Ceratopogonidae (Diptera: Nematocera) of the foothill of the Yungas forest of Tucumán: ecology and distribution

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Many genera of the Ceratopogonidae family transmit numerous diseases to humans and animals, while other genera act as important pollinators of tropical crops. In the Yungas region of Argentina, previous systematic and ecological research on Ceratopogonidae focused on *Culicoides*, since they are the main transmitters of mansonelliasis in northwestern Argentina.; however, few studies included the genera *Forcipomyia*, *Dasyhelea*, *Atrichopogon*, *Alluaudomyia*, *Echinohelea*, and *Bezzia* Therefore, the objective of this study was to determine the presence and abundance of Ceratopogonidae in this region, their association with meteorological variables, and their variation in areas disturbed by human activity. Monthly collection of specimens took place from July 2008 to July 2009 using CDC miniature light traps deployed for two consecutive days. A total of 361 specimens were collected, including *Dasyhelea* (47.92%) as the most abundant, followed by *Forcipomyia* (26.86%). Bivariate analyses showed significant differences in the abundance of the genera in different sampling sites and climatic conditions, with the summer season and El Corralito site showing the greatest abundance of specimens. Accumulated rainfall was the variable most closely related to the abundance of *Culicoides* (10.52%), while temperature was the variable most closely related to the abundance of *Forcipomyia*, *Dasyhelea*, and *Atrichopogon*

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ABSTRACT

Many genera of the Ceratopogonidae family transmit numerous diseases to humans and animals, while other genera act as important pollinators of tropical crops. In the Yungas region of Argentina, previous systematic and ecological research on Ceratopogonidae focused on *Culicoides*, since they are the main transmitters of mansonelliasis in northwestern Argentina.; however, few studies included the genera *Forcipomyia*, *Dasyhelea*, *Atrichopogon*, *Alluaudomyia*, *Echinohelea*, and *Bezzia* Therefore, the objective of this study was to determine the presence and abundance of Ceratopogonidae in this region, their association with meteorological variables, and their variation in areas disturbed by human activity. Monthly collection of specimens took place from July 2008 to July 2009 using CDC miniature light traps deployed for two consecutive days. A total of 361 specimens were collected, including *Dasyhelea* (47.92%) as the most abundant, followed by *Forcipomyia* (26.86%). Bivariate analyses showed significant differences in the abundance of the genera in different sampling sites and climatic conditions, with the summer season and El Corralito site showing the greatest abundance of specimens. Accumulated rainfall was the variable most closely related to the abundance of *Culicoides* (10.52%), while temperature was the variable most closely related to the abundance of *Forcipomyia*, *Dasyhelea*, and *Atrichopogon*

Subjects Entomology, Ecology, Epidemiology

Keywords Montane forests, Anthropic areas, Argentina.

INTRODUCTION

Ceratopogonidae family constitutes a much diversified and globally widespread group of nematocerans. Nowadays, it is represented by 6180 species, 111 living genera and 4 subfamilies (Ceratopogoninae, Leptoconopinae, Forcipomiinae and Dasyheleinae) (Borkent 2014).

Austroconops Wirth & Lee (only one australian species), *Culicoides* Latreille*,* *Leptoconops* Skuse and *Forcipomyia* Meigen (subgenus *Lasiohelea*) are implied in the transmission of arbovirus, parasites and protozoa which affect humans and animals (Mellor *et al.* 2000; Borkent & Spinelli 2007; Veggiani Aybar *et al.* 2010a, 2015b). Other genera proportion important services in ecological systems, with *Forcipomyia* y *Dasyhelea* and, to a lesser extent, *Atrichopogon*, *Culicoides* y *Stilobezzia* as potential pollinators of different crops, such as cocoa (*Theobroma cacao*), rubber (*Hevea brasiliensis*) and mango (*Mangifera indica*) in tropical regions (Borkent & Spinelli 2007; Bravo *et al.* 2011); while some species of *Forcipomyia* and *Culicoides* are ectoparasites of insects, by sucking the lymph of lepidopterans, coleopterans, odonata, phasmids, neuropterans and hemipterans (Borkent 2004). Also, *Ceratopogon*, *Bezzia*, *Brachypogon*, *Monohelea, Serromyia, Stilobezzia*, *Palpomyia* (Bernotienė 2006), *Allohelea* (Werner & Kampen 2010), *Ceratoculicoides* (Huerta & Borkent 2005), *Alluaudomyia* and *Echinohelea* (Borkent & Spinelli 2007) are predators of small flying insects of the same or smaller size. However, the relevance of this family is given by *Culicoides* genus, which is vector of the bluetongue virus, the equine encephalitis virus, the Schmallenverg virus, among others, affecting ovine and bovine cattle (Mellor *et al.* 2000; Carpenter *et al.* 2013); and of the transmission to humans of the Oropouche virus, the nematode *Mansonella* and the Trypanosomatidae *Leishmania* (Mellor *et al.* 2000; Ronderos *et al.* 2003; Borkent 2004; Slama *et al*. 2014).

