Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 8- Survey of adult behaviour of Spissipes Section species of *Culex (Melanoconion)* in South-Eastern Brazil

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A study of Spisslpes Section of *Culex (Melanoconion)* adults behaviour was carried out from August 1992 through December 1993 in human dominated (anthropic) environment in the Ribeira Valley, S.Paulo State, Brazil. By sampling at several sites it the dominance of *Culex ribeirensis* and *Cx. sacchettae* became evident even through a total number of ten species was recorded. Those two mosquitoes showed a clear tendency to frequent the domiciliary environment where they were caught, both indoor and outdoor, through the use of the human bait. In the outside environments, the residual patchy forests seems to display a concentration role, from which these adults spread to the open land and reach the dwellings. As their vector competence has been demonstrated through the virus isolations in natural conditions, it is advisable to pay attention to the presence of these mosquitoes in the man-made environment.

Culicidae. Ecology, vectors. Culex.

Introduction

As it is well known Culex (Melanoconion) are noctumal mosquitoes of the Americas, inhabiting mainly forest environments and most active at dusk and dawn. They feed on the blood of vertebrates including humans, when available. Their competence to transmit various arboviruses has been demonstrated both through isolations from naturally infected mosquitoes and laboratory transmission trials. Thus, they are considered as important vectors of arboviruses in their sylvan natural cycle, specially the group of the Spissipes Section. Among these agents, the encephalitis viruses transmission has been seen as deserving of special treatment.

The southern geographical distribution of Melanoconion mosquitoes corresponds to the southern Brazil and northern Argentina. As far as is known, the Spissipes Section is there represented by eleven widely species spread. Five of these were found naturally infected with arboviruses. In Argentina, several bunyaviruses have been isolated from Culex delpontei and Cx. ocossa besides alphaviruses represented by strains of the Venezuelan equine encephalitis (VEE) and the western equine encephalitis (WEE) viruses (Calisher³, 1985; Mitchell et al.^{20,21}, 1985, 1987). In S. Paulo State, Southern Brazil, strains of VEE and a number of bunyaviruses have been isolated from Cx. ribeirensis (formerly confounded with Cx. crybda and Cx. epanastasis), Cx. sacchettae

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and *Culex* sp.VR (hitherto misidentified as *Cx. taeniopus*) (Calisher et al.^{1,2}, 1982, 1983; Sallum²⁴, 1994). Thus, the proportion of the isolations from these *Melanoconion* mosquitoes presages the incrimination species of that Section as important vectors of arboviruses.

During the undertaking of an investigation program in the Ribeira Valley region of S. Paulo State, mosquito behaviour was observed. In that area an encephalitis epidemic started earlier in 1975-1976 thereafter decreasing gradually until 1983 (Iversson¹⁹, 1988). Among others, mosquitoes belonging to the species referred above were focused mainly as regards their biting activities. peridomiciliary habits and its occurrence in primitive and residual woods in an anthropic environment (Forattini et al.4.5.6.9.10, 1981, 1986, 1990, 1991). The results of these observations were that Cx. ribeirensis and Cx. sacchettae appeared to be widespread in the region while Cx. delpontei, Cx. ocossa and Culex VR appeared to the limited to certain geographical areas, at least where they were found in larger numbers. Nevertheless with regard to the general anthropic and the particular dwelling environments, the two former species seems better adapted and quite attracted to human bait.

During the 1992-1993 period, a continuation of the above-mentioned investigation program, adult mosquito collections were performed through the use of human bait and the Shannon type traps. Considering the epidemiological importance of the *Culex (Melanoconion)* species the results of the research will be presented in this paper for the purpose of contributing to knowledge about their biting behavior in the anthropic environment.

Study areas

The data here reported are the results of sampling carried out at several sites on the Experimental Station (ES) and at the "Galiléia Farm" (GA) of the Pariquera-Açu County, S. Paulo State, Brazil, which were already described elsewhere (Forattini et al. 12.15, 1993, 1994). According to the criteria adopted to mapping world ecosystems these sites were recognized as corresponding to disturbed environments (Hannah et al. 18, 1994). The ES was included in the human dominated (anthropic) type where the agriculture is permanent and prevails over the primary vegetation that remains as limited remnants patches. As regards the GA environment, it was defined as partially disturbed, with secondary

vegetation without intensive agricultural activity or any artificial irrigation system. The domiciliary environment was represented by the rural dwellings of the "Sitio Barra do Capinzal" (BC) that is included within the ES area.

