Classification of Immature Stage Habitats of Culicidae (Diptera) Collected in Córdoba, Argentina

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In order to classify mosquito immature stage habitats, samples were taken in 42 localities of Córdoba Province, Argentina, representing the phytogeographic regions of Chaco, Espinal and Pampa. Immature stage habitats were described and classified according to the following criteria: natural or artificial; size; location related to light and neighboring houses; vegetation; water: permanence, movement, turbidity and pH. Four groups of species were associated based on the habitat similarity by means of cluster analysis: Aedes albifasciatus, Culex saltanensis, Cx. mollis, Cx. brethesi, Psorophora ciliata, Anopheles albitarsis, *and* Uranotaenia lowii *(Group A);* Cx. acharistus, Cx. quinquefasciatus, Cx. bidens, Cx. dolosus, Cx. maxi *and* Cx. apicinus *(Group B);* Cx. coronator, Cx. chidesteri, Mansonia titillans *and* Ps. ferox *(Group C);* Ae. fluviatilis *and* Ae. milleri *(Group D). The principal component analysis (ordination method) pointed out that the different types of habitats, their nature (natural or artificial), plant species, water movement and depth are the main characters explaining the observed variation among the mosquito species. The distribution of mosquito species by phytogeographic region did not affect the species groups, since species belonging to different groups were collected in the same region.*

Key words: *Aedes* - *Anopheles* - *Culex* - *Mansonia* - *Psorophora* - *Uranotaenia* - immature stage habitats - classification - Argentina

Large outbreaks of equine encephalitis have been documented for the temperate zone of Argentina, caused by western equine (WEE) and eastern equine encephalitis (EEE) virus (Mitchell et al. 1985, 1987). Other arboviruses of potential medical importance in Argentina include St. Louis encephalitis (SLE) virus, Rocio virus and Venezuelan equine encephalitis (VEE) virus (Sabattini et al. 1985). At present, only *Aedes albifasciatus* from Córdoba Province (Argentina) has been incriminated as an experimental vector of WEE virus (Avilés et al. 1990).

The ecology of arboviruses remains far obscure due to the few studies carried out in Argentina. To determine the host preferences, feeding patterns were studied for mosquitoes collected in Chaco, Santa Fe and Río Negro Provinces (Mitchell et al. 1985, 1987) and in Córdoba Province (Almirón & Brewer 1995a). The discovery of eggs, larvae and pupae of several mosquito species in natural field environments during the autumn-winter period in Córdoba Province suggested that immatures continue to develop throughout these seasons (Almirón & Brewer 1994). In addition, studies on seasonal distribution of immature stages and females were conducted in Córdoba Province (Almirón & Brewer 1995b). The species collected as females and/or larvae were, from the most to the least abundant, *Cx. quinquefasciatus, Cx. apicinus, Cx. dolosus, Cx. saltanensis, Cx. bidens, Cx. brethesi, Cx. maxi, Ae. albifasciatus, Cx. spinosus, Cx. acharistus* and *Cx. chidesteri. Ae. albifasciatus* was mainly captured in summer and fall while *Culex* species were collected throughout the year, although peaks of mosquito abundance were from September to March.

The World Health Organization has recommended that public health personnel concentrate on vector control, including biological control and environmental management (Rejmankova et al. 1991). The knowledge of relationships between habitats, environmental factors and occurrence of mosquito larvae is essential for an efficient application of mosquito control methods. In a coastal lowland farm in Rio de Janeiro (Brazil), Oliveira et al. (1986) collected several species of *Aedes, Anopheles, Culex, Limatus, Mansonia, Phoniomyia, Psorophora, Uranotaenia* and *Wyeomyia,* describing their immature stage habitats. The majority of species preferred to breed on the ground, specially natural sites, but they also developed in urban areas. Some species were more abundant in temporary breeding places while oth-

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ers occurred usually in permanent ones. Some species were collected in natural recipients, others in bromeliads, in tree-holes, in plant leaf axils, and in artificial containers. Field surveys of mosquito larval sites carried out on the Pacific coast of southern Chiapas, Mexico, found several quantified environmental factors to be promising as predictors of anopheline larval occurrence (Rejmankova et al. 1991). One of the most important factors was the vegetation that favors the remote sensing and geographic information system technologies to monitor and predict larval densities (Savage et al. 1990, Rejmankova et al. 1991, 1992, Rodriguez et al. 1993).

No research on this subject has been conducted in Argentina. Del Ponte and Blaksley (1945/1948), Prosen et al. (1960), Bachmann and Casal (1962) reported the type of breeding sites where they collected immature stages in Buenos Aires and Córdoba Provinces, and the associated species, providing no information on the habitat features. As part of the mosquito fauna studies of Córdoba Province, we carried out field surveys of possible mosquito breeding sites in order to describe them and to classify the mosquito species according to the similarity of their habitats.

MATERIALS AND METHODS

Study site - Córdoba is a mediterranean province located between 29°29'-35°1'LS and 32°54'-61°46'LW. The climate in this province is temperate (Capitanelli 1979). Freezing temperatures are recorded from April through September, July being the month with the majority of days (6.5) with frost. The mean temperatures are 17°C in the SE and 20°C in the NE. Mean annual rainfall in the area is between 380-900 mm. The principal rainy season is in March, with a secondary one from October through December. Phytogeographically, Córdoba Province is divided into different regions (Luti 1979). The Chaco region is divided into the western and eastern woodlands, and shows the greatest physiognomical variety in the province. The annual precipitation is between 550-600 mm. In the Espinal region grassland and woodland are mixed, with about 800 mm of annual precipitation. The Pampa region is a wide flat grassland, subhumid in the east with 900 mm of annual rainfall, and semi-arid in the southwest with 500 mm.

