Journal of Mammalogy, 89(3):744–754, 2008

EVOLUTIONARY HISTORY OF THE GENUS *RHOGEESSA* (CHIROPTERA: VESPERTILIONIDAE) AS REVEALED BY MITOCHONDRIAL DNA SEQUENCES

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Evolutionary relationships among bats of the genus *Rhogeessa* (Chiroptera: Vespertilionidae) are poorly understood because of the morphological similarity of many of the species and the limited resolution of karyotypes and allozymes in previous studies. Previous karyotypic studies reported several populations that differ by Robertsonian centric fusions, which led to a proposed mechanism of speciation called speciation by monobrachial centric fusions. Here, we present a molecular phylogenetic analysis of 8 of the 10 currently recognized species of *Rhogeessa* using the mitochondrial DNA gene cytochrome *b* as well as new karyotypic data. The results are generally consistent with speciation by monobrachial centric fusions because karyotypically distinct populations typically comprise monophyletic maternal lineages. One exception was 2 individuals that were possible hybrids between *R. tumida* (2n = 34) and *R. aeneus* (2n = 32). We found ostensible species-level differentiation among 3 karyotypically identical (2n = 34) but geographically separated populations of *R. tumida*. Examination of new karyotypic data shows a population from western Ecuador to have 2n = 42 and study of molecular data shows it to be phylogenetically distinct from both the karyotypically identical *R. genowaysi* from Mexico and the South American *R. io* (2n = 30) to which it was previously allocated. We recognize this population by its available name, *R. velilla*. We also found an unexpectedly close relationship between *Baeodon alleni* and *R. gracilis*.

Key words: cytochrome *b*, karyotype, molecular phylogeny, monobrachial centric fusion, speciation, taxonomy

The genus *Rhogeessa* (Chiroptera: Vespertilionidae) exhibits unusual karyotypic diversity in comparison to other New World vespertilionid bats (Bickham 1979b). Karyotypic diversity in *Rhogeessa* is characterized by species having unique sets of chromosomal fusions. Previously, these fusion events were proposed to be the cause of speciation within the *R. tumida* species group (Baker et al. 1985), a hypothesis known as speciation by monobrachial centric fusions (Baker and Bickham 1986). This speciation model states that centric fusions (a common form of chromosomal rearrangement in mammals) are not per se an effective isolating mechanism. However, if 2 populations become fixed for biarmed chromosomes that differ by having 1 arm in common but not the other (monobrachial homology), they will be reproductively isolated from each other because of the failure of meiosis in hybrids. In the hybrids, complex chains or rings of biarmed chromosomes differing by monobrachial centric fusions are formed in the 1st meiotic division. The chromosomes that comprise these multivalents fail to assort properly, which causes sterility and results in virtually instantaneous speciation. Under this model, populations differing by monobrachial rearrangements are expected to be reproductively isolated from one another, whereas those having no monobrachial rearrangements should be capable of interbreeding.

Currently, there are 10 recognized species of *Rhogeessa* (Table 1), 5 of which belong to the *R. tumida* species complex (*R. tumida*, 2n = 34; *R. aeneus*, 2n = 32; *R. io*, 2n = 30; *R. genowaysi*, 2n = 42; and *R. hussoni*, 2n = 52—Bickham and Baker 1977; Genoways and Baker 1996). Members of the *R. tumida* complex historically were considered to be conspecific because of their morphological similarities (LaVal 1973). The remaining species, all of which are morphologically distinguishable (Laval 1973), are: *R. parvula*, 2n = 44 (Bickham and Baker 1977); *R. alleni*, 2n = 30 (Volleth and Heller 1994;

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TABLE 1.—*Rhogeessa* species. The names provided in this table reflect taxonomic status before our study, with the exception of *R. velilla*, which we recognize here. Species marked with an asterisk (*) belong to the *R. tumida* complex.

