap material.

All analyses were run in Python 3 with NumPy, pandas and SciPy(Van Rossum & Drake Jr, 1995).

3. Results

3.1. Phase 1: Sabethini colonization and egg brooding by *Tr. digitatum*

All six ovitraps were colonized, with positivity per inspection between 50% and 100%. In total, 20 eggs and 63 larvae of *Tr. digitatum* and 19 larvae of *Li. durhamii* were collected; both species co-occurred in one trap (16.7%). From ten laboratory-reared larvae of each species, four *Tr. digitatum* and three *L. durhamii* adults emerged. *Li. durhamii* larvae were most abundant at the second inspection, when *Tr. digitatum* larvae were scarcer in the shared trap.

On the last inspection day, a *Tr. digitatum* female was found standing on the water surface over her egg raft, with the eggs beneath her body (**Fig. 3**; **Video 1-3**). She was observed from 07:30 to 14:50 h. She stayed on the raft when the trap was moved 30 m, and when the raft was removed with a pipette and returned to the water she moved back. The female then left the raft, and the 10 eggs were preserved in ethanol 70%. The same trap contained larvae of earlier cohorts and remains of adults.

3.2. Phase 2: egg collection by trap material and attractant

Over 28 days, the 83 ovitraps collected 43,764 *Aedes* eggs (**Table 1**).

Trap positivity. Seventy-two traps (86.7%) were positive at least once, with no difference among materials (clay 22/23, 95.7%; plastic 24/30, 80.0%; bamboo 26/30, 86.7%; χ^2 test, $p = 0.25$). Detection differed only at the start: on day 1 a single plastic trap was positive (16 eggs), and by 48 h 10 traps (12.0%) were positive, 4 clay (17.4%) and 6 plastic (20.0%) but no bamboo traps (Fisher's exact test, bamboo vs. other materials, $p = 0.012$). Weekly positivity then rose from 37.3% on day 7 to 68.7% on day 21 and was 60.2% on day 28, without differences among materials (mean weekly OPI: clay 53.3%, plastic 53.3%, bamboo 58.3%; Wald $\chi^2 = 0.50$, $df = 2$, $p = 0.78$) (**Figure 4A**).

Number of eggs. Material did affect the number of eggs. Clay traps collected 4,415 eggs (mean ± SD 192 ± 205 per trap; median 101), against 18,117 in plastic (604 ± 799; median 255) and 21,232 in bamboo traps (708 ± 951; median 298) (**Figure 4B**). In the negative binomial model of totals (LR $\chi^2 = 7.85$, $df = 2$, $p = 0.020$), plastic traps collected 3.09 times (95% CI 1.62–5.87) and bamboo traps 3.61 times (1.91–6.84) more eggs than clay traps, and plastic and bamboo did not differ (RR = 0.85, 0.44–1.66, $p = 0.64$). This difference was consistent over time: in the model of weekly collections (days 7–28), the material × collection interaction was not significant (Wald $\chi^2 = 9.04$, $df = 6$, $p = 0.17$). The difference persisted among positive weekly collections: plastic and bamboo traps held

about 3.4 times more eggs than clay traps (plastic RR = 3.47, 2.13–5.64; bamboo RR = 3.38, 2.07–5.52; both $p < 0.001$). The EDI of clay traps rose from 47.5 eggs on day 7 to 110.6 on day 28, whereas that of plastic and bamboo traps peaked on day 21 (329.9 and 443.5 eggs) (**Figure 4C**). The attractant had no effect on totals (RR = 0.92, 0.54–1.57, p = 0.75) or on eggs per positive trap (RR = 0.96, 0.62–1.50, p = 0.86), and there was no material × attractant interaction (LR $\chi^2 = 1.27$, df = 2, p = 0.53).