Collections - Eggs, larvae and pupae were sampled in 42 localities which included the three phytogeographic regions (Table I). A total of 252 samples were collected and analyzed.

Immature stage habitats were classified using the following criteria: natural or artificial; size; type; location in relation to light (sunlight, partial shade and complete shade) and houses; breeding depth; water permanence (permanent, semipermanent and temporary); water movement (stagnant or in motion), although the speed was not measured; turbidity was estimated visually either as turbid or clear (turbid samples were those where the white dipper background could not be seen); pH (papers MN-Macherey-Nagel D-5160) and vegetation (present or absent).

Identification - Immature stages were identified using species descriptions and keys by Lane (1953), Forattini (1962, 1965a,b) and Darsie (1985). Identification was based on fourth instar larvae and/or adults, so eggs, first to third instar larvae and pupae collected were reared to fourth instars or adults for identification. The specimens were deposited in the mosquito collection of Centro de Investigaciones Entomológicas de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba.

Vegetation sampling - A botanical survey was conducted to collect and identify the plant species in the habitats that were visited. Plants were sent to the Botanical Museum of the National University of Córdoba for identification. Plant species appearing after the preliminary survey was completed, or whose identification was in question were collected, and preserved for subsequent identification.

Data analysis - Data were analyzed according to the following steps (Crisci & López Armengol 1983): operative taxonomic units (OTUs) were chosen; a basic matrix of data (BMD) was developed; the similarity was calculated for each pair of OTUs; a similarity matrix (SM) was developed; and mosquito species were grouped.

Since the purpose was to group species with similar breeding features, the operative taxonomic units (OTUs) chosen were the species collected (Table I). The recorded characteristics were analyzed to identify common patterns of immature stage habitats where the different species were collected. Both quantitative (size, distance from breeding sites to the nearest house, pH, and depth) and qualitative (natural or artificial, type, shade, water permanence, water movement, turbidity, and vegetation) characters were codified as 1/0 (= presence/absence). Quantitative data were subdivided into intervals (see depth, for instance, in Table III), each one being considered as a character.

A BMD was developed based on the codified data, consisting of 19 rows for the mosquito species and 86 columns for all the characters. Values within the cells represented 1 or 0 if each character was recorded for each species or not respectively. This table was used for analysis to calculate the similarity for all possible pairs of OTUs, and is not presented here.

The similarity for all possible combinations of

species pairs (OTUs) was calculated using the Dice or SD association coefficient (Crisci & López Armengol 1983). The coefficients obtained were used to develop the SM (Table II). OTUs were grouped by similarity using cluster analysis. The UPGMA (unweighted pair group method using arithmetic averages) was used as a linkage technique, representing the groups by a phenogram (Sneath & Sokal 1973).

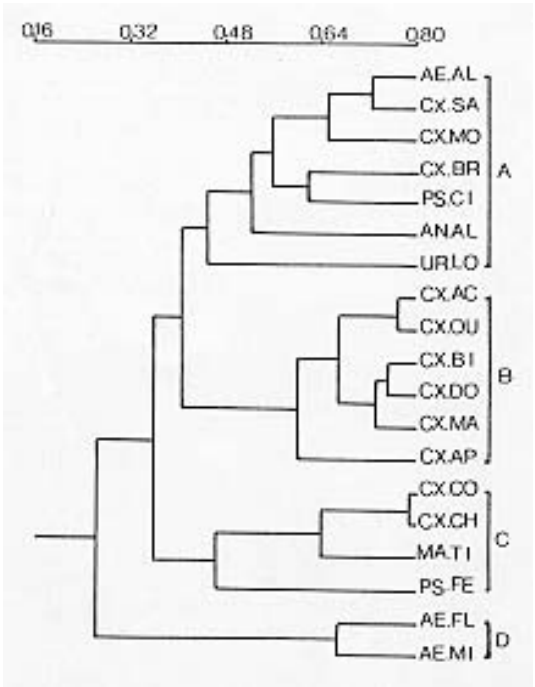
The principal component analysis (ordination method) was used to examine the relationships among all the OTUs based on their similarity. The SM was used to represent all the characters by a reduced number of principal components according to a linear model, where each component contains part of the total variability of the characters (Crisci & López Armengol 1983). The NTSYS program (Rohlf 1988) was used for this analysis.

RESULTS AND DISCUSSION

Four groups of species can be distinguished in the phenogram (Fig.) according to the association coefficients obtained (Table II): *Ae. albifasciatus, Cx. saltanensis, Cx. mollis, Cx. brethesi, Ps. ciliata, An. albitarsis* and *Ur. lowii* (Group A), *Cx. acharistus, Cx. quinquefasciatus, Cx. bidens, Cx. dolosus, Cx. maxi* and *Cx. apicinus* (Group B), *Cx. coronator, Cx. chidesteri, Ma. titillans* and *Ps. ferox* (Group C), *Ae. fluviatilis* and *Ae. milleri* (Group D). *Cx. coronator* and *Cx. chidesteri* showed the highest association coefficient (0.785), being grouped together (Group C). The lowest coefficient (0.160) was for *Cx. mollis* and *Ae. milleri* (Table I), so they were included in the group A and D respectively (Fig.). Species of group B are clearly separated from the other groups. Species of groups A, B and C were collected from two or three phytogeographic regions (Table I), so their geographical distribution did not affect the formation of the groups.

