Mosquitoes in Bromeliads at Ground Level of the Brazilian Atlantic Forest: the Relationship Between Mosquito Fauna, Water Volume, and Plant Type

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ABSTRACT Water accumulating in the axils of bromeliads provides habitat for numerous invertebrates, frequently among them, immature mosquitoes. To evaluate mosquito richness in bromeliads and the relationship between mosquito presence and biotic and abiotic variables, we performed a study in the Parque Nacional do Itatiaia, Rio de Janeiro, Brazil. Mosquitoes of genus *Culex* were the most abundant and varied in species richness, among which nine belonged to subgenus *Microculex*, *Culex* (*Microculex*) *neglectus* Lutz and *Culex ocellatus* Theobald being the most frequent species. Sabethines of genera *Wyeomyia* and *Runchomyia* were found in low numbers. *Wyeomyia* (*Spilonympha*) *airosai* Lane and Cerqueira and *Wyeomyia* (*Spilonympha*) *finlayi* Lane and Cerqueira tend to proliferate in bromeliads of the genus *Bilbergia* which hold less than 50 ml of water and grow either alone or with *Runchomyia frontosa* (Theobald). The larger the volume of water, the greater the chance of finding *Culex*, *Anopheles* as well as *Wyeomyia* (*Phoniomyia*) species, which seems to be the more generalist as it is present in different bromeliad types with a large range of plant water holding capacities.

KEY WORDS bromeliad, rain forest, phytotelmata, *Wyeomyia*

The family Bromeliaceae is native to the Neotropics, where its species are distributed from the southern United States to Argentina (Frank 1983). Bromeliads grow in all Brazilian biomes, especially in the Atlantic Forest. Many bromeliad species are able to stock water in the leaf base. There are many organisms that thrive in the water contained in the bromeliad leaf axils, and immature forms of insects from the Culicidae family may constitute the dominant invertebrate community (Richardson 1999). Indeed, around 200 species of Culicidae have been recorded in bromeliads (Frank and Lounibos 2008), and among which a considerable number of Neotropical species are bromeliad specialists (e.g., Shannon 1931, Pittendrigh 1950). Many factors can affect the presence of mosquitoes in these plants such as plant location, exposure to sunlight, environment where the plant is fixed, water volume held by the axils of leaves and the amount of organic debris accumulated in this water tanks (Frank et al. 1976, Frank and O' Meara 1985). These bionomic parameters seem to influence competition among the associated fauna, leading to different community compositions in bromeliads.

Several studies have assessed bionomic aspects of bromeliad-inhabiting invertebrates, although identifications have usually been limited to Family and Order levels (Cotgreave et al. 1993, Richardson 1999, Armbruster et al. 2002). There have been significant contributions to the knowledge on the bionomics and ecology of bromeliad-inhabiting mosquitoes in North America, specifically the species of genus *Wyeomyia* from Florida. Accordingly, the relationship of bromeliad size and sun exposure with the number of immature as well as the larval feeding strategy, oviposition behavior, and biology of these mosquitoes (Frank and Curtis 1977a,b, 1981; Frank 1983, Frank and O' Meara, 1985, Frank et al. 1985). In contrast, there are few data on the bromeliads and their mosquito-inhabiting species in the Neotropical region where species richness and diversity is much higher. During our fieldwork concerned with *Wyeomyia* immature form collection in Brazil for taxonomic studies, we suspected that large bromeliads were usually negative for this genus, except for the species of subgenus *Phoniomyia*. Thus, we decided to design and conduct a field study to test this hypothesis in the Atlantic rainforest. Indeed, in spite of the low number of *Wyeomyia* collected during the fieldwork reported herein, we could confirm this hypothesis for the first time. As other mosquito genus and species were collected and identified, we extended our analysis to include these species, exploring a few biological and ecological parameters. In the present study, we describe the results of a longitudinal survey of natural bromeliads located at the ground level, in a preserved Brazilian Atlantic coast rain forest to identify the colonizing mosquito species and investigate their correlation with bromeliad water volume and type of bromeliad.

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Materials and Methods

The field study was conducted from January to December 2007 in the Parque Nacional do Itatiaia (PARNA – Itatiaia), situated in southeastern Brazil, Rio de Janeiro State. The ecotype is the Atlantic Rain Forest and the climate is mesothermal (Cwa, according to Köppen's classification), where the hottest months are rainy and the coldest are dryer, the annual average temperature and precipitation being 19°C and 1.356 mm/ year. The study site, approximately 1,100 m altitude along a non-paved narrow road in the forest closed to visitors, consisted of a forest patch of about 200m^2, subdivided into two subareas, named subarea A and B (S 22°25′55.5″ W 44°37′16.0″), where subarea A, was on a branch of the main road and subarea B a section of the main road itself (Fig. 1). The vegetation coverage of subarea A was less dense than subarea B, thus the bromeliads selected at the former site were generally more exposed to sunlight.

