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Mosquito community composition in dynamic landscapes from the Atlantic Forest biome (Diptera, Culicidae)

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ABSTRACT. Mosquito community composition in dynamic landscapes from the Atlantic Forest biome (Diptera, Culicidae). Considering that some species of Culicidae are vectors of pathogens, both the knowledge of the diversity of the mosquito fauna and how some environment factors influence in it, are important subjects. In order to address the composition of Culicidae species in a forest reserve in southern Atlantic Forest, we compared biotic and abiotic environmental determinants and how they were associated with the occurrence of species between sunset and sunrise. The level of conservation of the area was also considered. The investigation was carried out at *Reserva Natural do Morro da Mina*, in Antonina, state of Paraná, Brazil. We performed sixteen mosquito collections employing Shannon traps at three-hour intervals, from July 2008 to June 2009. The characterization of the area was determined using ecological indices of diversity, evenness, dominance and similarity. We compared the frequency of specimens with abiotic variables, *i.e.*, temperature, relative humidity and pluviosity. Seven thousand four hundred ten mosquito females were captured. They belong to 48 species of 12 genera. The most abundant genera were *Anopheles*, *Culex*, *Coquillettidia*, *Aedes* and *Runchomyia*. Among the species, the most abundant was *Anopheles cruzii*, the primary vector of *Plasmodium* spp. in the Atlantic Forest. Results of the analyses showed that the abiotic variables we tested did not influence the occurrence of species, although certain values suggested that there was an optimum range for the occurrence of culicid species. It was possible to detect the presence of species of Culicidae with different epidemiologic profiles and habitat preference.

KEYWORDS. Diversity; Insecta; malaria; Shannon trap; tropical rain forest.

Although the Brazilian Atlantic Forest has been classified as one of the five most important biodiversity hotspots on earth, it continues to be the target of anthropogenic activity (Myers *et al.* 2000). Even after centuries of destruction, however, this biome is still home to more than 8,000 endemic species of vascular plants, amphibians, reptiles, birds and mammals (Maack 1981; Ab'Saber 2003).

The Culicidae of the Atlantic Forest are represented by 23 genera and approximately 90 species (Forattini 2002; Harbach 2007; Guedes 2012). In the state of Paraná, there are 205 species of Culicidae of 18 genera. This corresponds to approximately 10% of the culicid fauna of the Neotropical Region (Lozovei & Luz 1976; Lozovei 2001; Barbosa *et al.* 2003; Bona & Navarro-Silva 2006; Guedes 2012; Müller *et al.* 2012).

In this study we addressed the composition of a culicid community that occurs in a forest reserve area situated in the southern Atlantic Forest of the state of Paraná. The region had been under intense human exploitation for natural resources. Mosquitoes present in the area have never been sampled. Additionally, we assessed the circadian and seasonal characteristics of the Culicidae community to address species occurrence patterns and to provide information for environmental conservation and entomological monitoring.