In northwestern Argentina, studies focused mainly in *Culicoides* genus due to its epidemiologic relevance as the vector of filaria *Mansonella ozzardi* (Shelley & Coscarón 2001; Veggiani Aybar *et al.* 2015a, 2016); however, the study of other families of Ceratopogonidae in the region is of great need. Thus, the aim of this study was to determine the presence and abundance of the main genera of Ceratopogonidae in piedmont forests of Tucumán province, and to determine the effect of meteorological variables in their distribution

MATERIALS AND METHODS

Characterization of the study area

This study took place at Juan Bautista Alberdi department (27°35’05.89”S; 65°37’11.70”O; 400 masl), Tucumán province (Fig. 1). The area corresponds to Yungas phytogeographic region, specifically to the altitudinal tier of piedmont forest.

The piedmont forests extends between 700 and 1000 masl and exhibits a subtropical climate, with mean rainfall ranging from 700-1000 annual mm and concentrated in summer months (November to April); a mean maximum temperature of 27.6° C and a mean minimum temperature of 15.4° C (Brown & Grau 1995; Malizia *et al.* 2012). Native vegetation is arboreal of closed canopy, while near roads and at the edges of streams vegetation is open. Among canopy trees, *Blepharocalyx salicifolius*, *Enterolobium contorsiliquum*, *Juglans australis* and *Parapiptadenia excelsa* are the more frequent, while in the undergrowth *Piper tucumanum*, *Eugenia uniflora*, *Urera baccifera* and *Solanum riparium* are found; also, there are many species of lianas of Bignoniaceae, Ulmaceae and Amarantaceae families, and vascular plants with epiphyte habits, belonging to Polipodaceae, Asplaniaceae, Piperaceae and Bromeliaceae. In open areas, the most common arboreal species are *Tipuana tipu, Jacaranda mimosifolia,* *Anadenanthera colubrina var. cebil, Tabebuia avellanedae, Heliocarpus popayanensis, Fagara coco, Tecoma stans, Salix humboldtiana* and *Carica quercifolia* (Grau 2005; Brown *et al.* 2006).

Despite the climatic variability, rises in mean annual rainfall in the last years have been detected, as a consequence of the replacement of native vegetation and increases in extensive crops (sugarcane, tobacco, fruit trees, among others), which caused important modifications in the landscape (Brown & Malizia, 2004).

Collecting sites

Based on environmental and socio-demographic characteristics and operational accessibility, a total of 10 households were considered for sampling (five paired sampling sites, Fig. 1). The following sampling sites were selected: El Corralito (EC1: 27°37’25,2”S; 65°42’59,9”O and EC2: 27°37’56,9”S; 65°41´23,9”O), El Badén (EB1: 27°37’13,9”S: 65°41’39,6”O and EB2: 27°37’27,2”S; 65°41’32,0”O), Yánima (YA1: 27°37’58,8”S; 65°39’13,7”O and YA2: 27°37’49,4”S; 65°39’19,2”O), Bajo Marapa (BM1: 27°37’30,3”S; 65°38’00,5”O and BM2: 27°37’28,4”S; 65°38’07,1”O) and Marapa Central (MC1: 27°36’46,6”S; 65°38’01,2”O and MC2: 27°36’45,1”S; 65°38’08,1”O).

Households were georeferenced and characterized through an *ad-hoc* survey, using the criteria of “worst scenario”. Such methodology is employed for the study of Phlebotominae subfamily and defines sites with features such as shade presence, moist soils, organic detritus, and epidemiological records, among others; with higher probability of finding the individuals of interest (Feliciangeli *et al.* 2004; Correa Antonialli *et al*. 2007).

Collection and processing of specimens

Adult specimens were collected monthly from July, 2008 to July, 2009 with CDC mini light traps (Sudia & Chamberlain 1962), placed from 18 pm to 07 am for two consecutive days.

