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Biodiversity, bugs, and barcodes: the Cicadellidae associated with grassland and phytoplasmas in the Sabana de Bogotá, Colombia

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Abstract

The leafhoppers (Cicadellidae) are a diverse and cosmopolitan group of Hemiptera that feed on plants, and occasionally cause losses due to the direct damage they inflict to their hosts, or by transmission of viruses and phytoplasmas. Phytoplasmas are plant pathogenic bacteria that can adversely affect many plant families. In the Sabana de Bogotá, Colombia, several species of urban trees, potatoes, and strawberry crops are affected by phytoplasma diseases. The family Cicadellidae contains the largest number of known vectors of phytoplasmas, but in Colombia, knowledge of their biology is scarce. The objective of this work was to characterize the diversity of Cicadellidae associated with kikuyu grass *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone (Poaceae) in the grasslands of the Sabana de Bogotá, at an urban site of the Universidad Nacional de Colombia and a semirural site at the Universidad Militar Nueva Granada, located in Bogotá and Cajicá, Colombia, respectively. Species richness, abundance, and cicadellid dominance were compared for the 2 sampling sites, using alpha and beta diversity estimators. In total, 3,334 leafhoppers were collected, represented by subfamilies Deltocephalinae (82.3%), Cicadellinae (15.8%), Aphrodinae (1.2%), Iassinae (0.6%), Idiocerinae (0.03%), and Typhlocybinae (0.03%). Of the 15 leafhopper species present, 6 were identified to the species level. DNA barcodes were established for 12 morphotypes, including 6 species and 6 superior taxa by amplification of the *COI* gene. In both locations, the sampling effort was deemed insufficient. Species richness at Universidad Militar Nueva Granada was greater (*n* = 13) than at Universidad Nacional de Colombia (*n* = 10), but abundance was higher at Universidad Nacional de Colombia (*n* = 1,982) than at Universidad Militar Nueva Granada (*n* = 1,352). The 2 most abundant species were *Amplicephalus funzaensis* Linnavuori and *Exitianus atratus* Linnavuori (both Hemiptera: Cicadellidae), both recorded as vectors of phytoplasmas. Other common leafhopper species in collections were *Haldorus* sp. and *Dalbulus* sp. (both Hemiptera: Cicadellidae) and are considered possible phytoplasma vectors. Because *C. clandestinus* is host to several cicadellid species, it may play an important role in the epidemiology of phytoplasma transmission in the Sabana de Bogotá.

Key Words: DNA barcodes; insect vectors of phytoplasmas; biodiversity; *Haldorus* sp.; *Dalbulus* sp.

Resumen

Los saltahojas (Cicadellidae) son un grupo diverso y cosmopolita de Hemiptera, que se alimenta de plantas, y ocasionalmente causan pérdidas debido al daño directo que producen a sus huéspedes o por transmisión de virus y fitoplasmas. Los fitoplasmas son bacterias patogénicas de plantas que pueden afectar adversamente muchas familias de plantas. En la Sabana de Bogotá, Colombia, varias especies de árboles urbanos, cultivos de papa y fresa son afectados por enfermedades fitoplásmicas. La familia Cicadellidae contiene el mayor número de vectores conocidos de fitoplasmas, pero en Colombia el conocimiento de su biología es escasa. El objetivo de este trabajo fue caracterizar la diversidad de Cicadellidae asociados con pasto kikuyu *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone (Poaceae) en pastizales de la Sabana de Bogotá en un sitio urbano en la Universidad Nacional de Colombia y en uno semirural en la Universidad Militar Nueva Granada, localizados en Bogotá y Cajicá, Colombia, respectivamente. La riqueza, abundancia y dominancia de los cicadélidos se comparó para los sitios de muestreo usando estimadores de diversidad alfa y beta. En total, se colectaron 3,334 saltahojas que eran representados por las subfamilias Deltocephalinae (82.3%), Cicadellinae (15.8%), Aphrodinae (1.2%), Iassinae (0.6%), Idiocerinae (0.03%), y Typhlocybinae (0.03%). De las 15 especies presentes, 6 se identificaron hasta nivel de especie. Se establecieron códigos de barras de ADN para 12 morfotipos incluyendo 6 especies y 6 taxones superiores por amplificación del gen *COI*. En ambas locaciones, el esfuerzo de muestreo fue considerado insuficiente. La riqueza de las especies en la Universidad Militar Nueva Granada fue mayor (*n* = 13) que en la Universidad Nacional de Colombia (*n* = 10) pero la abundancia fue mayor en la Universidad Nacional de Colombia (*n* = 1,982) que en la Universidad Militar Nueva Granada (*n* = 1,352). Las 2 especies más abundantes fueron *Amplicephalus funzaensis* Linnavuori (Hemiptera: Cicadellidae) y *Exitianus atratus* Linnavuori (Hemiptera: Cicadellidae), ambas reportadas como vectores de fitoplasmas. Otras especies de saltahojas comunes en las colectas fueron *Haldorus* sp. (Hemiptera: Cicadellidae) y *Dalbulus* sp. (Hemiptera: Cicadellidae) que son consideradas posibles vectores de fitoplasmas. Debido a que *C. clandestinus* es huésped de varias especies de Cicadellidae, este puede jugar un rol importante en la epidemiología de la transmisión de fitoplasmas en la Sabana de Bogotá.

