Conservation units of *Pteronotus davyi* (Chiroptera: Mormoopidae) in Mexico based on
phylogeographical analysis

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The analysis of genetic diversity is routinely used to identify divergent intraspecific units and contribute to the knowledge base of biodiversity. In this study we used genetic diversity to propose three management units (MUs) for the Davy's naked-backed bat, an insectivorous forest-dwelling species that is distributed in tropical and subtropical areas of America. We analyzed a 555 bp segment of the mitochondrial DNA (mtDNA) control region in 144 individuals from 18 localities spread across the species distribution range in Mexico. Our results demonstrated that the genetic diversity of *P. davyi* is distributed in three management units (MUs), namely Gulf North, Pacific-Veracruz and Southeastern, with conservation priority, due to either the high genetic diversity or the high proportion of unique haplotypes, for the following populations: Playa de Oro, Arroyo del Bellaco and Catemaco in the Pacific-Veracruz region, and Agua Blanca, Sardina, Calakmul, Calcehtok and Kantemó from the Southeastern region. The Gulf North unit shows signs of the recent loss of genetic variability. These proposed conservation units could be considered a generalized model of conservation for other species of cave-dwelling bats that share the same habitats.

INTRODUCTION

Phylogeographic studies are very useful in identifying the principles and processes governing the geographical distributions of genealogical lineages, especially those at the intraspecific level (Avice *et al.*, 1987), aspects that are essential for biodiversity conservation (Moritz, 2002). The identification of evolutionary divergent populations is a priority for maintaining genetic intraspecific diversity (Avice, 2004), and molecular characters provide a rich source of information for the analysis of this intraspecific history and the delineation of population units, such as the Evolutionary Significant Unit (ESU) and, more closely related to conservation, the Management Unit (MU) (Moritz, 1994). An ESU can be defined as a group of individuals or populations with reciprocal monophyly for mitochondrial markers. ESUs represent historically isolated lineages that cover the evolutionary diversity of a taxon and therefore have a high priority for conservation. MUs consist of one or more populations with significant differentiation in their mitochondrial haplotypes, regardless of the monophyly of the alleles; in operational terms they represent populations exhibiting low gene flow and consequently some demographic independence (Moritz, 1994).

The Davy's naked-backed bat, *Pteronotus davyi* Gray 1838, is a forest-dwelling insectivorous bat (Adams, 1989) belonging to the family Mormoopidae that is distributed mainly in tropical and subtropical areas of America. In Mexico all naked-backed bats are included within the subspecies *P. d. fulvus*, which is distributed along two separated narrow strips that extend along the Pacific coast (from Sonora to Chiapas) and the Gulf of Mexico (from Tamaulipas to Tabasco) that converge in the lowlands of the Isthmus of Tehuantepec and then penetrate into the Yucatan Peninsula (Smith, 1972).

Due to its typically large population sizes, Davy's naked-backed bat plays an essential regulatory role in forest insect populations, important even for the maintenance of these tropical ecosystems (Bateman and Vaughan, 1974; Adams, 1989). Nevertheless, and mainly due to human activities, the tropical forests are continuously shrinking. The official document "Mexico's REDD+ vision" (CONAFOR, 2010), states that the country lost on average 155,152 hectares of forest extent each year for the period 2002-2007, highlighting the fact that for the last 5 years 99.9% of the deforestation has occurred in the tropical forests, the main habitat of *P. davyi*. If this trend continues it is very likely that in the near future populations of this forest bat species will need active management to prevent the loss of genetic diversity.

In this study we analyzed the evolutionary history of *P. davyi* in Mexico, focusing on its genetic geographic variation and establishing dates for the main recent evolutionary events. We then utilized this information to identify ESUs and MUs for *P. davyi* in Mexico. The MUs proposed are used to determine priorities for the conservation of the genetic diversity of this species. Finally, *P. davyi* is employed as a model species to define conservation priorities for other forest-dwelling fauna whose shared habitat is being similarly affected by human activities.