Material and Method

Sampling was done in accordance with the procedures described elsewhere (Forattini et al.¹⁵, 1994). Thus Shannon traps operated by a two-man team were used fortnightly from 17:00 to 20:00 hours at ES and GA sites, as follows:

Period	Site	Place	Shannon trap
January - December 1993	ES	remnant forest A	Shan 1
		remnant forest B rice-paddy margin	Shan 2
		(open land)	Shan E
August 1992 - December 1993		margin of Pariquera-Açu river	Shan GA

As Shan 1 and Shan 2 traps operated alternat, the total number of mosquitoes caught corresponded to the fortnightly rhythm.

Sampling the biting mosquitoes catches made in the domiciliary environment was done using human bait. Two collectors volunteers vacinated against yellow fever caught the females as they came to feed and alighted on their exposed legs during two hour crepuscular period, from 17:30 to 19:30 or from 18:00 to 20:00 hours according to the season (Forattini et al.4,11,15, 1981, 1993, 1994). The collections were simultaneously performed indoors and outdoors, by one man in each place using a batteryoperated aspirators to catch landing/biting females. Catches were made at BC from August 1992 through December 1993, following the fortnightly rhythm in a regular manner. The biting activity was estimated by the Williams media (\bar{x}_w was originally used (Haddow^{16,17}, 1954, 1960).

Regarding the degree of domiciliation, the synanthropic index of Nuorteva was calculated for both species, according to the percentages of specimens collected at the different places. In this manner an estimation of the synanthropy of these mosquitoes was proposed (Nuorteva²², 1963; Povolny²³, 1971; Forattini et al.¹³, 1993). For this

purpose the various collection places were considered as follows:

$$S = \frac{2a + b \cdot 2c}{2}$$

Where:

a = percentage collected in the domiciliary environment (BC).

b = percentage collected in the anthropic and partially disturbed environments (Shan E and Shan GA).

c = percentage collected in the remnant patchy forest environments (Shan 1 and Shan 2).

s = Nuorteva index.

Thus the comparisons were undertaken within the disturbed anthropic environment and covered its various aspects here studied. To obtain a general over-view of Cx, ribeirensis's and Cx, sacchettae's degrees of synanthropy, the relations between the total numbers of specimens collected at each of the places referred to above were calculated as synanthropic ratios (sr).

Results

A total number of 6,631 adult mosquitoes (6,344 females and 287 males) of the Spissipes Section was collected. Of that number 5,602 (84.5%) were caught with the Shannon traps whereas 764 (11.5%) with the use of human bait. Ten species were obtained and their specific distribution was as follows:

Species	N	%
Cx. delpontei	9	0.1
Cx. faurani	17	0.3
Cx. lopesi	1	(*)
Cx. ocossa	139	2.1
Cx. pedroi	33	0.5
Cx. pereyrai	1	(*)
Cx. ribeirensis	3,093	46.6
Cx. sacchettae	3,266	49.3
Cx. spissipes	22	0.3
CulexVR	50	0.8
Total	6,63	100.0

(*) less than 0.1

The results are presented, in accordance with the several samples obtained at the ES and GA sites, and the methods employed, in Table 1. Thus Cx. ribeirensis and Cx. sacchettae jointly accounted for 95.9% of the Spissipes Section specimens collected. The behavior of these two species called for particular attention in the present paper for that reason.

Shannon traps.- The monthly distributions of Cx. ribeirensis and Cx. sacchettae are presented in Tables 2 and 3. Only females were obtained by the Shan GA. Considering the agricultural anthropic environment of the ES area it seems that those mosquitoes were mainly produced in the months of February-April when a total of 74.6% of the adults were caught. However, as a general feature, the remnant forest patches (Shan 1 and Shan 2) produced

Tabela 1 - Number of adult mosquitoes of the Spissipes Section of *Culex (Melanoconion)* collected in the anthropic (ES) and partially disturbed environments (GA) August 1992 through December 1993.