Palabras Clave: Códigos de barras genéticos; insectos vectores de fitoplasmas; biodiversidad; *Haldorus* sp.; *Dalbulus* sp.

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Cicadellidae is a worldwide cosmopolitan group of about 20,000 species (Dietrich 2013a). Because leafhoppers feed from the xylem, mesophyll, or phloem of their hosts they can have a significant negative impact on their host plants, reducing plant growth, producing damage by oviposition, and acting as vectors of viruses and phytoplasmas (Nielson 1968; Ammar & Nault 2002; Weintraub & Beanland 2006). The ecologic interactions of pathosystems that involve phytoplasmas, insect vectors, suitable plant hosts, and a conducive environment are very complex (Lee et al. 2000; Weintraub & Beanland 2006). In order to successfully control phytoplasmal diseases, 2 aspects should be addressed: strengthening plant defense mechanisms and a reduction in transmittal efficiency of the pathogen by the insect vectors (Weintraub & Wilson 2010).

The Sabana de Bogotá consists of a plateau located in the center of the Colombian Andes that is interspersed with urban, rural, and forested areas where plant diseases associated with phytoplasmic groups 16SrI-B and 16SrVII-A have been described. The presence of phytoplasmas has been reported in native and introduced symptomatic urban trees from these areas, such as *Acacia melanoxylon* R.Br. (Fabaceae), *Croton* spp. (Euphorbiaceae), *Eugenia neomyrtifolia* M. Sobral (Myrtaceae), *Fraxinus uhdei* (Wenz.) Lingelsh. (Oleaceae), *Liquidambar styraciflua* L. (Altingiaceae), *Magnolia grandiflora* L. (Magnoliaceae), *Pittosporum undulatum* Guill. (Pittosporaceae), *Populus nigra* L. (Salicaceae), and *Quercus humboldtii* Bonpl. (Fagaceae) (Perilla-Henao et al. 2012; Perilla-Henao & Franco-Lara 2013; Franco-Lara & Perilla-Henao 2014; Franco-Lara et al. 2017). In addition, physiological alterations associated with phytoplasma infection have been described in potato and strawberry crops in the Sabana de Bogotá (L. Franco-Lara unpublished data). In addition to the environmental and economic cost of replacing infected urban trees, a considerable amount of concern also has arisen regarding the potential negative impact of phytoplasmas in the food production and urban environment. Information regarding the diversity and abundance of Cicadellidae inhabiting the Sabana de Bogotá is scarce but needed to define strategies for surveillance and management of phytoplasma diseases. The objective of our study was to characterize the species richness and composition of grassland Cicadellidae of the Sabana de Bogotá, as well as generate DNA barcodes for these species in order to complement their taxonomic identification for future studies.

Materials and Methods

INSECT SAMPLING AND IDENTIFICATION

Leafhoppers were collected at 2 sites of the Sabana de Bogotá. The first site was an urban location in Bogotá, Colombia, on the campus of the Universidad Nacional de Colombia (4.640333°N, 74.081500°W; 2,559 masl) with an average temperature of 13.6 °C and average relative humidity of 79.6%. The habitat sampled encompassed 121.3 ha of which about 80% was covered in *Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone (Poaceae) grassy areas with 13% trees and bushes. The second site was a semirural location at Cajicá, Cundinamarca, Colombia, on the campus of Universidad Militar Nueva Granada (4.944055°N, 74.008722°W; 2,447 masl). The Universidad Militar Nueva Granada campus encompasses 78 ha with an average annual temperature of 13.9 °C and average relative humidity of 87.9%. The predominant plant species is *C. clandestinus*, which covers about 70% of the campus, less than 10% is dedicated to trees and bushes.

Insect sampling was carried out twice per mo from Feb 2016 to Jan 2017, between 11:30 A.M. and 12:30 P.M. On each campus, 1 location was sampled that consisted of three 25 m^2 quadrants further subdivided into five 1 m × 5 m sub-quadrants. On each sample date, 3 randomly selected sub-quadrants were sampled. Sampling sites at both campuses corresponded to areas with little disturbance except in cases where the grass was cut; in those cases new quadrants were established near the original location. At Universidad Nacional de Colombia, the first quadrant was sampled from Feb to Apr 2016, the second quadrant from May to Jun 2016, and the third from Jul 2016 to Jan 2017. At Universidad Militar Nueva Granada, the first quadrant was sampled from Feb to May 2016, the second from May to Oct 2016, and the third from Oct 2016 to Jan 2017. Leafhoppers were captured with an entomological sweep net and mouth aspirator, sweeping 25 times in each sub-quadrant for a total of 900 samples per site. Specimens were preserved in 90% ethanol. Adults were identified based on male external morphological characters with male genitalia cleared in 10% KOH (Oman 1949) based on Dietrich (2005). The following taxonomic keys were used for identifications: Linnavuori (1959), Young (1977), Dietrich (2005), Zahniser & Dietrich (2008, 2013), Beltrán et al. (2011), and Krishnankutty et al. (2016). Some morphotypes were not identified to the species level because only females were collected, or due to insufficient taxonomic keys for the Cicadellidae of Colombia.