MATERIAL AND METHODS

Samples and study area

We sampled 18 *P. davyi* populations (names and acronyms are provided in Appendix 1) throughout the whole distribution area of the species in Mexico. Specimens were captured using harp traps, measured, and then biopsied from wing membranes using a three-mm

biopsy puncher (Fray Products Corp., Buffalo, New York). Tissue samples were stored in 70% ethanol. The sampled bats were then immediately released, except for a few specimens which were preserved as vouchers and deposited in the scientific collections at the Estación Biológica de Doñana (CSIC) (catalogue numbers: EBD12750, EBD21346, EBD25382 and EBD25383). For appropriate ethics we followed our institutional protocol (Anonymous, 2010) and Sikes *et al.* (2011).

DNA extraction, amplification and sequencing

A total of 144 bats were used (accession numbers EF989018-EF989084, JN375694-JN375710). Total DNA extraction was performed using the DNeasy Tissue Kit (QIAGEN, Inc., Valencia, California). Primers, amplification conditions and sequencing of the hypervariable domain (HVII) of the control region followed the methods described by Guevara-Chumacero *et al.* (2010).

Genealogical analyses and genetic structure

We explored global genetic structure using Spatial Analysis of Molecular Variance (SAMOVA), version 1.0 (Dupanloup *et al.*, 2002). This method defines k as continuously homogenous but genetically differentiated populations where the proportion of total genetic variance (F_{CT}) is maximized. We ran SAMOVA with 1000 simulated annealing processes for a range of k values from two to four. An analysis of molecular variance (AMOVA) was then used to examine the amount of genetic variability partitioned within and among populations, as well as among groups of populations, using Arlequin, version 3.11

(Excoffier *et al.*, 2005). To explore the relationships among haplotypes a median-joining network was built using Network, version 4.5.1.6 (Bandelt *et al.*, 1999).

Average genetic distances among regional groups were evaluated using the Tamura–Nei substitution model (TrN—Tamura and Nei, 1993) implemented in Mega, version 4 (Tamura *et al.*, 2007). We used this model because it is suitable for comparisons among closely related taxa (Palma *et al.*, 2005; Guevara-Chumacero *et al.*, 2010).

Polymorphism levels were estimated by haplotype diversity (H) and nucleotide diversity (π), which were determined within geographic regions using DNAsp, version 4 (Rozas *et al.*, 2003).

Finally, linear regressions were generated with NCSS, version 2000 (Hintze, 1999) to examine the relationship between latitude and nucleotide diversity for each geographic region (Gulf, Pacific and Southeastern) to help evaluate possible post-Pleistocene events.

Divergence time and migration. The isolation with migration (IM) coalescent model, as implemented in the program IM (update 2009; Hey and Nielsen, 2007), was used to estimate the divergence times (t) and migration rates in both directions (m_1 and m_2), among the groups of populations of *P. davyi* previously defined by SAMOVA. To calculate these parameters we estimated the effective population sizes of the current (θ_1 and θ_2) and ancestral populations (θ_A) using the same software. The model uses Metropolis–Hasting Markov Chain Monte Carlo (MCMC) simulations to find the optimal combination of values for these demographic parameters that best fit the dataset. The inheritance scalar was set to 0.25, and the model of evolution was set to Hasegawa–Kishino–Yano (HKY) (Hasegawa *et al.*, 1985) nucleotide substitution model, as recommended for mitochondrial DNA (IM manual). We initially performed exploratory runs to define the minimum range

of bounded priors for demographic parameters that ensure the incorporation of full marginal posterior probability densities. The prior values used for the final analyses were as follows: $t=20$, $\theta_1=400$, $\theta_2=300$, $\theta_A=100$ and $m_1=m_2=2$. The convergence was assessed through two independent MCMC runs (with different seed numbers carried out with 20,000,000 recorded steps after a burn-in of 2 million steps), and by monitoring the ESS values, the update acceptance rates and the trend lines.

A divergence rate of 10% per million years was used for the control region, following Wilkinson and Fleming (1996). We converted t to real time (t) using $t = t/\mu$. The effective number of female migrants between populations was calculated using the formulae: $M_1 = \theta_1 * m/2$ and $M_2 = \theta_2 * m/2$ (Hey and Nielsen, 2004).