Species	Diploid no.	Geographic range	Relevant literature
*R. tumida**	2n = 34	Widespread from Tamaulipas, Mexico, to northern Panama	Vonhof 2000
*R. genowaysi**	2n = 42	Single locality in Chiapas, Mexico	Baker 1984; Roots and Baker 1998
*R. aeneus**	2n = 32	Yucatan region of Mexico and Belize	Audet et al. 1993
*R. io**	2n = 30	Southern Panama into South America	Genoways and Baker 1996
R. minutilla	Unknown	Northern Colombia and Venezuela	
*R. hussoni**	2n = 52	Suriname into Brazil	Genoways and Baker 1996
R. parvula	2n = 44	Pacific coast of Mexico	LaVal 1973; Roots and Baker 2007
R. alleni	2n = 30	Mountains of western Mexico from Jalisco to Oaxaca	LaVal 1973
R. mira	Unknown	Michoacan, Mexico	Arroyo-Cabrales and Polaco 1997
R. gracilis	2n = 30	Pacific coast of Mexico from Jalisco to Oaxaca	Jones 1977
*R. velilla**	2n = 42	Southern Pacific coast of Ecuador	This paper

Volleth et al. 2006); *R. gracilis*, 2n = 30 (this study); *R. minutilla*, 2n unknown; and *R. mira*, 2n unknown. Of the 10 *Rhogeessa* species, 3 occur in South America and the remainder in Mexico and Central America (Fig. 1; Table 1). Members of this genus exhibit unusually small, parapatric ranges, whereas most other New World vespertilionids have large, overlapping distributions. The descriptions of many *Rhogeessa* species are based on karyotypic differences rather than morphological differences. This contrasts with most vespertilionid genera that are composed of morphologically distinct species having little or no chromosomal variability (Bickham 1979b).

Baker et al. (1985) showed that members of the *R. tumida* complex differ in their karyotypes through a series of centric fusions (although they did not examine *R. hussoni*). *R. tumida*, *R. aeneus*, and *R. io* all have monobrachial differences from