The Group A species were mainly collected in natural habitats, though *Ae. albifasciatus, Cx. saltanensis* and *An. albitarsis* were also found in artificial containers (Table IV). Ground pools ranging in size from small to large represent the principal habitat of this group. Aquatic plants were either present or absent. Other characteristics included: either shade or sunlight; either close to far away from houses; either clear or turbid water; a range of pH from 6.4-8.0; shallow; stagnant water; and either temporary or permanent water source. *Ae. albifasciatus-Cx. saltanensis* and *Cx. brethesi-Ps. ciliata* form the two clusters that share the greatest number of habitat features (Fig.).

The species of group B were exclusively in the genus *Culex*. They were found both in natural and artificial habitats and in a great variety of small to large places. Aquatic vegetation was an important



Phenogram of 19 operative taxonomic units (*Aedes albifasciatus, Culex saltanensis, Cx. mollis, Cx. brethesi, Psorophora ciliata, Anopheles albitarsis, Uranotaenia lowii, Cx. acharistus, Cx. quinquefasciatus, Cx. bidens, Cx. dolosus, Cx. maxi, Cx. apicinus, Cx. coronator, Cx. chidesteri, Mansonia titillans, Ps. ferox, Ae. fluviatilis, Ae. milleri*) resulting from cluster analysis. The scale 0.16-0.80 indicates the range of the association coefficients obtained.

feature, although it was not found in every site. Habitats were located in either shade or sunlight, both close to and far away from houses. Temporary and permanent habitats showed clear to turbid water, pH (6.4->8), and depths 0.03-1.8 m. All species were collected in stream margins except *Cx. bidens*, those species being found in slowly-moving water in the presence of aquatic plants. This group shows higher uniformity of habitat features compared with groups A and C (Table III). Two nuclei can be recognized: *Cx. acharistus-Cx. quinquefasciatus* and *Cx. bidens-Cx. dolosus*. The differences between the nuclei are found in breeding type, vegetation and degree of illumination. *Cx. maxi* is closely related to the second nucleus sharing more types of habitats, while *Cx. apicinus* is clearly separated from the other species (Fig.). *Cx. apicinus* and *Cx. quinquefasciatus* were found more frequently (67 and 71% respectively) in artificial containers (Table III). The type of habitat, size and location (closely related to human environment) distinguish *Cx. apicinus* from the other species.

The Group C species were collected only in

TABLE I

Mosquito species collected in Córdoba as immature stages

Species	Localities			N	Months
	Chaco	Espinal	Pampa		
Group A					
Ae. albifasciatus	CO	CR, EC, JC, SI, SJ, VA, VR		38	2, 3, 4, 6, 7, 10,12
Cx. saltanensis	CO, HU, PC, QF, VM	AG, GU, MI, VA, VR		15	1-5
mollis		RR		2	3
brethesis		MI	PA	6	2, 8
Ps. ciliata		CU, VR		2	2, 12
An. albitarsis	LA	VR		6	4-6, 8
Ur. lowii		MI, VR		2	2, 5
Group B					
Cx. acharistus	CO, VM	AG, CC, CR, GU, JM, LC, VA, VR, VW		76	1, 2, 4-12
quinquefasciatus	CO, HU, LP, QF, RC, VM	AG, BV, CC, CR, DE, GU, JM, LC, PI, RR, VA, VR		78	2-12
bidens	AC, CG, CO, HU, LP, RP, VS, VG	AG, BV, CC, JC, JM, LC, MI, RR, VA, VR		27	1-5, 9, 12
dolosus	AC, CE, CO, EP, RP, TA, VS, VG	AG, BV, CC, CR, GU, JM, LC, MI, RR, VA, VR, VI, VW	LB, WE	138	1-12
maxi	CA, CG, CE, CO, HU, LA PC, QF, RP, VC, VM, VS	AG, BV, CR, GU, JC, LC, MI, RR, VR	LB	40	1-5, 7-7, 11, 12
apicinus	CO	AG, CR, GU, PI, RS, VA, VI		35	1, 4-9, 11, 12
Group C					
Cx. coronator	CO			6	2, 4, 12
chidesteri	CO	LC		3	8
Ma. titillans		CR		1	5
Ps. ferox		VR		1	4
Group D					
Ae. fluviatilis	CO			1	7
milleri	CO			1	12