RESULTS

Nucleotide sequences and haplotypes

A 555-base pair fragment that covered the complete HVII domain of the mtDNA control region of *P. davyi* was analyzed. In this fragment, 61 positions were variable, of which 65.6% was parsimony informative. Eighty-three different haplotypes were identified, with haplotype 53 the most common, occurring in 14.4% of the sampled individuals. A total of 17 (20.5%) shared haplotypes and 66 (79.5%) unique haplotypes were observed (Fig. 1).

Insert Fig. 1....

Genealogical analyses and genetic structure

The hierarchical and spatial analysis of genetic structure (SAMOVA) identified three units ($K=3$, $F_{CT} = 0.52$, $P = 0.000$) as the most fundamental subdivisions: Gulf North (GULN) that covers TR, TA, and PU populations; Pacific-Veracruz (PAC-VER) which includes TI,

SD, FR, VI, AM, OR, PO, LA, CA, and AR populations; and the Southeastern Unit (SOU) that comprises the populations of AB, SA, CK, KA, and CAL.

The distinction of the three groups is supported by a haplotype network, in which six haplotypes belonging to the GULN region, 43 to the PAC-VER region, 32 to the SOU region, and two haplotypes shared among regions (Fig. 2).

The Analysis of Molecular Variance (AMOVA) among these three groups revealed a significantly higher percentage of genetic variance between regional groups than among populations within regional groups (36.2% and 6.6%, respectively), with the largest fraction (57.2%) due to differences within populations (fixation indices: $F_{SC} = 0.10318$, $F_{ST} = 0.42791$, $F_{CT} = 0.36209$, all P -values significant at < 0.05).

Genetic distances based on the Tamura–Nei substitution model (TrN) indicated that the greatest difference was between regions PAC-VER and SOU and GULN and SOU (2.02% and 1.84%, respectively); differentiation between PAC-VER and GULN was 1.52%. The PAC-VER and SOU regions presented similar levels of haplotype diversity, which was lower in the GULN region. Nucleotide diversity also was lower in the GULN region (Table 1).

Insert Table 1....

There was a significant negative correlation between nucleotide diversity and latitude from Pacific ($R^2 = 0.73$, $P = 0.015$) and Gulf ($R^2 = 0.87$, $P = 0.021$) geographic regions. For the Southeastern region this correlation also was negative, although not significant ($R^2 = 0.27$, $P = 0.367$) (Fig. 3).

The isolation with migration coalescent model (IM) suggests that the number of migrants between PAC-VER and SOU regions are effectively low, and also among regions PAC-

VER, GULN and SOU. The posterior distribution in these regions was 0.033 and 0.041, respectively. The migration estimates were greater than one for the PAC-VER–GULN groups, $m_{\text{PAC-VER vs GULN}} = 0.155$ [90% highest posterior density interval (HPD) = 0 to 1.075] and $m_{\text{GULN vs PAC-VER}} = 0.505$ (90% HPD = 0 to 1.359) (Table 2).

Divergence time isolation between PAC-VER, GULN and SOU geographical groups during the Pleistocene was 67,500 years ago [90% HPD = 50,200–84,100 years ago]. Divergence time isolation between PAC-VER and GULN geographical groups was shorter [$t = 21,100$; 90% HPD = 8,500–36,600 years ago], (Table 2).

Insert Table 2....

DISCUSSION

Our results indicate a phylogeographic structure with three lineages: Gulf North (GULN), Pacific-Veracruz (PAC-VER), and Southeastern (SOU), genetically isolated but with a common Pleistocene origin.

Insert Fig. 2....

The isolation with migration results suggest that populations from PAC-VER and GULN regions diverged from populations of the Southeastern region at least 67,000 years ago; a date corresponding to the Mid-Wisconsin interglacial period (Hall, 2005). A similar date was obtained by Guevara-Chumacero *et al.* (2010) for the divergence between PAC–GUL and SOU groups, indicating a long period of isolation supported by very low levels of gene flow, which corroborates the estimates obtained from the IM model (Table 2). Despite the clear differentiation between populations of *P. davyi* on both sides of the Isthmus of Tehuantepec (1.97%) - a transition zone between the Nearctic and Neotropical faunal

regions (Marshall and Liebherr, 2000; Morrone and Márquez, 2001) - they are not monophyletic groups due to the presence of some shared haplotypes and they cannot be considered evolutionary significant units *sensu* Moritz (1994).

