Diversity of Biting Midges of the Genus *Culicoides* Latreille (Diptera: Ceratopogonidae) in the Area of the Yacyretá Dam Lake between Argentina and Paraguay

María M Ronderos/+, Nancy M Greco*, Gustavo R Spinelli

Departmento Científico de Entomología, Museo de La Plata, Paseo del Bosque s/no., 1900 La Plata, Argentina
*CEPAVE (UNLP-CONICET), La Plata, Argentina

The Culicoides *communities have been analyzed between 1993/1998 in the area influenced by the Yacyretá Dam Lake (Paraná River, Argentina-Paraguay). Adults of* Culicoides *were collected monthly by using CDC light traps exposed for 24 h in 9 sampling sites located at both margins of the river; 21 species were recorded. Highest values of species richness were recorded during 1993/1994, being Quiteria and Corpus the sites with the higest number of species (10 and 11, respectively). The species diversity was elevated in Quiteria, Zaimán, Candelaria, Santa Tecla, Capitán Meza and Corpus (Shannon's diversity index 1.0-1.9) while Corateí, Ituzaingó and Aguapey showed less richness and diversity. The more abundant species were* C. insignis, C. venezuelensis, C. leopoldoi, C. limai, C. flinti, C. debilipalpis, C. paraensis *and* C. guttatus. C. insignis, *potential vector of bluetongue virus (BTV) to domestic and wild rumiants in the Neotropical region, is the predominant species in the area and was the only species widely distributed.* C. paraensis, *a proven vector of Oropouche virus to humans, is a common and abundant species.* C. pusillus *and* C. lahillei, *potential vectors of BTV and a filarial parasite, respectively, were occasionally collected. The taxonomic structure of communities was constant during the study period. The occasional species were not characteristic to one particular site and their presence could be related to non-intrinsic conditions.*

Key words: diversity - *Culicoides* - Yacyretá Dam Lake - Argentina - Paraguay

The largest area influenced by the Yacyretá Dam Lake, located in the Paraná River between Argentina and Paraguay, offers adequate breeding sites for hematophagous nematocerous Diptera. The ceratopogonid biting midges of the genus *Culicoides* Latreille, which are well known as notorious blood sucking pests on man and animals throughout the world (Wirth et al. 1988, Greiner et al. 1990), are represented in the area by 22 species (Ronderos & Spinelli 1998, in press). Some of these species are proven vectors of viruses in the Neotropical region, e.g. *C. paraensis* (Goeldi) transmitting "oropouche" virus (OROV) to humans (Pinheiro et al. 1982, Linley et al. 1983, Degallier et al. 1998, Mellor et al. 2000). Other species are potential vectors, e.g. *C. insignis* Lutz and *C. pusillus* Lutz for the bluetongue virus (BTV) to domestic and wild ruminants (Greiner et al. 1984, 1990, Homan et al. 1990, Tanya et al. 1992, Sáenz & Greiner 1994, Mellor et al. 2000) and *C. lahillei* (Iches) for the filarial parasite *Mansonella ozzardi* Manson to humans in Northern Argentina (Shelley & Coscarón 2001). Many of them, e.g. *C. debilipalpis* Lutz, *C. lahillei* and *C. paraensis* are also well known by the inhabitants of the area by their annoying disturbance of fishermen, farmers, and tourists at recreational resorts.

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+Corresponding author. Fax: +54-221-425727. E-mail: ronderos@museo.fcnym.unlp.edu.ar
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In spite of the sanitary and economic relevance of the genus, and the adequate achieved knowledge on its taxonomy for the region (Ronderos & Spinelli 1998), there is no information available on the major features of their communities. The area is characterized by the existence of several different breeding environments, so local variations in *Culicoides* assemblages are expected. Likewise, at the community level, the temporal structure of an assemblage may greatly change in terms of both, numbers and relative abundance of the species. Analyses of temporal constancy from assemblages are desirable in order to determine whether the patterns observed up to date are representative.