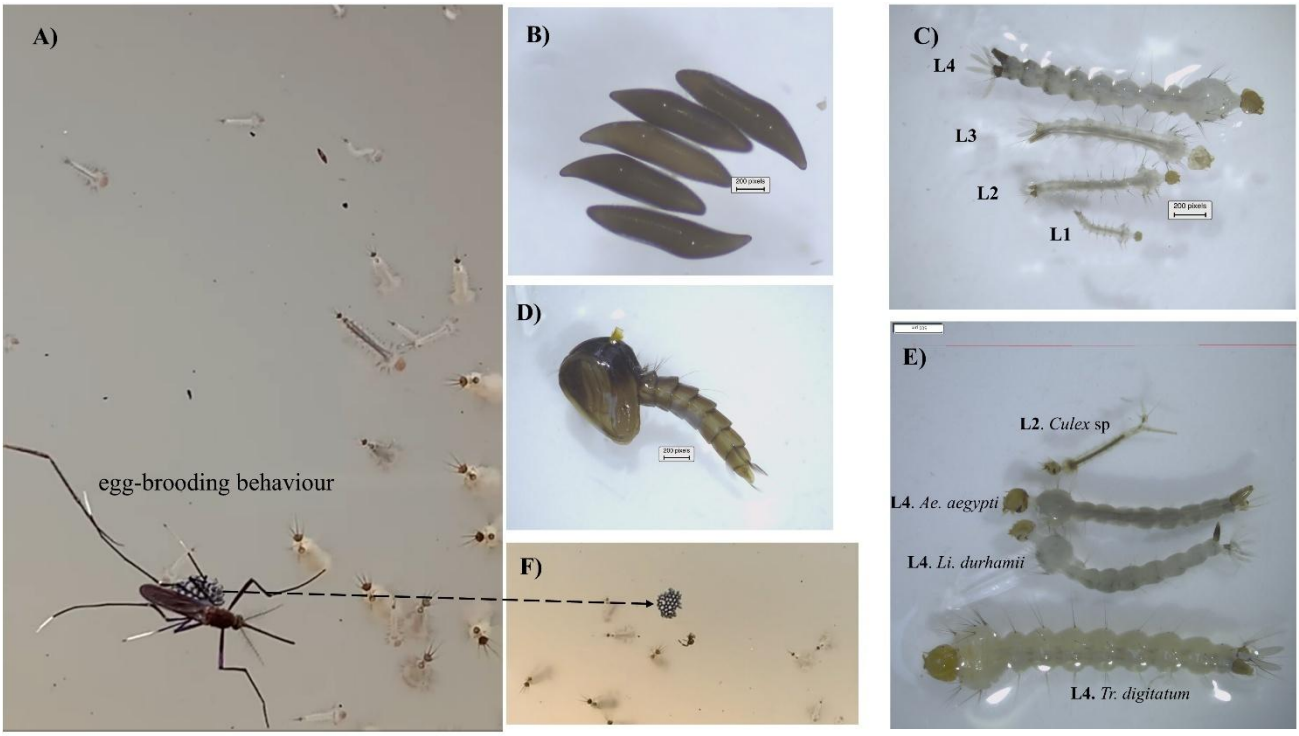


Figure 3. Egg brooding and immature stages of *Tr. digitatum* collected in OviCol ovitraps in the Sierra Nevada de Santa Marta forest (Phase 1). (**A**) Female standing over her egg raft on the water surface of a plastic ovitrap (still from Supplementary Videos 1-3); larvae of earlier cohorts surround her. (**B–E**) Stereomicroscope images: (**B**) *Tr. digitatum* Eggs. (**C**) *Tr. digitatum* larval instars L1–L4 reared from field-collected. (**D**) *Tr. digitatum* Pupa. (**E**) Fourth-instar larvae of the Sabethini *Tr. digitatum* and *Li. durhamii* compared with a fourth-instar *Ae. aegypti* and a second-instar *Culex* sp. (**F**) The same egg raft on the water surface after the female left (arrow).

Table 1. *Aedes* eggs collected in 28 days (11 June–9 July 2025) by artisanal ovitraps of three materials and two attractants on the Universidad del Magdalena campus (Phase 2).

Material	Attractant	Traps	Positive at least once, n (%)	Positive at 0–48 h, n (%)	Mean weekly OPI (%)	Total eggs	Eggs per trap, mean ± SD	Median	Rate ratio (95% CI)
Clay	Canary seed	11	11 (100.0)	1 (9.1)	47.7	1,533	139 ± 120	88	
	Molasses + yeast	12	11 (91.7)	3 (25.0)	58.3	2,882	240 ± 257	170	
	All	23	22 (95.7)	4 (17.4)	53.3	4,415	192 ± 205	101	1 (reference)
Plastic	Canary seed	15	14 (93.3)	3 (20.0)	63.3	10,359	691 ± 800	393	
	Molasses + yeast	15	10 (66.7)	3 (20.0)	43.3	7,758	517 ± 816	193	
	All	30	24 (80.0)	6 (20.0)	53.3	18,117	604 ± 799	255	3.09 (1.62–5.87)
Bamboo	Canary seed	15	13 (86.7)	0 (0.0)	56.7	12,425	828 ± 1158	331	
	Molasses + yeast	15	13 (86.7)	0 (0.0)	60.0	8,807	587 ± 708	219	
	All	30	26 (86.7)	0 (0.0)	58.3	21,232	708 ± 951	298	3.61 (1.91–6.84)
Total		83	72 (86.7)	10 (12.0)	55.1	43,764	527 ± 776	206	

OPI, ovitrap positivity index (traps with ≥ 1 egg); mean weekly OPI is the mean over the collections of days 7, 14, 21 and 28. The 0–48 h count is the cumulative count on day 2. Rate ratios are from a negative binomial model of total eggs per trap (days 0–28) with material and attractant as factors (robust 95% CI). Material: LR $\chi^2 = 7.85$, df = 2, p = 0.020; plastic vs. bamboo: RR = 0.85 (0.44–1.66), p = 0.64; attractant (molasses + yeast vs. canary seed): RR = 0.92 (0.54–1.57), p = 0.75; material × attractant: LR $\chi^2 = 1.27$, df = 2, p = 0.53. Positivity at least once: χ^2 test, p = 0.25; positivity at 0–48 h, bamboo vs. other materials: Fisher's exact test, p = 0.012