Bromeliads. Ninety bromeliads were randomly selected, 60 from subarea A and 30 from subarea B, attempting to include a variety of bromeliad types and sizes. The lack of crucial diagnostic structures in some bromeliads throughout the sampling period precluded their taxonomic identification. Each bromeliad was labelled with a plastic numbered tag (1–90). Mosquito communities in bromeliads may depend upon the plant support (epiphytic, terrestrial or lithophytic) as well as the height of its location in the forest (ground level or canopy) (Frank and Lounibos 2008). In the present study, however, samplings were limited to plants located close to ground level. Most of the sampled bromeliads were terrestrial or lithophytic growing on small rocks, and epiphytic plants were at most 1 m above ground. The total sample was randomly divided into three groups of 30 bromeliads which were investigated through specimen collection at three month intervals in a rotation (Mocellim et al. 2009). This procedure resulted in four collections per bromeliad during the survey. At each inspection, the total water volume held by each bromeliad was aspirated by a manual suction pump, measured (ml) with measuring cylinder, poured into a tray for the search and isolation of predators, such as immature forms of Odonata, Chaoboridae and mosquitoes of genera *Toxorhynchites* and *Runchomyia*, and subsequently stored in individual plastic bags with

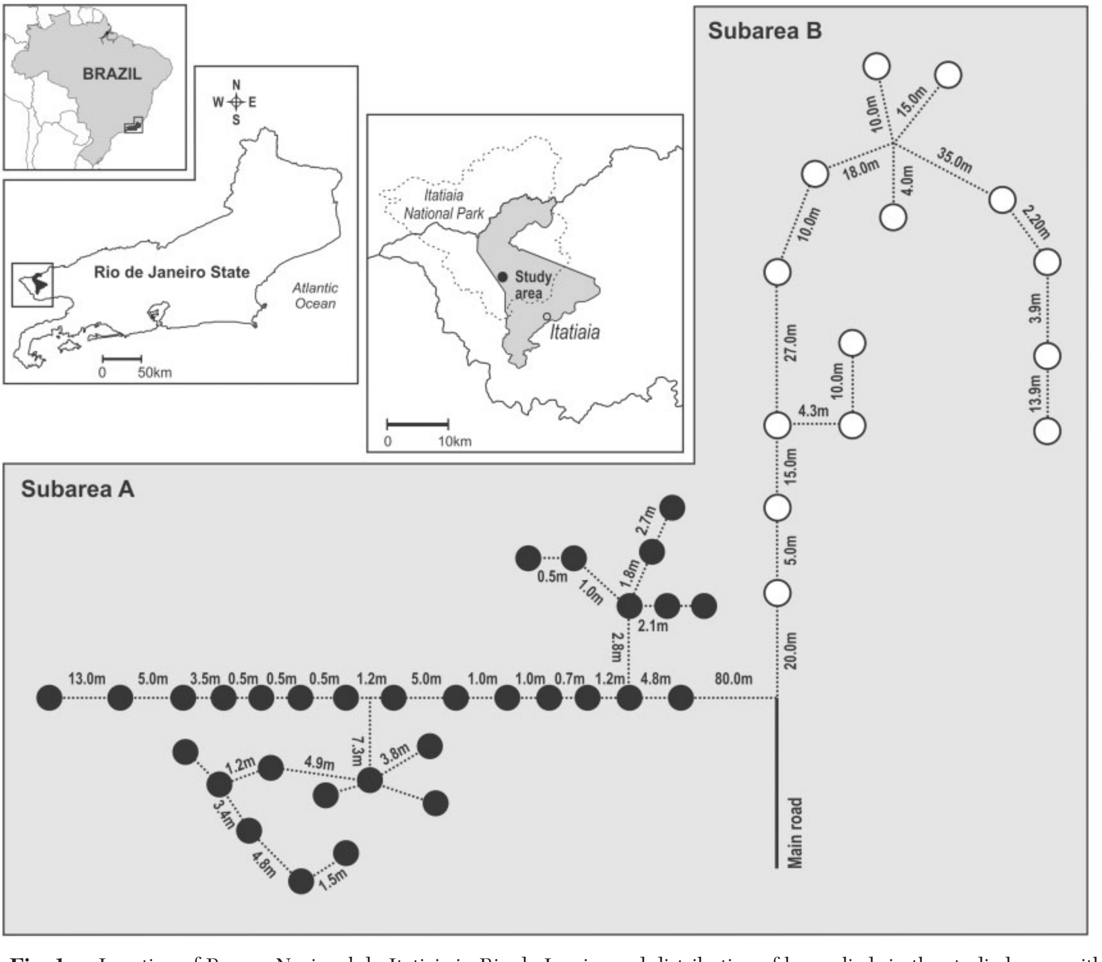

Fig. 1. Location of Parque Nacional do Itatiaia in Rio de Janeiro and distribution of bromeliads in the studied area, with distance between them. The black circle represents subarea A and the white subarea B.

the bromeliad identification number and collection date.

Successively, all collected materials were kept in a cool box and carried to the laboratory. In the laboratory, a portion of the fourth-instar larvae and pupae were reared to adults, larval and pupal skins preserved individually in 80% ethyl alcohol. The majority of the fourth-instar larvae were killed and identified in this stage. Identifications were based on morphological characteristics of immature stages and emerged adults, including male genitalia (Lane 1953; Forattini 1965, 2002; Lane and Whitman 1951; Corrêa and Ramalho 1956; Wilkerson and Peyton 1992; Cotrim and Galati 1977; Motta and Lourenço-de-Oliveira 2005). Species identification were confirmed by comparison with specimens deposited in two entomological collections [Coleção de Culicidae (CCULI), Instituto Oswaldo Cruz, FIOCRUZ, and Coleção Entomológica de Referência da Faculdade de Saúde Pública, Universidade de São Paulo (FSP-USP) Brazil]. The adults and the larval and pupal skins were deposited at CCULI.