Localities: *Chaco*: Arroyo La Candelaria (AC), Cabana (CA), Campo Grande (CG), Cerro Colorado (CE), Cosquín (CO), Estancia El Puente (EP), Huascha (HU), La Cañada (LA), Las Pencas (LP), Puesto de Castro (PC), Quebracho Flojo (QF), Rayo Cortado (RC), Río Pinto (RP), Tanti (TA), Villa Candelaria (VC), Villa de María de Río Seco (VM), Villa de Soto (VS), Villa Giardino (VG); *Espinal*: Alta Gracia (AG), Bell Ville (BV), Colonia Caroya (CC), Córdoba City (CR), Cuatro Esquinas (CU), Despeñaderos (DE), El Cortijo (EC), Guiñazú (GU), Jerónimo Cortés (JC), Jesús María (JM), La Calera (LC), Miramar (MI), Pilar (PI), Río Segundo (RS), Ruta 36 and Río Segundo (RR), San Isidro (SI), San José de la Quintana (SJ), Villa Allende (VA), Villa del Rosario (VR), Villa Rivera Indarte (VI), Villa Warcalde (VW); *Pampa*: Laborde (LB), Pascanas (PA), Wenceslao Escalante (WE). Months: 1-12 (January-December).

natural habitats, medium to large in size, mainly ground pools, with or without aquatic plants, partially illuminated, relatively close to houses; in clear to turbid water, pH between 7-7.6, 0.1-0.2 m depth, always stagnant; and in temporary to permanent places. The *Culex* species in Group C form a nucleus which displays the highest similarity coefficient (0.785) (Fig.).

Larvae of *Ae. fluviatilis* and *Ae. milleri* (Group D) were only found in small artificial containers, without vegetation, in partial to complete shade, with clear, stagnant water, pH 6.7-6.8, 0.2 m depth, and in temporary habitats located in a house.

Ae. albifasciatus, Cx. acharistus, Cx. apicinus, Cx. bidens, Cx. brethesi, Cx. dolosus, Cx. maxi and *Cx. quinquefasciatus* were often collected together and with other species (Table IV); they were also found as unique species from a single sample. The most frequent associations (based on 252 samples) were *Cx. acharistus-Cx. dolosus* (11.9%), followed by *Cx. apicinus-Cx. quinquefasciatus*, and *Cx. dolosus-Cx. quinquefasciatus* (2.77% for both combinations), and *Cx. bidens-Cx. maxi* (2.38%). All these species belong to group B. Except for *Cx. bidens*, the other species of this group were found at least once all together in the same habitat. The least frequent associations were *Ae. albifasciatus-Ps. ciliata, Ae. fluviatilis-Cx.*

TABLE II

Similarity matrix for the OTUs calculated using the SD association coefficient

	Ae. al	*Ae. fl*	*Ae. mi*	*An. al*	*Cx. ac*	*Cx. ap*	*Cx. bi*	*Cx. br*	*Cx. co*	*Cx. ch*	*Cx. do*	*Cx. ma*	*Cx. mo*	*Cx. qu*	*Cx. sa*	*Ma. ti*	*Ps. ci*	*Ps. fe*	*Ur. lo*
Ae. al	1.000																		
Ae. fl	0.333	1.000																	
Ae. mi	0.342	0.666	1.000																
An. al	0.545	0.400	0.344	1.000															
Cx. ac	0.500	0.241	0.210	0.393	1.000														
Cx. ap	0.508	0.311	0.318	0.490	0.592	1.000													
Cx. bi	0.580	0.250	0.212	0.428	0.666	0.563	1.000												
Cx. br	0.564	0.240	0.166	0.545	0.295	0.333	0.392	1.000											
Cx. co	0.400	0.230	0.240	0.411	0.387	0.367	0.500	0.551	1.000										
Cx. ch	0.368	0.250	0.260	0.375	0.333	0.340	0.360	0.444	0.785	1.000									
Cx. do	0.552	0.225	0.229	0.457	0.755	0.564	0.750	0.369	0.424	0.312	1.000								
Cx. ma	0.586	0.272	0.186	0.500	0.600	0.686	0.742	0.425	0.458	0.391	0.714	1.000							
Cx. mo	0.650	0.230	0.160	0.529	0.387	0.408	0.461	0.620	0.533	0.571	0.393	0.416	1.000						
Cx. qu	0.500	0.241	0.210	0.363	0.765	0.592	0.619	0.393	0.322	0.266	0.734	0.625	0.354	1.000					
Cx. sa	0.723	0.303	0.250	0.536	0.492	0.500	0.610	0.611	0.486	0.400	0.575	0.618	0.648	0.550	1.000				
Ma. ti	0.222	0.272	0.285	0.333	0.241	0.222	0.291	0.400	0.692	0.583	0.258	0.272	0.384	0.172	0.363	1.000			
Ps. ci	0.540	0.173	0.181	0.451	0.237	0.304	0.285	0.615	0.444	0.400	0.285	0.311	0.592	0.271	0.411	0.260	1.000		
Ps. fe	0.400	0.380	0.400	0.344	0.210	0.272	0.297	0.333	0.480	0.434	0.262	0.325	0.320	0.210	0.437	0.476	0.363	1.000	
Ur. lo	0.368	0.333	0.260	0.500	0.233	0.255	0.280	0.518	0.428	0.384	0.281	0.260	0.428	0.233	0.400	0.416	0.480	0.434	1.000

Aedes albifasciatus, Ae. fluviatilis, Ae. milleri, Anopheles albitarsis, Culex acharistus, Cx. apicinus, Cx. bidens, Cx. brethesi, Cx. coronator, Cx. chidesteri, Cx. dolosus, Cx. maxi, Cx. mollis, Cx. quinquefasciatus, Cx. saltanensis, Mansonia titillans, Psorophora ciliata, Ps. ferox, Uranotaenia lowii.

acharistus-Cx. apicinus-Cx. dolosus-Cx. q*uinquefasciatus, An. albitarsis-Cx. dolosus-Cx. maxi-Cx. saltanensis,* and *Cx. bidens-Cx. brethesi-Ur. lowii,* found only once in these combinations. To find species of different genera in the same habitat suggests a similarity of habitat requirements.