Posteriorly, specimens were taken to the laboratory, where they were separated and identified following Spinelly & Wirth (1993) and *Spinelli et al.* (2005) taxonomic keys.

Data analysis

The obtained data was spread in a sheet for their analysis with InfoStat 2016e version statistical software (Di Rienzo *et al.* 2016). Genera abundance by season and sampling site was compared and bivariate statistical analysis (chi squared test) were applied. In all cases, Cramer V coefficient was used to measure the association or independence among the considered variables, taking values between 0 (weak association) to 1 (strong association). Posteriorly, multiple regression analyses (stepwise method) were performed. This method is based on the study of the possible relation between a response Y variable (dependent variable) and two or more X variables (independent or predictor variables). Thus, it analyzes how changes in predictor variables affect the response variables through the adjustment of a model for such relationship:

Multiple regression equation:

$$\mathbf{Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \ldots\ldots + \beta_k X_{ki} + \varepsilon_i}$$

Where,

Yi: is the dependent variable, which is interpreted as a lineal combination of a set of *k* independent variables (Xk), each of which is accompanied by a βk coefficient.

β_0: is a constant which represents the intercept (the point where the straight line intercepts the vertical axis).

β_k: are the unknown parameters which represent the change rates of Y under one unit change in X_1, X_2..., X_k, respectively. It indicates the relative weight of each predictor variable in the equation and represents the slope of the straight line.

X_k: represent the independent variables.

ε_i: is the random error term, which encompasses all the variation that independent variables fail to explain (Balzarini *et al*. 2008)

Mean abundance values (dependent variable) where standardized using log (n+2). The meteorological variables (independent variables) considered in this study were: temperature (T), rainfall (R), relative humidity (Rh), wind speed (Ws) and maximum wind speed (maxWs), which were monthly averaged. Meteorological data was obtained from the Agro-meteorology department of Estación Experimental Agroindustrial Obispo Colombres, Tucumán province. All the results were considered as significative if $p \leq 0.05$.

RESULTS

A total of 361 Ceratopogonidae specimens, belonging to *Alluaudomyia, Atrichopogon, Bezzia, Culicoides, Dasyhelea*, *Echinohelea* and *Forcipomyia* genera were collected.

Of these seven genera, the most abundant were *Forcipomyia* (26.86%), followed by *Culicoides* (10.52%), which represented 40% of the total, while the other 60% corresponded to *Dasyhelea* (47.92%), *Atrichopogon* (13.85%), and *Alluaudomyia*, *Echinohelea* y *Bezzia* (0.38%, respectively) (Table 1).

Chi-squared Analysis

When considering specimens total abundance, it was higher in El Corralito (EC, 41%), followed by Marapa Central (MC, 18.56%) and El Badén (EB, 17.73%). Yánima (YA) and Bajo Marapa (BM) exhibited similar abundances (11.63% and 11.08%, respectively).

For the bivariate analyses, the four more abundant genera were considered. Significant differences among sampling sites, seasons and genera were observed (Table 2).

Dasyhelea genus was the most abundant in autumn, winter and summer, and at El Corralito, El Baden, Bajo Marapa and Marapa Central sites, followed by *Forcipomyia*, which overcame it in spring and at Yánima site. In turn, *Atrichopogon* was the most abundant genus in autumn, winter and summer at El Corralito, El Baden and Bajo Marapa site; while *Culicoides* was more abundant in spring and at Yánima and Marapa Central sites. Finally, there was an increase in the abundance of the seven genera in all the sampling sites compared to warmer seasons along the study period.

Regression analyses

The multiple regression analyses allowed obtaining the following descriptive models: for *Culicoides* genus, the regression analysis between the abundance of specimens and climatic variables determined a significant correlation with accumulated rainfall ($R^2 = 0.46$; $P < 0.0157$) (Fig. 2A); while temperature was the strongest related variable with the abundance of *Forcipomyia* ($R^2 = 0.32$; $P < 0.0561$) (Fig. 2B), *Dasyhelea* ($R^2 = 0.59$; $P < 0.0035$) (Fig. 2C) and *Atrichopogon* species ($R^2 = 0.42$; $P < 0.0221$) (Fig. 2D).

Partial and predicted residuals

From the partial residuals (Fig. 3 A-D), a positive lineal relation was observed between *Culicoides* abundance and accumulated rainfall, while the same relation was observed between temperature and *Forcipomyia*, *Dasyhelea* and *Atrichopogon*, although less marked in the latter. In turn, standardized versus predicted residuals (Figs. 4 A-D) determined a dispersed point cloud, which indicated that the used model was valid for three of the four studied genera. Finally, the points trend of *Atrichopogon* was negative, indicating that the model was not suitable for the regressor variable retained by the model.