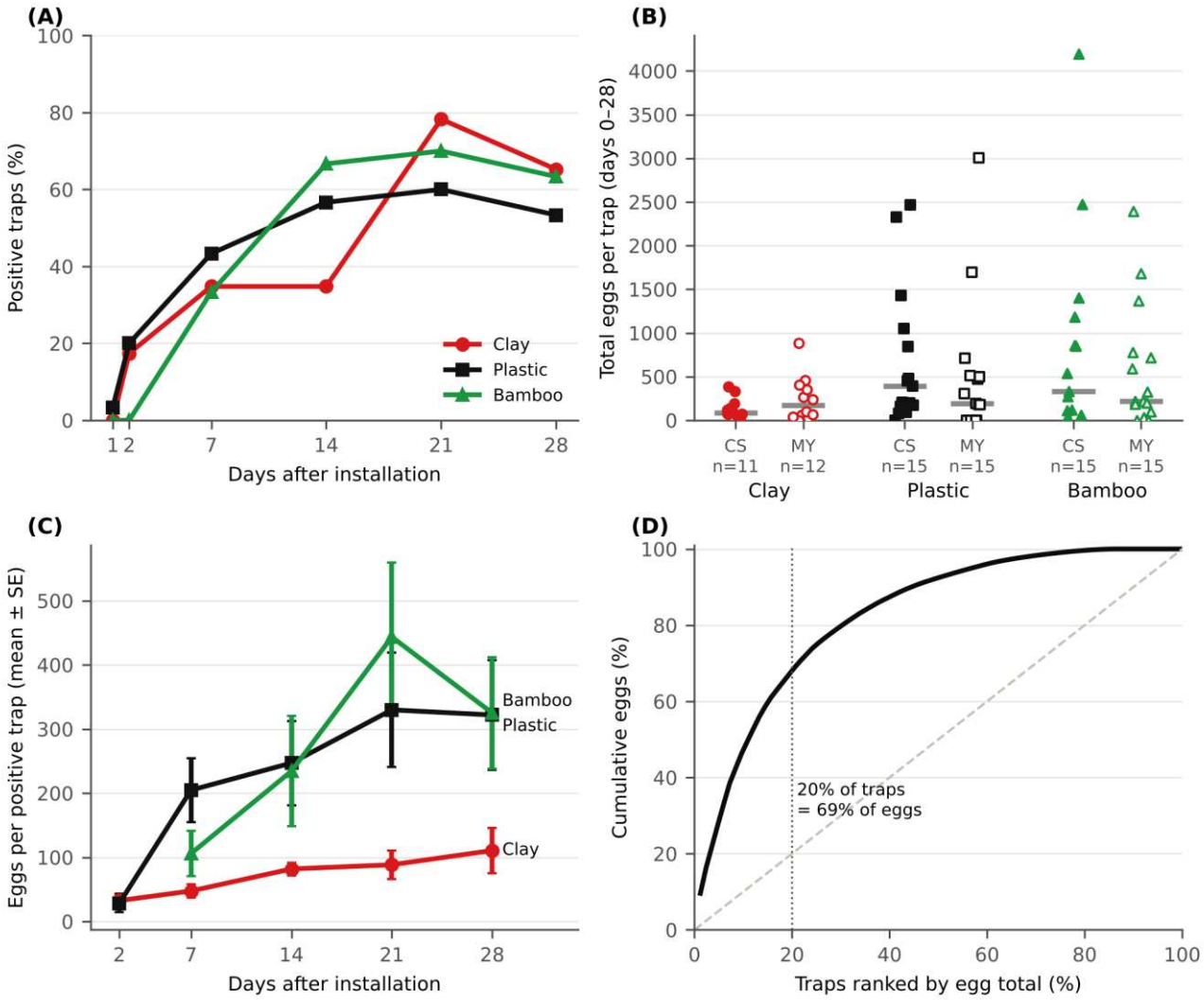


Figure 4. *Aedes* oviposition in clay, plastic and bamboo ovitraps on the Universidad del Magdalena campus (Phase 2). (**A**) Percentage of positive traps (≥ 1 egg) at each collection. (**B**) Total eggs per trap (days 0–28) by material and attractant (CS, canary seed; MY, molasses + yeast); points are traps and grey bars are medians. (**C**) Eggs per positive trap at each collection (mean ± SE). (**D**) Cumulative percentage of eggs against traps ranked by egg total; the dashed line marks an even distribution.

Concentration and persistence. Egg collection was concentrated in few traps: 20% of the traps held 69% of the eggs (**Figure 4D**), and the five most productive traps (three bamboo and two plastic) collected between 2,395 and 4,195 eggs each. This concentration was similar within materials (top 20% of traps: clay 56%, plastic 66%, bamboo 64% of the eggs). The same traps tended to remain productive: the Spearman correlation between consecutive collections rose from 0.08 (0–48 h vs. day 7; $p = 0.46$) to 0.28 ($p = 0.012$), 0.42 ($p < 0.001$) and 0.69 (day 21 vs. day 28; $p < 0.001$). The number of traps with ≥ 75 eggs per collection doubled from 18 on day 7 to 37 on day 28, while traps without eggs fell from 52 to 33 (**Figure 5A–D**). Nineteen traps reached ≥ 75 eggs in at least three of the four weekly collections and together accounted for 62% of all eggs. Of these, 10 were bamboo, 8 plastic and 1 clay, and four traps (three plastic and one bamboo) did so in every collection (**Figure 5E**). In contrast, 26 traps never reached 75 eggs, including 10 of the 23 clay traps. Spatially,

oviposition was concentrated in the west-central sector of the campus, where kernel density peaked at about 11,400 eggs ha⁻¹; the 32 traps within 100 m of this peak collected 69% of the eggs (**Figure 5F**). Even there, neighbouring traps differed widely: two traps less than 35 m from the peak collected no eggs, while others nearby collected more than 2,000.