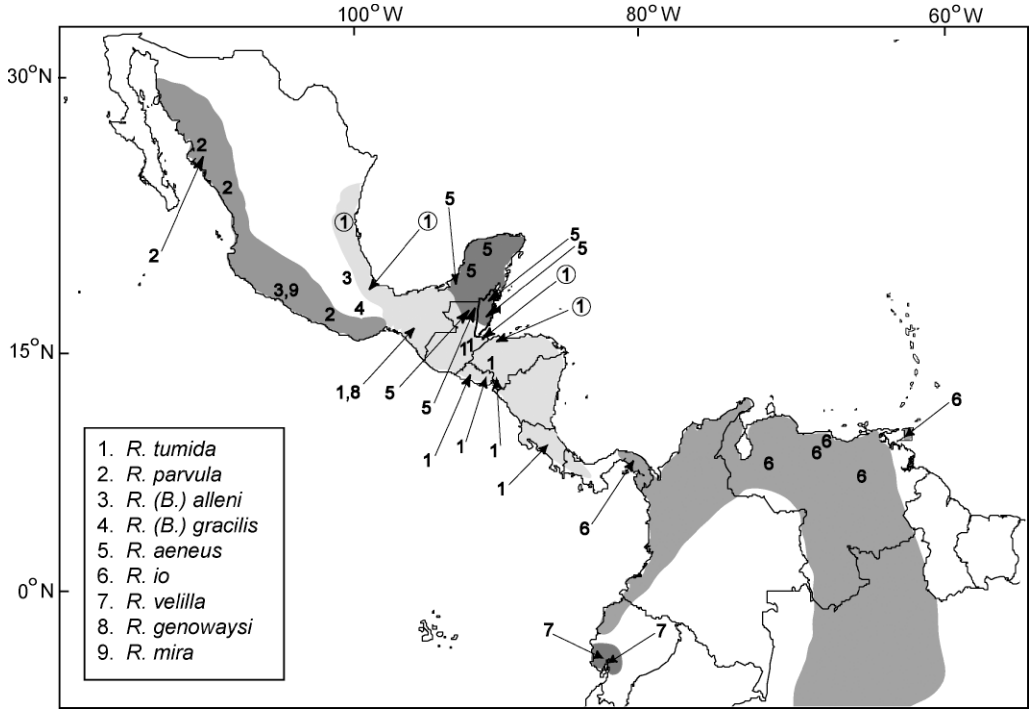

FIG. 1.—Locations of samples of *Rhogeessa* included in this study. Collection localities in close proximity are not shown. Circled localities for *R. tumida* represent sites where specimens group with the Atlantic clades; noncircled localities for *R. tumida* contain individuals from the Pacific clade. The southernmost circled localities (Guatemala and Honduras) are where the 2 *R. tumida* that group with *R. aeneus* are from, along with *R. tumida* that group with the Central American Atlantic clade. Shading represents approximate ranges for *R. tumida*, *R. parvula*, *R. aeneus*, *R. io*, and *R. velilla*, *R. genowaysi* and *R. mira* are known from only 2 localities, near our samples indicated here. *R. gracilis* and *R. alleni* have overlapping ranges in western Mexico (*R. gracilis* from northern Jalisco to central Oaxaca; *R. alleni* from central Jalisco to central Oaxaca) and overlap the eastern part of the range limit of *R. parvula*.

one another and therefore would be expected to be reproductively isolated from one another under the model of speciation by monobrachial centric fusions (Baker and Bickham 1986). In contrast, *R. genowaysi* has a different set of fusions relative to these species, but no monobrachial differences from them (and may be capable of interbreeding with any of those species according to the model). Based on these karyotypic observations, it should be expected that *R. tumida*, *R. aeneus*, and *R. io* represent distinct genetic lineages.

Although the current taxonomic status of most species of *Rhogeessa* is currently uncontroversial, the status of *R. alleni* has been viewed differently by various authors. Corbet and Hill (1991) and Duff and Larson (2004) placed it in a separate genus, *Baeodon*, whereas Honacki et al. (1982), Jones et al. (1988), Hall (1981), and LaVal (1973) considered it to be a member of *Rhogeessa*. Hoofer and Van Den Bussche (2003) placed it in the genus *Baeodon*, sister to *Rhogeessa*, based on molecular data. The karyotype of *R. alleni* was reported by Volleth and Heller (1994; see also Volleth et al. 2006). We have included *R. alleni* in our study of *Rhogeessa* for further study of its generic placement.

Very little molecular work has been done showing the degree of divergence within *Rhogeessa*. Baker et al. (1985) showed that allozymes distinguished several of the members of the *R. tumida* complex. Hoofer and Van Den Bussche (2003) included several *Rhogeessa* species (1 specimen of each) in their broader study investigating phylogenetic relationships within Vespertilionidae. Included in their study were *R. aeneus* (Belize), *R. mira* (Mexico: Michoacan), *R. parvula* (Mexico: Sonora), *R. tumida* (Honduras: Valle), and *R.* (*Baeodon*) *alleni* (Mexico: Michoacan). Their analysis of mitochondrial 12S/16S rRNA genes was able to differentiate each *Rhogeessa* species and supported the following topology for *Rhogeessa*: (*R. alleni*, (*R. mira*, *R. parvula*), (*R. aeneus*, *R. tumida*)). This tree does not reject the hypothesis of monophyly for the *R. tumida* complex as we have defined it.

Given its unusual karyotypic diversity, the *R. tumida* complex is an ideal system in which to investigate the relationships between chromosomal evolution and speciation. By studying this system using molecular data, we can test previous hypotheses of speciation within the genus, investigate chromosomal evolution in the group, and determine phylogenetic relationships among species. The goals of this study are to examine the relationships of as many *Rhogeessa* species as possible and determine whether different karyotypic forms represent unique species and mitochondrial DNA (mtDNA) lineages. Additionally, we will examine whether the *R. tumida* complex is a monophyletic group in which chromosomal speciation may have taken place. We also present the karyotypes of *R. gracilis* and an isolated population of *Rhogeessa* from western Ecuador.

Materials and Methods

Sampling.—We sampled tissues from 90 individuals representing of 8 of the 10 recognized species of *Rhogeessa* (Appendix I). Sampling covered much of the geographic range of these species, including an isolated population of putative *R. io* from western Ecuador (Fig. 1). Species not represented in this study are *R. minutilla* and *R. hussoni*, as well as the 2n = 32 population of *R. tumida* from Nicaragua (Baker et al. 1985). Field procedures followed guidelines approved by the University of Texas, which follow recommendations of the Animal Care and Use Committee of the American Society of Mammalogists (Gannon et al. 2007). *Plecotus auritus* (2n = 32; GenBank accession number AY665169), *Antrozous pallidus* (2n = 46—Baker and Patton 1967), and *Bauerus dubiaquercus* (2n = 44—Engstrom and Wilson 1981) were used as outgroups in the phylogenetic analysis, based on the relationships among these taxa presented by Hoofer and Van Den Bussche (2003).