DISCUSSION

In the present work, the abundance of Ceratopogonidae genera in a strongly modified and human transformed Yungas area was registered. The abundance of specimens varied among seasons and study sites, with a differential pattern observed mainly in the warm season and at El Corralito, Marapa Central, El Badén, Yánima and Bajo Marapa sites. Such differences in abundance could be due to that suggested by Borkent & Spinelli (2007), who mentioned that most members of Ceratopogonidae family require of environments with high humidity for their development, with the humid season as the more suitable for them to complete their life cycle; since many of the genera go through winter in the later larval stage, similarly to what occurs at temperate regions of the north. Also, the available food sources in the study area, such as barnyard animals (chicken, pigs and horses) or humans in the case of hematophagous genera; and vegetal food sources such as flowers and fruits for pollinators should be considered; as well as the suitable environments for the development of immature stages, which can be aquatic, semi-aquatic or terrestrial.

Regarding seasonality of *Culicoides* genera in northwest Argentina, Veggiani Aybar *et al.* (2010a, 2012) determined population peaks during summer, autumn and spring to a lesser extent in Tucumán province, and during spring and summer but gradually diminishing towards winter in Salta province; in agreement with that reported in the present study.

Among the collected genera, *Culicoides* and *Forcipomyia* exhibit public health significance; the former excelling as the main vector of *M. ozzardi* in the region. However, *Forcipomyia* genus (*Lasiohelea* subgenus), scarcely studied in the province, might be involved not only in the transmission of Mansonelosis but also in the transmission of other viruses and protozoa, which would represent a potential risk for the region, especially considering their abundance both in the present and in other studies carried out in Northwest Argentina (Veggiani Aybar *et al.* 2010b, 2015b). On the other hand, it is worth mentioning that in the last years, the global infection of *Culicoides* species with *Leishmania infantum* (LV) has been determined (WHO, 2010). In the study area, Salomon et al. (2006) determined the spatial and temporal distribution of risk and the regional epidemiological trends of tegumentary leishmaniasis (TL), other type of *Leishmania* which is endemic of argentine Yungas. The here presented data in addition to other research in the area (Veggiani Aybar *et al.* 2010a, 2012, 2015a) is a starting point for continuing with the study of this genus in areas where its distribution matches that of the family Psychodidae, which are the main vectors of this parasite in the region (Córdoba Lanús & Salomón 2002; Salomón *et al.* 2006; Quintana *et al.* 2012).

Regarding the influence of climatic variables over the abundance of medical and veterinarian important genera, accumulated rainfall and temperature were significantly important for *Culicoides* and *Forcipomyia*, respectively. In relation to this, Veggiani Aybar *et al.* (2010b, 2011) also observed that in Tucumán province, the higher incidence of *Culicoides* was mainly associated with accumulated rainfall, followed by relative humidity, wind speed and mean temperature, although the last were not significant in the present study. On the other hand, other studies in Salta province determined that the abundance of *Culicoides* positively correlated with both temperature and relative humidity (Veggiani Aybar *et al.* 2012). Also, several authors have informed the direct relation between temperature, rainfall and humidity with the abundance of *Culicoides* in Brazil, due to the influence of these climatic variables over the life cycles of the species or the alteration in their breeding sites (Sherlock & Guitton 1964; Santos da Silva *et al.* 2001; De Barros *et al.* 2007).

About *Forcipomyia*, *Dasyhelea* and *Atrichopogon*, as it has previously been mentioned, studies assessing ecological aspects of these species in northwest Argentina are scarce. However, Veggiani Aybar *et al.* (2010b, 2015b) reported the presence and abundance of these genera in Argentine and Bolivian Yungas, as well as the presence of *Brachypogon, Monohelea*, *Stilobezzia* and *Clinohelea.* It is worth mentioning that their importance as pollinators of economical important crops has not been evaluated in the region, although several studies in America Latina corroborate it (Kaufmann 1975; Young 1983; Bravo *et al.* 2011; Córdoba *et al.* 2013). Such background highlights the importance of these genera, not only for the natural ecosystem but also for agricultural systems of northwest Argentina, where the production of a wide variety of fruit crops is registered, which might be pollinated by these species. Finally, *Bezzia*, *Echinohelea* and *Alluaudomyia*, which are predators of insects, might act as controllers of pests insects associated to crops.