Data Analyses. To analyze the distribution of mosquito taxon between bromeliads, we initially calculated some descriptive statistics as the proportion of bromeliads with each taxon, and the minimum, median and maximum number of larvae per positive bromeliad. To test for the aggregation of mosquito taxa within bromeliads, we first computed the proportion of positive bromeliads (p). Then, we computed the expected probability of observing the same bromeliad positive 0 to 4 times, according to a binomial distribution with probability p. This expectation was compared with the observed frequency using a chi-square test with critical P-value = 0.01. Deviation from the expectation was interpreted as evidence of aggregation.

Regression analyses were carried out to assess the effect of trail (A and B), distance from each trail entrance (in meters), bromeliad's water volume and month on mosquito abundance. A Poisson regression model was chosen because the response variable is a count and there was weak evidence of overdispersion. The full model included fixed terms (trail, distance within trail, log [water volume], and month), and a random effect (bromeliad) to take into account the longitudinal structure of the data. Assessment of effect was done by likelihood ratio tests, that is, by comparing models with fixed effects to a reference model with no fixed effect. These models were fitted using the glmer function in library (lmer4) from R 3.1.0 (R Core Team 2014, Vienna, Austria). Model comparison used the ANOVA function. Differences in taxa abundance between were further compared with Kruskal–Wallis.

Because water volume was the strongest predictor of mosquito abundance, further modeling was carried out to capture the relationship between water volume and the probability of finding the main mosquito groups. An initial exploratory analysis suggested a nonlinear relationship between volume and the proportion of bromeliads with a specific taxon, and a generalized additive regression model with a logistic distribution was chosen. The response variable was the presence or absence of individuals of the mosquito taxa (*Anopheles*, *Culex*, *Spilonympha*, and *Phoniomyia*) while log (water volume) was the explanatory variable. Using the generalized additive regression model, we obtain a smooth nonparametric curve for the relationship between the logit (presence) and the explanatory variable. For more details, consult Zuur et al. (2007). The function GAM (library mgcv) in the software R 3.1.0 was used. At last, we use multivartiate correspondence analysis to describe the structure of the bromeliad mosquito communities. Only the most abundant mosquito species or group ($N = 13$) were considered. Presence–absence of each species per bromeliad is used as input for the model. The result is presented in the form of a dendrogram. The function hclustvar (library ClustOfVar) in the software R 3.1.0 was used.

Results and Discussion

Bromeliad Mosquito Community Composition. In total, 1,932 immature Culicidae from 16 species were collected in the 90 inspected bromeliads (Table 1). Only 3.70% of the collected mosquitoes were from the tribe Sabethini ($n = 71$), and *Anopheles* (*Kerteszia*) spp. represented 4.76% of the total. On the other hand, mosquitoes of genus *Culex* (91.49%) were the most abundant and exhibited the highest species richness group, with 1,768 specimens belonging to 10 species (Table 1). Besides Culicidae, immature forms of other Diptera and Odonata were also observed, but they were not included in the study dataset.

Five sabethine species were collected: *Wyeomyia* (*Phoniomyia*) *theobaldi* Lane and Cerqueira, *Wyeomyia* (*Spilonympha*) *airosai* Lane and Cerqueira, *Wyeomyia* (*Spilonympha*) *finlayi* Lane and Cerqueira as well as *Wyeomyia* (*Phoniomyia*) *pilicauda* (Root) and *Runchomyia frontosa* (Theobald). Among the *Culex*, *Culex ocellatus* Theobald was the most frequent, followed by *Culex* (*Microculex*) *neglectus* Lutz (Table 1). The number of sabethine species collected in our study was lower than expected. In fact, the subgenus *Phoniomyia*, which includes 22 nominal species and develops almost exclusively in bromeliads, has been reported frequently in other mosquito bromeliad surveys carried out at other sites in the Atlantic Forest (Correa and Ramalho 1956, Müller and Marcondes 2006, Marques and Forattini 2008, Mocellin et al. 2009). However, Marques et al. (2012) and Müller and Marcondes (2007) were also surprised by the low number of *Wyeomyia* (*Phoniomyia*) spp. in sampled *Nidularium* sp., or even in *Vriesea* sp. bromeliads, in southeastern Brazilian forests, where biting rates of these mosquitoes were high.

The species richness and abundance of *Wyeomyia* (*Spilonympha*) spp. was also low in our survey, compared with the results of both Marques and Forattini (2008) and Palacio et al. (2010). Indeed, except for *Wyeomyia* (*Spilonympha*) *bourrouli* Lutz and *Wyeomyia* (*Spilonympha*) *forcipenis* Lourenço-de-Oliveira and Silva, other *Spilonympha* species (*Wy. airosai*, *Wy. finlayi* and *Wyeomyia howardi* Lane and Cerqueira) harbored in bromeliads are usually found in low

Table 1. Number and percentage of Culicidae species collected in 90 sampled bromeliads according to two subareas at Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007