	ES	remn	ant fore	st	E\$ ope	n land	BC c	iwell	lings (hb)	G/	4			
Species	Shan 1		Shan 2		Shan E		Outdoor		Indoor		Shan GA		Total		
	f	m	f	m	f	m	f	m	f	m	f	m	f	m	Т
Cx. delpontei	1	-		1	5	2	_				_	-	6	3	9
Cx. faurani	2	12	2	1	-	•	-	-	-	-	-		4	13	17
Cx. lopesi	-	1	-	-	-	-	-	•	-	-	-	-	-	1	1
Cx. ocossa	23	-	2	•	102	7	3	1	-	•	1	-	131	8	139
Cx. pedroi	3	25	2	3	-	•	-	-	-	-	-	-	5	28	33
Cx. pereyrai	•	1	•	-	-	-	-	-	-	-	-	-	-	1	1
Cx. ribeirensis	1,436	63	533	22	403	4	134	1	92	-	405		3,003	90	3,093
Cx. sacchettae	1,113	59	979	78	338	5	274	•	258	-	162	-	3,124	142	3,266
Cx. spissipes	14	-	7	1	-	-	-	-	-	-	-		21	1	22
Cx. VR	19	-	29	-	-	-	1	•	•	•	1	-	50	-	50
Total	2,611	161	1,554	106	848	18	412	2	350	-	569	-	6,344	287	6,631

f - female

hb - human bait

I Station GA - "Galilléia Farm"

m - male Shan - Shannon trap BC - "Barra do Capinzal" Farm

ES - Experimental Station

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Table 2 - Monthly distribution of *Culex ribeirensis and Cx. sachettae* collected in Shannon traps in the anthropic environment (ES) area, January through December 1993.

		C	x. ribe	is	Cx	Cx. sacchettae				Total					
Monthy	Shan	fem	ale	ale m		fem	ale	m	ale	fema	ale	male		Т	
		n	%	n	%	n	%	n	%	n	%	n	%		
January	1	15	0.6	-	-	28	1.1	-	-	43	0.9		-	43	0.9
-	2	54	2.3	1	1.1	13	0.5	2	1.4	67	1.4	3	1.3	70	1.4
	Ε	34	1.4	-	-	6	0.2	-	-	40	8.0	•	-	40	8.0
February	1	659	27.8	41	46.0	97	4.0	5	3.5	756	15.7	46	19.9	802	15.9
	2	103	4.3	3	3.4	14	0.6	3	2.1	117	2.4	6	2.6	123	2.4
	Ë	3	0.1	-	-	2	0.1	-	-	5	0.1	-	-	5	0.1
March	1	313	13.2	18	20.2	237	9.7	30	21.1	550	11.4	48	20.8	598	11.9
	2	222	9.3	11	12.3	273	15.3	42	29.6	595	12.4	53	22.9	648	12.9
	Ε	152	6.4	4	4.5	241	9.9	2	1.4	393	8.2	6	2.6	399	7.9
April	1	149	6.3	•	-	598	24.6	16	11.3	747	15.6	16	6.9	763	15.2
	2	57	2.4	3	3.4	289	11.9	13	9.1	346	7.2	16	6.9	362	7.2
	Ε	32	1.3	•	-	25	1.0	-	-	57	1.2	•	-	57	1.1
Мау	1	66	2.8	-	-	96	3.9	3	2.1	162	3.4	3	1.3	165	3.3
	2	13	0.5	1	1.1	142	5.8	5	3.5	155	3.2	6	2.6	161	3.2
	E	21	0.9	-	-	8	0.3	-	-	29	0.6	-	-	29	0.6
June	1	9	0.4	-	-	-	-	-	-	9	0.2	•	•	9	0.2
	2	1	• • • •	-	-	-	-	-	-	1		-	-	1	
	E	29	1.2	-	-	9	0.4	-	-	38	8.0	-	-	38	0.8
July	1	67	2.8	1	1.1	9	0.4	2	1.4	76	1.6	3	1.3	79	1.6
	2	3	0.1	-	-	-	-	-	-	3		•	-	3	0.1
	E	22	0.9	•	-	4	0.2	-	-	26	0.5	-	-	26	0.5
August	1	19	8.0	-	-	7	0.3	-	-	26	0.5	-	-	26	0.5
	2	5	0.2	1	1.1	2	0.1	-	-	7	0.1	1	0.4	8	0.2
	E	1		-	-	-	-	-	-	1		•	-	1	
September	1	51	2.1	2	2.2	14	0.6	-	-	65	1.3	2	0.9	67	1.3
	2	24	1.0	-	-	109	4.5	10	7.0	133	2.8	10	4.3	143	2.8
	E	1		-	-	-	-	-	-	1		•	-	1	
October	1	22	0.9	-	-	7	0.3	3	2.1	29	0.6	3	1.3	32	0.6
	2	13	0.5	-	-	16	0.6	3	2.1	29	0.6	3	1.3	32	0.6
	E	25	0.2	-	-	15	0.6	3	2.1	40	0.8	3	1.3	43	0.9
November	1	51	2.1	-	-	13	0.5	-	-	64	1.3	-	-	64	1.3
	2	18	0.7	-	-	8	0.3	-	-	26	0.5	-	-	26	0.5
	E	54	2.3	-	-	10	0.4	-	-	64	1.3	•	•	64	1.3
December	1	15	0.6	1	1.1	7	0.3	-	-	22	0.5	1	0.4	23	0.4
	2	20	8.0	2	2.2	13	0.5	-	-	33	0.7	2	0.9	35	0.7
	E	29	1.2	•	•	18	0.7	•	•	47	1.0	-	-	47	0.9
Total	1	1,436	60.5	63	70.8	1,113		59	41.5		53.0	122	52.8	2,671	53.1
	2	533	22.4	22	24.7	979	40.2	78	54.9	1,512	31.4	100	43.3	1,612	
	E	403	17.0	4	4.5	338	13.9	5	3.5	741	15.4	9	3.9	750	14.9
Т		2,372	99.9	89	100.0	2,430	99.9	142	99.9	4,802	99.8	231	100.0	5,033	100.0