From the obtained results in this study, emerges the need of developing further research in other areas of northwest Argentina, in order to upgrade the knowledge of both taxonomic and distributional aspects of Ceratopogonidae family, and their relevance as disease vectors and pollinators of commercial crops.

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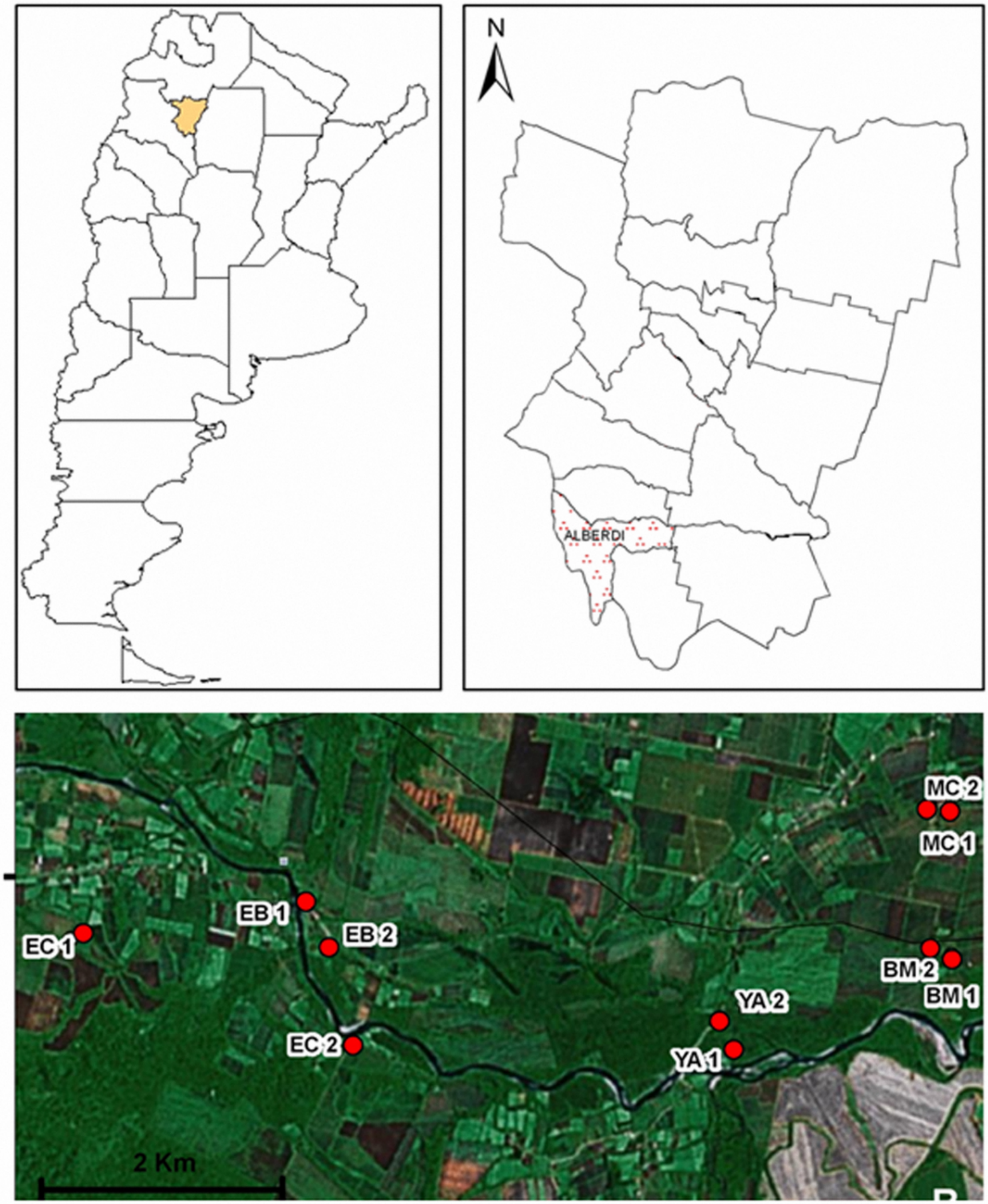
Figure 1

Geographic distribution of sampling sites in Juan Bautista Alberdi Department, Tucumán. *EC*= El Corralito, *EB*= El Badén, *YA*= Yánima, *BM*= Bajo Marapa, *MC*= Marapa Central. Map data © 2006 Google Earth.


