

Mosquito community structure in phytotelmata from a South American temperate wetland

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ABSTRACT: Phytotelmata, or plant-held waters, are considered to be good model systems for the study of community ecology. The fauna of these natural container habitats, particularly the mosquitoes, have been extensively investigated in tropical regions, but there is little known about them in temperate South America. We assessed the structure of immature mosquito communities in leaf axils, tree holes, and bamboo stumps from a temperate wetland of Argentina. A total of 4,330 immature mosquitoes were collected among the 2,606 phytotelmata inspected. Leaf axils of eight plant species and tree holes were larval habitats for nine mosquito species belonging to the genus *Culex*, *Wyeomyia*, *Isostomyia*, and *Toxorhynchites*. The mosquito communities showed richness ranging from one to four species. Marked differences were detected in the plant specificity for the species collected. Some of them were exclusively found in one plant species (*Isostomyia paranensis* in *Scirpus giganteus*), whereas others were collected in up to five plant species but belonging to the same phytotelm class, the leaf axils. Those from tree holes are well-known dwellers of artificial containers and ground water habitats, such as *Culex pipiens*. Our results support the idea of low mosquito richness in phytotelmata from temperate regions in comparison with tropical areas, but the observed specificity patterns echo the findings of tropical forests. *Journal of Vector Ecology* 36 (2): 437-446. 2011.

Keyword Index: Culicidae, leaf axils, richness, South America, temperate wetlands, tree holes.

INTRODUCTION

Phytotelmata, or plant-held waters, are natural microcosms with inherent attributes of small size, amenable to manipulation, short generation times, contained structure, and hierarchical spatial arrangement that make them great model systems for the study of community ecology (Maguire 1971, Kitching 2001, Srivastava et al. 2004, Jocque et al. 2010). The great variety of known phytotelmata can be classified in five to seven major classes, depending on author criteria, with tree holes, bamboo internodes, leaf axils, flowers, and modified leaves the most common (Kitching 1971, 2001, Greeney 2001). This classification is mainly based on their position in the plant and on the nature of the liquid they contain. Different phytotelmata could be associated with arthropod communities with different attributes in terms of families or species composition, diversity, and/or abundance (Jabiol et al. 2009, Campos 2010).

Among 70 families from 11 orders of insects that have been reported from phytotelmata, mosquitoes (Diptera: Culicidae) are the most studied (Greeney 2001). This is probably due in part because mosquitoes are among their most prolific dwellers, but more importantly because of their role as disease vectors. Among 3,000 known mosquito species, more than 400 from 15 genera have been estimated to occur in phytotelmata (Fish 1983). There are many clear examples of phytotelm-breeding mosquitoes of medical concern, as *Aedes aegypti* (L.), *Anopheles bellator* Dyar and Knab, and *Haemagogus janthinomys* Dyar, vectors

of dengue, malaria, and sylvan yellow fever, respectively (WRBU 2011). Besides the interest in medically important species, many baseline studies have focused on the entire assemblage of mosquitoes using plant-held waters as larval habitats (Lounibos 1979, Machado-Allison et al. 1986, Derraik 2009). Baseline information on mosquito community structure is necessary to address questions dealing with ecological interactions involving phytotelm mosquitoes (Lounibos and Machado-Allison 1993, Sunahara and Mogi 2002, Yee and Juliano 2006).

In Argentina, earlier publications about phytotelm-breeding mosquitoes dealt with species records and original descriptions of immature stages (Martínez et al. 1959, Casal et al. 1966, Casal and García 1966, 1967a,b, García and Casal 1967, 1968a,b). Despite the great number of papers about mosquitoes of phytotelmata recently published, the information regarding these larval habitats in temperate regions of South America is scanty. During the last decade, only a few ecologic studies have focused on mosquitoes inhabiting water contained in plants of this vast region; *Isostomyia paranensis* (Brethes) in *Scirpus giganteus* (Marti et al. 2007) and *Culex* (*Phytotelmatomyia*) spp. Rossi and Harbach in *Eryngium* spp. (Campos and Lounibos 1999). The objective of the present study was to characterize the mosquito communities of a wide range of phytotelmata present in the main temperate wetland of Argentina. With this aim, the structures of the communities found in leaf axils, tree holes, and bamboo stumps were assessed in terms of percentage of occupied habitat, richness, species composition, abundance and density of immatures, and

diversity and evenness measures.