ES - Experimental Station

Shan - Shannon trap

85.1% of the total adults caught during the period, while 14.9% was obtained on the open land (Shan E).

In the partially disturbed environment of the GA site, the distribution pattern was quite irregular and no significant difference was observed.

Domiciliary environment.- Through the use of human bait, a total of 758 females mosquitoes was caught of which 226(29.8%) belonged to Cx. ribeirensis and 532(70.2%) to Cx. sacchettae.

With respect to collection sites the results were as follows (Tables 1 and 4):

	Indoor	Outdoor	Total
Cx. ribeirensis Cx. sacchettae			
Total	350	408	758

Table 3 - Monthly distribution of female *Culex ribeirensis* and *Cx. sachettae* collected in Shannon trap in the partially disturbed environment (GA), August 1992 through December 1993.

		Çx. rib	eirensis	Cx. sac	cchettae	Total		
	Months	n	%	n	%	n	%	
1992		_						
	August	73	18.0	2	1.2	75	13.2	
	September	141	34.8	3	1.8	144	25.4	
	October	5	1.2	3	1.8	8	1.4	
	November	16	3.9	24	14.8	40	7.1	
	December	-	-	58	35.8	58	10.2	
1993								
	January	15	3.7	9	5.6	24	4.2	
	February	1	0.2	2	1.2	3	0.5	
	March	25	6.2	20	12.3	45	7.9	
	April	40	9.9	11	6.8	51	9.0	
	May	6	1.5	-	-	6	1.0	
	June	4	1.0	1	0.6	5	0.9	
	July	5	1.2	1	0,6	6	1.0	
	August	45	11.1	10	6.2	55	9.7	
	September	6	1.5	5	3.1	11	1.9	
	October	1	0.2	3	1.8	4	0.7	
	November	20	4.9	•	-	20	3.5	
	December	2	0.5	10	6.2	12	2.1	
Total		405	99.8	162	99.8	567	99.7	

GA - "Galiléia Farm"

Table 4 - Monthly distribution of female *Culex ribeirensis* and *Cx. sachettae* collected by means of human bait in the domiciliary environment (BC), August 1992 through December 1993.