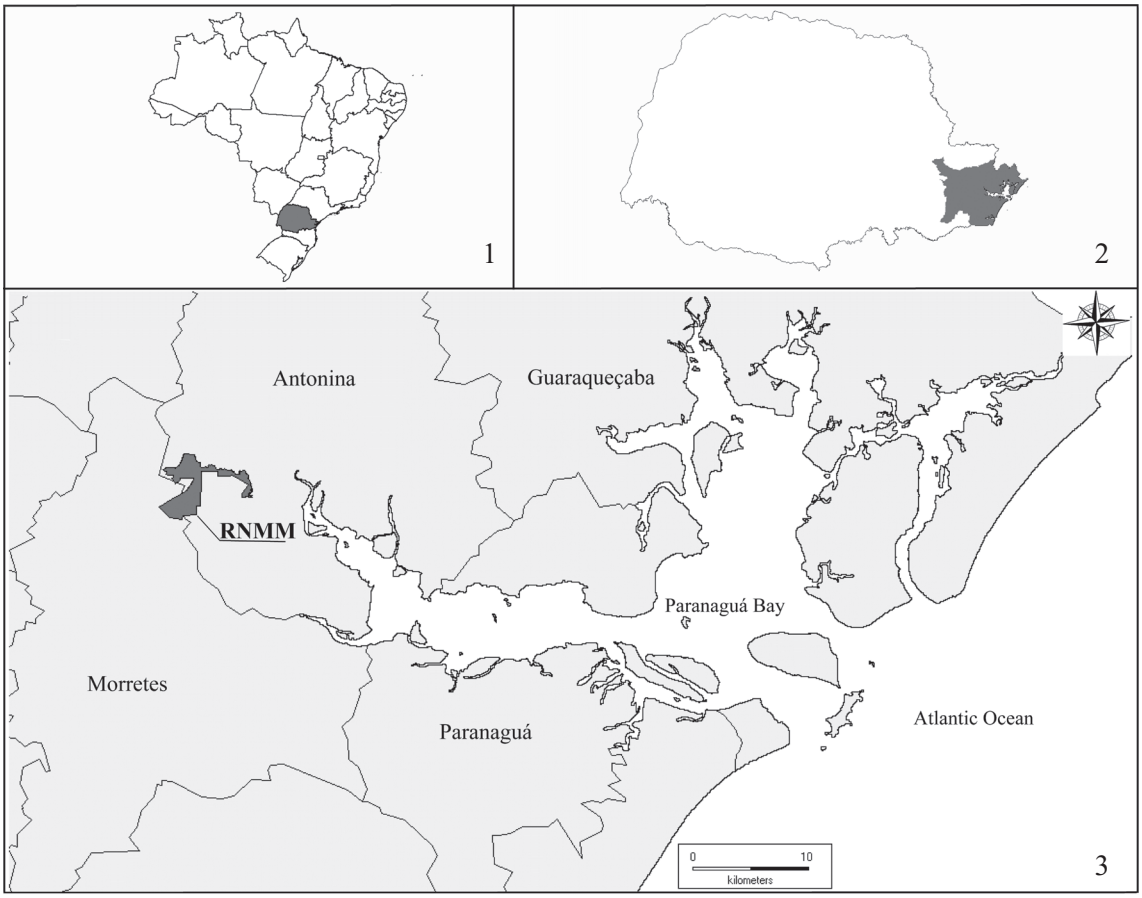
MATERIAL AND METHODS

Culicid adults were captured in sixteen field trips between July 2008 and June 2009 at the Atlantic Forest of the *Reserva Natural do Morro da Mina*, in Antonina, state of Paraná, Brazil (Figs. 1–3). According to the Köppen's classification, the local climate is Af type (Köppen 1936).

The study area had been under intense ecological pressure caused by the exploitation of natural resources and unplanned extractivism. From 1930 to the mid-1980s there was extensive extraction of iron ore in the region. After that, trees were cut off for charcoal production. The area came under government protection in 1995 and is now under the administration of the *Sociedade de Pesquisa em Vida Selvagem e Educação Ambiental* (SPVS), which seeks to preserve and to regenerate the local forest (IAP 2013).

We used a Shannon trap (Shannon 1939) modified with a light source to capture females of Culicidae. One trap was installed about 50 m from the forest border into the forest (25°22'54"S, 48°47'03"W). In order to avoid the intrusion effect we installed the traps one hour before we started collecting. Each sampling interval, equivalent to a total of nine hours, was divided into three phases, as following: evening twilight (one hour and a half before and one hour and a half after sunset), nocturnal (three hours between evening twilight and morning twilight) and morning twilight (one hour and a half before and one hour and a half after sunrise). Each three-hour phase was divided into intervals of thirty-minutes, in the end of which culicids were aspirated, totaling six captures in each phase.

The nomenclature and abbreviations adopted herein for all Culicidae genera and subgenera followed WRBU (2013)



Figs. 1–3. Geographic location of the study site in southern Brazil. 1, Map of Brazil, with indication of the State of Paraná (in gray), 2, Map of the State of Paraná, with emphasis on the municipalities along the coast of the state. 3, Location of the study site, *Reserva Natural do Morro da Mina* (RNMM), in the coastal plain of the Paranaguá bay, in Paraná.

and Reinert (2001). Voucher specimens are deposited in the *Coleção Entomológica Padre Jesus Santiago Moure* (DZUP) of the *Departamento de Zoologia* at UFPR.

