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Snail shells as larval habitat of *Limatus durhamii* (Diptera: Culicidae) in the Yungas of Argentina

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ABSTRACT

The shells of dead snails collect water from rainfalls producing aquatic microenvironments called gastrotelmata. These habitats are small and hold simple detritus based on animal communities, being rotifers and culicids the most studied. Although a high diversity of aquatic microhabitats has been reported as larval habitats of mosquitoes in Argentina, the shell of snails has not been investigated yet. We report the shells of three species of native *Megalobulimus* genus as larval habitats of a neotropical mosquito and suspected vector of bunyaviruses, *Limatus durhamii*, and describe these microhabitats in the Yungas forest of Argentina.

1. Introduction

The shells of dead snails are small receptacles that act as containers which collect water from rainfalls, giving rise to aquatic microhabitats called gastrotelmata (Gastro = Gastropoda, telma = pool) (Janetzky et al., 1995). Due to the small size of these containers, the biological communities that inhabit them are simple, with rotifers and larvae of Diptera the invertebrates most frequently found. These communities are based on detritus provided by residues of snail bodies, plant matter and invertebrate remains. Snail shells retain water longer than any other natural container, providing a relatively permanent habitat, in which food is probably limited, leading to larval density dependent competitions (Lounibos, 1980).

Little is known about the inhabitants of the snail shells, being the rotifers (e.g. Koste et al., 1993, 1995) and culicids the most studied. Most reports of mosquitoes breeding in shells come from Africa. Unidentified species of the genus *Aedes (Stegomyia)* Theobald and *Eretmopodites* Theobald were reported developing in snail shells in Mombasa, Kenya (Wiseman et al., 1939); *Aedes aegypti* L., *Aedes simpsoni* (Theobald) and *Eretmopodites quinquevitatus* Theobald were the main inhabitants in empty shells of *Achatina fulica* Bowdich (Gastropoda: Achatinidae) in the coast of Dar es Salaam, Tanzania (Trpis, 1972, 1973; Rao et al., 1973). *Eretmopodites subsimplicipes* Edwards, *E. quinquevittatus* and *E. silvestris conchobius* Edwards, were studied at the Kenya coast and larvae were observed breeding in shells more frequently than in other microhabitats (Lounibos, 1980). Immature of the *Eretmopodites* group and *Aedes* Meigen were reported from the shell of *Archachatina marginata* (Swainson) (Achatinidae) in Ekpoma, Nigeria (Igbinosa, 1989), and *Aedes albopictus* from Southern Cameroon (Fontenille and Toto, 2001). In the Americas, few species of mosquitoes use shells as larval habitats.

Limatus Theobald is a genus of forest mosquito that occurs in Central America, eastern South America and the West Indies.

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Immature stages develop in phytotelmata such as bamboo, tree holes, coconut husks, cacao pods, fallen leaves and spathes, and other types of aquatic microhabitats such as snail shells, rock holes and a variety of small artificial containers (Lane, 1953). Of the eight known species, *Limatus durhamii* Theobald was the only one reported for Argentina, being the southernmost geographic distribution in South America. *Limatus durhamii* is a suspected vector of Orthobunyavirus (Bunyaviridae) (Barajas et al., 2013; Berger, 2016). Immature stages were collected from fallen bracts of *Euterpe edulis* Martius (Arecales: Arecaceae) in a subtropical forest on the northeast of the country (Campos et al., 2011). It has occasionally been found in artificial containers in urban areas during several surveys of *Aedes aegypti*.

Although a high diversity of aquatic microhabitats has been reported as larval habitats of mosquitoes in Argentina (Campos et al., 2011), the shell of native snails has not been investigated yet. The main purpose of the present study is to report the shells of three species of *Megalobulimus* Miller as larval habitats of a native mosquito, and to describe this microhabitat in the Yungas forest of Argentina.

2. Materials and methods

2.1. Study area

Field studies were conducted in the Yungas forest near the city of San Ramon de la Nueva Orán (23°08′S, 64°20′W, elevation 337 m.a.s.l.), in northwest Argentina (Fig. 1). The Yungas region is located in the Pedemontane rain-forest floor, where *Calycophyllum multiflorum* Griseb (Rubiaceae) ("Palo Blanco"), *Phyllostylon rhamnoides* (Poisoon) Taub. (Ulmaceae) ("Palo Amarillo") and several species of vines predominate (Brown et al., 2001). The climate is subtropical, with a mean temperature of 27.7° C in summer and 16.4° C in winter; annual rainfall is 1000 mm and occurs mostly during the warmer months (October to April).

Sampling was carried out in three sites in the native Yungas, far from the city. Site 1 was located 0.5 km to the northeast (23°7.123′S, 64°18.517′W), site 2, 7 km to the east (23°9.089′S, 64°13.759′W), and site 3, 4 km to the southeast (23°11.635′S, 64°18.107′W) (Fig. 1).

2.2. Sampling design

An arbitrary area of 50 × 50 m^2 in each sampling site was delimited and ten to twelve snail shells per site were collected in January (only site 1) and February (all sites) of 2012, when rainfall was most frequent.