The pair-wise F_{ST} , SAMOVA and network analyses indicate a significant subdivision of *P. davyi* into three population groups, PAC-VER, GULN and SOU, and that these populations merit assignment to different management units (MUs), which are defined as “one or more populations with significant differentiation in their mitochondrial haplotypes, regardless of the phylogenetic distinctiveness of the alleles” (Moritz, 1994). The three MUs are the result of the complex recent evolutionary history of *P. davyi*, with different expansion episodes occurring during the Pleistocene. The significant negative correlation between genetic diversity and latitude among bat populations inhabiting both the Pacific and Atlantic coasts (Fig. 3) corresponds to a rapid geographic expansion from southern refugia to formerly unsuitable areas (Merilä *et al.*, 1996; Ruedi and Castella, 2003), through a process of repeated founder events (and loss of alleles) during the range expansion of the populations (Hewitt, 2000).

Insert Fig. 3....

The identified MUs are important in terms of conservation, but given their high genetic diversity and high proportion of unique haplotypes (above 70% by population) (Fig. 1), conservation efforts should be focused foremost on bat populations from Playa de Oro, Arroyo del Bellaco and Catemaco (PAC-VER region), as well as those from Agua Blanca, Sardina, Calakmul, Calcehtok and Kantemó (SOU region). The populations of Agua Blanca and Sardina are located geographically close to the contact area between the regions PAC-VER and SOU, a zone that should be considered under some conservation plan given that

the infrastructure for generating wind energy has had a strong negative impact on bat populations, similar to what occurred in many parts of North America (Johnson, 2005; Kunz *et al.*, 2007; Arnett *et al.*, 2008). A recent study of this contact area in Mexico reported 20 different bat species dead under wind turbines with *Pteronotus davyi* the most commonly killed species (INECOL, 2009).

Due to their loss of genetic variability, the populations of Taninul, Troncones and Pujal (GULN region) should also be considered primary targets of conservation. In this region other bat species with low levels of genetic diversity have also been identified (*Natalus mexicanus*, López-Wilchis *et al.*, 2012; *Pteronotus personatus*, Guevara-Chumacero *et al.*, unpublished data), and similarly low levels of genetic diversity have been identified in plants (González-Astorga *et al.*, 2006). The status of genetic diversity of bats and plants populations in this zone is possibly due to the geographic isolation of the GULN region. As Eckert *et al.* (2008) showed, nucleotide diversity is reduced in marginal areas with respect to central areas of the species distribution. Furthermore, results from IM analysis indicate that *P. davyi* bat populations in the GULN region diverged from the PAC-VER region at least 21,000 years ago, corresponding to the last glacial maximum (LGM). Isolation between GULN and PAC-VER regions is probably amplified by the loss of habitat due to deforestation (Jahrsdoerfer and Leslie, 1988; Ortega-Huerta and Peterson, 2004). In addition, it has been shown that among the causes of decline of genetic diversity in bats are recent changes in land-use, as observed for example in the trefoil horseshoe bat *Rhinolophus trifolius*, papillose woolly bat *Kerivoula papillosa* (Struebig *et al.*, 2011) and Seba's short-tailed bat *Carollia perspicillata* (Meyer *et al.*, 2009). An urgent conservation goal should be to manage the populations of the GULN region with actions

focused on maintaining their current demographic and genetic diversity, given that isolated populations are likely to become extinct in the near future (Templeton *et al.*, 1990), which recently occurred, for example, to the Chihuahuan meadow vole, *Microtus pennsylvanicus chihuahuensis* (List *et al.*, 2010).