DNA BARCODES

Half of leafhopper abdomens were used for DNA extraction while the remainder was preserved as voucher specimens. Extractions were performed using the protocol of Hung et al. (2004) with minor modifications. The DNA pellet was air dried and finally re-suspended in 25 µL of TE (10 mM Tris HCl pH 8.0, 1 mM EDTA). DNA extracts were stored at −20 °C. The PCR amplification of the *COI* gene was performed with primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), which produce an expected band of about 710 bp (Folmer et al. 1994). Samples that did not amplify with primers LCO1490/HCO2198 were tested with primers LepF2_t1 (5′-TGTAAAACGACGGCCAGAATCATAARGATATYGG-3′) and LepR1 (5′-TAAACTTCTGGATGTCCAAAAAATCA-3′), which produce an expected band of about 658 bp (Hebert et al. 2004; Foottit et al. 2014). Reactions were carried out at a final volume of 15 µL, with 1X reaction buffer, 2.5 mM Cl_2Mg, 0.3 mM dNTPs, 0.3 mM of each set of primer, 2 µL of DNA template, 0.05 U of Taq DNA polymerase (Bioline, London, United Kingdom), and MiliQ (Merk KGaA, Darmstadt, Germany) sterile water. The thermic cycle for both pairs of primers was: 1 cycle at 95 °C 10 min; 35 cycles of denaturation at 95 °C for 60 s, annealing at 45.6 °C for 45 s, and extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. When using LCO1490 and HCO2198 in the Idiocerinae sp. samples, the annealing temperature was increased to 48.4 °C in order to obtain a single band. Amplicons were analyzed by standard gel agarose at 1% in 0.5X TBE buffer (Tris-Borate-EDTA). Samples used for sequencing were amplified by PCR as described, but at final volume of 30 µL.

PCR products were cleaned with the "UltraClean PCR Clean-up" kit (MoBio, Carlsbad, California, USA) and sequenced bi-directionally at Macrogen, Seoul, Korea. Sequences were edited manually, and the forward and reverse sequences aligned with MUSCLE (Edgar 2004) using the Geneious 10.2.3 software (Kearse et al. 2012) to generate consensus sequences of each sample. The sequences were compared by BLASTn with the NCBI (https://www.ncbi.nlm.nih.gov/) and BOLD (Barcode of Life Data System) databases. Genetic distances were estimated and a sequence tree constructed with the neighbor joining algorithm using the Kimura-2-parameters model (Kimura 1980) with 1,000 bootstrap (Felsenstein 1985) in Mega 7.0.26 software (Kumar et al. 2016), using as an outgroup *Tylocentrus quadricornis* Funkhouser (Hemiptera: Membracidae). The sequences produced in this work

were aligned with sequences of Cicadellidae species obtained from BOLD. A Barcode gap analysis was performed for these sequences using Mega 7.0.26 to estimate the intra- and interspecific genetic distances. Voucher specimens were placed in the entomological collection of Universidad Militar Nueva Granada and the DNA barcode sequences submitted to BOLD.

BIODIVERSITY STUDIES

Cicadellid males and females were used in all biodiversity analyses. The alpha diversity of the 2 sampling sites was calculated as the relative abundance per mo using the Margalef index (DMg), the dominance index Simpson (λ), and the equity index Shannon-Wiener (H´), using the PAST 3.15 software (Hammer et al. 2001). To determine if sampling effort was adequate for each site, accumulation curves for the collected species were built with EstimateS 9.1.0 (Colwell 2013) and compared with the accumulative curves of non-parametric estimators Chao 2, Jackknife 1, Jackknife 2, and Bootstrap (Magurran 2004; Chao et al. 2005). Hill numbers N0, N1, and N2 were calculated to determine the effective number of species. Beta diversity was based on Bray-Curtis (Ss), Jaccard (IJ), and Sørensen (IS) indices (Magurran 2004).

Results

LEAFHOPPER DIVERSITY

During the period of study, a total of 3,334 leafhopper adults and 1,175 nymphs were captured at both sample sites (Universidad Nacional de Colombia: *n* = 1,982 adults, *n* = 856 nymphs; Universidad Militar Nueva Granada: *n* = 1,352 adults, *n* = 919 nymphs). The most abundant subfamily was Deltocephalinae (82.3%), followed by Cicadellinae (15.8%), Aphrodinae (1.2%), Iassinae (0.6%), Idiocerinae (0.03%), and Typhlocybinae (0.03%) that belonged to 15 taxonomic groups (Table 1). Identification to species level was possible for 6 of them: *Amplicephalus funzaensis* Linnavuori; *Borogonalia impressifrons* Signoret; *Draeculacephala soluta* Gibson; *Exitianus atratus* Linnavuori; *Paracatua rubrolimbata* (Signoret), and *Xestocephalus desertorum* Berg (all Hemiptera: Cicadellidae). The sex ratio of these species is presented in Table 1. Images of the collected morphotypes are presented in the Supplementary Material.

The semirural environment of Universidad Militar Nueva Granada contained a greater number of taxonomic groups (*n* = 13) than the urban environment of Universidad Nacional de Colombia (*n* = 10); however, the Chao 2 index estimated *n* = 13 for both sampling sites. For Universidad Militar Nueva Granada, Jackknife 1 and Jackknife 2 estimators calculated an expected number of species of *n* = 15, and for Universidad Nacional de Colombia *n* = 13 and *n* = 16, respectively (Fig. 1). The Simpson index showed that Universidad Nacional de Colombia species dominance was greater than Universidad Militar Nueva Granada due to the fact that *A. funzaensis* was an abundant species (*n* = 1,334). For both sites the similarity was > 53% (Table 2).