The snail shells were carried to the laboratory where the presence and volume of water, detritus and number of immature mosquitoes were recorded. Water content from each snail shell was overturned and examined in a white plastic tray, from which larvae and pupae were collected with a pipette. Once the original water content was removed from shells and measured, additional water was added on them to remove larvae and pupae that may have remained stuck inside the shells. A visual qualitative inspection of detritus was made and recorded as: Absent (0); scarce (few small particles) (1), and abundant (mostly plant and other clearly visible detritus) (2), and whether it was mostly plant or animal detritus. Fourth instar larvae were killed and stored in 80% ethanol, while 1st, 2nd and 3rd instars and pupae were reared either to the 4th instar or to adult emergence. Snails were determined by a specialist to the species level based on shell characteristics. Taxonomic determinations of mosquitoes were based on morphological characteristics of 4th instar larvae and/or adults, using Darsie (1985) keys. Immature stages of other arthropods were determined to family level.

In order to explore whether there were unhatched eggs in the walls of shells, after removing the original water, snail shells were allowed to dry for up to 7 days, after which they were immersed in dechlorinated tap water for two weeks to stimulate the hatching.

2.3. Data analysis

Samples from January were only for mosquito and snail taxonomic determinations. Data on mosquito larvae and pupae from February were pooled together for analyses and referred as immature stages.

Fig. 1. Location of study area and sampling site.

Relationships between snail species, site, water volume (categorized as low ±0.5 ml and high ±10 ml), and detritus type (plant or plant and animal detritus) on mosquito abundance were analyzed by generalized linear models considering a Poisson distribution. For all tests, a p value < 0.05 was considered to represent significant differences. Throughout the text, the results are presented as the mean and standard errors.

3. Results

3.1. Snail shells as aquatic microhabitats

Snails from site 1 were identified as *Megalobulimus oblongus* (Müller), from site 2 as *Megalobulimus lorentzianus* (Doering) and from site 3 as *Megalobulimus oblongus musculus* (Bequaert) (Gastropoda: Strophocheilidae). Snail shells width and length of aperture ranged between 2 and 2.5 cm and between 3.5 and 4 cm for *M. oblongus,* respectively; between 2.5 and 3 cm and between 4 and 7 cm for *M. lorentzianus* and between 1.5 and 4 cm and between 2.5 and 5 cm for *M. oblongus musculus.* Of all the collected shells (n = 44), 40% (n = 21), 46% (n = 13) and 100% (n = 10) in sites 1, 2 and 3 respectively, held water. Average and standard deviation of water volume was 2.9 ± 2.38 ml for *M. oblongus* and 3.7 ± 2 ml for *M. lorentzianus* and *M. oblongus musculus*. Water volume per shell was either approximately 10 ml or 0.5 ml or less. For *M. oblongus*, approximately 75% held 10 ml of water; 67% of *M. lorentzianus* and the same percentage of *M. oblongus musculus* shells also held close to 10 ml, while the remaining shells for each species each held ≤ 0.5 ml, and thus we considered two water volume categories (i.e., 0.5 ml and 10 ml) for further data analyses. Plant detritus were found in 100% of the three snail species, while animal detritus were found in 25% of *M. oblongus* shells, 100% of *M. lorentzianus* and 60% of *M. oblongus musculus* shells, mainly exoskeletons from insects or other arthropods, such as dead spiders, ants, and exuviae of mosquitoes and/or Psychodidae.

3.2. Macroinvertebrate community

The aquatic fauna inhabiting the shells was composed of *Li. durhamii* immature (95% larvae, 5% pupae), and Psychodidae larvae. Out of 44 shells examined, *Li. durhamii* were found in 75% and Psychodidae in 13.6% as unique inhabitants, while in 11.3% both taxa were collected together. All Psychodidae were recorded in January from site 1. The average number of *Li. durhamii* immature per snail shell was 11.6 (SD ± 2.3) and ranged from 1 to 27, being the range of 1–7 larvae the most frequent.

3.3. Abiotic parameters effects on *Limatus durhamii* abundance

Since only one species of snail was found per site, interactions between snail species and site cannot be sorted out; then effect of snail species, regardless of collection site, on mosquito abundance was analyzed. Water volume had a significant effect on mosquito abundance (p = 0.0001), being significantly higher in snails holding 10 ml (Mean 17.9, DS ± 2.7) compared to 0.5 ml (Mean 9.7, DS ± 1.1). Also, significant interactions were detected between water volume and detritus type (categorized as only plant or plant and animal detritus) (p < 0.0001) and between detritus type and snail species (p = 0.0001). A higher *Li. durhamii* abundance was detected in *M. oblongus musculus* containing plant and animal detritus compared to other snail species and detritus content (Fig. 2a). Effects of the interaction of detritus type and water volume did not show a clear pattern (Fig. 2b).