				Cx. ribe	irens	is	С	x. sacc	hetta	e			Tota		
	Months i		In	door	Out	tdoor	Inc	door	Out	door	Ind	door	Ou	tdoor	Т
			n	%	n	%	n	%	n	%	n	%	n	%	
1992															
	August	2	-	-	12	8.9	1	0.4	-	-	1	0.3	12	2.9	13
	September	2	3	3.2	-		1	0.4	1	0.4	4	1.1	1	0.2	5
	October	2	-	-	-	-	-	-	-	•	•		-	-	-
	November	2	30	32.6	23	17.2	6	2.3	8	2.9	36	10.3	31	7.6	67
	December	3	1	1.1	19	14.2	8	3.1	52	19.0	9	2.6	71	17.4	80
1993					•										
	January	2	14	15.2	19	14.2	5	1.9	25	9.1	19	5.4	44	10.8	63
	February	2	26	28.3	23	17.2	5	1.9	36	13,1	31	8.9	59	14.5	90
	March	2	12	13.0	10	7.5	171	66.3	23	8.3	183	52.3	33	8.1	216
	April	2	2	2.2	2	1.5	32	12.4	55	20.0	34	9.7	57	14.0	91
	Мау	2	1	1.1	2	1.5	23	8.9	42	15.3	24	6.9	44	10.8	68
	June	3	-	-	2	1.5	2	0.8	3	1.1	2	0.6	5	1.2	7
	July	2	1	1.1	1	0.7	1	0.4	5	1.8	2	0.6	6	1.5	8
	August	2	1	1.1	-	-	-	-	-	-	1	0.3	-	-	1
	September	1	-	-	1	0.7	-	•	1	0.4	-	-	2	0.5	2
	October	2	-	-	7	5.2	-	-	13	4.7	-	-	20	4.9	20
	November	3	-	-	6	4.5	3	1.2	6	2.2	3	0.9	12	2.9	15
	December	2	1	1.1	7	5.2		-	4	1.5	_1	0.3	11	2.7	12
Total		34	92	100.0	134	100.0	258	100.0	274	99.8	350	180.2	408	100.0	758

N = Number of catches

BC = "Barra do Capinzal" Farm

The Williams' general means values were as follows:

	Indoor	Outdoor
Cx. ribeirensis	0.46	0.53
Cx. sacchettae	0.50	0.57

The individual values applied for the estimation of the Williams' means were compared by the Mann-Witney text, which showed the absence of differences, at the level of 5.0%, between Cx. ribeirensis and Cx. sacchettae.

Thus biting females of both species may be caught in the domiciliary environment, with similar frequencies (Table 4). As a general feature the peak of abundance corresponded to the December-April period when 540 (71.2% of the total) females were collected. That peak roughly corresponds to that obtained through the Shannon trap sampling (Table 2) in the ES area when 3,566 (74.2% of the total) females were collected during the February-April period.

Synanthropy - As has already been mentioned the three environments studied were compared by means of the synanthropic indices (s) for both the mosquito species collected in these habitats. The results obtained were as follows:

	a(%)	b(%)	c(%)	\$
Cx. ribeirensis	7.5	26.9	65.6	
Cx. sacchettae	17.0	16.0	67.0	

Considering the general overview of this man-made environment, both mosquitoes appeared, in large measures, to retain their sylvatic habits.

Regarding the synanthropic ratios (sr) the overall value in the ES area, related to the remnant forest samples (Shan 1 and Shan 2) was 0.35 suggesting that these residual patches retain a greater number of both mosquitoes than the open land (Shan E) and the domiciliary environment (BC). Then, relating these latter two, the ratio obtained was 1.01, which shows no overall differences between these environments as regards the two culicids. Nevertheless, when the two species were considered separately the ratios obtained were the following:

Cx. ribeirensis
$$sr = \frac{Shan E}{Shan 1 + Shan 2} = 0.20$$
$$sr = \frac{BC}{Shan E} = 0.56$$
$$Cx. sacchettae
$$sr = \frac{Shan E}{Shan 1 + Shan 2} = 0.15$$
$$sr = \frac{BC}{Shan E} = 1.55$$$$

Thus, in the domiciliary environment (BC) Cx. sacchettae showed a higher synanthropy than Cx. ribeirensis when compared with the open land (Shan E) sampling.

