

1 **Treeholes as relevant larval habitats for *Aedes aegypti***
2 **surveillance: urban, suburban and forest spatial distribution in**
3 **a dengue affected area**

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5 C. Mangudo^{1,2}, J. P. Aparicio^{1,2,3} and R. M. Gleiser^{4*}

6
7 ¹*Instituto de Investigaciones en Energía No Convencional (INENCO, UNSa*
8 *- CONICET), Universidad Nacional de Salta, Av. Bolivia 5150, A4400FVY,*
9 *Salta, Argentina* ²*Instituto de Investigaciones en Enfermedades Tropicales, Sede Regional*
10 *Orán, Universidad Nacional de Salta, Alvarado 751 Orán, 4530 Salta, Argentina,*
11 ³*Mathematical, Computational and Modeling Sciences Center, Arizona State University,*
12 *PO Box 871904, Tempe, AZ 85287-1904 and* ⁴*Centro de Relevamiento y Evaluación de*
13 *Recursos Agrícolas y Naturales-IMBIV (CONICET-UNC), Facultad de Ciencias*
14 *Agropecuarias, Av. Valparaíso sn (5016) Córdoba, Argentina* *Cátedra de Ecología,*
15 *Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba. Av.*
16 *Vélez Sársfield 299 (5000) Córdoba, Argentina.*

17
18 ***Corresponding author:** Raquel M. Gleiser. *Centro de Relevamiento y Evaluación de*
19 *Recursos Agrícolas y Naturales-IMBIV (CONICET-UNC), Facultad de Ciencias*
20 *Agropecuarias, Av. Valparaíso sn (5016) Córdoba, Argentina. Tel: +54 (0351)*
21 *4334105/16/17 FAX +54 (0351) 4334118. Email: rgleiser@crean.agro.uncor.edu*

23 **Running head: Treeholes as *Aedes aegypti* larval habitat**

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25

26 **Abstract**

27 *Aedes aegypti* (L.) (Diptera: Culicidae), the main vector of dengue and urban yellow
28 fever in the world, is highly adapted to the human environment. Artificial containers are its
29 most commonly used larval habitat, but it may develop in tree holes and other
30 phytotelmata. This study assessed whether tree holes in San Ramón de la Nueva Orán, a
31 city located in subtropical montane moist forest where dengue outbreaks occur, are relevant
32 as larval habitat for *Ae. aegypti* and if the species may be found in natural areas far from
33 human habitations. Water holding tree holes were sampled during three years once a month
34 along the rainy season using a siphon bottle, in urban and suburban sites within the city and
35 in adjacent forested areas. Larvae and pupae were collected and the presence and volume of
36 water in each tree hole were recorded. Finding *Ae. aegypti* in forested areas was an isolated
37 event; however, the species was frequently collected from tree holes throughout the city
38 and along the sampling period. Moreover, larvae were collected in considerably high
39 numbers, stressing the importance of taking into account these natural cavities as potential
40 reinfestation foci within dengue control framework.

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42 **Keywords:** Culicidae, landscape, phytotelmata, population ecology, vector

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Introduction

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47 *Aedes aegypti* (*Stegomyia aegypti*) (L.) (Diptera: Culicidae) is the main vector of dengue
48 and urban yellow fever in the world (Gubler, 2004). *Aedes aegypti* is highly adapted to the
49 human environment, and artificial containers are its most commonly used larval habitat
50 (Forattini, 2002). Nevertheless, in some countries in Africa, where the species might have
51 originated, it is a regular component of the tree hole fauna (Anosike *et al.*, 2007).
52 Observations of *Ae. aegypti* developing in tree holes and other natural water containers are
53 also occasionally reported from other regions of the world. In Cuba, immature stages were
54 found in tree holes, coconut shells, and axils of banana leaves (Marquetti *et al.*, 2005). In
55 Brazil, larvae were found in native bromeliads on rocky slopes (Malta Varejao *et al.*, 2005)
56 and in public and private gardens (Mocellin *et al.*, 2009).
57 In Argentina, in the province of Misiones, the National Coordination of Vector Control
58 (Ministry of Health of Argentina) has reported finding larvae in the axils of banana leaves
59 Stein *et al.*, (2011) and Campos *et al.* (2011) observed the species in the axils of Araceae, at
60 the Puerto Iguazú National Park. Stein *et al.* (2013) found *Ae. aegypti* larvae in the epiphyte
61 *Aechmea distichantha* Lemaire (Poales: Bromeliaceae) both in semi-urban and rural
62 localities of pedemontane forest of the subtropical mountainous Yungas rainforest in the
63 province of Tucumán, north western Argentina. In Aguaray city, Salta province, we
64 detected larvae and pupae using tree holes as larval habitat (Mangudo *et al.*, 2011). Reports
65 of *Ae. aegypti* breeding in natural habitat in forested areas far from urbanization are less
66 frequent.

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163

164 **Table 1.** Tree species that were positive for *Aedes aegypti* immature stages (larvae and/or
 165 pupae) in sidewalks and public access areas in urban, suburban and Yunga Forest sites in
 166 San Ramón de la Nueva Orán, Salta province, Argentina.

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Tree species	Trees with holes ¹	Positive holes	Immature culicids
<i>Delonix regia</i> (Bojer) Raf. (Fabales: Fabaceae)	8 / 38	6	3095
<i>Bauhinia sp.</i> L. (Fabales: Fabaceae)	7 / 54	6	824
<i>Thevetia nereifolia</i> Juss. (Gentianales: Apocynaceae)	1 / 76	1	358
<i>Morus sp.</i> L. (Rosales: Moraceae)	3 / 51	2	306
<i>Ficus sp.</i> L. (Rosales: Moraceae)	4 / 69	1	41
<i>Broussonetia papyrifera</i> (L.) Vent. (Rosales: Moraceae)	2 / 28	2	3
<i>Jacaranda sp.</i> Juss. (Lamiales: Bignoniaceae)	5 / 45	4	170
<i>Citrus sinensis</i> Osbeck (Sapindales: Rutaceae)	3 / 41	3	155
<i>Lagerstroemia indica</i> (L.) Pers (Myrtales: Lythraceae)	3 / 30	2	95
<i>Mangifera indica</i> L. (Sapindales: Anacardiaceae)	1 / 41	1	6
Undetermined sp.	3 / 3	1	9
Total	40 (476)	29	5062

168 ¹ Trees with water holding holes in relation to trees examined.

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170

171 **Figure Legend**

172 **Figure 1.** Location of study area and tree holes that harboured *Aedes aegypti* larvae and/or
173 pupae on every sample (black figures), at least once (grey figures), or never (white figures).

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