Abundance of monthly leafhopper capture rates varied for different species depending on mo and site. *Amplicephalus funzaensis*, the most abundant species, was collected infrequently at Universidad Nacional de Colombia during the first sample period, but the number of captured individuals increased up to 4 times from Oct 2016 to Jan 2017; at Universidad Militar Nueva Granada the number of captures was similar throughout the yr (Fig. 2A). *Exitianus atratus*, the second most frequent species, had a high rate of capture at Universidad Nacional de Colombia from Apr to May and Nov. Individuals of this species at Universidad Militar Nueva Granada were most abundant during Feb to Oct with a decrease in the last of mo of the yr (Fig. 2B). For *B. impressifrons*, collections from Universidad Nacional de Colombia were very low but in Universidad Militar Nueva Granada this species was abundant from Nov to Jan (Fig. 2C). Capture rates of *Dalbulus* sp. at Universidad Nacional de Colombia were very high during Feb, but remained very low the following mo until a peak was observed from Nov to Dec. At Universidad Militar Nueva Granada, no individuals of this genus were collected in sweep net samples obtained during Feb to Sep but peaked in Nov (Fig. 2D). *Haldorus* sp. collections at Universidad Militar Nueva Granada peaked in Sep with a lesser peak in May and very low captures in Jul, Aug, Nov, and Dec 2016, and Jan 2017, which appeared to coincide with the precipitation periods. At Universidad Nacional de Colombia, more leafhoppers of this genus occurred in samples during May and Jun (Fig. 2E). In both sample locations,

Table 1. Number of Cicadellidae captured at Universidad Nacional de Colombia (UNAL) and Universidad Militar Nueva Granada (UMNG) and their taxonomic identification.

Subfamily	Tribe	Species or specimens	UNAL total	M	F	Sex ratio (M:F)	UMNG total	M	F	Sex ratio (M:F)
Aphrodinae	Portanini	*Portanini* sp.	1	0	1	—	2	1	1	1:1
Aphrodinae	Xestocephalini	*Xestocephalus desertorum*	33	27	6	4.5:1	4	4	0	—
Cicadellinae	Cicadellini	*Borogonalia* sp.	224	120	104	1.2:1	0	0	0	—
Cicadellinae	Cicadellini	*Borogonalia impressifrons*	8	5	3	1.7:1	198	99	99	1:1
Cicadellinae	Cicadellini	*Draeculacephala soluta*	0	0	0	—	91	30	61	1:2
Cicadellinae	Cicadellini	*Paracatua rubrolimbata*	0	0	0	—	7	2	5	1:2.5
Deltocephalinae	Chiasmini	*Exitianus atratus*	204	80	124	1:1.6	312	141	171	1:1.2
Deltocephalinae	Deltocephalini	*Deltocephalini* sp.	0	0	0	—	2	2	0	—
Deltocephalinae	Deltocephalini	*Amplicephalus funzaensis*	1334	535	799	1:1.5	305	117	188	1:1.6
Deltocephalinae	Deltocephalini	*Amplicephalus* sp.*	1	0	1	—	1	0	1	—
Deltocephalinae	Deltocephalini	*Haldorus* sp.	34	26	8	3.3:1	225	160	65	2.5:1
Deltocephalinae	Macrostelini	*Dalbulus* sp.	142	79	63	1.3:1	185	115	70	1.6:1
Iassinae	Gyponini	*Gyponini* sp.	0	0	0	—	19	6	13	1:2.1
Idiocerinae	—	*Idiocerinae* sp.	1	1	0	—	0	0	0	—
Typhlocybinae	Empoascini	*Empoasca* sp.*	0	0	0	—	1	0	1	—
		Abundance per site	1,982				1,352			
		Total abundance	3,334							

*Morphospecies where only females were collected. M = males, F = females.