According to the principal component analysis, the first three components explain 52.4% of the variation observed among the different mosquito species. The type of breeding place, aquatic plants, habitat nature (artificial or natural), and water movement contributed the most to explain the variation in the first component. Water depth is the most important character in the second component (Table III). Type of breeding place and vegetation are the most important in the third component. Relationships between vegetation and immature stages were reported by several authors (Savage et al. 1990, Rejmankova et al. 1991, 1992, Rodriguez et al. 1993), who investigated spatial and seasonal variations on anopheline larval densities and their plant associations in Mexico, finding that larval abundance was related to the presence of certain types of vegetation.

Ae. albifasciatus has also been found in swamps (Del Ponte & Blaksley 1945/1948), in hypersalty water (NaCl 31.7-49.8 g/l, Na_2SO_4 11.32-19.44 g/l, $CaCO_3$ 7.5-12.8 g/l) with the aquatic plant *Atriplex* (Bachmann & Casal 1962), and in habitats at 2,300 m above sea level (Forattini 1965a), displaying considerable diversity in habitats. Our data on *Cx. saltanensis* agree with those reported by Oliveira et al. (1986) who found immature stages in partially shaded natural and artificial habitats, with clear to turbid water.

Very little is knwon about the habitat of the immature stages of *Cx. brethesi*. Bachmann and Casal (1962) found it breeding in hypersalty water (NaCL 2.5 g/l, $CaCo_3$ 730 mg/l). *Ps. ciliata* larvae were also found in Bromeliaceae feeding on mosquito larvae of *Aedes, Ae. taeniorhynchus, Culex, Ps. (Janthinosoma), Ps. ferox and Ps. confinnis* (Prosen et al. 1962/1963, Forattini 1965a).

Cx. mollis was collected in permanent natural ground pools in our study. However, it can also be found in temporary habitats, Bromeliaceae, drinking places, tanks, barrels, cans, fountains, banana tree leaf axils and small pools, associated with *Cx. coronator* and some species of *Aedes* (Forattini 1965a, Clark-Gil & Darsie 1983).

An. albitarsis can breed in several habitats such as lagoons, dikes, drains, swamps, floodlands, stream margins, in stagnant or slow-moving water, with abundant or scarce debris, in fresh or salty water, and associated with one or more of the following: *An. albimanus, An. argyritarsis, An. darlingi, An. noroestensis, An. pseudopunctipennis, An. strodei,* and *An. triannulatus* (Deane et al. 1948, Forattini 1962).

Ur. lowii was collected in the margins of lakes, lagoons, in very sunny places, usually with aquatic plants, and associated with *An. albimanus* and *Cx. coronator* (Carpenter & La Casse 1955, Clark-Gil & Darsie 1983). Oliveira et al. (1986) found larvae in a small plastic receptacle. We also collected this species in shaded habitats, without vegetation.

Cx. acharistus and *Cx. quinquefasciatus* are closely related species (similarity coefficient = 0.765) (Fig.), though *acharistus* was mainly collected in natural habitats (76%) and *quinquefasciatus* in artificial containers (71%) (Table III). No information was found on *Cx. acharistus* to compare with our results. Our data on *quinquefasciatus* agree with those by Dyar (1922), Del Ponte and Blaksley (1945/1948), Carpenter and La Casse (1955), Prosen et al. (1960), Forattini (1965a), Ishii and Sohn (1987), Kulkarni and Naik (1989); these authors also found this species in barrels, dikes, drains, sewages, and less frequently in crabholes, bamboo internodes and Bromeliaceae.

Cx. dolosus and *Cx. bidens* are members of a nucleus. The first species was collected in artificial containers and stream margins whereas *Cx. bidens* preferred ground habitats with aquatic plants. This observation is in concordance with that reported by Oliveira et al. (1986).

According to Forattini (1965a) *Cx. apicinus* can breed in highlands, in small stream bed pools. In our study, this species was mainly collected in artificial receptacles (67%), domestic or peridomestic, including domiciliary water tanks. Based on these data this species can be considered domestic as well, at least in these regions of Argentina.

Cx. coronator and *Cx. chidesteri* were collected in ground pools and swamps. However, the first species has also been found in artificial containers, drains, hoof-prints, tree holes, Bromeliaceae, fruit shells, in clear to polluted water, shaded to sunny places, and associated with some species of *Aedes, Anopheles, Culex* and *Ps. confinnis* (Carpenter & La Casse 1955, Forattini 1965a, Clark-Gil & Darsie 1983). Larvae of *Cx. chidesteri* were also collected in lagoons, ponds, cement pools, streams, breeding habitats with or without plants, and associated with *An. argyritarsis* and *An. pseudopunctipennis* (Forattini 1965a, Clark-Gil & Darsie 1983, Oliveira et al. 1986).

Ma. titillans is close to the *Cx. coronator-Cx. chidesteri* nucleus because they were all collected in large breeding habitats. *Ma. titillans* was found associated with *Pistia* sp., and in addition it can also be found in ground pools with Araceae, in dikes, and in stream margins attached to *Eicchornia*, *Ipomea* and grass roots (Prosen et al.