Pluviometric data are presented as the sum of two successive periods of collecting; in analyses, we used the maximum, minimum and the average values of temperature and relative humidity. The seasons were divided as follows: from June 21st to September 23rd, winter (four collections); from September 23rd to December 21st, spring (four collections); from December 21st to March 21st, summer (four collections) and from March 21st to June 21st, fall (four collections).

To describe the faunal composition we used the following ecological indices: evenness (Pielou – J'), dominance (Berger-Parker) and diversity (Shannon – H). Simple linear correlation tests were conducted to verify the existence of a possible interference of abiotic conditions on the numbers of Culicidae collected. For this we used the mean values and the sum of two collections of rainfall, temperature and relative humidity (since they are percentage values of relative humidity they were transformed into arcsine values to become comparable).

The computer program Paleontological Statistics Software Package for Education and Data Analysis version 1.88 (PAST) was used to analyze the data (Hammer *et al.* 2001).

RESULTS

The Shannon diversity index for the Culicidae was H = 1.5. This value reflects the 7,410 female specimens captured belonging to 12 genera and 48 species (Table I).

During the spring, a low equitability rate was detected (Table II). About 78% of the species and more than half of the number of individuals collected were recorded during this season, when *Anopheles* (*Kerteszia*) *cruzii* Dyar & Knab, 1908 was very abundant. During the winter, the local species diversity was more elevated because a fewer number of individuals was collected. Fall was characterized by a higher evenness, whereas in the summer we observed a higher diversity and a lower species dominance (Table II).

Anopheles (*Ker*.) *bellator* Dyar & Knab, 1906, *An*. (*Ano*.) *intermedius* (Peryassú, 1908), *Li*. *flavisetosus* de Oliveira Castro, 1935, *Ru.* (*Run*.) *reversa* (Lane & Cerqueira, 1942) and *Ru.* (*Run*.) *theobaldi* (Lane & Cerqueira, 1942) were collected throughout the year, whereas other species were rare, occurring in less than four samples: *Anopheles* (*Nys*.) *evansae* (Brethés, 1926), *An*. (*Nys*.) *triannulatus* (Neiva & Pinto, 1922), *Culex* (*Mel*.) *sacchettae* Sirivanakarn & Jakob, 1982, *Cx*. (*Phe*.) *corniger* Theobald, 1903, *Wyeomyia* (*Pho*.) *galvaoi*

Table I. Species composition and abundance of Culicidae collected in the Reserva Natural do Morro da Mina, Paraná, southern Brazil, in the period of July 2008 to June 2009, with Shannon trap.