The aim of this paper is to describe and analyse the major features of the *Culicoides* communities, determining the species composition and the relative abundance and constancy of the potential vector species in the area influenced by the Yacyretá Dam Lake.

MATERIALS AND METHODS

Study area - The study area, located between 27ºS and 28ºS along the Paraná River (Fig. 1), is characterized by high relative humidity and an annual average temperature of 21.5ºC (ranging between 15ºC and 27ºC). Nine sampling sites were selected at both margins of the Paraná River, currently transformed in a dam lake, between Candelaria and Ituzaingó on its left shore (Argentina), and Capitán Meza and Corateí on its right shore (Paraguay). Most of them were located in natural environments of the subtropical forest corridors with abundant streams, being the ones located in Ituzaingó, Quiteria and Zaimán periurban or urban localities.

The stations were selected in order to obtain an adequate representation of both sides of the river, also con-

Fig. 1: area of the Yacyretá Dam Lake (Argentina, Paraguay). Sampling sites: Corateí, Ituzaingó, Aguapey, Santa Tecla, Quiteria, Zaimán, Candelaria, Capitán Meza and Corpus.

cerning their locations at similar stretches. In the particular case of Corpus and Corateí, they were selected considering that construction of an hydroelectric complex is projected between these two sites in the near future, so the obtained data could be available for future impact assessment.

Sampling of Culicoides - Adults of *Culicoides* were monthly collected by using CDC light traps (Service 1976) exposed at each sampling station for 24 h. A portable automobile battery supplied a 12v/15w light source. Biting midges were also attracted to the trap by a constant rate of CO_2 liberation of 500 ml/min. The individuals collected were preserved in isopropylic alcohol. The sampling periods ranged from July 1993 to June 1994 (9 sites), from July 1995 to June 1996 (9 sites), and 1997 and 1998 from February to September (4 sites). The abundance of the species in the four sites surveyed during the hole collected period (Corateí, Santa Tecla, Aguapey and Corpus) was analyzed for the study of temporal variations in the community structure. The collected specimens were deposited in the collection of the Museo de La Plata, La Plata, Argentina.

Data analysis - Relative abundance of *Culicoides* species for each study site and year was calculated as the abundance of each one in relation to the total abundance of all collected species. The species richness (total number of species in the communities) at each site for each year was estimated. The diversity was assessed by Shannon's diversity index (Begon et al. 1996).

In order to examine the distribution of the species in hierarchy groups over the entire region, they were ranked by frequency of site-year presence. The proportion of the species that were uncommon (present at < 25 % of the 26 total site-years), intermediate (present at > 25 and < 75 % of the 26 total site-years) and widespread (present at ≥ 75 % of the 26 total site-years) distributed was computed. The relative abundance of species in each hierarchy group was compared by the Mann-Whitney test (Zar 1996).

The constancy in the taxonomic structure of these communities was measured using Kendall's Coefficient of concordance (W) (Siegel 1991), based on the ranks of the abundance of the top four species among years in the four sites that were sampled during all the study period (Corateí, Santa Tecla, Aguapey and Corpus).

A binary matrix challenging the 21 species and the 9 collecting sites was organized based on the presence or absence of the *Culicoides* species in a given site. Euclidean distances were used to measure the distances among

Culicoides communities. To explore the existence of a grouped distribution pattern among the species, a cluster analysis was conducted using the unweighted pair of group's method (Sneath & Sokal 1973).

RESULTS

Twenty one species of *Culicoides* were captured during the entire sampling period. During 1993/1994 the predominant species were *C. insignis*, *C. leopoldoi*, *C. limai*, *C. flinti* and *C. venezuelensis* (98%); in 1995/1996 *C. insignis*, *C. leopoldoi*, *C. venezuelensis* and *C. limai* (71%); in 1997 *C. insignis*, *C. debilipalpis*, *C. leopoldoi*, *C. paraensis* (98%), and in 1998 *C. insignis* and *C. limai* (96%). Relative abundance, richness and diversity of species are presented in Tables I and II. Those species that only appeared in 1993/1994 showed a low relative abundance. Species richness and diversity were variable between years and sample sites. The highest values of species richness were recorded in Quiteria, Corpus, Santa Tecla and Capitán Meza. The species diversity was highest in Quiteria, Corpus, Santa Tecla, Capitán Meza, Zaimán and Candelaria. Corateí, Ituzaingó and Aguapey showed less richness and diversity. Richness and diversity decreased during 1998 at all sites.