N
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EC 1
EB 2
BM 2
BM 1
YA 2
EC 2
YA 1
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Figure 2

Mean relative abundance of A. *Culicoides* B. *Forcipomyia* C. *Dasyhelea* D. *Atrichopogon* versus regressor variables.

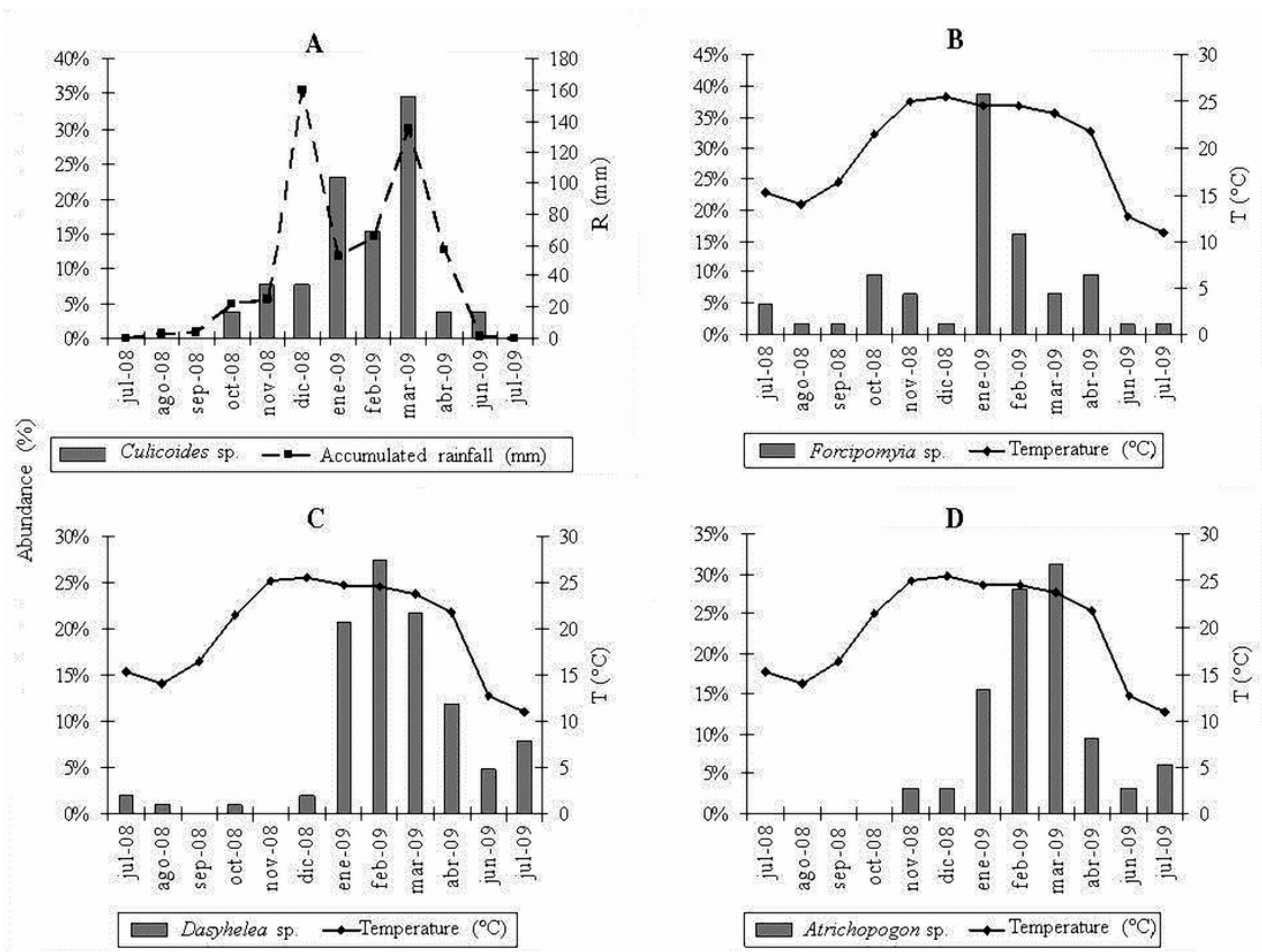

Figure 3

Partial residuals of A. *Culicoides* B. *Forcipomyia* C. *Dasyhelea* D. *Atrichopogon* and retained variables by the statistical model.