MATERIALS AND METHODS

Study area

The study was conducted in a section of the Lower Delta of the Paraná River that belongs to Buenos Aires Province, Argentina. This area is included in the districts of Campana (34°10'S, 58°57'W), San Fernando (34°26'S, 58°33'W), and Tigre (34°25'S, 58°34'W) and comprises around 1,850 km². Its hydrologic regime is mainly affected by moon and wind tides of the De la Plata River and by the seasonal regime of the Paraná and Uruguay rivers (Kandus et al. 2006). The climate is sub-humid temperate with a mean annual temperature of 16.7° C (min. 6° C, max. 30° C) and annual precipitation of 1,073 mm. This area is composed of pan-shaped islands characterized by a mosaic of forests, lowlands temporarily or permanently flooded, and domestic areas (small human settlements). Forest habitats are dominated by introduced species, *Populus* spp. (poplars) and *Salix* spp. (willows), and remnants of *Erythrina crista-galli* (seibo) are located in highlands. Wetland grasses such as *Scirpus giganteus* and *Schoenoplectus californicus* occupy central depressions (Kandus and Malvárez 2004). Regarding the knowledge of mosquito fauna, it is mainly restricted to collections from the 1960s (Prosen et al. 1960, García and Casal 1965) and a recent survey of human-attracted mosquitoes (Loetti et al. 2007).

Data collection and mosquito identification

Mosquito collections were carried out bi-monthly from November, 2008 to September, 2009 (six surveys) in ten islands. In each survey, between 45 and 64 transects of 50 m randomly selected were used. Six quadrats of 4x4 m were equidistantly placed in each transect and up to five phytotelmata, the ones closer to the center, were inspected. All the plants with leaf axils inspected were at ground level and only tree holes and bamboo placed lower than 2 m were included in the survey (Figure 1). An additional, not at random, survey out of transect was done at each site and survey in order to ensure covering the heterogeneity of environments and the different phytotelm habitats.

All the water contained in each plant was extracted using pipettes and/or plastic tubes for siphoning (Silver 2008). The water was returned to the plant at least twice in order to remove the organic mater and specimens from the bottom. Although pipetting could be considered an inefficient method for studies that focus on the entire community, it provides a good collection method for mosquitoes (Jocque et al. 2010). The water volume extracted from each plant was recorded. Larvae were fixed in ethanol 70%. Pupae were transported alive to the laboratory with a small amount of habitat water and reared until adult emergence.

Third and 4th instar larvae and adults were identified using dichotomical keys (Darsie 1985, Rossi et al. 2002) and specific descriptions (Lane and Ramalho 1960, Casal and García 1967a, b, Forattini 2002, Rossi and Harbach 2008, Campos and Zavortink 2010). Several specimens of



Figure 1. Pictures of different classes of phytotelmata inspected; (a) *Scirpus giganteus* and (b) *Eryngium* sp. are leaf axils, (c) bamboo and (d) tree hole.

Culex collected in *Eryngium* spp. could not be identified and were sent to Prof. Gustavo Rossi, taxonomic specialist on *Phytotelmatomyia*, a new subgenus of *Culex* recently proposed (Rossi and Harbach 2008). These specimens belong to a presumed new species and are mentioned as *Culex* sp. 1 in the present study.

Data analysis

We considered *a priori* the mosquito assemblage of each plant species as different mosquito communities, with the exception of the trees *Platanus hispanica*, *Salix* sp., and *Populus* sp. that were grouped as tree holes. The structure of each community was characterized by the following attributes: percentage of occupied habitat, number of species (richness), species composition, abundance and density of immatures, and diversity and evenness measures. The percentage of occupied habitat was estimated as the percentage of water-held plants with mosquitoes, following the reasoning used for dengue vectors in man-made container habitats (CI = container index) (Silver 2008). For each plant species, an overall CI and mosquito species-specific CIs were estimated. Diversity estimations were made using Simpson's Index due to the low number of species detected in each community under study (Magurran 2004).