The community structure is summarized in the Fig. 2, combining frequency and relative abundance of all collected species. There was a high number of species narrowly distributed (occasionally present), whose relative abundance ranged between 0.001 and 0.75. Likewise, the intermediate species distribution (common and very common) showed significantly higher relative abundance ($U = 15$, $P = 0.015$, $n_1 = 13$, $n_2 = 7$). *C. insignis* was the only species widely distributed (abundant).

The analysis of the relative abundance between 1993 and 1998 (Table III) showed a little variation among the four top ranked species, *C. insignis* being the most abundant. Considering only these four species, the taxonomic structure of communities was constant along the studied period, except for Santa Tecla (Corateí: $W = 0.69$, $P < 0.05$; Aguapey: $W = 0.93$, $P < 0.01$; Santa Tecla: $W = 0.53$, $P > 0.05$ and Corpus: $W = 0.74$, $P < 0.05$).

According to the presence of the different species along the sampling period, the cluster analyses indicated two groups (Fig. 3). One of them showed an increasing gradation of sites and the distance between them was determined by the distribution of occasional species. The other group, represented by the nearest localities Corateí and Ituzaingó, is characterized by the presence of the three common and abundant species (*C. insignis*, *C. venezuelensis* and *C. paraensis*) showing low richness as well as low diversity.

DISCUSSION

A high percentage (64.7%) of the 34 species recorded from Argentina by Ronderos and Spinelli (in press) is represented in the studied communities herein.

From the results, and considering the species real or potential involvement in virus transmission, it is clear that *C. insignis* is the dominant species. *C. paraensis* is a common and abundant species associated to *C. insignis* and *C. venezuelensis* in Corateí and Ituzaingó. *C. pusillus* is narrowly distributed (Candelaria, Capitán Meza and Corpus) and shows a moderate relative abundance. *C. lahillei* is also occasional (Santa Tecla, Quiteria, Capitán Meza and Corpus).