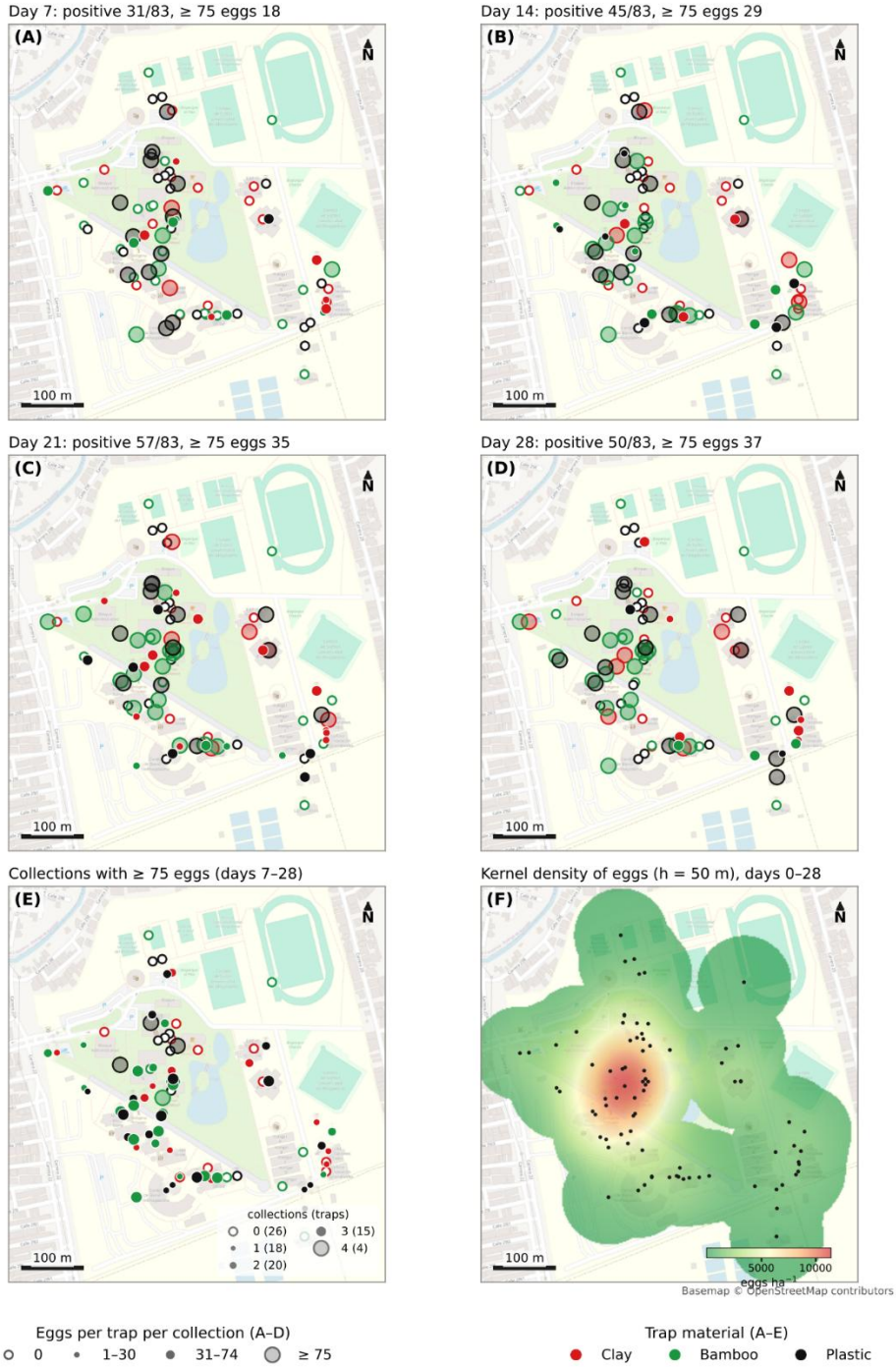


Figure 5. Spatial distribution of *Aedes* oviposition on the Universidad del Magdalena campus (Phase 2). (**A–D**) Eggs per trap on days 7 to 28. Colour indicates trap material and symbol size the egg class (0, 1–30, 31–74, ≥ 75 eggs). (**E**) Number of weekly collections (days 7–28) in which each trap held ≥ 75 eggs. (**F**) Gaussian kernel density of total eggs per trap (days 0–28; bandwidth 50 m), in eggs ha⁻¹.

Automated counting. On the 50 positive day-28 substrates, Col-Ovo and manual counts were strongly correlated (Spearman ρ = 0.89; CCC = 0.79), but Col-Ovo counted fewer eggs (mean difference −78.1 eggs; MAE = 81.1), recording 70% of the manual total (Wilcoxon signed-rank test, $p < 0.001$). Agreement was highest for clay (CCC = 0.97; MAE = 21.5), intermediate for bamboo (0.84; 81.6) and lowest for plastic substrates (0.62; 136.4). Most of the disagreement came from two heavily loaded substrates (one plastic and one bamboo, with 1,234 and 1,340 eggs by manual count), the only values outside the Bland–Altman limits of agreement (−408 to +252 eggs). Without them, agreement rose to CCC = 0.95 (MAE = 49.3; Col-Ovo/manual = 0.79), and to 0.88 for plastic and 0.98 for bamboo substrates. The absolute error increased with egg load (ρ = 0.74, $p < 0.001$), whereas the relative error did not (ρ = 0.07, p = 0.65); agreement was poor for substrates with > 500 eggs (n = 7; CCC = 0.15) and good for those with ≤ 500 eggs (n = 43; CCC = 0.83) (**Figure 6**). Visual inspection of the images showed that agreement decreased on substrates with a high egg load, aggregated (overlapping) eggs and broken eggs (**Figure S1**).