The overall CIs were compared with a χ^2 test for multiple independent proportions (Fleiss et al. 2003).

Then, multiple pairwise comparisons by Tukey procedure were done to identify groups of similar values (Zar 1999). When a mosquito species was found in more than one community, its CIs were also compared. The abundance of immatures (total and for each mosquito species) was characterized in each community by the median number of individuals per infested plant. These values were compared by Kruskal-Wallis or Mann-Whitney tests depending on the number of plant species involved. If the former showed a significant difference, Dunn's test was applied for multiple pairwise comparisons (Daniel 1990, Zar 1999). Densities of immatures in infested plants (expressed as median numbers per 10 ml of water) were also compared by the Kruskal-Wallis and Dunn's tests.

Finally, to assess whether two mosquito species breeding in the same plant species co-occurred more (or less) frequently than would be expected on a random basis, the C_8 coefficient of interspecific association (Hurlbert 1969) was calculated when appropriate. This coefficient based on presence-absence data varies between 1 and -1, meaning maximum positive and negative associations, respectively.

RESULTS

Out of 4,602 plants inspected, 2,606 had water collected from them. Fourteen plant species were identified as follows: *Scirpus giganteus* (1,714 inspected, 75.9% with water), *Iris pseudacorus* (300, 100%), *Eryngium pandanifolium* (459, 54%), *E. serra* (584, 42.1%), *E. eburneum* (95, 78.9%), *Typha latifolia* (300, 48%), *Sagittaria montevidensis* (163, 44.8%), tree hole (100, 69%), *Zizaniopsis bonariensis* (55, 85.5%), *Alocasia* sp. (41, 97.6%), *Musa* sp. (26, 73.1%), *Philodendron* sp. (14, 100%), *Zantedeschia aethiopica* (5, 100%), and the golden bamboo *Phyllostachys aurea* (746, 3.3%). Mosquitoes were never collected in *I. pseudacorus*, *Musa* sp., *Philodendron* sp., *Z. aethiopica*, and bamboo. Among the nine plant species acting as mosquito habitats, 28% (627/2,243) of the water-held plants were found harboring mosquito immatures.

A total of 4,330 immatures of nine mosquito species were identified. The mosquito species composition and other attributes of the communities of the nine plant species are summarized in Table 1. A relative low richness was observed in all the communities; four plant species harbored only one mosquito species and the maximum was four. The maximum diversity and evenness measures were 2.2 and 0.7, respectively. Some mosquito species were found exclusively in one plant species (*Is. paranensis*), whereas others occurred in up to five plant species (e.g., *Culex hepperi* Casal and García).

The percentage of occupied habitat differed significantly ($\chi^2_{(8)} = 246.6$, $p < 0.001$) among plant species (Table 1). CIs for *E. serra* and *E. eburneum* reached around 60% and were significantly higher than those of other plants, at $p < 0.001$. Regarding the comparison of CIs for each mosquito species among different plant species, those of *Cx. castroi* Casal and García and *Cx. hepperi* also differed among the plants infested by each one ($\chi^2_{(3)} = 75.59$, $p < 0.001$; $X^2_{(4)} =$

83.17, $p < 0.001$, respectively). *Cx. castroi* occurred more frequently in *E. eburneum* than in *E. serra* ($p < 0.05$) and in both plants more frequently than in *E. pandanifolium* and *S. montevidensis* ($p < 0.001$). *Cx. hepperi* recorded similar CIs in *E. eburneum* and *E. serra* but higher than in the other three plants ($p < 0.05$). CIs of *Wyeomyia leucostigma* Lutz were not significantly different between the two plant species in which they were collected. Sample sizes of *Cx. renatoi* Lane and Ramalho and *Culex* sp. 1 were too low for this analysis.