C. insignis has also been reported previously as predominant species in the area of Salto Grande Dame Lake

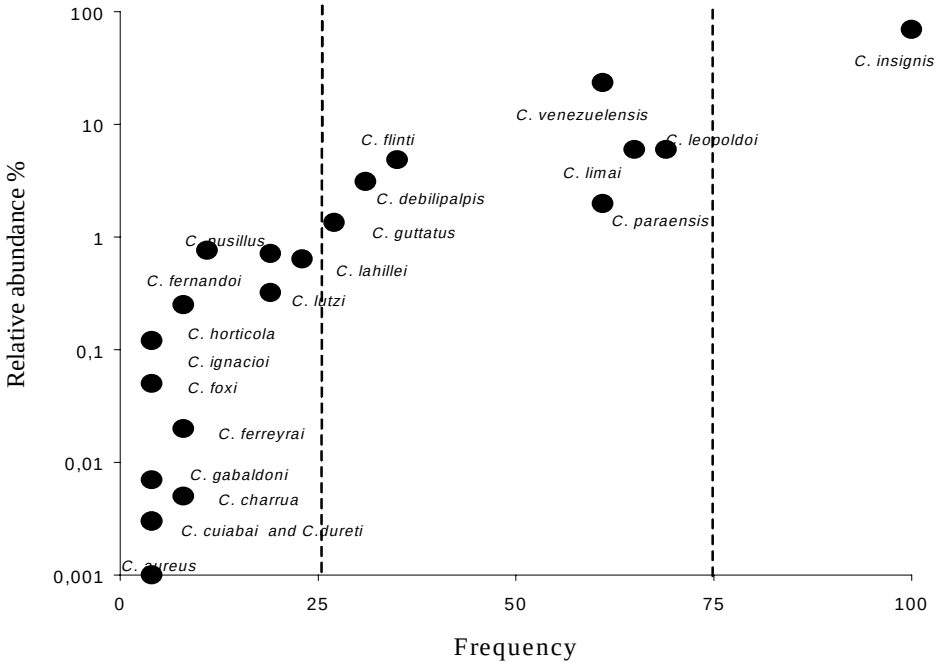

Fig. 2: frequency and relative abundance of *Culicoides* species in the area of the Yacyretá Dam Lake. Frequency of 0-25%, 25-75% and 75-100% correspond to occasionally, common and very common, and abundant species, respectively.

TABLE I

Relative abundance of collected *Culicoides* spp. at each sampling site of the area of the Yacyretá Dam Lake in 1993/1994 and 1995/1996

Years	1993/1994									1995/1996								
Sites	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Species																		
C. aureus		0.03																
C. charrua			0.08						0.05									
C. cuiabai		0.01																
C. debilipalpis											0.36	0.23		1.47	5.00		2.27	12.50
C. dureti					0.12													
C. fernandoi				6.40			9.10											4.16
C. ferreyrai						0.70			0.05									
C. flinti						48.90	0.90	55.80	3.90	1.72			5.42	5.80			2.27	
C. foxi																		
C. gabaldoni					0.18													
C. guttatus					0.44		1.80		5.60					2.94		17.30	6.81	
C. horticola				6.40					1.10									
C. ignacioi				3.20														
C. insignis	90.00	99.90	91.70	38.70	57.30	47.60	46.70	30.80	79.70	65.00	99.00	83.40	85.20	34.00	45.00	36.90	36.30	41.70
C. lahillei				3.20	0.60			0.80	0.05					2.90				
C. leopoldoi			3.60		16.40		22.20	10.80	1.70	5.20		10.20	0.80	2.90	15.00	19.50	34.10	8.30
C. limai			2.10	22.50	18.20	2.00	17.30		4.50			5.80	0.80	5.80	10.00	17.30	4.50	20.80
C. lutzi			1.90	3.22		0.70		0.80		1.70								
C. paraensis	3.00	0.02		12.90	1.50			0.80	0.05	1.70	0.40		0.80	8.80		2.20	2.30	4.16
C. pusillus							1.50		1.10			0.20				6.50	9.10	
C. venezuelensis	6.10	0.02	0.50	3.22	4.90		0.40			24.00			6.90	34.00	25.00		2.30	8.30
No. of individuals	65	11,223	1,236	30	1,582	147	451	120	177	58	273	429	139	70	20	46	44	24
Richness	3	6	6	9	10	5	8	6	11	6	3	5	6	9	5	6	9	7
Diversity	0.37	0.01	0.39	1.76	1.21	1.00	1.90	1.53	0.58	0.98	0.05	0.58	0.59	1.65	1.37	1.55	1.65	1.63

Sampling sites: 1: Corateí, 2: Ituzaingó, 3: Aguapey, 4: Santa Tecla, 5: Quiteria, 6: Zaimán,7: Candelaria, 8: Capitán Meza, 9: Corpus

TABLE II

Relative abundance of collected *Culicoides* spp. at each sampling site of the area of the Yacyretá Dam Lake in 1997 and 1998. Sampling sites: 1: Corateí, 3: Aguapey, 4: Santa Tecla, 9: Corpus