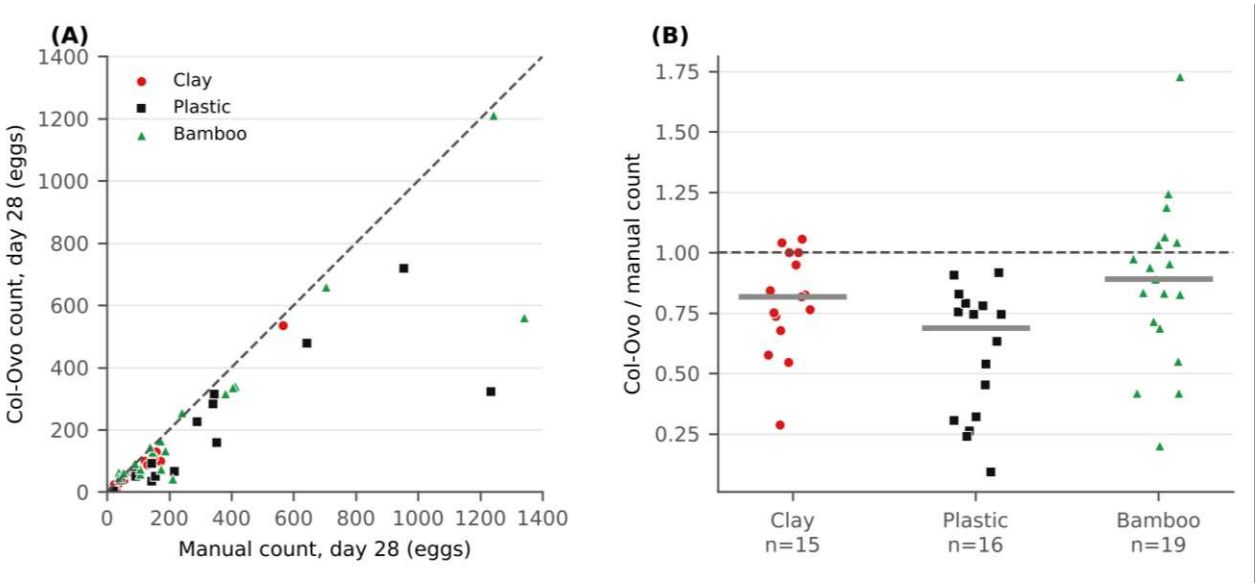


Fig 6. Agreement between Col-Ovo and manual counts of *Aedes* spp. eggs by trap material. (**A**) Col-Ovo count against manual count by trap material; the dashed line is the 1:1 line. (**B**) Ratio of Col-Ovo to manual count by trap material; grey bars are medians and the dashed line marks perfect agreement (ratio = 1).

3.3. Phase 2: larval assemblage on the urban campus

Thirty-one larval subsamples yielded 431 culicid larvae of seven taxa, 16 larvae of *Clogmia* sp. (Psychodidae) and one unidentifiable adult that emerged in ethanol. The most frequent taxa were *Culex quinquefasciatus* (191 larvae; 14 subsamples) and *Ae. aegypti* (158; 11), both found in all three materials. *Culex* (*Phenacomyia*) *corniger* (28; 3) also occurred in all materials. *Culex* (*Carrollia*) cf. *bihaicola* (32; 2) was found in a single bamboo trap, *Culex* (*Culex*) cf. *nigripalpus* (2; 1) in a clay trap, and one *Culex* larva could not be identified to species. A subsample from the city centre contained only *Ae. aegypti* (**Figure 7**).

Aedes albopictus was identified in three subsamples (19 larvae) from different traps: two bamboo traps (one with canary seed and one with molasses) and one clay trap with canary seed.

3.4. Phase 3: forest assemblage in the SNSM

In the SNSM, 15 larval subsamples yielded 114 larvae of eight taxa, and no *Aedes* were found (**Figure 7** and **8).** Sabethini dominated: *Tr. digitatum* (55 larvae; 5 subsamples), *Li. durhamii* (34; 5), *Wyeomyia* (*Phoniomyia*) sp. (1; 1) and *Wyeomyia* sp. (1; 1). The remaining taxa were *Orthopodomyia* cf. *fascipes* (16; 2), the predator *Toxorhynchites* (*Lynchiella*) sp. (1; 1), *Culex* (*Culex*) *nigripalpus* (4; 2) and *Culex* (*Culex*) sp. (2; 1).

Clay traps were placed on the ground because their weight prevented suspension, whereas plastic and bamboo traps were suspended at 5 m or ≥ 10 m. Trap material and height were therefore confounded. Ground-level clay traps provided most subsamples and taxa (10 subsamples, 5 taxa, 77 larvae), followed by suspended bamboo (3, 3, 26) and plastic traps (2, 2, 11). *Limatus durhamii*, both *Wyeomyia* taxa and *Culex* (*Culex*) sp. were collected only in clay traps on the ground. *Orthopodomyia* cf. *fascipes* and *Toxorhynchites* sp. (5 m) were collected only in suspended bamboo traps, and *Cx. nigripalpus* only in suspended plastic traps. *Tr. digitatum* occurred at both levels and in all three materials. Molasses-baited traps yielded 6 taxa in 10 subsamples and canary-seed traps 4 taxa in 4 subsamples; one subsample had no attractant recorded. The only taxon shared by the urban and forest assemblages was *Cx. nigripalpus* (recorded as cf. *nigripalpus* in the city) (**Supplementary Atlas S1).**