Regarding the median number of mosquito immatures per infested plant, the overall value was 2 (quartile 1 = 1, $q_3 = 6$). This abundance differed significantly among plant species ($H_{(8,627)} = 286.4$; $p < 0.001$), ranging between 1 and 9 (Table 2). Multiple comparisons by Dunn's test showed that abundance in *S. giganteus* was significantly lower than in the others at $p < 0.05$ (except with *S. montevidensis*), and in *E. serra* and *E. eburneum* was significantly higher than in *T. latifolia* and *S. montevidensis* ($p < 0.05$). Specific abundance of *Cx. hepperi* also differed among the five plant species infested ($H_{(4,253)} = 12$, $p < 0.05$); significant differences were observed between *S. montevidensis* and both *E. serra* and *E. eburneum*. On the contrary, the abundances in different plant species were similar for *Cx. castroi* ($H_{(3,95)} = 4.01$, $p > 0.05$) and *Wy. leucostigma* ($U = 34.5$, $p > 0.05$).

Regarding mosquito species sharing the same plant species, the medians of immatures were similar in *E. pandanifolium* ($H_{(3,79)} = 5.32$, $p > 0.05$) and in *S. montevidensis* ($U = 4.5$, $p > 0.05$). On the contrary, some differences were detected in *E. serra* ($H_{(2,198)} = 11.37$, $p < 0.01$) and *E. eburneum* ($H_{(3,72)} = 8.69$, $p < 0.05$), in which *Cx. renatoi* abundances were significantly lower than those of *Cx. hepperi* and *Cx. castroi* ($p < 0.05$). In tree holes, an overall significant difference was also found ($H_{(2,16)} = 6.54$, $p < 0.05$); abundance was lower for *Toxorhynchites theobaldi* Dyar and Knab than for *Cx. eduardoi* Casal and García and *Cx. pipiens* L. ($p < 0.05$). Notwithstanding those average values of immatures per infested plant, the relative contribution of immatures within each habitat showed that *Cx. hepperi* was the most abundant in five plant species, and *Cx. eduardoi* in tree holes (Figure 2).

Water volume extracted from leaf axils ranged from 2 to 205 ml (median = 5, $q_1 = 3$, $q_3 = 10$) and in tree holes from 2 to 2000 ml (median = 200, $q_1 = 60$, $q_3 = 300$) (Table 1). Densities of immatures differed significantly among plant species ($H_{(8,607)} = 133.16$; $p < 0.001$) (Figure 3). Multiple comparisons by Dunn's test resulted as follows: tree hole vs each of the others ($p < 0.05$); *S. giganteus* vs each *Eryngium* sp. ($p < 0.01$) and vs *T. latifolia* ($p < 0.05$); *S. montevidensis* vs *E. serra* and *E. eburneum* ($p < 0.05$); and *E. pandanifolium* vs *E. serra* ($p < 0.05$).

The two mosquito species most frequently recorded, *Cx. castroi* and *Cx. hepperi*, shared breeding habitats in the three species of *Eryngium*. The C_8 coefficients of interspecific association indicated that there is a strong positive association between these species in *E. pandanifolium* ($C_8 = 0.86$; $p < 0.001$) and a relative weak association in *E. serra* ($C_8 = 0.36$; $p < 0.05$).

Table 1. Median water volume of phytotelmata and attributes of the mosquito communities identified in the Lower Delta of the Paraná River, Argentina. Container index (CI) means percentage of water-filled plants with mosquitoes; sample size within brackets, and q1-q3 means quartiles 1 and 3, respectively.

	<i>Eryngium eburneum</i> (75)	<i>Eryngium pandanifolium</i> (248)	<i>Eryngium serra</i> (246)	<i>Sagittaria montevidensis</i> (73)	<i>Alocasia</i> sp. (40)	<i>Scirpus giganteus</i> (1301)	<i>Zizaniopsis bonariensis</i> (47)	<i>Typha latifolia</i> (144)	Tree hole (69)
Median water volume (ml)	15 q1:10-q3:30	5 3-20	10 5-25	5 3-10	10 5-20	5 3-10	10 5-20	5 3-10	200 60-300
Species richness	4	4	3	2	1	1	1	1	3
Simpson's diversity (1/D)	2.2	1.3	1.5	1.4	-	-	-	-	1.6
Evenness ($E_{1/D}$)	0.6	0.3	0.5	0.7	-	-	-	-	0.5
Overall CI (%)	60.0	27.4	63.4	9.6	20	23.7	17.0	9.7	18.8
<i>Culex castroi</i>	38.7	4.0	22.4	2.7					
<i>Culex hepperi</i>	45.3	26.2	56.5	9.6	20				
<i>Culex renatoi</i>	5.3	0.8	2.0						
<i>Culex</i> sp. 1	6.7	0.8							
<i>Wyeomyia leucostigma</i>							17.0	9.7	
<i>Culex eduardoi</i>									11.6
<i>Culex pipiens</i>									2.9
<i>Toxorhynchites theobaldi</i>									8.7
<i>Isostomyia paranensis</i>						23.7			

Table 2. Abundance of immatures per infested plant [median (quartile 1- quartile 3)] for each mosquito species in phytotelmata found in the Lower Delta of the Paraná River, Argentina.