Species	Subarea A (*N*)	Subarea B (*N*)	Total	Percentage
Anopheles (*Kerteszia*) *cruzii* Dyar and Knab	54	26	80	4.14
Anopheles (*Kerteszia*) sp.	12	0	12	0.62
Culex ocellatus Theobald	86	518	604	31.26
Culex (*Microculex*) *neglectus* Lutz	532	0	532	27.54
Culex (*Microculex*) sp.	122	38	160	8.28
Culex (*Microculex*) *reducens* Lane and Whitman	161	0	161	8.33
Culex (*Microculex*) *inimitabilis* Dyar and Knab	95	0	95	4.91
Culex (*Microculex*) Serie *imitator*	79	12	91	4.71
Culex (*Microculex*) *dubitans* Lane and Whitman	31	25	56	2.88
Culex (*Microculex*) *consolator*Dyar and Knab	47	0	47	2.43
Culex (*Microculex*) *worontzowi* Pessoa and Galvão	7	0	7	0.36
Culex (*Microculex*) Serie *pleuristriatus*	6	0	6	0.32
Culex (*Microculex*) *aphylactus* Root	5	0	5	0.26
Culex (*Microculex*) *davisi* Kumm	3	0	3	0.16
Culex (*Microculex*) *intermedius* Lane and Whitman	1	0	1	0.05
Wyeomyia (*Spilonympha*) *airosai* Lane and Cerqueira	15	3	18	0.94
Wyeomyia (*Spilonympha*) *finlayi* Lane and Cerqueira	5	0	5	0.26
Wyeomyia (*Phoniomyia*) *theobaldi* (Lane and Cerqueira)	28	0	28	1.45
Wyeomyia (*Phoniomyia*) *pilicauda* Root	3	0	3	0.16
Wyeomyia (*Phoniomyia*) spp.	13	1	14	0.73
Runchomyia (*Runchomyia*) *frontosa* (Theobald)	3	0	3	0.16
Toxorhynchites sp.	1	0	1	0.05
Total	1,309	623	1,932	100%

Table 2. Percentage of positive bromeliads, median, minimum, and maximum number of larvae per bromeliad sampled at Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007

Species	% Positive bromeliads	Larvae/positive bromeliad	
		Median	Range
Anopheles (*Kerteszia*) *cruzii* Dyar and Knab	15.8*	1	1–4
Anopheles (*Kerteszia*) sp.	2.5*	1	1–2
Culex ocellatus Theobald	19.2*	5	1–75
Culex (*Microculex*) *neglectus* Lutz	18.3*	3.5	1–54
Culex (*Microculex*) sp.	16.1*	2	1–15
Culex (*Microculex*) *reducens* Lane and Whitman	3.0*	10	1–66
Culex (*Microculex*) *inimitabilis* Dyar and Knab	6.9*	3	1–12
Culex (*Microculex*) Serie *imitator*	3.6*	7	1–15
Culex (*Microculex*) *dubitans* Lane and Whitman	2.7*	4	1–15
Culex (*Microculex*) *consolator*Dyar and Knab	3.6*	2	1–14
Culex (*Microculex*) *worontzowi* Pessoa and Galvão	0.8*	2	2–3
Culex (*Microculex*) Serie *pleuristriatus*	0.3	6	6–6
Culex (*Microculex*) *aphylactus* Root	0.5*	2.5	2–3
Culex (*Microculex*) *davisi* Kumm	0.5*	1.5	1–2
Culex (*Microculex*) *intermedius* Lane and Whitman	0.3	1	1–1
Wyeomyia (*Spilonympha*) *airosai* Lane and Cerqueira	3.0*	1	1–4
Wyeomyia (*Spilonympha*) *finlayi* Lane and Cerqueira	1.1	1	1–2
Wyeomyia (*Phoniomyia*) *theobaldi* (Lane and Cerqueira)	4.7	1	1–3
Wyeomyia (*Phoniomyia*) *pilicauda* Root	0.8	1	1–1
Runchomyia (*Runchomyia*) *frontosa* (Theobald)	0.8	1	1–1
Toxorhynchites sp.	0.3	1	1–1
Total	48.9	5	1–13

Asterisks indicate that the mosquito type tend to aggregate within individual bromeliads (X2 test: * $P < 0.01$).

numbers per plant. Actually, only few *Spilonympha* larvae are normally encountered in each bromeliad leaf axil (Motta and Lourenço-de-Oliveira 2005, Marques and Forattini 2008, Mocellin et al. 2009).

The high abundance and species richness of *Culex* (*Microculex*) spp. found in the present study is in accordance with those by Lane and Whitman (1951), Marques and Forattini (2008), and Mocellin et al. (2009) at other sites in the Atlantic Forest. Surely, *Culex* (*Microculex*) spp. and other related *Culex* species are the most abundant Culicidae group in Brazilian coastal bromeliads (Müller and Marcondes 2006, 2007). For instance, *Cx. ocellatus* has been one of most abundant species in bromeliads from sites located both in the lowland areas (Marques and Forattini 2008, Mocellin et al. 2009) and mountains covered by the Atlantic Forest, as shown in our survey.

All species tended to aggregate within the same bromeliads. That is, the probability of finding the same species in the same bromeliads at subsequent visits was

Table 3. Factors associated with the abundance of the main mosquito species and mosquito groups in the bromeliads from two trails in the Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007

Species	Trail distance	Trail	Water volume	Months
Anopheles (*Kerteszia*) *cruzii* Dyar and Knab			**	**
Anopheles (*Kerteszia*) sp.	**		**	
Culex ocellatus Theobald	**	**	**	**
Culex (*Microculex*) *neglectus* Lutz		**	**	
Culex (*Microculex*) sp.			**	
Culex (*Microculex*) *reducens* Lane and Whitman			**	**
Culex (*Microculex*) *inimitabilis* Dyar and Knab		**	**	
Culex (*Microculex*) Serie *imitator*			**	**
Culex (*Microculex*) *dubitans* Lane and Whitman			**	
Culex (*Microculex*) *consolator* Dyar and Knab		**	**	
Wyeomyia (*Spilonympha*) *airosai* Lane and Cerqueira				**
Wyeomyia (*Phoniomyia*) *theobaldi* (Lane and Cerqueira)		**	**	
Mosquito groups				
Genus *Anopheles*			**	**
Genus *Culex*			**	**
Subgenus *Phoniomyia*			**	
Subgenus *Spylonympha*				**

Trail distance refers to the distance from the entrance; water volume refers to the bromeliad water content, and months refers to the four samplings carried out in each bromeliad.
Asterisks indicate the variables that were significantly associated with $P < 0.01$.