Subfamily/Tribe	Species	Abundance	%
Anophelinae	*Anopheles* (*Anopheles*) *fluminensis* Root, 1927	10	0.1
	Anopheles (*Anopheles*) *intermedius* (Peryassú, 1908)	34	0.5
	Anopheles (*Anopheles*) *mediopunctatus* s.l.	94	1.3
	Anopheles (Kerteszia) *bellator* Dyar & Knab, 1906	60	0.8
	Anopheles (*Kerteszia*) *cruzii* Dyar & Knab, 1908	4,924	66.5
	Anopheles (*Kerteszia*) sp.	11	0.1
	Anopheles (*Nyssorhynchus*) *evansae* (Brethés, 1926)	2	0
	Anopheles (*Nyssorhynchus*) *triannulatus* (Neiva & Pinto, 1922)	6	0.1
Culicinae			
Aedini	*Ochlerotatus* (*Ochlerotatus*) *scapularis* (Rondani, 1848)	102	1.4
	Ochlerotatus (*Protoculex*) aff. *hastatus* (Dyar, 1922)	14	0.2
	Ochlerotatus (*Protoculex*) aff. *serratus* (Theobald, 1901)	164	2.2
	Psorophora (*Janthinosoma*) *champerico* (Dyar & Knab, 1906)	2	0
	Psorophora (*Janthinosoma*) *ferox* (von Humboldt, 1819)	16	0.2
	Psorophora (*Janthinosoma*) *lutzii* (Theobald, 1901)	15	0.2
Culicini	*Culex* (*Culex*) aff. *bidens* Dyar,1922	304	4.1
	Culex (*Culex*) *chidesteri* Dyar, 1921	10	0.1
	Culex (*Culex*) *coronator* complex	109	1.5
	Culex (*Culex*) aff. *dolosus* (Lynch Arribálzaga, 1891)	38	0.5
	Culex (*Culex*) aff. *maxi* Dyar, 1928	3	0
	Culex (*Culex*) ssp.	162	2.2
	Culex (*Melanoconion*) *pedroi* Sirivanakarn & Belkin, 1980	2	0
	Culex (*Melanoconion*) aff. *pedroi* Sirivanakarn & Belkin, 1980	2	0
	Culex (*Melanoconion*) *ribeirensis* Forattini & Sallum, 1985	208	2.8
	Culex (*Melanoconion*) *sacchettae* Sirivanakarn & Jakob, 1982	10	0.1
	Culex (*Melanoconion*) section of *Melanoconion*	29	0.4
	Culex (*Phenacomyia*) *corniger* Theobald, 1903	15	0.2
	Lutzia (*Lutzia*) *bigoti* (Bellardi, 1862)	4	0.1
Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *albicosta* (Peryassú, 1908)	636	8.6
	Coquillettidia (*Rhynchotaenia*) *chrysonotum* (Peryassú, 1922)	48	0.6
	Coquillettidia (*Rhynchotaenia*) *hermanoi* (Lane & Coutinho, 1940)	165	2.2
	Coquillettidia (*Rhynchotaenia*) *venezuelensis* (Theobald, 1912)	12	0.2
	Mansonia titillans (Walker, 1848)	2	0.0
Sabethini	*Limatus flavisetosus* de Oliveira Castro, 1935	38	0.5
	Runchomyia (*Runchomyia*) *reversa* (Lane & Cerqueira, 1942)	49	0.7
	Runchomyia (*Runchomyia*) *theobaldi* (Lane & Cerqueira, 1942)	57	0.8
	Trichoprosopon pallidiventer (Lutz, 1905)	1	0
	Wyeomyia (*Menolepis*) aff. *leucostigma* Lutz, 1904	3	0
	Wyeomyia (*Phoniomyia*) *galvaoi* (Corrêa & Ramalho, 1956)	7	0.1
	Wyeomyia (*Phoniomyia*) *incaudata* (Root, 1928)	5	0.1
	Wyeomyia (*Phoniomyia*) *pilicauda* Root, 1928	2	0
	Wyeomyia (*Phoniomyia*) *quasilongirostris* (Theobald, 1907)	5	0.1
	Wyeomyia (*Phoniomyia*) sp.	1	0.0
	Wyeomyia (*Prosopolepis*) *confusa* (Lutz, 1905)	22	0.3
	Wyeomyia (*Spilonympha*) aff. *mystes* Dyar, 1924	2	0
	Wyeomyia (subgenus uncertain) *negrensis* Gordon & Evans, 1922	2	0
Uranotaeniini	*Uranotaenia* (*Uranotaenia*) *geometrica* Theobald, 1901	1	0
	Uranotaenia (*Uranotaenia*) aff. *pallidoventer* (Theobald, 1907)	1	0
	Uranotaenia (*Uranotaenia*) *lowii* Theobald, 1901	1	0
Total		7,410	100

(Corrêa & Ramalho, 1956), *Wy*. (*Pho*.) *incaudata* Root, 1928 and *Wy*. (*Pho*.) *quasilongirostris* (Theobald, 1907).

Most individuals of *Oc*. *scapularis* (Rondani, 1848) were collected from August to December. *Coquillettidia* (*Rhy*.) *hermanoi* (Lane & Coutinho, 1940) was captured in all seasons but was more frequent in September and October. Although occurring in lower frequency, *Cq*. (*Rhy*.) *albicosta* (Peryassú, 1908) had an expressive presence, peaking in December (Fig. 4).

Specimens in the *Cx*. (*Cux*.) *coronator* complex were more frequent from November to June, whereas *Cx*. (*Mel*.) *ribeirensis* Forattini & Sallum, 1985 was collected throughout the year, mainly during the last months of the spring. *Anopheles mediopunctatus* s.l. was captured during the spring. However, the species was found more frequently and in higher number from October to December (Fig. 4).