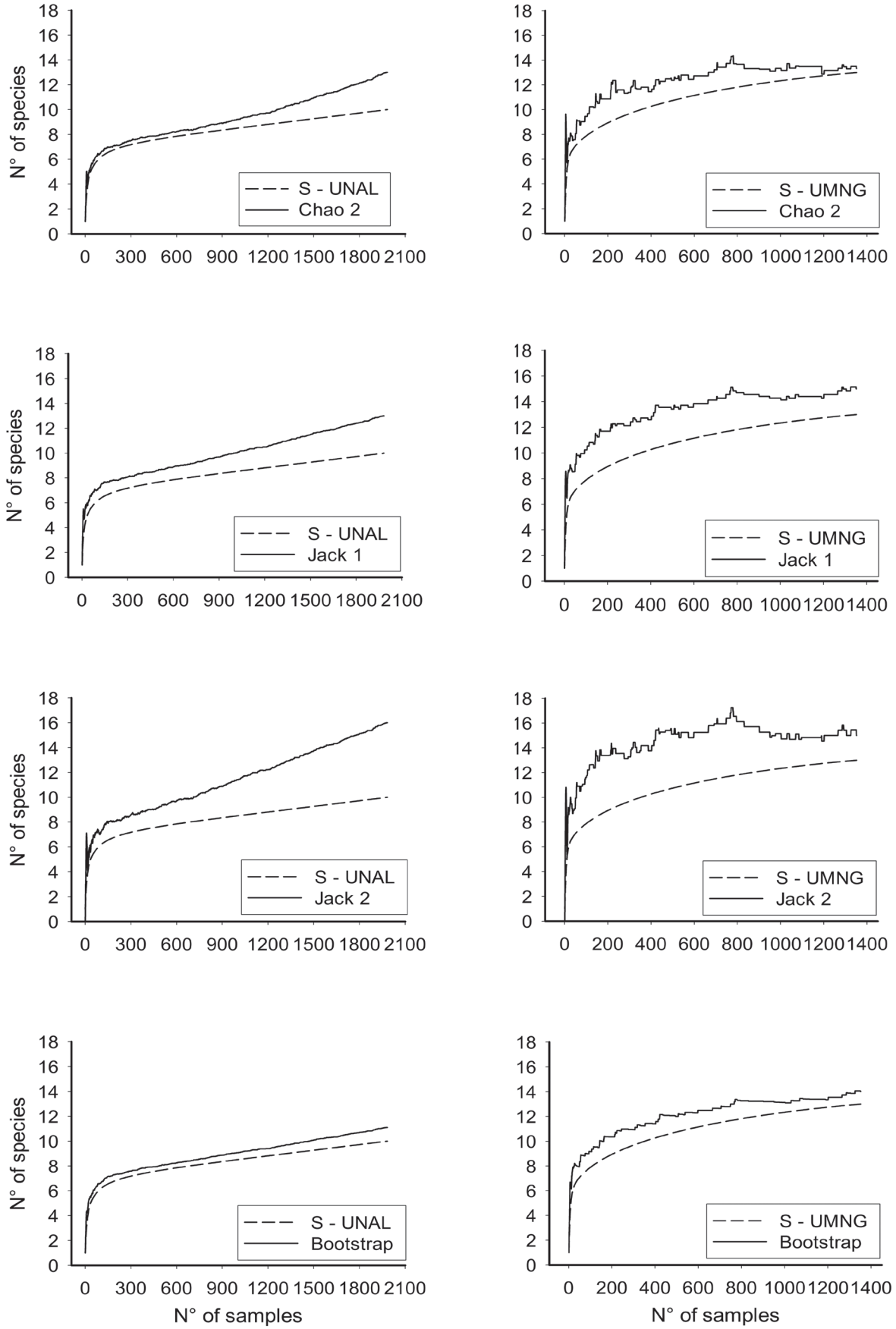

Fig. 1. Accumulation curves for the observed species (S) and for the non-parametric estimators Chao 2, Jack 1 = Jackknife 1, Jack 2 = Jackknife 2, and Bootstrap from Universidad Nacional de Colombia (UNAL) and Universidad Militar Nueva Granada (UMNG).

Table 2. Index values for specific richness, dominance, equitability, effective number of species (N0, N1, and N2), similarity and dissimilarity for Universidad Nacional de Colombia (UNAL) and Universidad Militar Nueva Granada (UMNG).

	Number samples	Margalef (specific richness)	Simpson (dominance)	Shannon-Wiener (equitability)	N_0	N_1	N_2	Jaccard (similarity)	Sørensen (similarity)	Bray-Curtis (dissimilarity)
UNAL	1,982	1.19	0.48	1.11	10	3	2	0.53	0.70	0.41
UMNG	1,352	1.66	0.18	1.84	13	6	5			

abundance of the remaining species was too low in both locations to determine seasonality.

During the study, 3 closely located quadrants were sampled at each location. Interestingly, the spatial distribution of *B. impressifrons*, *Dalbulus* sp. and *D. soluta* was not homogeneous. These 3 species were collected together from 1 quadrant, unlike *E. atratus* and *Haldorus* sp. where they occurred in any quadrant. *Amplicephalus funzaensis* was an interesting case because at Universidad Militar Nueva Granada, the number of collected individuals was more or less homogenous among quadrants, but at Universidad Nacional de Colombia, a marked heterogeneity was observed (Table 3).