Years	97				98			
Sites	1	3	4	9	1	3	4	9
Species								
C. aureus								
C. charrua								
C. cuiabai								
C. debilipalpis			50.00	11.00				
C. dureti								
C. fernandoi								
C. ferreyrai								
C. flinti								2.20
C. foxi	1.40							
C. gabaldoni								
C. guttatus		0.09						
C. horticola								
C. ignacioi								
C. insignis	93.00	98.00	33.00	44.00	94.90	94.00	88.80	94.70
C. lahillei				11.00				
C. leopoldoi	1.40	1.00	8.30	11.00		6.00		1.30
C. limai		0.30	8.30				11.10	1.30
C. lutzi								
C. paraensis	4.00	0.10		11.00				
C. pusillus								
C. venezuelensis		0.50		11.00	5.10			0.40
Number of individuals	73	1,092	32	41	39	133	29	227
Richness	4	6	4	6	2	2	2	5
Diversity	0.32	0.12	1.13	1.58	0.20	0.23	0.35	0.27

TABLE III

Relative abundance of predominant *Culicoides* spp. in Corateí, Aguapey, Santa Tecla and Corpus

Species	Mean	SD
	Coratei	
C. insignis	85.70	13.90
C. venezuelensis	8.80	10.50
C. leopoldoi	2.44	1.64
C. paraensis	2.18	1.72
	Aguapey	
C. insignis	91.78	6.13
C. leopoldoi	5.21	3.93
C. limai	2.04	2.68
C. venezuelensis	0.26	0.30
	Santa Tecla	
C. insignis	61.45	29.70
C. debilipalpis	12.50	25.00
C. limai	10.69	9.05
C. leopoldoi	5.54	4.63
	Corpus	
C. insignis	65.02	26.34
C. limai	6.65	9.62
C. leopoldoi	5.58	4.84
C. venezuelensis	4.93	5.57

between Argentina and Uruguay (Spinelli and Ronderos 1991). Besides, communities associated to cattle showed *C. insignis* as the most important species (Kramer et al. 1985, Sáenz and Greiner 1994).

The highest values of species richness and diversity were registered in the localities situated in the northern part of the study area. This could be related to the existence in the mentioned zone of subtropical forest habitats housing different kinds of breeding sites for *Culicoides* spp. Mullen and Hribar (1988), Hribar and Mullen (1991), Murphree and Mullen (1991), and Hribar (1993) reported tree holes, stump holes, and other rotting woods as common environments for immature stages of several species of the genus.

Common (and rare) species tended to be typically common (and rare) from year to year, during the entire study. Each assemblage was reasonably predictable. Some species were not characteristic of any particular site and their presence could be related to non-intrinsic conditions (i.e., high temperature and abundant rains in 1993-1994).

More ecological studies at the population and community levels are necessary to describe and to explain the abundance and relative importance of the potential vectors species in the communities of this region, especially considering the roles of *C. insignis* and *C. paraensis* as vectors of BTV and OROV, respectively.

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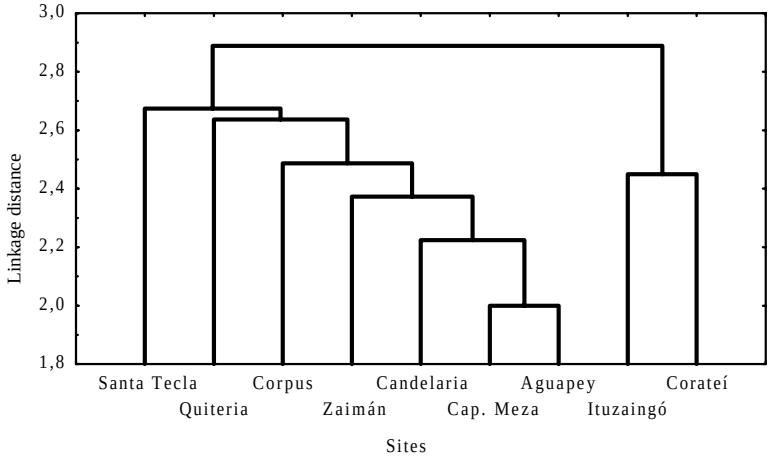

Fig. 3: Tree diagram (Euclidean distances) for presence or absence of the *Culicoides* species in the sampling sites of the area of the Yacyretá Dam Lake.

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