	<i>Eryngium eburneum</i>	<i>Eryngium pandanifolium</i>	<i>Eryngium serra</i>	<i>Sagittaria montevidensis</i>	<i>Alocasia</i> sp.	<i>Scirpus giganteus</i>	<i>Zizaniopsis bonariensis</i>	<i>Typha latifolia</i>	Tree hole
<i>Culex castroi</i>	6 (4-11)	8 (2.25-12)	5 (3-10)	1.5 (1.25-1.75)					
<i>Culex hepperi</i>	6 (3-10.75)	4 (2-7)	6 (3-12.5)	2 (1.5-2.5)	3 (1.75-7)				
<i>Culex renatoi</i>	1 (1-1)	1 (1-1)	1 (1-1)						
<i>Culex</i> sp. 1	3 (2-10)	4 (3-5)							
<i>Wyeomyia leucostigma</i>						5 (2-11.75)	2 (2-3)		
<i>Culex eduardoi</i>								33.5 (2.5-41)	
<i>Culex pipiens</i>								27.5 (15.75-39.25)	
<i>Toxorhynchites theobaldi</i>								1 (1-1)	
<i>Isostomyia paranensis</i>						1 (1-2)			
Overall	9 (5-17)	4.5 (2-7)	7 (3-15.25)	2 (1.5-3.5)	3 (1.75-7)	1 (1-2)	5 (2-11.75)	2 (2-3)	2 (1-32)

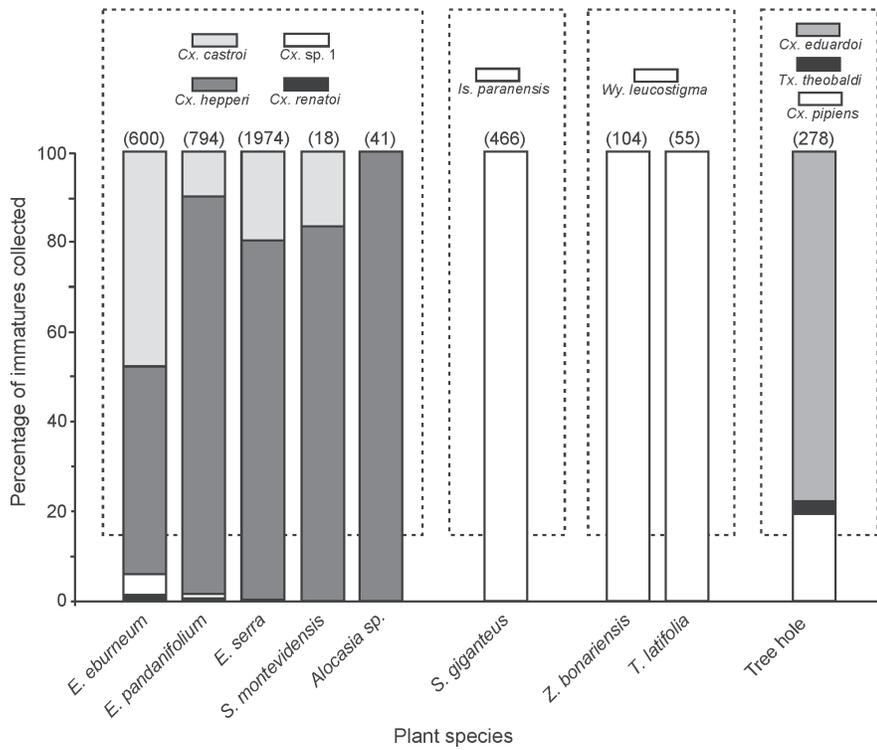


Figure 2. Relative abundances of mosquito species inhabiting phytotelmata in the Lower Delta of the Paraná River (Argentina); total number of immatures collected in each plant species within brackets.