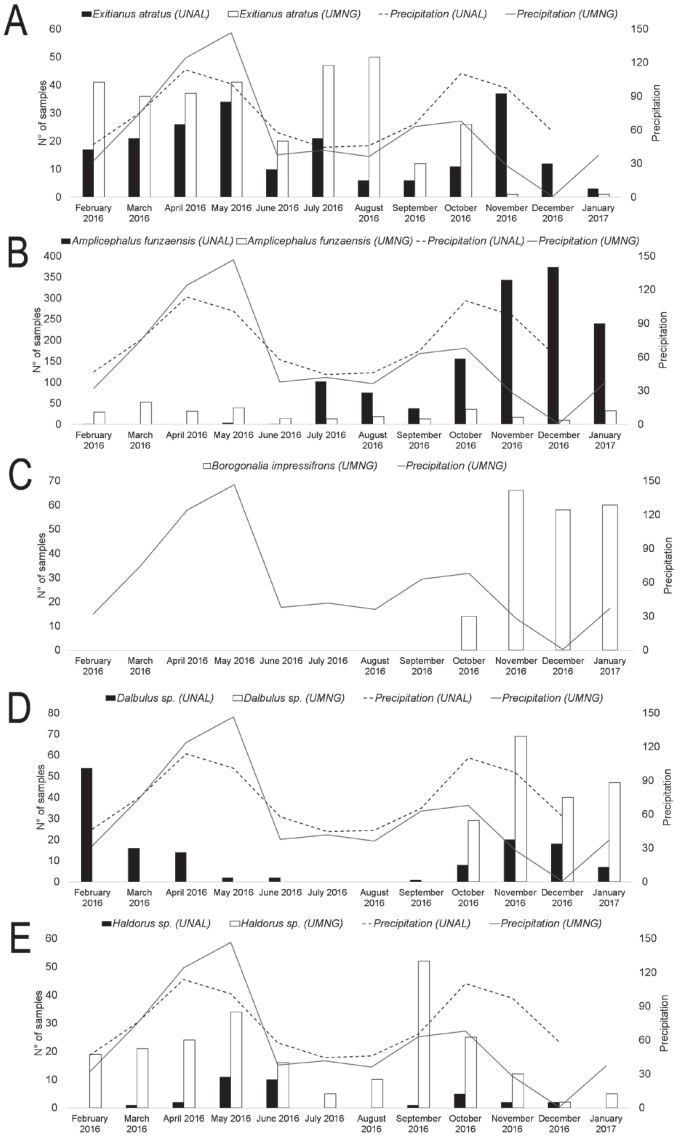

Fig. 2. Capture rate per month of *Exitianus atratus*, *Amplicephalus funzaensis*, *Borogonalia impressifrons*, *Dalbulus* sp., and *Haldorus* sp. at Universidad Nacional de Colombia (UNAL) and Universidad Militar Nueva Granada (UMNG).

DNA BARCODES

PCR reactions using primers LCO1490/HCO2198 produced amplicons of approximately 600 bp for *A. funzaensis*, *Dalbulus* sp., *D. soluta*, *E. atratus*, *Haldorus* sp., *P. rubrolimbata*, and *X. desertorum*, including those of Portanini sp., Gyponini sp., and Idiocerinae sp. For *B. impressifrons* and *Empoasca* (Hemiptera: Cicadellidae) sp., LepF2_t1/LepR1 produced amplicons or about 600 bp. However, with these 2 primer sets no amplification of the *COI* gene was observed for individuals of *Amplicephalus* sp., *Borogonalia* sp., or Deltocephalini sp. In some cases, the LCO1490/HCO2198 primers amplified sequences of the bacteria *Wolbachia* spp. (Rickettsiales: Anaplasmataceae) from *A. funzaensis* and *Dalbulus* sp. From 45 sequenced *COI* amplicons, 36 showed good quality reads. For *B. impressifrons*, *D. soluta*, *E. atratus*, *Haldorus* sp., and *P. rubrolimbata*, 2 haplotypes per species were found, but for the rest of the taxonomic groups only 1 haplotype was obtained. Sequences, trace files, collection data, and specimen photographs were deposited in the Barcode of Life Data System (BOLD, http://www.boldsystems); processes ID are shown in fig. 3.

A neighbor joining sequence tree was constructed including 12 sequences of leafhoppers from Universidad Nacional de Colombia, 24 from Universidad Militar Nueva Granada, with 42 sequences downloaded from BOLD (Fig. 3). In all cases, sequences belonging to the same taxa clustered together. Furthermore, all sequences of Deltocephalinae species clustered in a single clade, and the Cicadellinae, Typhlocybinae, and Idiocerinae were grouped in a second clade. A third clade contained the sequences from Portanini sp. that belong to the subfamily Aphrodinae. A fourth clade contained the subfamily Iassinae, whereas a fifth clade contained individuals of the tribe Xestocephalini that belong to subfamily Aphrodinae.

In order to evaluate the utility of sequences as DNA barcodes, a Barcode gap analysis was performed. The average intraspecific divergence of sequences was 0.3%, with a minimum value of 0% and a maximum of 2.4%; average interespecific divergence was 25.1% with a minimum of 10.7% and a maximum value of 33.7%. Sequences of Portanini sp., Gyponini sp., and Idiocerinae sp. showed an intraspecific divergence of 0% while that of *B. impressifrons*, *D. soluta*, *E. atratus*, *Haldorus* sp., and *P. rubrolimbata* was of 0.08%, 0.1%, 0.04%, 0.1%, and 0.3%, respectively. All sequences used in our analyses fulfilled the barcode gap criterion (results not shown).

Discussion

We found that the biodiversity of Cicadellidae in Sabana de Bogotá showed greater richness at the semirural Universidad Militar Nueva Granada campus, compared with the urban Universidad Nacional de Colombia campus. This geographic area is located in a tropical zone where meteorological seasons do not exist, maximum and minimum daily temperatures are similar throughout the yr with a bimodal precipitation regime from Apr to May, and Sep to Nov the rainiest mo

Table 3. Number of leafhoppers captured per quadrant, per site at Universidad Nacional de Colombia (UNAL) and Universidad Militar Nueva Granada (UMNG).