Table 4. Number of bromeliads in subareas A and B, percentage of each bromeliad group, maximum mean value of water volume from four periodic collections from each bromeliad type, and total of immature forms collected in each bromeliad type in Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007

Bromeliads	Subarea A (N)	Subarea B (N)	Number and percentage of bromeliads	Maximum mean water volume (ml)	Total of mosquito specimens (%)
Quesnelia sp.	23	5	28 (31.11)	276.6	402 (20.81)
Vriesea sp.	14	7	21 (23.33)	1,649.0	1,182 (61.18)
Bilbergia sp.	10	6	16 (17.78)	78.0	145 (7.51)
Nidularium sp.	2	4	6 (6.67)	60.0	60 (3.11)
sp. 1	0	3	3 (3.33)	28.2	57 (2.95)
sp. 2	7	5	12 (13.34)	70.3	48 (2.48)
sp. 3	1	0	1 (1.11)	498.0	37 (1.91)
sp. 4	3	0	3 (3.33)	15.5	1 (0.05)
Total	60	30	90 (100)		1,932 (100)

greater than pure chance. Table 2 indicates with an asterisk those taxa with significantly aggregated distributions. Those without an asterisk had too few individuals to allow testing. Table 3 shows the most significant factors associated with the abundance of each taxa per bromeliad. Overall, bromeliad water volume was the main factor for all taxa. Differences between trails and collections are observed for some species.

The majority of mosquito species were more abundant in subarea A (Table 1). However, neither the species richness nor abundance (X2) differed significantly. Sabethine abundance and richness were greatest in subarea A, the more sunlight exposed site (Table 1). For instance, *Wy.* (*Pho.*) *theobaldi*, *Wy.* (*Pho.*) *pilicauda*, *Cx.* (*Mcx.*) *neglectus*, *Culex* (*Microculex*) *inimitabilis* Dyar and Knab, and *Culex* (*Microculex*) *reducens* Lane and Whitman were only found in subarea A, while *Cx. ocellatus* was more abundant in subarea B (Tables 1 and 2). The other *Culex* species were only or mainly in subarea A, except for *Culex dubitans* Lane and Whitman that was equally distributed in both subareas (Table 1). Sun-exposed and -shaded bromeliads differ in several ways, shaded plants containing many dead leaves and detritus, whereas exposed plants bear algae (Frank 1983). A study developed in Florida, comparing the preference of two mosquito species for bromeliad environments reported that *Wy. vanduzeei* was more numerous in a sun-exposed habitat, while *Wy. mitchellii* was absent (Frank and O'Meara 1985). Immature forms of *Wy.* (*Spi.*) *howardi* were observed only in sun-exposed bromeliads (Palacio et al. 2010). Perhaps sun exposure may have influenced the mosquito species distribution in Itatiaia forest.

Relationships Between Bromeliad Water Volume, Type, and Mosquito Richness and Abundance. The great majority of the 90 sampled bromeliads could be identified at the genus level: *Vriesea* sp., *Quesnelia* sp., *Nidularium* sp., and *Bilbergia* sp. The genus of 19 bromeliads could not be determined, thus these plants were called sp. 1, sp. 2, sp. 3, and sp. 4 (Table 4). Further identification of the bromeliads was limited by the lack of key structures, as flower at the sampling times.

Among the 90 sampled bromeliads, 18 were negative for immature mosquitoes in all collections, and all but one of the 18 held water at the sampling times. The range of volumetric capacity of the sampled bromeliads was very large, from 1 to 2,585 ml, and the maximum mean value of water volume from four periodic collections from each bromeliad type is displayed in Table 4. The Poisson model with month and bromeliad type as fixed effects and bromeliad as random effect was the best model to represent the data in comparison to reduced models, suggesting that there is variation in volume along months and between bromeliad types. *Vriesia* sp. presented the largest water volumes, followed by bromeliad sp. 3 and *Quesnelia* sp. and *Bilbergia* sp. The greatest number of mosquito specimens was observed in bromeliads of genus *Vriesea*, which were the second most common bromeliad and held the greatest water volume (1,649.0 ml). Accordingly, 1,182 mosquitoes were collected in *Vriesea* sp. (61.18% of the total). In contrast, bromeliad sp. 4 held the least average water volume (7.5 ml) and harbored only 0.05% of the collected mosquitoes (Table 4). There was a positive correlation between the water volume held by the bromeliad and mosquito species richness (Fig. 2). This positive relationship was pointed out in other bromeliad studies (Marques 2008, Jabiol et al. 2009, Hammill et al. 2014), as when considering different organisms other than mosquitoes with bromeliad size (Cotgreave et al. 1993).