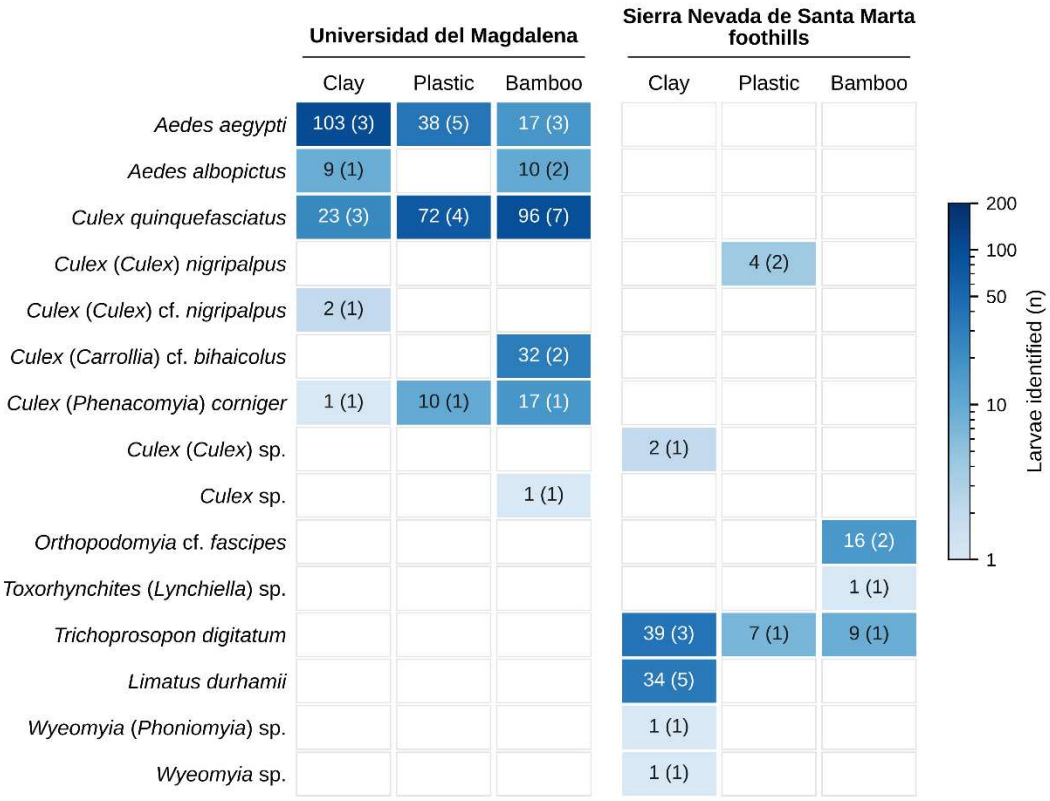


Figure 7. Mosquito larvae (Culicidae) identified by setting and ovitrap material. Values indicate the number of larvae identified, with the number of subsamples in which each taxon was recorded in parentheses. *Clogmia* sp. is not a culicid and is listed for completeness. A subsample from the city centre (*Ae. aegypti*, 3 larvae) is not included.



Fig. 8. Culicid larval assemblages in clay, plastic and bamboo ovitraps in urban and forest settings, Colombia.

4. **Discussion**

We evaluated artisanal ovitraps made of plastic, bamboo and clay as tools for the surveillance and control of culicid mosquitoes in urban and forest settings. Baited with common organic attractants, the three trap types detected the two main urban arbovirus vectors, an urban *Culex* community, a sylvatic Sabethini community and a natural predator. To our knowledge, the same traps also provided the first evidence of *Ae. albopictus* in the city of Santa Marta(Perdomo-Balaguera et al., 2026) and the first video record of egg brooding by *Tr. digitatum*. These results show that artisanal ovitraps can serve as a common, low-cost tool across the urban–forest gradient, where entomological surveillance usually relies on separate and more expensive methods.

Beyond detection, the repeated egg counts on the urban campus allowed us to follow the oviposition behavior of *Aedes* under field conditions. Skip oviposition, the distribution of a single clutch among several containers, was first demonstrated genetically in a natural population of *Ae. aegypti* (Colton et al., 2003), who used codominant markers to show that single females lay their eggs in several containers in the field. Under laboratory and semi-field conditions, de Abreu et al (de Abreu et al., 2015) later found that a female lays about 72–74 eggs per gonotrophic cycle (range 40–142) and spreads them among four to six breeding sites (maximum 11), with one "favourite" site receiving 40% or more of the eggs. Our observations are consistent with this behavior. On day 1, a single trap held 16 eggs, and at 48 h the nine positive traps held a median of 33 eggs (range 3–94), values mostly compatible with single visits by one or two females. Later collections exceeded 75 eggs, roughly the output of one complete gonotrophic cycle, in a growing number of traps, which implies visits by several females. As the study progressed, the ranking of traps also became more stable: the correlation between consecutive collections rose from 0.08 to 0.69, and 19 traps that repeatedly held ≥ 75 eggs accumulated 62% of all eggs. Gravid females therefore spread their eggs widely but return to the same productive sites, which explains why positivity plateaus (60–69% from day 14) while egg counts keep increasing. In practice, a small number of persistent "hot" traps could concentrate egg removal and breeding-site searches, making egg-based control more efficient.

Entomological surveillance exists to turn vector counts into decisions, yet ovitrap indicators remain unstandardized. The most widely used ones measure whether eggs are present, not how many. In Hong Kong, the Area Ovitrap Index (the percentage of positive traps per area) is read in four alert levels: <5%, 5–<20%, 20–<40% and ≥40%. Vector-control operations are stepped up as the level rises (FEHD, 2018). Mexico adopted ovitrap surveillance as a national program in 2013(CENAPRECE, 2023; Ortega-Morales et al., 2018), and Brazil recently extended it nationwide(Ministerio da Saúde, 2025). Both protocols compute egg-level indicators, but neither sets egg-based risk thresholds. Brazil uses the Egg Density and Vector Density indices without numerical cut-offs and intensifies control within 200 m of a trap when indicators rise relative to the previous survey. Mexico treats weekly eggs per city block as the relative risk of transmission and mentions reference values for optimal, good, deficient, alarm and emergency control, but does not state them.

Our data suggest a simple egg-based alternative. The classes used here (0, 1–30, 31–74 and ≥ 75 eggs per trap per collection) separate occasional visits from sustained oviposition by several females, and persistence at ≥ 75 eggs across collections identified the traps that concentrated most eggs. There is evidence that egg abundance carries epidemiological information beyond positivity: in Indonesia, egg density (eggs per positive trap) was the best predictor of monthly dengue cases ($r = 0.67$; 44% of variance explained), ahead of ovitrap positivity (42%) and the positive house index (34%) (Sasmita et al., 2021). Egg classes are easy to compute in the field, but they remain candidate indicators. Future studies should aim to validate egg-based thresholds against adult density and dengue

incidence and to calibrate them across settings and trap designs, so that egg counts can be translated into standardized risk levels for vector-control decisions.

These results also have an operational implication. For decades, *Aedes* surveillance and control have been the responsibility of the health sector almost exclusively. Low-cost ovitraps make it possible to share this responsibility with other sectors, especially education. Universities host a large floating population that commutes daily from many neighbourhoods and municipalities, and any of the three trap types evaluated here could be incorporated into their pest-management plans. In this study, all collected eggs were inactivated with hot water alone, a method that achieved 100% ovicidal efficacy without insecticides (Arrieta-Ángel et al., 2025). The same approach could then move from the campus to the home through an "adopt an ovitrap" scheme, in which students and staff maintain traps in their own households, multiplying the number of traps and the number of eggs removed. To support such participation, we have made available a platform (https://reportes.chilloa.org/) that integrates Col-Ovo AI (Almanza & Montenegro, 2026): any user, anywhere, can photograph the substrate before inactivating the eggs, report it, and have the eggs counted automatically. Each contribution thus becomes both an act of control and a georeferenced surveillance record, which could extend egg-based control well beyond the reach of health programs.