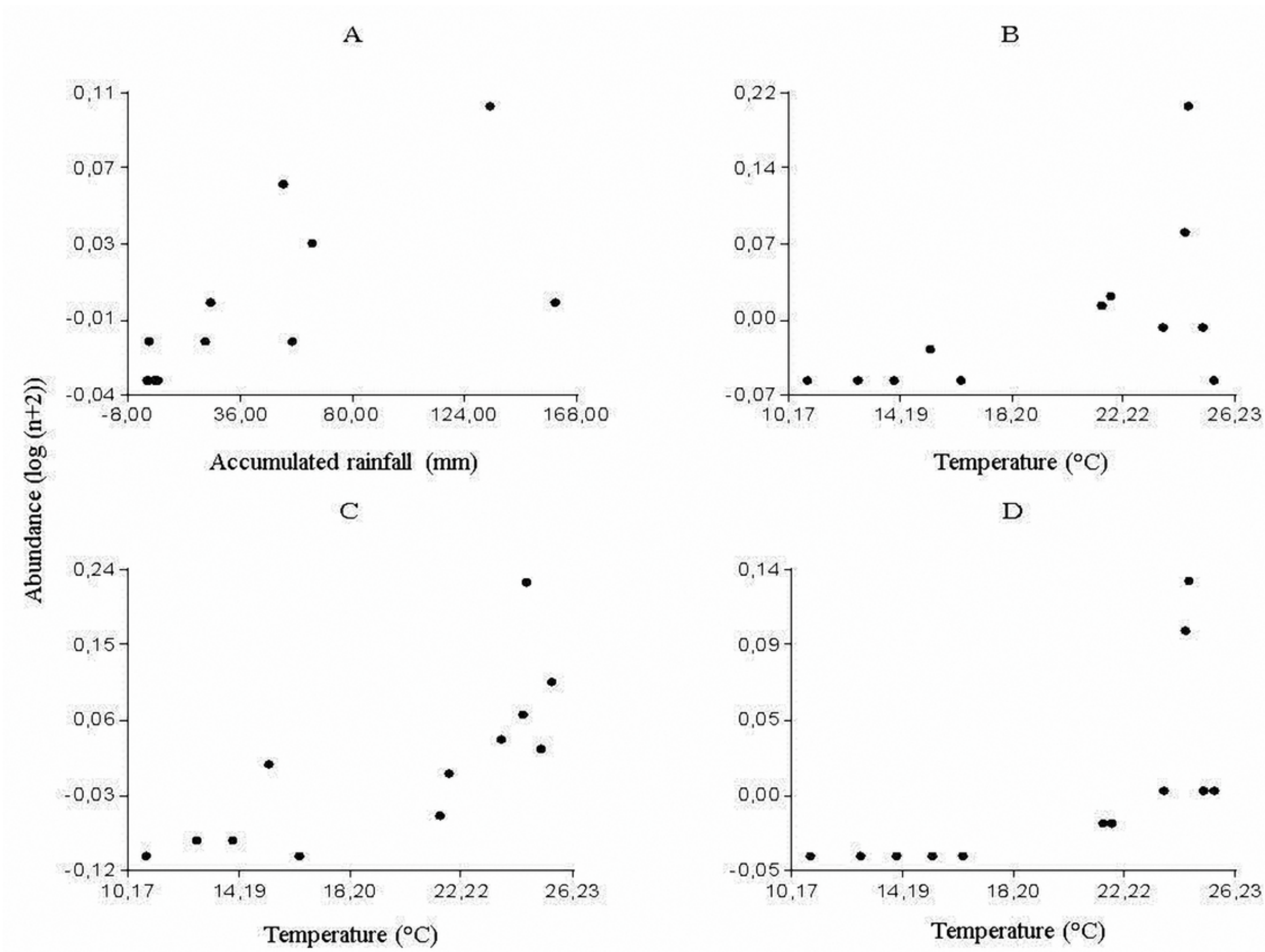

Figure 4

Standardized vs. predicted residuals for A. *Culicoides* B. *Forcipomyia* C. *Dasyhelea* D. *Atrichopogon.*