The importance of considering the proposed MUs for a forest-dwelling bats in Mexico, such as *P. davyi*, lies in the fact that the species typically inhabits areas where habitat fragmentation has increased dramatic in recent years due to exceptionally high rates of deforestation for agricultural transformation and other human activities (Bray and Klepeis, 2005; Newton, 2007). In addition, the proposed conservation units in this study can probably be generalized to other species of forest-dwelling bats, such as *Pteronotus personatus*, *P. parnellii*, *Mormoops megalophylla* and *Natalus mexicanus*, which in many cases share habitat and shelters (Bayona-Miramontes and Sánchez-Chávez, 2007), and for its large population sizes are important ecosystem service providers and help control insect populations, essential even for the maintenance of tropical ecosystems (Guevara-Chumacero, 2008; Kunz *et al.*, 2011). Conservation management in Davy's naked-backed bats should aim to maintain connectivity between populations to guarantee the observed high levels of gene flow among populations.

In conclusion, this study identifies three different management units (MUs) for *P. davyi*, all of conservation concern, due to the loss of Mexican forests, the main habitat for this species, and the impact caused by human activities. The management of *P. davyi* populations as MUs proposed in this study can be used as a guideline in making decisions concerning the conservation of these forest-dwelling bats, as well as populations of other bat species that exhibit similar ranges and ecological requirements. The importance of the

reduction in deforestation rates and the design of connecting geographic corridors are highlighted as the main ways of preserving genetic diversity not only for this and other species of bats, but also for the remaining Mexican forest flora and fauna.

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FIGURE LEGENDS

Figure 1. Distribution of haplotypes for 18 populations of *Pteronotus davyi* sampled across Mexico. Each circle represents the haplotype diversity of one population, with the relative size of each wedge proportional to the frequency of each haplotype in the population. Unique haplotypes are represented by different numbers in black wedge. The two letter acronym signifies the population (see Appendix 1). Management units are presented and priority populations for conservation are indicated with acronyms listed in red.

Figure 2. Median-joining network of 83 haplotypes of *Pteronotus davyi* in Mexico. Circles represent haplotypes and the size of circle is proportional to the number of individuals sharing that haplotype. Each line is connecting a circle or square (hypothetical internode, i.e. presumed unsampled or missing intermediate haplotypes) and numbers near a branch indicate the number of mutations when greater than one.

Figure 3. Linear regressions, correlation values and significance of nucleotide diversity of *Pteronotus davyi* populations in relation to latitude for the Pacific and Gulf versants, and the Southeastern region. The maps on the bottom represent the populations included for each linear regression.

425 Table 1. Genetic diversity indices [sample size (n), number of segregating sites (S),
 426 nucleotide diversity (π), number of haplotypes (h), and haplotype diversity (H)] for
 427 populations within the proposed management units of *Pteronotus davyi*.

Statistics	PAC-VER	GULN	SOU
n	73	30	41
S	50	13	34
π	0.0166	0.0029	0.0087
h	45	8	33
H	0.963	0.740	0.977

428 Table 2. Results of the isolation with migration model implemented in IM for a fragment of the control region to estimate divergence
429 of *Pteronotus davyi*. Parameter estimates are presented for each pairwise analysis where both the divergence time, t , and migration
430 rate, m , are scaled by the mutation rate (see Methods). The pairwise analysis are PAC-VER vs GULN, PAC-VER vs SOU, and PAC-
431 VER,GULN vs SOU. Mean, 90Low, and 90High represent the mean and 90% confidence limits from the marginal posterior
432 probability distribution.

Pairwise analysis	Migration	Migration	Time
	$m_{\text{PAC-VER vs GULN}}$	$m_{\text{GULN vs PAC-VER}}$	$t_{\text{PAC-VER vs GULN}}$
PAC-VER vs GULN	0.155 (0.000-1.075)	0.505 (0.000-1.359)	21,100 (8,500–36,600)
	$m_{\text{PAC-VER vs SOU}}$	$m_{\text{SOU vs PAC-VER}}$	$t_{\text{PAC-VER vs SOU}}$
PAC-VER vs SOU	0.033 (0.000-0.105)	0.041 (0.000-0.129)	69,133 (51,444-86,823)
	$m_{\text{PAC-VER,GULN vs SOU}}$	$m_{\text{SOU vs PAC-VER,GULN}}$	$t_{\text{PAC-VER,GULN vs SOU}}$
PAC-VER,GULN vs SOU	0.033 (0.000-0.103)	0.039 (0.000-0.121)	67,500 (50,200– 84,100)

433 APPENDIX 1. List of localities, acronyms, GenBank accession numbers (D-Loop), haplotypes of the sequences, and references of the
 434 samples used in the study.