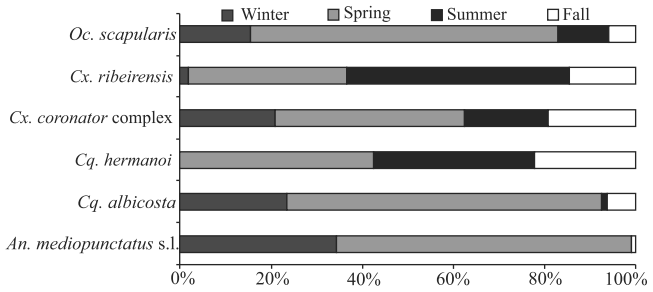


Fig. 4. Species of Culicidae with relative occurrence between 1% and 10% captured along the seasons in the *Reserva Natural do Morro da Mina*, Paraná, southern Brazil, in the period of July 2008 to June 2009.

Anopheles cruzii was the dominant species, representing 66% of total Culicidae captured. The greatest numbers of individuals were obtained in October (n = 335), November (n = 328) and December (n = 1,940) (Fig. 5).

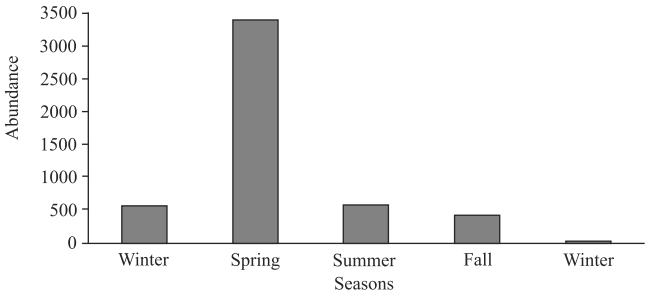


Fig. 5. Occurrence of *Anopheles* (*Kerteszia*) *cruzii* along the seasons from July 2008 to June 2009, in the *Reserva Natural do Morro da Mina*, Paraná, southern Brazil.

The mean precipitation and mean temperature, for the sum of two successive periods of collecting, during the twelve months of the study (July 2008 to June 2009) were 6.1 mm (± 11.4) and 20°C (± 3.4), respectively. The highest mean temperatures were recorded in March (29°C), May (31°C) and July (29°C), whereas the highest temperature range (±19.4°C) was found in July. The average relative humidity during the period of the collections was 90% (± 5.78).

The results of linear regression analyses did not reveal a significant correlation between the number of mosquitoes and the following daily or monthly average variables: temperature, precipitation and relative humidity.

The highest richness and the greatest numbers of individuals were detected in the evening twilight, when 33 species and about 3,700 individuals were obtained. The ecological indices depicted an increased ecological diversity and evenness of species over the three time slots and a reduction in species dominance from the dusk to the morning (Table II).

Table II. Ecological indices applied to the species of Culicidae sampled during the seasons and to the species captured at different sampling times in the Reserva Natural do Morro da Mina, Paraná, southern Brazil, in the period of July 2008 to June 2009.

	Season				Intervals		
Index	Winter	Spring	Summer	Fall	CV	IN	CM
Shannon (H)	1.21	0.81	1.16	1.31	1.41	1.44	2.03
Berger-Parker	0.65	0.78	0.49	0.52	0.71	0.64	0.51
Pielou (J')	0.55	0.33	0.50	0.57	0.37	0.44	0.58

CV, evening twilight interval; IN, night interval; CM, morning twilight interval.