Discussion

In a first attempt at an interpretation of the results obtained by the present research, the prevalence of Cx. ribeirensis and Cx. sacchettae among the Spissipes Section members must be considered. This fact was already noticed in previously published papers (Forattini et al. 5.6.13,14, 1986, 1993). Nevertheless this pattern must be taken as regional because in others regions of the Ribeira Valley others species may be prevalent. This happens in the Iguape area where Cx. delpontei, Cx. occssa and Culex VR, jointly with Cx. ribeirensis and Cx. sacchettae, showed a high frequency in the amount collected (Forattini et al. 9, 1990).

The main objective was to compare the adult abundance at the several sites. Two different techniques for sampling were employed, Shannon traps in the ouside environment and human bait in the domicilary one. This different aspects maybe influenced the results. However, according to the local circunstances, human bit was the more suitable sampling method that was used in dwellings.

The two residual patchy woods (Shan 1 and Shan 2) in the ES area accounted for the majority of the specimens of the two mosquito species caught. Jointly with the ES open land (Shan E), the monthly distribution showed a peak abundance corresponding to the February-April period. That corresponded to the results already reported (Forattini et al. 13, 1993) for Cx. ribeirensis caught at the same place. When the two environments are compared it would seem that the remnant forest represents something like an area of concentration from which adults of the two culicids disperse and reach the open land, seeing only immature stages

of *Cx*, *ribeirensis*, through as meaningless numbers, were found at the rice ES irrigation system (Forattini et al.¹², 1993).

As regards biting behavior in the domiciliary environment, the general values of Williams' mean showed no difference as between the two species, December-April being the period of peak abundance roughly corresponding with that at the outside ES areas. Nevertheless within the same period secondary peaks of Cx. sacchettae were observed regarding that remarkably appeared in high numbers in the March samples (Table 4). It is hard to find an adequate explanation for this, but perhaps, besides the human bait, peculiar conditions related to the attractiveness of the increased number of fowl kept there may contribute to explain it. As regards Cx. ribeirensis, a more regular behavior in this environment was observed, in accordance with the general pattern already described for the natural outside environment (Forattini and Gomes⁷, 1988).

Regarding the degree of synanthropy of these two mosquitoes, both indices suggest weak tendencies to adapt to the domiciliary environment. This seemingly disagrees with the estimate in relation to the primitive environment for which indices of +63.0 for *Cx. ribeirensis* and -3.0 for *Cx. sacchettae* were obtained (Forattini et al.¹³,

1993). Nevertheless the comparisons were made in the light of the human dominated environment as a whole. Indeed the anthropic one studied here included several different features each one with its own characteristics. Thus the remnant forests. the cultivated open land and the dwelling houses represente quite distinct aspects of the man-made organized environment. As was mentioned above the patches of residual forest may fulfil both the preserving and the concentration roles for these mosquito populations. So it is quite understandable that they retained their sylvatic behavior when compared with their behavior in others places. Despite the comparison of their synanthropic ratios (sr), it became clear that, relating the open land to the domiciliary environment, the two culicids showed a higher frequency in the latter. Cx. sacchettae at least seems to have preferred the houses over the open land. As a general aspect, both species have showed a clear tendency towards domiciliation (Forattini et al.8, 1989).

In conclusion, the present research permits to consider Cx. ribeirensis and Cx. sacchettae as potentially synanthropic, at least in the Ribeira Valley region. As their competence for virus transmission has been reported, it is justifiable to pay attention to these mosquitoes in the anthropic environment.

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Resumo

Relatam-se os resultados obtidos mediante a coleta regular de adultos representantes da Seção Spissipes de Culex (Melanoconion) realizada em ambiente antrópico do Vale do Ribeira, SP, Brasil, no período de agosto de 1992 a dezembro de 1993. Foi possível evidenciar a dominância de Culex ribeirensis e de Cx. sacchettae se bem que, no total, tenham sido identificadas dez espécies desse grupo. Esses dois mosquitos revelaram clara tendência a freqüentar as casas onde foram coletados, tanto no intra como no peridomicílio, mediante o emprego da isca humana. No ambiente extradomiciliar, ao que parece, as matas residuais desempenhariam o papel de concentração dessas populações, de onde os adultos sairiam para o terreno aberto cultivado e alcançariam as moradias. Como a competência vetora dessas espécies tem sido revelada mediante o isolamento de vírus em condições naturais, é de se recomendar que mereça atenção devida, a presença desses mosquitos no ambiente artificial humano.

Culicidae. Ecologia de vetores. Culex.