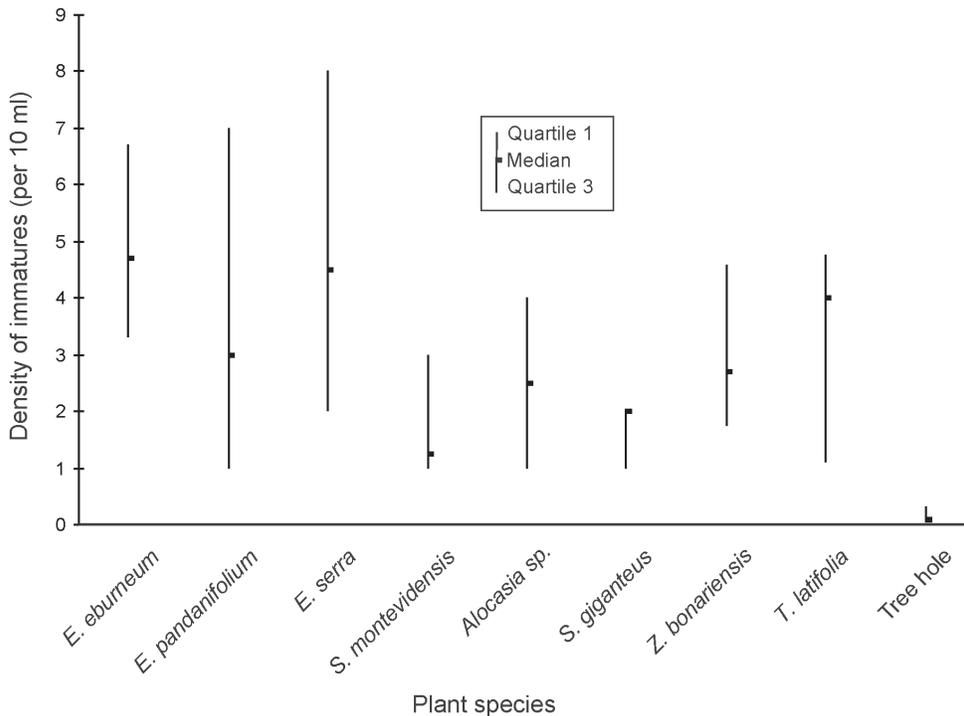


Figure 3. Density of mosquito immatures in phytotelmata found in the Lower Delta of the Paraná River, Argentina.

DISCUSSION

During this survey in the main temperate wetland of Argentina, only water-filled leaf axils and tree holes were found to be infested with mosquitoes. Although bamboo stumps are well-known mosquito habitats in many regions (Louton et al. 1996, Sota and Mogi 1996), the bamboo species found in the lower Delta seems not to be capable of retaining enough water; only 3.3% were found with water. Other phytotelm classes described in tropical regions, such as modified leaves and flowers, were not detected in the study area, and to our knowledge, never were reported in temperate South America.

Among 74 mosquito species known to be distributed in Buenos Aires Province (Rossi et al. 2006), we found eight of them breeding in phytotelmata, plus one presumably new *Culex* species. Matching with our findings, *Cx. castroi*, *Cx. hepperi*, *Cx. renatoi*, *Is. paranensis*, and *Wy. leucostigma* have been reported exclusively from water retained in leaf axils (Lourenço de Oliveira et al. 1986, Campos and Lounibos 1999, Marti et al. 2007). On the contrary, the tree hole dwellers *Tx. theobaldi*, *Cx. eduardoi*, and *Cx. pipiens* were previously collected in discarded tires, flower vases, and/or temporary ponds from temperate Argentina (Fontanarrosa et al. 2004, 2009, Vezzani and Albicocco 2009, Rubio and Vezzani 2011). The fact that mosquito species inhabiting natural tree holes also colonize artificial containers was previously noted in investigations from different regions (Sota et al. 1994, Derraik 2005). In summary, among phytotelm dwellers reported here, some mosquitoes showed high specificity for a plant species (*Is. paranensis* in *S. giganteus*), others occupied several plant species but from the same phytotelm class (e.g., *Cx. hepperi* in leaf axils of *Eryngium*, *Sagittaria* and *Alocasia*), and others could be considered as generalists in their larval habitats (*Cx. pipiens* in tree holes, ponds, and artificial containers). Similar general conclusions were previously outlined for tropical forests in Venezuela (Machado-Allison et al. 1986).