TABLE III

Variables that more contribute to explain the variation in the first two components

Group / Breeding characters	A							B						C				D	
	1	2	3	4	5	6	7	1	2	3	4	5	6	1	2	3	4	1	2
Artificial %	6	40				34		24	71	30	22	40	67					100	100
Natural %	94	60	100	100	100	66	100	76	29	70	88	60	33	100	100	100	100		
Breeding type:																			
Cement drinking place						x						x							x
Cistern									x										
Glass container									x										
Hoof print								x	x	x	x								
Lagoon																x			
Margin of : lagoon						x		x			x								
rivulet								x	x		x	x							
Metallic container								x	x	x	x	x	x					x	
Pool: in drain	x	x				x		x	x	x	x	x	x						
rivulet bed	x							x					x						
road	x	x								x	x	x					x		
road drain	x			x	x				x			x							
contact with drain				x					x	x	x			x					
river											x	x							
rivulet		x							x		x								
near at a drain								x	x		x				x				
lagoon						x	x				x								
river	x	x	x					x	x	x	x								
rivulet								x		x	x								
Spring				x			x												
Square fountain	x																		
Stream margin								x	x		x	x	x						
Swamp								x		x	x	x		x	x				
Swimming pool								x	x		x	x							
Domiciliary water tank													x						
Tank in disuse								x	x										
Tire								x	x		x								
Vegetation:																			
Alternanthera philoxeroides	x		x					x	x	x	x								
Alternanthera sp.		x																	
Azolla sp.										x									
Eichhornia sp.													x						
Equisetum sp.											x								
Hydrocotyle sp.	x		x					x	x	x	x	x	x	x	x				
Lemna sp.		x		x				x	x	x	x	x	x						
Ludwigia uruguayensis								x	x		x								
Ludwigia sp.	x	x	x					x	x	x	x	x	x						
Myriophyllum sp.						x	x	x	x	x	x	x							
Pistia sp.																x			
Polygonum hydropiperoides	x	x	x	x	x	x		x	x	x	x	x	x						
Riripa nasturtium-aquaticum								x	x	x	x	x	x						
Tyhpa sp.								x		x	x			x		x			
Veronica anagallis-aquatica								x	x		x								
Wolffia sp.								x	x				x						
Wolffiella sp.								x	x				x						
grass	x	x	x		x				x	x	x	x	x	x	x				
without vegetation	x	x					x	x	x	x	x	x	x						
Breeding depth up to: 0.1 m																	x		
0.15			x											x	x				
0.2				x	x		x									x		x	x
0.3						x													
0.4	x											x							
0.5		x																	
0.9								x											
1.0									x										
1.8										x	x	x							

Group A: 1 *Aedes albifasciatus*, 2 *Culex saltanensis*, 3 *Cx. mollis*, 4 *Cx. brethesi*, 5 *Psorophora ciliata*, 6 *Anopheles albitarsis*, 7 *Uranotaenia lowii*; Group B: 1 *Cx. acharistus*, 2 *Cx. quinquefasciatus*, 3 *Cx. bidens*, 4 *Cx. dolosus*, 5 *Cx. maxi*, 6 *Cx. apicinus*; Group C: 1 *Cx. coronator*, 2 *Cx. chidesteri*, 3 *Mansonia titillans*, 4 *Ps. ferox*; Group D: 1 *Ae. fluviatilis*, 2 *Ae. milleri*.

TABLE IV

Mosquito species associated as larvae and/or pupae, collected in natural and artificial immature habitats

Species	Associated species	
	Artificial habitats	Natural habitats
Ae. albifasciatus		*Cx. ac, Cx.ma, Cx. qu, Cx. sa, Ps.ci, Ps. fe*
Ae. fluviatilis	*Cx. ac, Cx. ap, Cx. do, Cx. qu*	
Ae. milleri	*Cx. ac, Cx. ap, Cx. do, Cx. qu*	
An. albitarsis	*Cx. ma*	*Cx. do, Cx. ma, Cx. sa*
Cx. acharistus	*Ae. fl, Ae. mi, Cx. ap, Cx. do, Cx. qu*	*Ae. al, Cx. ap, Cx. bi, Cx. co, Cx. ch, Cx. do, Cx. ma, Cx. qu, Cx. sa*
Cx. apicinus	*Ae. fl, Ae. mi, Cx. ac, Cx. bi, Cx. do, Cx. ma, Cx. qu, Cx. sa*	*Cx. ac, Cx. bi, Cx. br, Cx. do, Cx. ma, Cx. qu*
Cx. bidens	*Cx. ap, Cx. do, Cx. ma, Cx. qu*	*Cx. ac, Cx. ap, Cx. br, Cx. co, Cx. do, Cx. ma, Cx. mo, Cx. sa*
Cx. brethesi		*Cx. ap, Cx. bi, Cx. ma, Cx. qu, Cx. sa*
Cx. coronator		*Cx. ac, Cx. bi, Cx. do*
Cx. chidesteri		*Cx. ac, Cx. do*
Cx. dolosus	*Ae. fl, Ae. mi, Cx. ac, Cx. ap, Cx. bi Cx. ma, Cx. qu, Cx. sa*	*An. al, Cx. ac, Cx. ap, Cx. bi, Cx. co, Cx. ch, Cx. ma, Cx. qu, Cx. sa, Ur. lo*
Cx. maxi	*An. al, Cx. ap, Cx. bi, Cx. do, Cx. sa*	*Ae. al, An. al, Cx. ac, Cx. ap, Cx. bi, Cx. br, Cx. do, Cx. mo, Cx. qu, Cx. sa*
Cx. mollis		*Cx. bi, Cx. ma*
Cx. quinquefasciatus	*Ae. fl, Ae. mi, Cx. ac, Cx. ap, Cx. bi Cx. do, Cx. sa*	*Ae. al, Cx. ac, Cx. ap, Cx. bi, Cx. br, Cx. do, Cx. ma, Cx. sa*
Cx. saltanensis	*Cx. ap, Cx. do, Cx. ma, Cx. qu*	*Ae. al, An. al, Cx. ac, Cx. br, Cx. do, Cx. ma, Cx. qu*
Ma. titillans		
Ps. ciliata		*Ae. al*
Ps. ferox		*Ae. al*
Ur. lowii		*Cx. do*