	UMNG			UNAL		
Species or specimens	Q1	Q2	Q3	Q1	Q2	Q3
Amplicephalus sp.	0	0	1	1	0	0
A. funzaensis	129	106	70	1	56	1277
B. impressifrons	0	0	198	0	0	8
Borogonalia sp.	0	0	0	5	5	214
D. soluta	0	2	89	0	0	0
Dalbulus sp.	0	0	185	84	4	54
Deltocephalini sp.	2	0	0	0	0	0
E. atratus	135	175	2	64	63	77
Empoasca sp.	0	1	0	0	0	0
Gyponini sp.	16	3	0	0	0	0
Haldorus sp.	97	107	21	3	21	10
Idiocerinae sp.	0	0	0	0	1	0
P. rubrolimbata	0	0	7	0	0	0
Portanini sp.	0	0	2	1	0	0
X. desertorum	0	2	2	11	11	11

Q1 = quadrant 1, Q2 = quadrant 2, Q3 = quadrant 3.

(Guzmán et al. 2014). Cicadellid species, as well as abundance in collections fluctuated during the yr, but only in the case of *Haldorus* sp. did the fluctuation show a correlation with the dry and rainy periods. More observations and finer measurements of the climatic variables are needed to make correlations with the population dynamics of these species. However, observations indicate that for different mo, different vector species prevailed, and that during an entire yr there are insect vectors present to transmit phytoplasmas.

Non-parametric estimators showed that the sampling effort performed was not enough in either site. In a previous study (A. F. Silva-Castaño unpublished results), 13 species were collected from the Universidad Militar Nueva Granada campus, of which 4 (*Agalliana* sp., *Gyponana* sp., *Stehlikiana crassa* [Walker], and *Xerophloea* sp.) (all Hemiptera: Cicadellidae) were not captured in our current study. We found the diversity indices difficult to interpret from our datasets; therefore, the effective number of species was calculated (Magurran 2004). Hill series values indicated that at Universidad Nacional de Colombia, *A. funzaensis* was a dominant species, but at Universidad Militar Nueva Granada, greater equitability occurred because several abundant species were observed, such as *A. funzaensis*, *B. impressifrons*, *Dalbulus* sp. *E. atratus*, and *Haldorus* sp. We also found that the most abundant subfamilies represented in collections were Cicadellinae and Deltocephalinae. Both families have many representatives that are grass feeders (Dietrich 2000). The number of individuals of Idiocerinae, Iassinae, and Typhlocybinae, which are usually reported as tree and bush inhabitants, was low in our sweep net collections (Dietrich 2000; Dietrich 2013b). However, observations from the current study suggest that Idiocerinae sp. and *Empoasca* sp. may be casual visitors of *C. clandestinus*, because males and females of these species were observed during sampling in nearby *Eugenia neomyrtifolia* Sobral (Myrtaceae) and *Acacia decurrens* (J.C. Wendl.) Willd. (Fabaceae). A few individuals of *X. desertorum* were captured at Universidad Nacional de Colombia and Universidad Militar Nueva Granada. This species of Aphrodinae usually is associated with the roots of grasses (Cwikla & Blocker 1981).

The most abundant species in both sampling sites were *A. funzaensis* and *E. atratus*, both of which are known to transmit phytoplasmas of groups 16SrI-B and 16rVII-A under experimental conditions (Perilla-Henao et al. 2016). *Haldorus* sp. and *Dalbulus* sp. also were abundant in our sweep net collections. Perilla-Henao et al. (2016) showed that *Haldorus* sp. from the Sabana de Bogotá was infected with phytoplasmas of the 16SrI group. *Dalbulus* spp., on the other hand, has been reported as the vector of *Maize rayado fino virus* (Tymovirales: Tymoviridae) (Gámez 1973) maize bushy stunt phytoplasmas (group16SrI-B) (Nault 1980), and *Spiroplasma kunkelii* Whitcomb et al. (Entomoplasmatales: Spiroplasmataceae), the ethiological agent of corn stunt disease in maize in the US (Whitcomb et al. 1986). *Amplicephalus funzaensis*, *E. atratus*, *Haldorus* sp., and *Dalbulus* sp. are abundant inhabitants of the Sabana de Bogotá grassland and may be involved in the dispersal of phytoplasmas.

The ecosystem of the Sabana de Bogotá has changed dramatically from native low Andean forests in pre-Hispanic times, to the present situation of semirural areas intermingled with urban zones (Pérez-Preciado 2000). *Cenchrus clandestinus* was introduced into Colombia in the 1920s, becoming one of the predominant grass species of the Andes in urban and rural zones, replacing many native plant species (Mears 1970; Nepstad et al. 2013). In the Sabana de Bogotá, *C. clandestinus* is an asymptomatic host of phytoplasmas of 16SrI-A and 16SrVII-B groups (Perilla-Henao et al. 2016; L. Franco-Lara unpublished results). Therefore, *C. clandestinus* plays an important role in the epidemiology of phytoplasmas in the region because it serves as hosts to several Cicadellidae species incriminated as vectors.