Regarding the number of species developing in phytotelmata, it is widely assumed that the richest fauna is reached by far in wet tropics (Maguire 1971, Kitching 2001). Specifically, mosquito richness in tropical phytotelmata reaches maximum values equal or higher than 10 for some plant species (Machado-Allison et al. 1986, Yanoviak 1999, Delgado and Machado-Allison 2006). However, Sota et al. (1994) reported similar mosquito richness for tree holes on a temperate island of Japan, suggesting that the richness does not necessarily correlate with latitudinal gradients. Many other factors, such as microhabitat complexity and presence of predators, could be affecting the number of species in some types of phytotelmata. Our findings (maximum of four species) and previous studies from southern South America (Campos and Lounibos 1999, Marti et al. 2007, Campos 2010) support the idea of low mosquito richness in phytotelmata from temperate climates.

The percentages of occupied habitat and immature abundances differed among the studied communities. Among *Eryngium* species, *E. pandanifolium* grows mainly

on river shores and was frequently observed filled with mud (Albicocco, personal observation). Thus, the low percentages of infested plants of *E. pandanifolium* (in comparison with *E. serra* and *E. eburneum*) may be due to an unsuitable larval habitat because axils are regularly washed by moon and wind tides of the De la Plata River. An analogous larval mortality by habitat overflow due to the rainfall was described for different mosquito species developing in natural and artificial containers (Washburn and Anderson 1993, Koenraadt and Harrington 2008).

The specificity of *Is. paranensis* for *S. giganteus*, a plant species forming extensive marshes in the studied wetland, agreed with the results of Marti et al. (2007). This poorly known sabethine species was one of the less abundant per infested plant in our survey (median of one immature), partially supporting the idea that this species could be cannibal at 3rd and 4th instars (Campos and Zavortink 2010). Similarly to the observations of Marti et al. (2007), we frequently noted cannibalism in the collection tubes before preservation in alcohol. The other mosquito species with similar abundance per plant (one individual) belongs to the genus *Toxorhynchites*, a recognized group of predators and cannibals (Collins and Blackwell 2000).

The strong positive association between *Cx. castroi* and *Cx. hepperi* in *E. pandanifolium* matches with a previous study in temperate Argentina (Campos and Lounibos 1999). This relationship could be indicating that the presence of larvae or egg rafts of one species promotes the oviposition of the other, as was suggested through laboratory assays involving other mosquito species (Ríos et al. 1978). However, marked competitive interactions are well known for container-breeding mosquitoes due to the limited resource availability typical of these small habitats (Washburn 1995, Juliano 1998). Therefore, the selection of plants previously occupied by immatures of other mosquito species would seem hardly probable in natural containers with a very low water volume (median of 5 ml in our study). Another reasonable explanation would be that some plants have more suitable conditions for the oviposition of both species (a combination of pH, shade, and detritus), thus enhancing their coexistence.

Regarding the importance of these phytotelm-mosquitoes as vectors of diseases, *Cx. pipiens* has been implicated in the transmission of the dog heartworm, *Dirofilaria immitis*, and the St. Louis encephalitis virus in temperate Argentina (Diaz et al. 2006, Vezzani et al. 2011). Although this is the unique demonstrated vector species among the studied communities, the role of *Wyeomyia*, *Isostomyia* and other *Culex* spp. in the transmission of other parasites such as West Nile virus is truly unknown. Finally, it is noteworthy the absence of the dengue vector, *Ae. aegypti*, a very common species in urban temperate Argentina (Vezzani et al. 2011) that could use phytotelmata as larval habitat (WRBU 2011).

In summary, our research characterized the immature mosquito communities of phytotelmata in a temperate wetland where there was little previous information about this group of insects. This information could be used as a

basis for the assessment of the biotic and abiotic factors driving the community structures reported here.

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