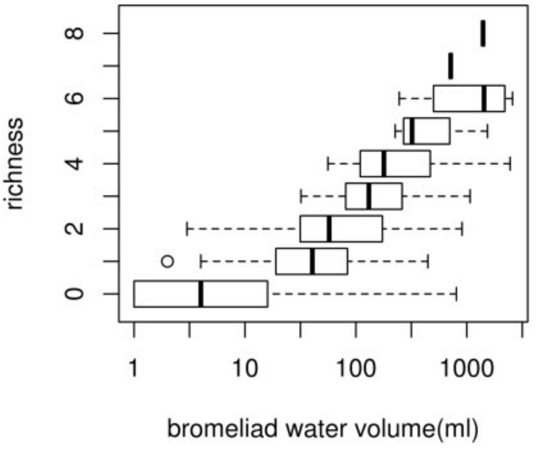

Fig. 2. Box-plot of bromeliad water volume for each value of species richness, in Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007.

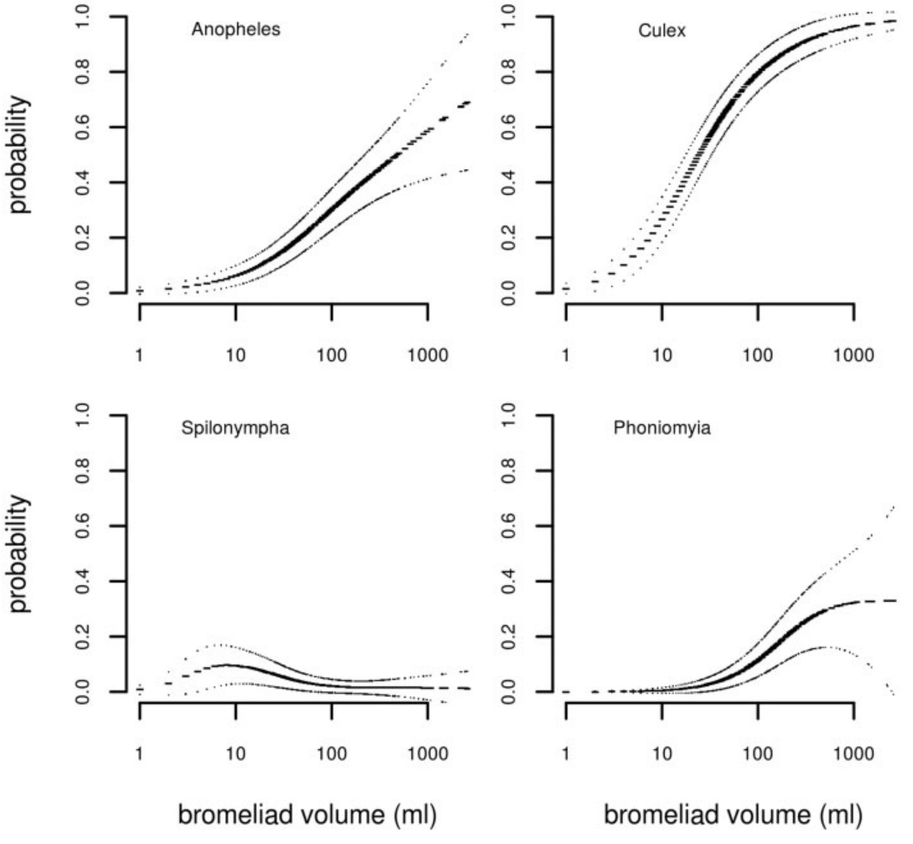

Fig. 3. Water volume-dependent probability of occurrence of species of *Anopheles*, *Culex*, *Wyeomyia* of subgenera *Spilonympha* and *Phoniomyia* mosquitoes in bromeliads in Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007. Lines indicate the expected probability and the 95% CI, according to the model described in the main text.