Col-Ovo was trained on fresh field substrates(Almanza & Montenegro, 2026), but here it was challenged with substrates whose eggs had dried for a week. Even so, agreement with manual counts was good (CCC = 0.79) and became almost perfect when two atypical substrates were excluded (CCC = 0.95). Col-Ovo underestimated counts by about 21%, a consistent bias that could be corrected with a calibration factor. Agreement fell sharply on substrates with more than 500 eggs. On the two most discordant substrates, each with more than 1,200 eggs, many eggs were aggregated in clusters and many were broken (**Figure S1**), egg formats that were absent from the training set. These conditions also make manual counting difficult, so at high egg loads the reference itself is uncertain. Overlapping eggs are a known limitation of image-based egg counting(Javed et al., 2023). In contrast, a third substrate with a similar load (1,241 eggs) (**Figure S1**), was counted almost exactly (1,211 eggs), which shows that density alone does not explain the error. Training the model with clustered and broken eggs, and validating it across the full range of densities, should precede its routine use.

Beyond the two urban vectors, the traps recorded a broad culicid fauna, which extends their use from vector surveillance to biodiversity sampling. On the urban campus, *Cx. quinquefasciatus* was the most abundant taxon in larval subsamples and occurred in all three materials, together with *Cx.* (*Phenacomyia*) *corniger*, *Cx.* (*Carrollia*) cf. *bihaicola* and *Cx.* cf. *nigripalpus*. This was expected, since organic infusions attract *Culex* females(Becker et al., 2020). We had previously shown that OviCol traps are not restricted to *Aedes*: in the Colombian Amazon, they also recorded *Culex* spp. and *Psorophora ferox*, a species not captured by BG-Sentinel traps (Mosquera et al., 2026).

No *Aedes* was found in the SNSM forest. Instead, the traps held a container-breeding Sabethini community (*Tr. digitatum*, *Li. durhamii* and *Wyeomyia* spp.), together with *Orthopodomyia* cf. *fascipes*, *Cx. nigripalpus* and the larval predator *Toxorhynchites* (*Lynchiella*) sp. Ovitraps have recovered similar faunas elsewhere in the Neotropics. In a Mesoamerican rainforest, they collected eight taxa, including *Tr. digitatum* and the sylvatic yellow fever vector *Sabethes chloropterus* (Chaverri et al., 2018). In Mexico, the national ovitrap program has collected 12 target and non-target species since 2013: five of *Aedes*, four of *Culex*, and one each of *Haemagogus*, *Limatus* and *Wyeomyia* (Ortega-Morales et al., 2018). Four of them (*Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus* and *Li. durhamii*) were also recorded in our traps.

Several of these taxa are also of public-health interest. *Cx. quinquefasciatus* is a vector of West Nile and St. Louis encephalitis viruses, and West Nile virus has been isolated from field-collected pools of this species(Elizondo-Quiroga et al., 2005). *Tr. digitatum* has been found naturally infected with St. Louis encephalitis, Bussuquara, Pixuna, Wyeomyia and Ilhéus viruses(Uribe-Soto & Suaza-Vasco, 2021). *Li. durhamii* uses a wide range of natural and artificial breeding sites, including artificial containers, bamboo stumps, tree holes and fruit husks (Chaverri et al., 2018), and is among the species that differentiate ground-level from elevated ovitraps in forest (Alencar et al., 2016). In contrast, *Tr. digitatum* was found alone in the ovitraps where it occurred, probably because of larval cannibalism (Chaverri et al., 2018), although both co-occurred in one Phase 1 trap. Artisanal ovitraps could therefore support eco-epidemiological studies beyond *Aedes* surveillance. Ovitraps reflect the relative abundance and species composition of container-breeding mosquitoes in the sampled area(Day, 2016), so they can track secondary and sylvatic vectors and describe how mosquito communities change along the urban–forest gradient. Because they attract gravid females, which have completed at least one gonotrophic cycle and are therefore more likely to be infected, oviposition-based traps are also valuable for virus surveillance (Day, 2016); the eggs and immature stages they collect could be screened for vertically transmitted arboviruses(Aitken et al., 1979).

The most unusual observation was a *Tr. digitatum* female brooding her egg raft inside an ovitrap (**Figure 3; Videos 1–3**). Maternal care is exceptional in mosquitoes. It was first reported in the Malayan *Armigeres flavus*, whose females carry their eggs on the hind legs until the larvae hatch(Strickland & Strickland, 1917). In *Tr. digitatum*, egg attendance was first noticed when the species was colonized in the laboratory in Trinidad: females stood guard over their raft for about 24 h, and could be dislodged only with considerable difficulty; rafts held about 60 eggs (up to 85), which hatched after 26–30 h (Aitken et al., 1968). In cacao husks in Venezuela, rafts were almost always guarded from oviposition until hatching (Lounibos & Machado-Allison, 1983), and that unguarded rafts broke up, leaving eggs stranded on the husk. Their experiments identified rainfall as the main selective pressure, showing that brooding prevents eggs from being dispersed by rain (Lounibos & Machado-Allison, 1986). Later field studies in Trinidad recorded 546 guarding females in nutshells and showed that oviposition occurs during the day, with two peaks (Chadee et al., 1989).