Location	Acronym s	D-Loop Hapl.	GenBank Accession N°	Reference	Location	Acronym s	D-Loop Hapl.	GenBank Accession N°	Reference
Arroyo del Bellaco	AR	H1 (1)	EF989018	Guevara- Chumacero <i>et</i> <i>al.</i> (2010)	Kantemó	KA	H44 (1)	EF989061	Guevara- Chumacero <i>et</i> <i>al.</i> (2010)
Laguitos	LA	H2 (1)	EF989019	Guevara- Chumacero <i>et</i> <i>al.</i> (2010)	Calcehtok	CAL	H45 (1)	EF989047	Guevara- Chumacero <i>et</i> <i>al.</i> (2010)
Frontera	FR	H3 (1)	EF989081	Guevara- Chumacero <i>et</i> <i>al.</i> (2010)	Catemaco	CA	H46 (1)	EF989063	Guevara- Chumacero <i>et</i> <i>al.</i> (2010)
Ortices	OR	H3 (1)	EF989020	Guevara-	Amatlán	AM	H47 (1)	EF989064	Guevara-

				Chumacero <i>et al.</i> (2010)	de Cañas				Chumacero <i>et al.</i> (2010)
Tigre	TI	H4 (1)	EF989021	Guevara-Chumacero <i>et al.</i> (2010)	Catemaco	CA	H48 (1)	EF989065	Guevara-Chumacero <i>et al.</i> (2010)
Santo Domingo	SD	H4 (3)	EF989021	Guevara-Chumacero <i>et al.</i> (2010)	Arroyo del Bellaco	AR	H49 (1)	EF989049	Guevara-Chumacero <i>et al.</i> (2010)
Frontera	FR	H4 (4)	EF989021	Guevara-Chumacero <i>et al.</i> (2010)	Ortices	OR	H50 (1)	EF989068	Guevara-Chumacero <i>et al.</i> (2010)
Viejas	VI	H4 (1)	EF989021	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H50 (1)	EF989068	This paper
Amatlán	AM	H4 (2)	EF989021	This paper	Ortices	OR	H51 (1)	EF989067	Guevara-

de Cañas									Chumacero <i>et al.</i> (2010)
Viejas	VI	H5 (1)	EF989022	Guevara-Chumacero <i>et al.</i> (2010)	Amatlán de Cañas	AM	H51 (1)	EF989067	This Paper
Amatlán de Cañas	AM	H6 (1)	EF989023	Guevara-Chumacero <i>et al.</i> (2010)	Taninul	TA	H52 (1)	EF989069	Guevara-Chumacero <i>et al.</i> (2010)
Catemaco	CA	H7 (1)	EF989024	Guevara-Chumacero <i>et al.</i> (2010)	Troncones	TR	H53 (2)	EF989070	Guevara-Chumacero <i>et al.</i> (2010)
Amatlán de Cañas	AM	H8 (2)	EF989025	Guevara-Chumacero <i>et al.</i> (2010)	Troncones	TR	H53 (1)	EF989070	This paper
Santo	SD	H9 (1)	EF989026	Guevara-	Taninul	TA	H53 (2)	EF989070	Guevara-