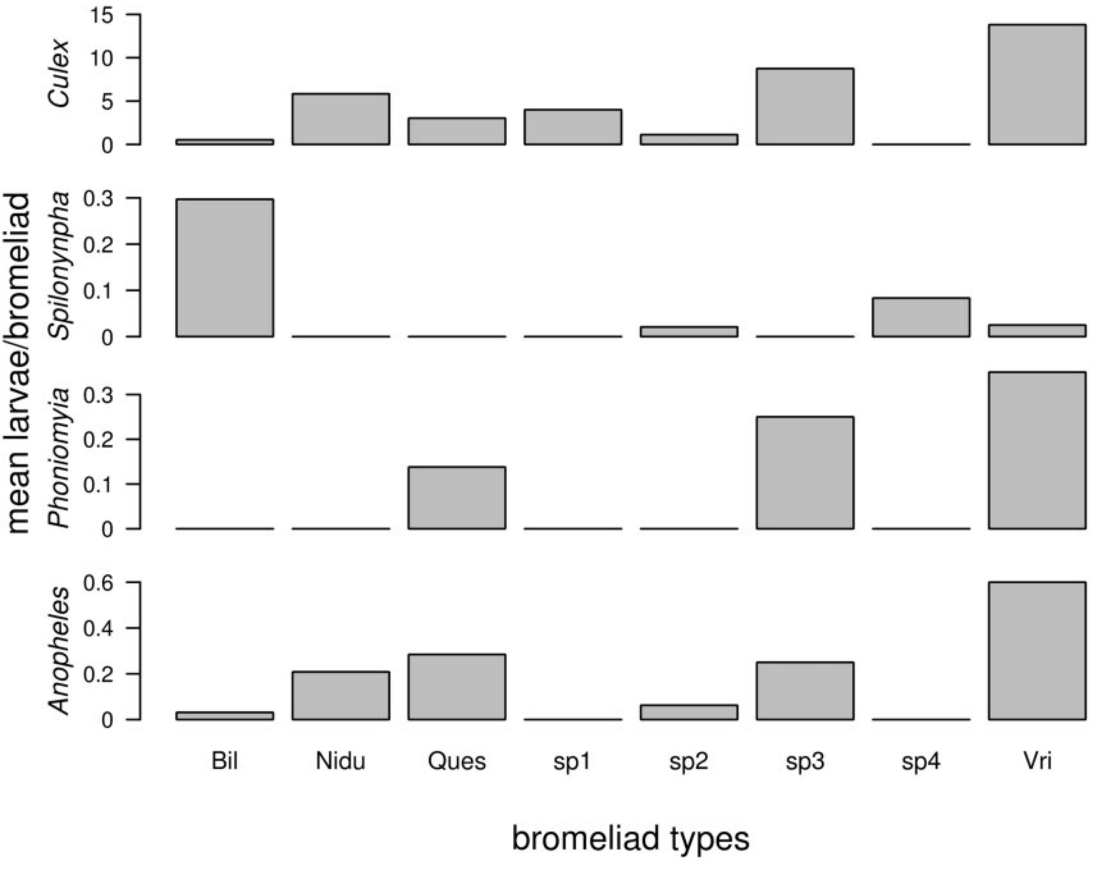

Fig. 4. Distribution of species of genera *Anopheles* and *Culex*, and *Wyeomyia* of subgenera *Spilonympha* and *Phoniomyia* in bromeliad groups in Parque Nacional do Itatiaia, Rio de Janeiro, from January to December 2007. Bil, *Bilbergia;* Nidu, *Nidularium*; Ques, *Quesnelia*; Vri, *Vriesea.*

To increase the robustness of comparisons between the bromeliad water volumes and considering the low frequency of Sabethines throughout the study, we further fit models comparing mosquito genera (*Anopheles* and *Culex*) or subgenera (*Spilonympha* and *Phoniomyia*) with the water volume held in the plant. The effect of the bromeliad water volume on the presence of mosquito groups was estimated by an additive logistic model. The additive model provides a nonlinear estimate of the relationship between water volume and the probability of finding the taxon. Figure 3 shows the result. For all four taxa, the water volume improved the model goodness-of-fit. The probability of finding bromeliads with immature forms of *Anopheles*, *Culex* and *Phoniomyia* increased with the increase of water volume held ($P < 0.001$). In contrast, *Spilonympha* mosquitoes were mostly found in bromeliads with water volume <50 ml (Fig. 3). An intermediate situation was evident for *Culex* and *Phoniomyia* mosquitoes, which were more associated with bromeliads holding water volumes >100 ml. Hammill et al. (2014) declared that the number of *Culex* larvae increased with bromeliad size (water volume) in Costa Rica, although plant genus did not affect their quantities. Frank et al. (1976) reported a significantly higher collection of eggs of *Wy.* (*Wyeomyia*) *vanduzeei* and *Wy.* (*Wyo.*) *medioalbipes* in larger *Tillandia utriculata* L. plants in Florida. Mocellin et al. (2009) also presented positive correlations between the amount of water in cultivated bromeliads in the Botanic Garden in Rio de Janeiro and the number of immature *Culex* (*Microculex*) spp. and *Wyeomyia* (*Phoniomyia*) spp.

There were Sabethines in six bromeliad types (Fig. 4). *Wyeomyia* (*Spilonympha*) spp. (*Wy. airosai* and *Wy. finlayi*) were collected in four different types of bromeliads, exhibiting a heterogeneous distribution of average number of specimens collected per bromeliad, although they were most commonly present in *Bilbergia* sp., representing 82.0% of the total number of mosquitoes collected ($KW = 38.97$; $df = 7$; $P < 0.001$), suggesting differences in the distribution among bromeliad types. Species of subgenus *Phoniomyia* were found in only three types of bromeliads, *Quesnelia* sp., *Vriesea* sp., and bromeliad sp. 3 (Fig. 4), being significantly more common in the first two plant types ($KW = 22.3619$; $df = 7$; $P = 0.0022$). Interestingly, *Ru. frontosa* was collected only in *Bilbergia* sp. The distribution of average number of *Culex* specimens