The main goal of the DNA Barcode system is to generate short DNA sequences that allow the identification of species by comparing them with a cured database such as BOLD (http://v3.boldsystems.org/) (Hebert et al. 2004). To complement the conventional taxonomic identification of the cicadellids from our collection, DNA barcodes for the 12 morphotypes were obtained. The neighbor joining tree built with those sequences clustered individuals of the same species and separate species in different clades, showing the usefulness of the barcodes. To our knowledge these are the first DNA barcode reports for Colombian Cicadellidae published in the BOLD database. However, no amplicons or sequences were obtained for the *Amplicephalus* sp., *Borogonalia* sp., or Deltocephalini sp. specimens. Failure to amplify those individuals with primers designed for the *COI* gene of invertebrates has been reported before (Palomera et al. 2012; Sharma & Kobayashi 2014). In 3 cases, the primers we used amplified the *COI* gene of *Wolbachia* from *A. funzaensis* and *Dalbulus* sp. samples. *Wolbachia* belongs to a genus of endosymbiotic bacteria of many arthropod species, which affects the sex-ratio and reproductive compatibility of its hosts. Insect *COI*

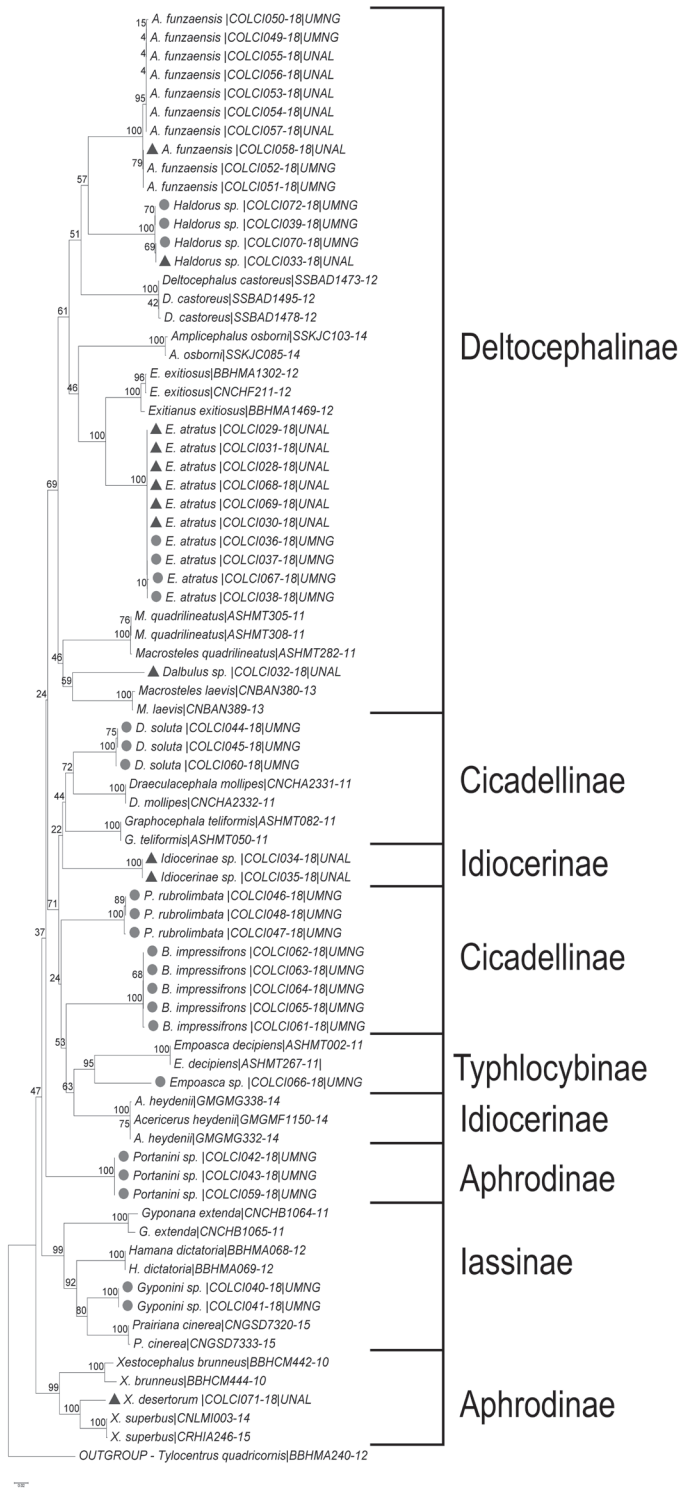

Fig. 3. *COI* gene neighbor joining tree using Kimura-two-parameter (K2P) with 1,000 Bootstrap. The tree includes 77 leafhopper sequences. Red Circles indicate the sequences of species captured at Universidad Militar Nueva Granada (UMNG) and triangles at Universidad Nacional de Colombia (UNAL).

primers can amplify the *Wolbachia COI* gene, but it is not considered to compromise the accuracy of the DNA barcode system (Smith et al. 2012). A Barcode gap analysis also was performed for sequences obtained in this work with additional Cicadellidae sequences retrieved from other databases. This analysis refers to the separation between mean intra- and interspecific sequence variability for congeneric *COI* sequences. If a gap exists between these 2, a cut value for the species delimitation can be established that would illustrate the usefulness of this method, as indicated by Meyer and Paulay (2005). We found that barcode gap analysis from all the taxonomic groups in our study met this criterion. However, this is a limited assessment because sequences of close phylogenetic species were not included because they are not available in the databases. This limitation has been pointed out, but it will be solved as the databases are being fed more information (Collins & Cruickshank 2012). DNA barcodes are useful for the routine identification of adult or immature arthropods, determination of cryptic or atypical species, including taxonomic and phylogenetic studies (Hajibabaei et al. 2007; Foottit et al. 2014; Čandek & Kuntner 2015). In our case, the availability of DNA barcodes will enhance our ability to study the biology of cicadellids, particularly vectors of phytoplasmas.

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