Using plastic cups in the forest, was showed that females prefer to lay where another female is already brooding and where food is abundant, but avoid containers with late-instar larvae, which cannibalize the young (Sherratt & Church, 1994). All these records relied on direct observation or still photographs(Lounibos, 1983); to our knowledge, ours is the first video record of the behavior. Through periodic visits, we followed the brooding female for at least 7 h. When we first found her, at about 07:00 h, the eggs were still white, indicating that they had been laid shortly before, in agreement with the early-morning oviposition peak reported in the field (Chadee et al., 1989). The female remained in the trap after the eggs were removed with a pipette and resumed brooding when they were returned, consistent with the difficulty of dislodging guarding females (Aitken et al., 1968). She did so in a trap already occupied by other cohorts, in line with the preferences described (Sherratt & Church, 1994), which suggests that the organic attractant of artisanal ovitraps may favour this species. However, she left the raft before the eggs hatched, as shown by subsamples taken after her departure. Our manipulation of the raft may have shortened brooding.

This study has several limitations. Phase 2 lasted 28 days at a single site and season, and eggs were not identified to species, so the material comparison refers to *Aedes* spp. and may combine *Ae. aegypti* and *Ae. albopictus*. Each clay trap weighed about 2 kg and could not be suspended, so material was confounded with height. Clay traps were also costlier to make and transport and had to be handled carefully to avoid breakage. Some plastic traps, in contrast, were displaced when dry,

which may have shortened their effective sampling time. Larval subsamples describe composition but not relative abundance, and some taxa were identified only to genus or as "cf." because diagnostic structures could not be examined in all specimens. In the forest, Phase 1 involved only six traps, Phase 3 was unbalanced for material, height and attractant, and maternal care was documented in a single female through periodic rather than continuous observation. Finally, the Col-Ovo comparison covered only positive day-28 substrates, and the manual counts used as reference were made by students who gained experience during the study and were not verified by an expert counter. Future work should replicate the material comparison across seasons and cities with all materials at the same height. It should also identify eggs or reared adults to species.

5. Conclusion

Artisanal ovitraps made from plastic, clay or bamboo are an inexpensive and versatile tool for integrated entomological surveillance and for egg-based control with low environmental cost. Plastic and bamboo traps maximise egg collection, clay traps are sufficient for presence–absence monitoring, and the attractant can be chosen on cost. Clay and bamboo add biodegradable alternatives to plastic. The same traps captured field evidence of *Ae. aegypti* oviposition behavior, provided the first evidence of *Ae. albopictus* in Santa Marta, revealed a sylvatic mosquito community of potential health relevance, and documented maternal care in *Tr. digitatum*. Simple tools can thus serve both public health and natural history.

6. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors were involved in the development of Col-Ovo, which was evaluated; this relationship is disclosed for transparency. To reduce potential bias, the manual egg counts used as the reference standard were performed by thesis students who did not participate in the AI-assisted counts and had no access to the platform outputs.

7. Author Contributions

FDR supported all Phase 2 field collections and performed the manual egg counts, and assisted in cutting the guadua (bamboo) used to build the bamboo ovitraps. JDAR conducted all larval biometry and photography. EP supported laboratory logistics, hatching assays, and taxonomic identification during Phase 1. CV provided administrative and logistical support and supervised thesis students during Phase 2. MC led the taxonomic diagnosis of all samples. JA developed the https://reportes.chilloa.org/ and Col-Ovo technological platforms and integrated the updated AI model into them. RGA provided administrative and laboratory support, including laboratory supplies for Phases 2 and 3. DM conceived and led the project, trained and retrained the Col-Ovo model, obtained laboratory supplies, performed the statistical analyses, and wrote the manuscript. All authors reviewed, edited, and approved the final version of the manuscript.

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Supplementary Video 1. Egg brooding by a female *Trichoprosopon digitatum* in an artisanal ovitrap, Caracolí farm, SNSM, December 2024.

Table S1. Comparison of two schemes for classifying the number of *Aedes* eggs per trap per collection (n = 83 traps, Universidad del Magdalena campus).

Collection	Classes	0	1–15	16–30	1–30	31–74	≥ 75	Classes with < 5 traps	Pielou's J	Cramér's V (material)	χ^2 p
0–48 h	5	73	4	1	–	4	1	4	0.32	0.27	0.140
	4	73	–	–	5	4	1	**2**	**0.35**	0.27	**0.063**
Day 7	5	52	2	3	–	8	18	2	0.66	0.29	0.095
	4	52	–	–	5	8	18	**0**	**0.74**	0.28	**0.038**
Day 14	5	38	3	4	–	9	29	2	0.77	0.22	0.432
	4	38	–	–	7	9	29	**0**	**0.85**	0.22	**0.257**
Day 21	5	26	5	5	–	12	35	0	0.84	0.34	0.014
	4	26	–	–	10	12	35	0	**0.91**	0.33	**0.005**
Day 28	5	33	1	4	–	8	37	2	0.72	0.27	0.140
	4	33	–	–	5	8	37	**0**	**0.81**	0.27	**0.064**

Agreement between consecutive collections (quadratic-weighted Cohen's kappa), five vs. four classes: 0–48 h → day 7, 0.00 vs. 0.01; day 7 → day 14, 0.20 vs. 0.22; day 14 → day 21, 0.33 vs. 0.33; day 21 → day 28, 0.60 vs. 0.59. Pielou's J = H′ / ln k, where H′ is the Shannon index of the distribution of traps among classes and k the number of classes. Cramér's V and χ^2 p refer to the association between trap material (clay, plastic, bamboo) and egg class; p values are approximate because some expected frequencies were low. Class limits follow the oviposition behavior of *Ae. aegypti* (about 73 eggs per clutch distributed among four to six sites; de Abreu et al., 2015): 1–30 eggs, about one female visit; 31–74, up to one complete clutch; ≥ 75, more than one female.

Revisores que recomendaremos

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