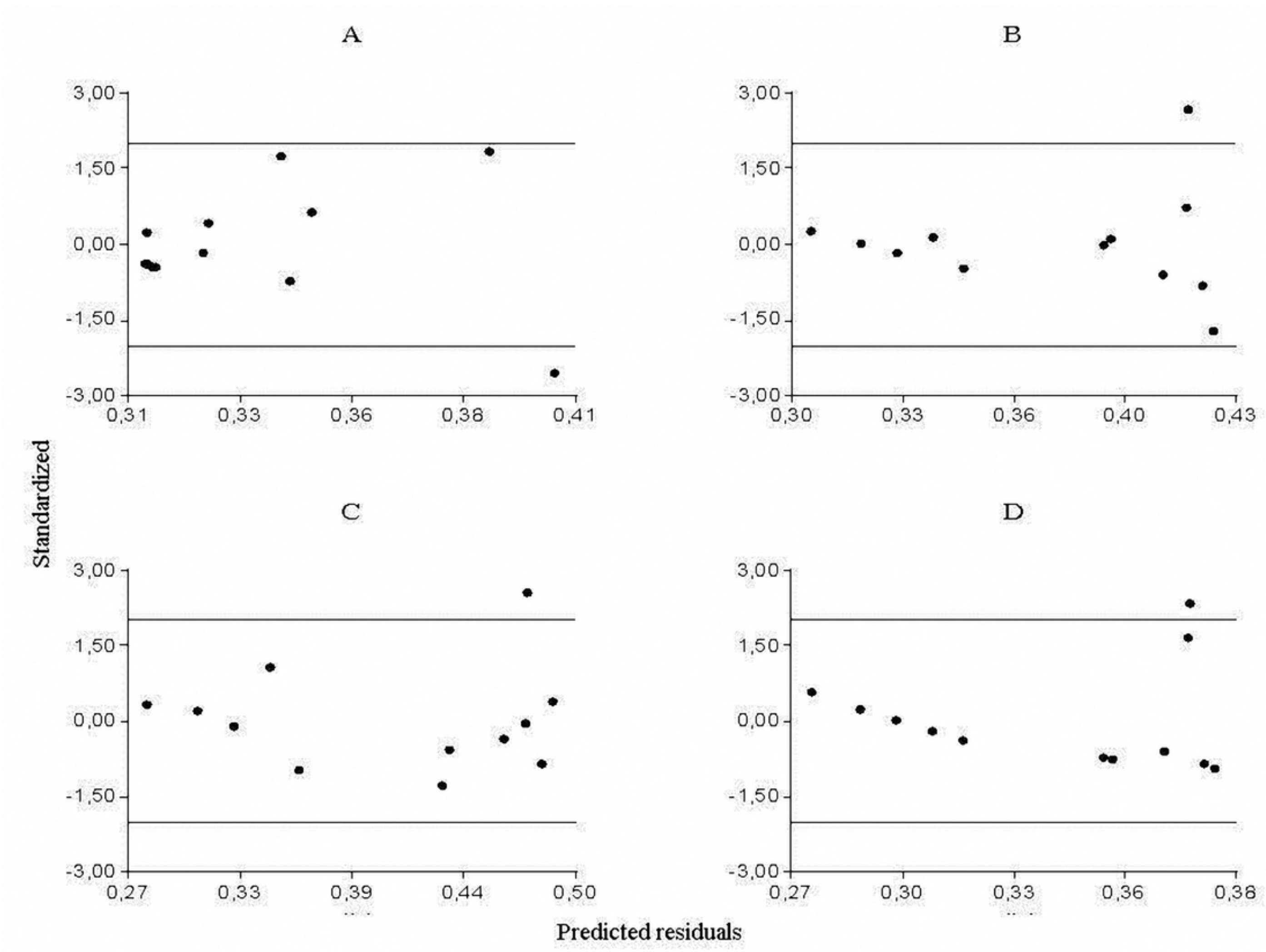

Table 1(on next page)

Absolute abundance of Ceratopogonidae during July, 2008- July, 2009, Juan Bautista Alberdi, Tucumán.

Genera	El Corralito	El Baden	Yánima	Bajo Marapa	Marapa Central	Total	(%)
Alluaudomyia	1	0	0	0	0	1	0.28
Atrichopogon	23	15	2	7	3	50	13.85
Bezzia	1	0	0	0	0	1	0.28
Culicoides	16	12	4	2	4	38	10.53
Dasyhelea	74	26	14	17	41	173	47.92
Echinohelea	1	0	0	0	0	1	0.28
Forcipomyia	32	11	22	14	19	97	26.87
Total	148	64	42	40	67	361	100

Table 2(on next page)

Chi-squared coefficient test table and V Cramer association coefficient for Ceratopogonidae, in relation to sampling sites and season.

Rows x columns	Chi-squared	g.l	p-value	x of table	Coef. V of Cramer
Sities x genera	38.92	12	0.0001	21.02	0.16
Seasons x genera	28.31	9	0.0008	16.91	0.14