Aedes albifasciatus (Ae. al), Ae. fluviatilis (Ae. fl), Ae. milleri (Ae. mi), Anopheles albitarsis (An. al), Culex acharistus (Cx. ac), Cx. apicinus (Cx. ap), Cx. bidens (Cx. bi), Cx. brethesi (Cx. br), Cx. coronator (Cx. co), Cx. chidesteri (Cx. ch), Cx. dolosus (Cx. do), Cx. maxi (Cx. ma), Cx. mollis (Cx. mo), Cx. quinquefasciatus (Cx. qu), Cx. saltanensis (Cx. sa), Psorophora ciliata (Ps. ci), Ps. ferox (Ps. fe), Ur. lowii (Ur. lo).

1960, Forattini 1965b).

Although in the present study *Ps. ferox* was collected only in a small ground pool without vegetation, it can also be found in swamps with abundant aquatic plants, shady places or breeding habitats where the water flow is slow (Forattini 1965a).

According to Anduze (1941), Forattini and Rabello (1960), and Forattini (1965a), *Ae. fluviatilis* breeds in artificial containers (barrels, metallic containers, flower vases in cemeteries) and tends to be domestic. Our findings agree with these data. In addition, we collected this species in natural breeding habitats consisting of fresh water contained in rock holes, and sunny places in stream margins in common with Dyar's (1922) findings. It has been found associated with *Ae. aegypti* in artificial receptacles (Anduze 1941). *Ae. milleri* was collected in an artificial container, but larvae of this species can be also found in fresh slow-moving water (Prosen et al. 1960) and in rock holes (Forattini 1965a).

The analysis of habitat features in the present study grouped the mosquito species into four clusters, two of them included species from different genera, suggesting a similarity of habitat requirements. Based on this supposition, finding one species at one habitat it is possible to predict what other species could be found in such habitat.

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REFERENCES

Almirón WR, Brewer ME 1994. Immature stages of mosquitoes (Diptera, Culicidae) collected during the autumn-winter period in Córdoba Province, Argentina. *Mem Inst Oswaldo Cruz 89:* 625-628.

Almirón WR, Brewer ME 1995a. Preferencia de hospedadores de Culicidae (Diptera) recolectados en el centro de la Argentina. *Rev Saúde Públ 29* (in press).

Almirón WR, Brewer ME 1995b. Distribución estacional de Culicidae (Diptera) en áreas periféricas de Córdoba (Argentina). *Ecol Austral 5:* 81-86.

Anduze PJ 1941. Anotaciones sobre los zancudos del Estado Carabobo, Venezuela (Diptera: Culicidae). *Rev San As Social 6:* 491-508.

Avilés G, Sabattini MS, Mitchell CJ 1990. Peroral susceptibility of *Aedes albifasciatus* and *Culex pipiens* complex mosquitoes (Diptera, Culicidae) from Argentina to western equine encephalitis virus. *Rev Saúde Públ 24:* 265-269.

Bachmann A, Casal O 1962. Mosquitos argentinos que crían en aguas salobres y saladas. *Rev Soc Entomol Arg 25:* 21-27.

Capitanelli RG 1979. Clima, p. 45-138. In JB Vázquez, RA Miatello, ME Roqué (eds). *Geografía Física de la Provincia de Córdoba*. Córdoba, Boldt, Rep. Argentina.

Carpenter SJ, La Casse WJ 1955. *Mosquitoes of North America*. Berkeley and Los Angeles, University of California Press, 360 pp.

Clark-Gil S, Darsie Jr RF 1983. The mosquitoes of Guatemala. Their identification, distribution and bionomics, with keys to adult females and larvae in english and spanish. *Mosq Syst 15:* 151-284.

Crisci JV, López Armengol MF 1983. *Introducción a la teoría y práctica de la taxonomía numérica*. Serie de Biología. Monografía N° 26. Programa Regional de Desarrollo Científico y Tecnológico. Secretaría General de la Organización de los Estados Americanos, 132 pp.

Darsie Jr RF 1985. Mosquitoes of Argentina. Part I. Keys for identification of adult females and fourth stages larvae in English and Spanish (Diptera: Culicidae). *Mosq Syst 17:* 153-253.