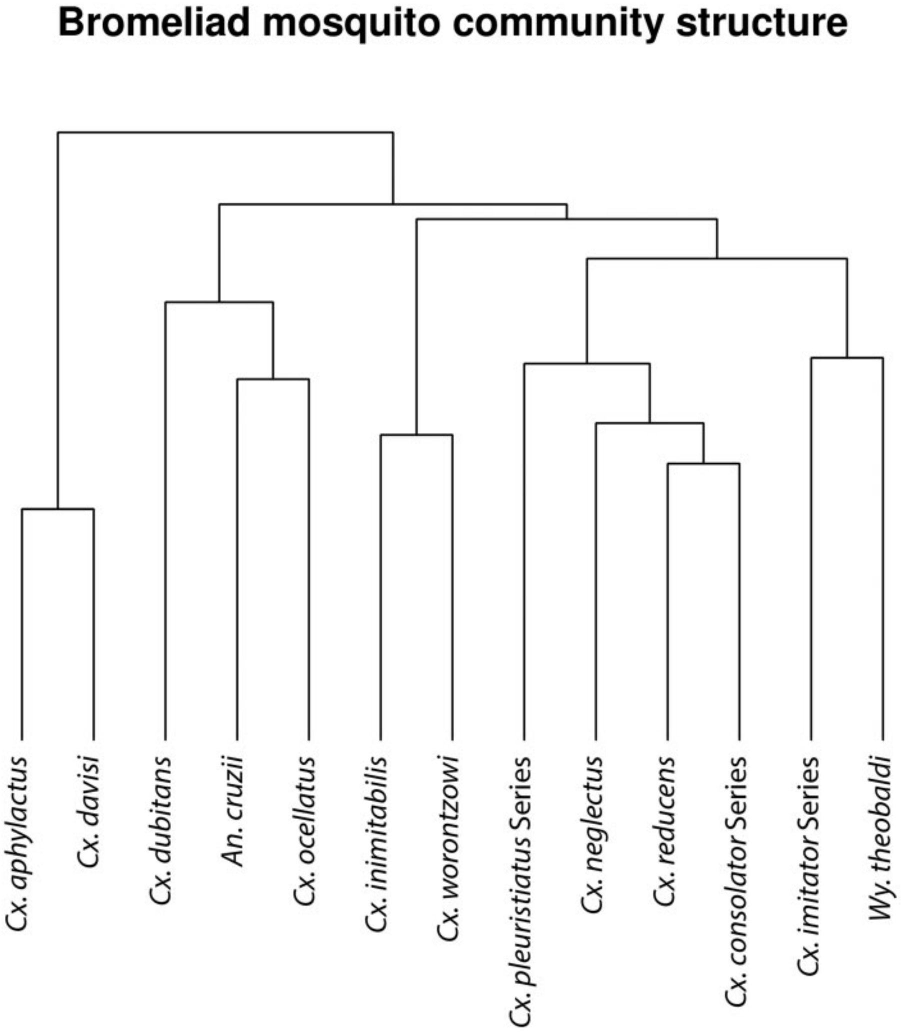

Fig. 5. Mosquito community structure in bromeliads, represented by a dendrogram of the most abundant mosquito species.

collected per bromeliad group presented significant variation (KW = 82.0915; df = 7; $P < 0.001$) with more concentration in *Vriesia* sp. and bromeliad sp. 3.

When studying mosquito fauna composition in bromeliads of genera *Vriesea* and *Nidularium* in the southeastern coast of Brazil, Marques et al. (2012) suggested that *Nidularium* plants are not an important larval habitat for the *Wyeomyia* (*Phoniomyia*) species, a hypothesis previously proposed by Müller and Marcondes (2007). Coincidently, no *Phoniomyia* was collected in *Nidularium* in the present survey. The premise of Marques et al. (2012) was reinforced by the contrasting high numbers of these mosquitoes in *Bilbergia nana* Pereira and *Neoregelia compacta* (Mez) by Mocellin et al. (2009). Jabiol et al. (2009) found that plant genus might not affect the presence of the species of genus *Wyeomyia*, as they were collected in five bromeiad species sampled in French Guiana despite being more frequent in *Aechmea melinonii* (Hooker) and *Vriesea* spp., bromeliads with low water volume (12–360 ml and 7–200 ml, respectively).

We observed that in 5 of the 11 occasions (45.4%) *Wyeomyia* (*Spilonympha*) spp. were the only species collected in the bromeliads. In two instances (18.2%), *Wyeomyia* (*Spilonympha*) spp. co-occurred with *Ru. frontosa* (18.2%) and only once simultaneously with *Culex* spp. or with both *Culex* spp. and *Ru. frontosa*. Contrarily, *Wyeomyia* (*Phoniomyia*) mosquitoes did not display a specific association pattern and frequently were together with other species (88.3% of positive samples for this mosquito co-occurring with species of *Culex* and *Anopheles*). It can be illustrated by the case of *Wy.* (*Pho.*) *theobaldi.* It clustered with five species of *Culex* subgenus *Microculex* in the dendrogram concerning bromeliad mosquito community structure constructed with data of the most abundant species (Fig. 5; right). In the dendrogram, other three major groups can be identified. One group (left) is composed by only two species – *Culex* (*Microculex*) *aphylactus* Root and *Culex* (*Microculex*) *davisi* Kumm. Interestingly, *Anopheles cruzii* Dyar and Knab formed a distinct major group with two other species of genus *Culex* – *Cx. ocellatus* and *Cx.* (*Mcx.*) *dubitans* (Fig. 5). Laporte and Sallum (2014) also described a significant positive association between mosquitoes of these two genera in bromeliads from the Atlantic Forest in southeast Brazil. Accordingly, there was a co-occurrence of *Anopheles bellator* Dyar and Knab and *Culex imitator* Theobald, in contrast to larvae of two co-subgeneric *Anopheles* species, *An. cruzii* and *An. bellator*, where coexistence was infrequent. These authors reported that although *Wy.* (*Pho.*) *quasilongirostris* (Theobald) and *Wy.* (*Pho.*) *muhelensis* Petrocchi may coexist in the same habitat, they usually are not present together in the same plant. We conclude that 1) The predominant mosquito fauna in bromeliads of Parque Nacional do Itatiaia is composed of the *Culex* species. 2) Bromeliads with greater water volume provide a greater chance of accommodating species of *Culex*, *Anopheles*, and *Phoniomyia.* 3) *Wyeomyia* (*Spilonympha*) *airosai* and *Wy.* (*Spi.*) *finlayi* tend to developed in bromeliads of genus *Bilbergia*, which accumulate <50 ml of water, and usually are found either alone or with *Ru. frontosa.* 4) The *Phoniomyia* subgenus species seems to be a generalist as these mosquitoes are encountered in different bromeliad types with various water holding capacities and are associated with different species of *Culex* and *Anopheles*.

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