Sabethini (*Limatus*, *Runchomyia* and *Trichoprosopon*) were not collected during the night. Specimens of *Mansonia* were captured only in the morning twilight and the species of the genus *Uranotaenia* were not recorded in this interval. The following species of *Culex* were more common in the evening twilight: *An*. (*Ker*.) *bellator*, *An*. (*Ano*.) *intermedius*, *Cq*. (*Rhy*.) *chrysonotum* (Peryassú, 1922) and *Ps*. (*Jan*.) *ferox* (von Humboldt, 1819). The two species of *Runchomyia*, *Ru*. (*Run*.) *reversa* (Lane & Cerqueira, 1942), *Ru*. (*Run*.) *theobaldi* (Lane & Cerqueira, 1942) and *Li*. *flavisetosus*, *Ps*. (*Jan*.) *champerico* (Dyar & Knab, 1906), *Ps*. (*Jan*.) *lutzii* (Theobald, 1901) were captured during morning and evening twilights. *Anopheles* (*Nys*.) *triannulatus*, *Tr*. *pallidiventer* (Lutz, 1905), *Uranotaenia* (*Ura*.) *geometrica* Theobald, 1901, *Ur*. (*Ura*.) *lowii* Theobald, 1901, *Wyeomyia* (*Pho*.) *galvaoi*, and *Wy*. (*Pho*.) *quadrilongirostris* were captured only in the evening twilight, whereas *Ma*. *titillans* (Walker, 1848) and *An*. *evansae* only in the morning twilight.

Anopheles mediopunctatus s.l. and *Cq*. (*Rhy*.) *hermanoi* are characterized as species that occur in the evening twilight, because 60% of the specimens were collected during that period. Species of the *Cx*. *coronator* complex are active at dusk and continue overnight. The activity period of *Ochlerotatus* (*Och*.) *scapularis* was constant during the intervals sampled (Fig. 6). *Anopheles* (*Ker*.) *cruzii* was captured in large number during the evening twilight, whereas *Cx*. (*Mel*.) *ribeirensis* and *Cq*. (*Rhy*.) *albicosta* were more frequent overnight (Fig. 6).

Because *An*. (*Ker*.) *cruzii* was found in great number and that 58% of the specimens were captured in the evening twilight (Fig. 7), we were able to address its activity pattern during the 9-hour period of Shannon collections. Accordingly, frequencies of specimens obtained in each 30-minute collection intervals were analyzed separate. Based on the time of sunset, *An*. *cruzii* is an eocrepuscular species (Fig. 8), peaking immediately after dusk. During the night (Fig. 9) the average number of mosquitoes was approximately 200 specimens collected every 30 minutes, decreasing during the twilight (Fig. 10). The frequency of *An*. *cruzii* was higher at the sunset (evening twilight-interval p = 0.183, r = 0.625) than at sunrise (morning twilight interval p = 0.019, r = 0.882).

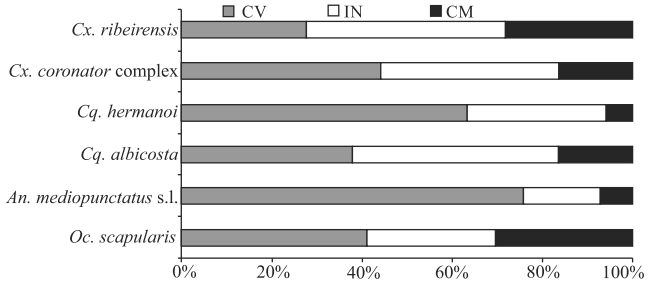


Fig. 6. Species of Culicidae with relative occurrence between 1% and 10% of Culicidae captured in each interval (CV-evening twilight interval; IN-night interval; CM-morning twilight interval), in the *Reserva Natural do Morro da Mina*, Paraná, southern Brazil, in the period of July 2008 to June 2009.