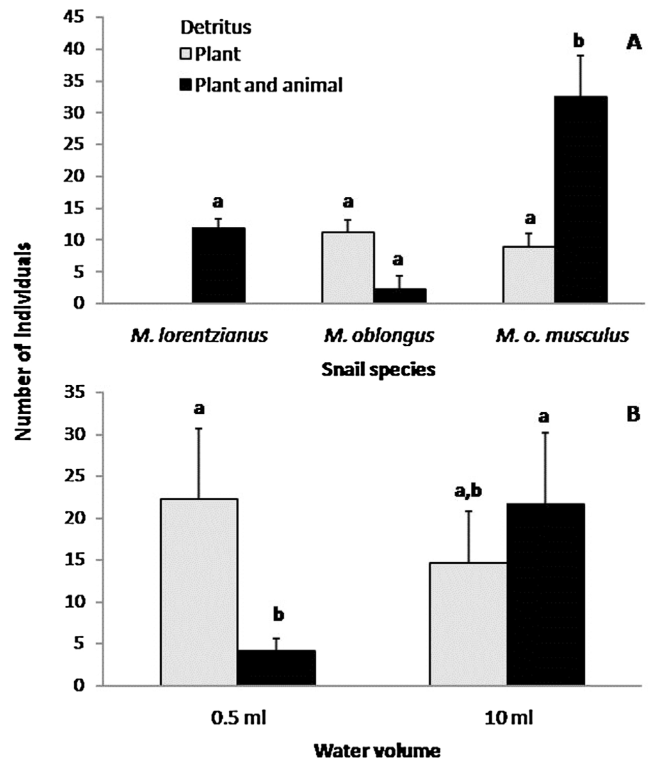

Fig. 2. Mean abundance (+standard error) of *Limatus durhamii* immature: A. per snail species and detritus type. M = *Megabulimus*; o = *oblongus*. All *M. lorentzianus* shells held plant and animal detritus; B. per detritus type and water volume. a,b Letters above bars represent significant differences (p < 0.001) between means as measured by generalized linear models.

4. Discussion

Limatus durhamii is a scarcely studied sylvan mosquito inhabitant of forested areas, even though a variety of natural and artificial micro aquatic environments were recognized as larval habitats. Immatures of *Li. durhamii* were found in *Bromelia* and *Xanthosoma* axils, treeholes and fallen leaves in Mexico (Ortega Morales et al., 2010). Like other mosquitoes inhabitants of phytotelmata, it was also found in artificial containers such as tires, buckets and tins in Costa Rica and Mexico (Calderón Arguedas et al., 2009; Ortega Morales et al., 2010), and in ovitraps in Brazil (Alencar et al., 2016). Other less frequent and rare sites where immature of *Li. durhamii* were occasionally found is in a pond (Ortega Morales et al., 2010) and in a percolation tank that received waste water from manure (Alencar et al., 2013). In Argentina *Li. durhamii* was collected from a watering hole, artificial containers (Rossi and Almiron, 2004) and a phytotelmata formed by floral bracts of the palm *E. edulis* in the forest of Misiones province (northeast) (Campos et al., 2011). Our finding in the Yungas (northwest) is the first report of snail shells as larval habitats of this species.

The species of snails occupied by *Li. durhamii* as larval habitats in the Yungas forest are also present in Misiones forest, but they have not been inspected in relation to mosquitoes until the present. Conversely, in the Yungas, several species of palms with leathery floral bracts (e.g. *Copernicia alba* Morong and *Bactris gasipaes* Kunth) (SIB, 2016) which have not been inspected, could be other natural habitats of *Li. durhamii* in this region.

Limatus durhamii larvae are saprophagous and in the absence of decaying matter they can behave like facultative predators of other mosquito species (Lopes, 1999). This nutritional behavior could be an advantage that allows larvae of *Li. durhamii* to live in such small places like the shells of snails, where the decaying matter is scarce. Psychodidae larvae could be the alternative preys which occasionally allow them to complete their development.

In the present study no other species of mosquitoes were found in the snail shells, despite the fact that *Ae. aegypti* was present in one of the sampling sites (Mangudo et al., 2015), and was reported breeding in these microhabitats in Tanzania (Trpis, 1973) and Kenya (Lounibos, 1980).

In a study from Costa Rica *Li. durhamii* was reported living with *Ae. aegypti* in the same artificial containers (Marín et al., 2009). In one of our sampling sites *Ae. aegypti* was found in a treehole (Mangudo et al., 2015), but despite the fact that these microhabitats are also used by *Li. durhamii* as larval habitat, it was not found associated with *Ae. aegypti*.

Trpis (1973) reported that shells of *A. fulica* (Giant African snail) may contain up to 250 ml of water (Mean: 56.5 ml) and found that the number of mosquito larvae per shell varies from 1 to 35 (Mean: 8.4). Due to the fact that *Megalobulimus* is a smaller snail, the average water volume retained was considerably lower (Mean: 3.5 ml); ranging from 0.5 to 10 ml, and the mean number of immature mosquitoes per snail shell was 1.4 time lower (Range: 1–27, mean: 11.6). In the same study, Trpis (1973) estimated that in order to attain the adult stage, each larva needs approximately 10 ml of water, depending on the availability of food, and other factors. Immatures of *Li. durhamii* collected in the Yungas developed in a considerably lower water volume, ranging from 0.02 ml to 2.2 ml per larvae.

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