Deane LM, Causey OR, Deane MP 1948. Notas sobre a distribuição e a biologia dos anofelinos das regioes Nordestina e Amazônica do Brasil. *Rev Serv Esp Saúde Publ 1:* 827-965.

Del Ponte E, Blaksley JC 1945/1948. Importancia sanitaria de los Culicidae de la ciudad de Buenos Aires. *Rev Inst Bact Arg 13:* 253-258.

Dyar HG 1922. The mosquitoes of the United States. *Proc Unit States Nat Mus 62:* 1-119.

Forattini OP 1962. *Entomologia Médica. I. Parte Geral, Diptera, Anophelini*. Faculdade de Saúde Pública. Depto. Parasitol., São Paulo, Brasil, 622 pp.

Forattini OP 1965a. *Entomologia Médica. II. Culicini: Culex, Aedes, Psorophora*. Universidade de São Paulo, Brasil, 506 pp.

Forattini OP 1965b. *Entomologia Médica. III. Culicini: Haemagogus, Mansonia, Culiseta. Sabethini. Toxorhynchitini. Arboviruses. Filariose bancroftiana. Genética.* Universidade de São Paulo, Brasil, 416 pp.

Forattini OP, Rabello EX 1960. Notas sobre Culicidae (Diptera). 2. A larva, a pupa e alguns dados biológicos de *Aedes (Finlaya) fluviatilis* Lutz, 1904. *Arquivos Fac Hig e Saúde Publ 14:* 87-94.

Ishii T, Sohn SR 1987. Highly polluted larval habits of the *Culex pipiens* complex in central Sweden. *J Am Mosq Control Assoc 3*: 276-281.

Kulkarni SM, Naik PS 1989. Breeding habitats of mosquitoes in Goa. *Indian J Malar 26:* 41-44.

Lane J 1953. *Neotropical Culicidae*. Ind. Graf. Siqueira S.A. São Paulo, Brasil, 1112 pp.

Luti R 1979. Vegetación, p.297-368. In JB Vázquez, RA Miatello, ME Roqué (eds). *Geografía Física de la Provincia de Córdoba*. Córdoba, Boldt, Rep. Argentina.

Mitchell CJ, Monath TP, Sabattini MS, Cropp CB, Daffner JF, Calisher CH, Jakob WL, Christensen HA 1985. Arbovirus investigations in Argentina, 1977-1980. II. Arthropod collections and virus isolations from argentine mosquitoes. *Am J Trop Med Hyg 34:* 945-955.

Mitchell CJ, Monath TP, Sabattini MS, Daffner JF, Cropp CB, Calisher CH, Darsie Jr RF, Jakob WL 1987. Arbovirus isolations from mosquitoes collected during and after the 1982-1983 epizootic of western equine encephalitis in Argentina. *Am J Trop Med Hyg 36:* 107-113.

Oliveira RL, Heyden R, Silva TF 1986. Alguns aspectos da ecologia dos mosquitos (Diptera: Culicidae) de uma área de planície (Granja Calábria), em Jacarepaguá, Rio de Janeiro. V. Criadouros. *Mem Inst Oswaldo Cruz 81:* 265-271.

Prosen AF, Martínez A, Carcavallo RV 1960. La familia Culicidae (Diptera) de la rivera fluvial de la provincia de Buenos Aires. *An Inst Med Reg 5:* 101-113.

Prosen AF, Carcavallo RV, Martínez A 1962/1963. Culicidae de Bolivia (Diptera). *An Inst Med Reg 6:* 59-124.

Rejmankova E, Savage HM, Rejmanek M, Arredondo-Jimenez JI, Roberts DR 1991. Multivariate analysis of relationships between habitats, environmental factors and occurrence of anopheline mosquito larvae *Anopheles albimanus* and *A. pseudopunctipennis* in Southern Chiapas, Mexico. *J Appl Ecol 28:* 827-841.

Rejmankova E, Savage HM, Rodriguez MH, Roberts DR, Rejmanek M 1992. Aquatic vegetation as a basis of classification of *Anopheles albimanus* Weideman (Diptera, Culicidae) larval habitats. *Environ Entomol 21:* 598-603.

Rodriguez AD, Rodriguez MH, Meza RA, Hernandez JE, Rejmankova E, Savage HM, Roberts DR, Pope KO, Legters L 1993. Dynamics of population densities and vegetation associations of *Anopheles albimanus* larvae in a coastal area of southern Chiapas, Mexico. *J Am Mosq Control Assoc 9:* 46-58.

Rohlf FJ 1988. *NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.4.* Exeter Publishing Ltd. New York, 146 pp.

Sabattini MS, Monath TP, Mitchell CJ, Daffner JF, Bowen GS, Pauli R, Contigiani MS 1985. Arbovirus investigations in Argentina, 1977-1980. I. Historical aspects and description of study sites. *Am J Trop Med Hyg 34:* 937-944.

Savage HM, Rejmankova E, Arredondo-Jimenez JI, Roberts DR, Rodriguez MH 1990. Limnological and botanical characterization of larval habitats for two primary malarial vectors, *Anopheles albimanus* and *Anopheles pseudopunctipennis*, in coastal areas of Chiapas State, Mexico. *J Am Mosq Control Assoc 6:* 612-620.

Sneath PHA, Sokal RR 1973. *Numerical taxonomy. The principles and practice of numerical classification.* Freeman, San Francisco, CA., XV, 573 pp.