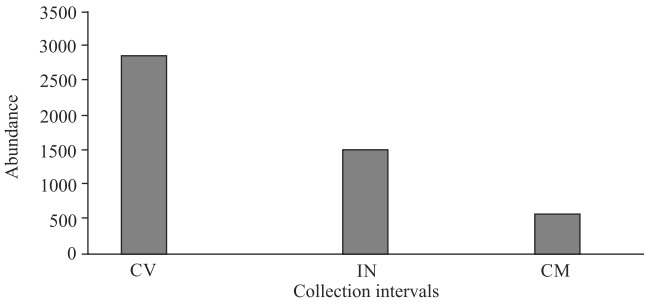
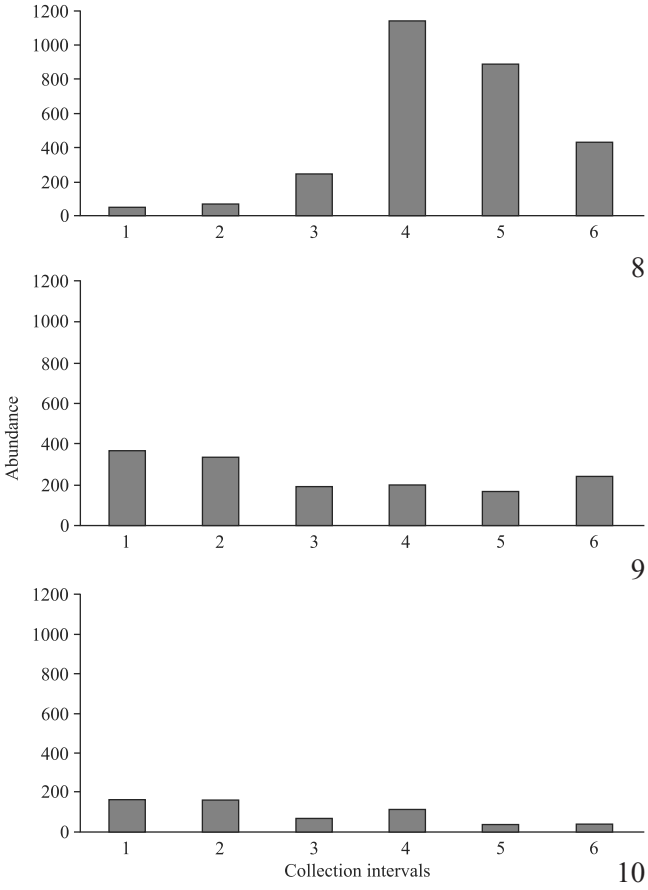


Fig. 7. Occurrence of *Anopheles* (*Kerteszia*) *cruzii* in the three collection intervals, (CV) Evening twilight interval, (IN) night interval, (CM) morning twilight interval, in the *Reserva Natural do Morro da Mina*, Paraná, southern Brazil, in the period of July 2008 to June 2009.



Figs. 8–10. Occurrence of *Anopheles* (*Kerteszia*) *cruzii* in collection intervals divided into six phases of thirty-minutes each, in the *Reserva Natural do Morro da Mina*, Paraná, southern Brazil, in the period of July 2008 to June 2009. 8, Evening twilight interval (numbers 3 and 4 are related to the time of evening twilight). 9, Night interval. 10, Morning twilight interval (numbers 3 and 4 are related to the time of morning twilight).

DISCUSSION

The floristic and the faunal composition of the *Reserva Natural do Morro da Mina* has characteristics that can potentially support a diverse community of Culicidae, providing distinct larval habitats, for instance, phytotelmatas, and several blood sources, such as birds and mammals (IPARDES 1990).

Studies conducted in different areas of the Atlantic Forest biome in the state of Paraná recorded 25 (Bona & Navarro-Silva 2008) and 31 (Santos Neto & Lozovei 2008) species of Culicidae. Despite the different sampling designs used, *An. cruzii* was dominant in both studies: 40% and 50% of the total samples, respectively. Even when the environment is conducive to the development of culicid species, the temporal dynamics of these organisms may be influenced by the length of the days and nights (Day 2005; Nagm *et al.*2007). In this study, we found that *An. mediopunctatus* s.l. and *Cq. hermanoi* are active in the evening twilight, whereas *Cx. ribeirensis* and *Cq. albicosta* are mostly active at night.

The temporal patterns, when analyzed at different scales, differ among species (circadian oscillations and seasonal variations) (Bradshaw *et al.* 2004). The occurrence of *An. cruzii* was high in all three intervals, indicating activity mostly in the evening twilight and potential activity throughout the evening, suggesting that the decreased brightness is one factor that influences the circadian rhythm of activity of this insect. It is known that this species can also occur during the morning in forested areas with similar floristic composition (Veloso *et al.* 1956; Aragão 1974; Bona & Navarro-Silva 2006).