Domingo				Chumacero <i>et al.</i> (2010)					Chumacero <i>et al.</i> (2010)
Santo Domingo	SD	H10 (1)	EF989027	Guevara-Chumacero <i>et al.</i> (2010)	Taninul	TA	H53 (2)	EF989070	This paper
Ortices	OR	H11 (1)	EF989028	Guevara-Chumacero <i>et al.</i> (2010)	Pujal	PU	H53 (3)	EF989070	Guevara-Chumacero <i>et al.</i> (2010)
Catemaco	CA	H12 (1)	EF989029	Guevara-Chumacero <i>et al.</i> (2010)	Pujal	PU	H53 (2)	EF989070	This paper
Arroyo del Bellaco	AR	H13 (1)	EF989030	Guevara-Chumacero <i>et al.</i> (2010)	Troncones	TR	H54 (2)	EF989071	Guevara-Chumacero <i>et al.</i> (2010)
Ortices	OR	H14 (1)	EF989031	Guevara-	Troncones	TR	H54 (2)	EF989071	This paper

				Chumacero <i>et al.</i> (2010)					
Kantemó	KA	H15 (1)	EF989032	Guevara-Chumacero <i>et al.</i> (2010)	Taninul	TA	H54 (1)	EF989071	Guevara-Chumacero <i>et al.</i> (2010)
Calakmul	CK	H16 (1)	EF989033	Guevara-Chumacero <i>et al.</i> (2010)	Taninul	TA	H54 (3)	EF989071	This paper
Arroyo del Bellaco	AR	H17 (1)	EF989034	Guevara-Chumacero <i>et al.</i> (2010)	Pujal	PU	H54 (2)	EF989071	This paper
Laguitos	LA	H18 (1)	EF989035	Guevara-Chumacero <i>et al.</i> (2010)	Taninul	TA	H55 (1)	EF989072	Guevara-Chumacero <i>et al.</i> (2010)
Sardina	SA	H19 (1)	EF989036	Guevara-	Troncones	TR	H56 (1)	EF989073	Guevara-

				Chumacero <i>et al.</i> (2010)					Chumacero <i>et al.</i> (2010)
Calakmul	CK	H20 (1)	EF989037	Guevara-Chumacero <i>et al.</i> (2010)	Pujal	PU	H56 (1)	EF989073	Guevara-Chumacero <i>et al.</i> (2010)
Sardina	SA	H21 (1)	EF989045	Guevara-Chumacero <i>et al.</i> (2010)	Arroyo del Bellaco	AR	H56 (1)	EF989073	Guevara-Chumacero <i>et al.</i> (2010)
Calcehtok	CAL	H22 (1)	EF989060	Guevara-Chumacero <i>et al.</i> (2010)	Agua Blanca	AB	H56 (1)	EF989073	Guevara-Chumacero <i>et al.</i> (2010)
Agua Blanca	AB	H23 (1)	EF989040	Guevara-Chumacero <i>et al.</i> (2010)	Laguitos	LA	H57 (1)	EF989074	Guevara-Chumacero <i>et al.</i> (2010)
Agua	AB	H24 (1)	EF989041	Guevara-	Arroyo del	AR	H58 (1)	EF989075	Guevara-

Blanca				Chumacero <i>et al.</i> (2010)	Bellaco				Chumacero <i>et al.</i> (2010)
Kantemó	KA	H25 (1)	EF989044	Guevara-Chumacero <i>et al.</i> (2010)	Laguitos	LA	H59 (2)	EF989076	Guevara-Chumacero <i>et al.</i> (2010)
Calcehtok	CAL	H26 (1)	EF989039	Guevara-Chumacero <i>et al.</i> (2010)	Arroyo del Bellaco	AR	H60 (1)	EF989077	Guevara-Chumacero <i>et al.</i> (2010)
Kantemó	KA	H27 (1)	EF989042	Guevara-Chumacero <i>et al.</i> (2010)	Catemaco	CA	H61 (1)	EF989078	Guevara-Chumacero <i>et al.</i> (2010)
Agua Blanca	AB	H27 (1)	EF989042	This paper	Tigre	TI	H63 (5)	EF989080	Guevara-Chumacero <i>et al.</i> (2010)
Sardina	SA	H28 (1)	EF989038	Guevara-	Viejas	VI	H63 (2)	EF989080	Guevara-

				Chumacero <i>et al.</i> (2010)					Chumacero <i>et al.</i> (2010)
Catemaco	CA	H29 (1)	EF989046	Guevara-Chumacero <i>et al.</i> (2010)	Amatlán de Cañas	AM	H63 (1)	EF989080	This paper
Calcehtok	CAL	H30 (1)	EF989043	Guevara-Chumacero <i>et al.</i> (2010)	Frontera	FR	H64 (1)	EF989020	Guevara-Chumacero <i>et al.</i> (2010)
Catemaco	CA	H31 (1)	EF989048	Guevara-Chumacero <i>et al.</i> (2010)	Amatlán de Cañas	AM	H64 (1)	EF989020	This paper
Calakmul	CK	H32 (1)	EF989049	Guevara-Chumacero <i>et al.</i> (2010)	Amatlán de Cañas	AM	H65 (1)	EF989082	Guevara-Chumacero <i>et al.</i> (2010)
Sardina	SA	H33 (1)	EF989050	Guevara-	Viejas	VI	H66 (1)	EF989083	Guevara-

				Chumacero <i>et al.</i> (2010)					Chumacero <i>et al.</i> (2010)
Calakmul	CK	H34 (1)	EF989051	Guevara-Chumacero <i>et al.</i> (2010)	Viejas	VI	H67 (1)	EF989084	Guevara-Chumacero <i>et al.</i> (2010)
Calcehtok	CAL	H35 (1)	EF989052	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H68 (1)	JN375694	This paper
Calakmul	CK	H35 (1)	EF989052	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H69 (1)	JN375695	This paper
Sardina	SA	H35 (1)	EF989052	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H70 (3)	JN375696	This paper
Calakmul	CK	H36 (1)	EF989053	Guevara-	Playa de	PO	H71 (1)	JN375697	This paper

				Chumacero <i>et al.</i> (2010)	Oro				
Sardina	SA	H37 (1)	EF989054	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H72 (1)	JN375698	This paper
Ortices	OR	H38 (1)	EF989055	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H73 (1)	JN375699	This paper
Santo Domingo	SD	H39 (1)	EF989056	Guevara-Chumacero <i>et al.</i> (2010)	Playa de Oro	PO	H74 (1)	JN375700	This paper
Tigre	TI	H40 (1)	EF989057	Guevara-Chumacero <i>et al.</i> (2010)	Troncones	TR	H75 (1)	JN375701	This paper
Santo	SD	H40 (1)	EF989057	Guevara-	Pujal	PU	H75 (1)	JN375701	This paper

Domingo				Chumacero <i>et al.</i> (2010)					
Frontera	FR	H40 (1)	EF989057	Guevara-Chumacero <i>et al.</i> (2010)	Troncones	TR	H76 (1)	JN375702	This paper
Viejas	VI	H40 (1)	EF989057	Guevara-Chumacero <i>et al.</i> (2010)	Kantemó	KA	H77 (1)	JN375703	This paper
Pujal	PU	H40 (1)	EF989057	Guevara-Chumacero <i>et al.</i> (2010)	Kantemó	KA	H78 (1)	JN375704	This paper
Kantemó	KA	H41 (1)	EF989058	This paper	Kantemó	KA	H79 (1)	JN375705	This paper
Calcehtok	CAL	H41 (1)	EF989058	Guevara-Chumacero <i>et al.</i> (2010)	Kantemó	KA	H80 (1)	JN375706	This paper

Calakmul	CK	H41 (1)	EF989058	Guevara- Chumacero <i>et al.</i> (2010)	Agua Blanca	AB	H81 (1)	JN375707	This paper
Agua Blanca	AB	H41 (1)	EF989058	Guevara- Chumacero <i>et al.</i> (2010)	Agua Blanca	AB	H82 (1)	JN375708	This paper
Sardina	SA	H41 (1)	EF989058	Guevara- Chumacero <i>et al.</i> (2010)	Agua Blanca	AB	H83 (1)	JN375709	This paper
Kantemó	KA	H42 (1)	EF989059	Guevara- Chumacero <i>et al.</i> (2010)	Agua Blanca	AB	H84 (1)	JN375710	This paper
Calcehtok	CAL	H43 (1)	EF989062	Guevara- Chumacero <i>et al.</i> (2010)					