Particularly in the southern and southeastern coastal area of Brazil the seasonal behavior of *An. cruzii* is somewhat well known and there may be some monthly and seasonal changes in its abundance (Forattini *et al.*1968; Forattini *et al.* 1986; Guimarães *et al.* 2000). Usually, culicid species are more abundant when temperature and precipitation values are greater, which in the Atlantic Forest occurs in the summer (Guimarães *et al.* 2001; Rona *et al.* 2009). However, in the present study, *An. cruzii* was more abundant in the spring, indicating that the seasonal pattern previously mentioned can change depending on the variations in the biotic and abiotic factors.

Culex ribeirensis occurs in regions adjacent to the coastal plains and slopes of the Atlantic rainforest (Forattini & Sallum 1985). This species has nocturnal activity and is commonly caught in automatic light traps (Forattini *et al.* 1991, Santos-Neto & Lozovei 2008). Its occurrence is associated to the pluviometric precipitation since the immature stages were

found in ground pool habitats where rainwater can accumulate (Forattini 2002).

The low abundance of representatives of the Sabethini in the present study may be due to the fact that most species are diurnal, peaking during the day (Lane & Cerqueira 1942). Diurnal collections were conducted in two periods of 1h30 minutes before and after sunrise. Moreover, a high richness of species of Sabethini may reflect a preserved environment (Dorvillé 1996), because the ecological characteristics present in preserved forest areas favor the occurrence of larval habitats and environmental conditions for the establishment of a diverse assemblage of species. The area occupied by the *Reserva Natural Morro da Mina* was under intensive ecological transformation caused by human activities, which included deforestation and mineral resources exploitation. Currently, the reserve is at a secondary stage of ecological succession (Cheung *et al*. 2009), supporting a floristic composition that may provide habitats for a less diverse community of Sabethini.

Some species of one of the subgenera with relevant epidemiological significance, *Culex* (*Culex*) are favored by anthropogenic environmental changes (Forattini *et al.*1990; Lopes & Lozovei 1995; Silva 2002; Alfonso *et al.* 2005). In our data, species in this subgenus occurred throughout the year, indicating that they were not influenced by the climatic factors utilized in the analyses.

In the lowland Atlantic Forest biome, periods marked by extremes in temperature and rainfalls do not occur over the different seasonal periods, providing suitable conditions for maintaining populations of Culicidae. The reduced range of environmental factors may favor the dominance of few species in different seasonal periods, as observed for *An*. *cruzii* (Tubaki *et al.* 1993; Guimarães *et al*. 2001).

During the evening twilight, species of *Psorophora*, *Anopheles* and *Coquillettidia* were predominant, whereas members of the tribe Sabethini were detected in low numbers, for instance *Wyeomyia confusa* (Lutz, 1905), *Wy. galvaoi*, *Wy. pilicauda* Root, 1928 and *Wy*. *quasilongirostris*. These species are diurnal (Lane & Cerqueira 1942) and their activities were extended beyond the expected.

Mosquito vector species of human pathogens are present in the studied area. The most important pathogens and their potential mosquito vectors found in our collections are: *Plasmodium* spp. (*An*. *cruzii*), arboviruses (*Oc. scapularis*, *Cx*. *ribeirensis* and *Ps*. *ferox*) (Casanova & Prado 2002; Guedes 2012). These mosquitoes, present in the forest, can acquire pathogens from sylvatic intermediate hosts and transmit them to humans when feeding on their blood (Reiter 2001; Lafferty 2009). Consequently, some mosquito species present in southern Atlantic Forest may participate of epidemiological cycles of reemergence of vector-borne diseases, which are mainly associated with environmental and climatic changes, as well as habitat fragmentation.

Despite the limitations of the mosquito sampling methods that were adopted in the study, we were capable of collecting more than 50% of the species that occur in the Atlantic Forest. They represent approximately 30% of the species of Culicidae already found in the state of Paraná (Guedes 2012). It is necessary to stress the presence, frequency and dominance of *An*. *cruzii* in the forest reserve area. The biological characteristics of this species, coupled with environmental and climatic changes, and also habitat fragmentation, may create new epidemiological scenarios of vector-borne diseases in the area, which justifies a constant entomological surveillance.

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