DNA extraction and sequencing.—DNA was extracted from frozen tissue samples using a Qiagen DNeasy kit (Qiagen, Valencia, California). The cytochrome-*b* (*Cytb*) gene was amplified in full using the primers LGL 765 forward (GAA AAA CCA YCG TTG TWA TTC AAC T) and LGL 766 reverse (GTT TAA TTA GAA TYT YAG CTT TGG G—Bickham et al. 1995, 2004). Polymerase chain reaction was performed using 25-μl reactions of the following reagents: 2.5 μl of 10x buffer, 2.5 μl of deoxynucleoside triphosphate mix, 1.25 μl of a 10-μM solution of each primer, 0.5 μl of Taq DNA polymerase, 13.5–14.5 μl of deionized water, and 1–2 μl of total genomic DNA. Thermal cycle conditions consisted of initial heating at 94°C for 1.5 min, then 35 cycles of denaturation at 94°C for 20 s, annealing at 48–50°C for 30 s, and extension at 72°C for 1 min, followed by an additional 7 min of extension at 72°C.

A single band was obtained using the primers listed above. Polymerase chain reaction products were purified using a Viogene gel extraction kit (Viogene, Sunnyvale, California) to obtain a clean polymerase chain reaction product. Purified products were subsequently used in standard sequencing reactions (with the same polymerase chain reaction primers) using Big Dye version 3.0 (Applied Biosystems, Foster City, California). Sequences were cleaned using Sephadex spin columns and samples were analyzed on an ABI3100 automated genetic analyzer (Applied Biosystems). Raw sequence data were analyzed using DNAstar software version 2 (DNAstar, Madison, Wisconsin) and aligned by eye using MacClade (Maddison and Maddison 2000). A total of 1,140 base pairs (bp; 1,088 bp of which contained no missing data across all samples) was used in the phylogenetic analysis. This fragment includes only the complete *Cytb* gene. All flanking sequences that amplified with the polymerase chain reaction primers were discarded before phylogenetic analysis.

Phylogenetic analysis.—Modeltest version 3.06 (Posada and Crandall 1998) was used to assess the appropriate model of evolution (HKY+I+Γ) for our data set under the Akaike information criterion. This model was implemented in a Bayesian analysis using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003), which generates posterior probability distributions through a Markov chain Monte Carlo process. We analyzed 3×10^6 generations of 1 cold and 3 heated Markov chains and discarded 100,000 burn-in generations based on

TABLE 2.—Maximum genetic (Kimura 2-parameter) divergence in the cytochrome-*b* gene measured within and between major clades of *Rhogeessa* and other bats examined in this study. The numbers along the diagonal represent divergence within a clade. Dashes indicate taxa for which only 1 specimen was examined.[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.014													
2	0.028	0.006												
3	0.038	0.025	0.010											
4	0.102	0.096	0.092	0										
5	0.108	0.105	0.108	0.099	0.007									
6	0.117	0.111	0.120	0.122	0.108	—								
7	0.113	0.111	0.123	0.115	0.121	0.131	0.016							
8	0.144	0.137	0.144	0.142	0.146	0.158	0.147	0.015						
9	0.157	0.156	0.155	0.160	0.158	0.161	0.016	0.108	—					
10	0.164	0.164	0.17	0.161	0.163	0.171	0.168	0.18	0.172	—				
11	0.202	0.198	0.196	0.191	0.201	0.211	0.2	0.201	0.223	0.186	—			
12	0.179	0.177	0.173	0.181	0.187	0.204	0.204	0.176	0.198	0.195	0.161	—		
13	0.167	0.169	0.173	0.17	0.166	0.176	0.168	0.187	0.181	0.017	0.186	0.197	0.011	
14	0.235	0.227	0.226	0.214	0.235	0.227	0.235	0.255	0.252	0.231	0.244	0.231	0.231	—

[a] 1 = *R. aeneus* (including 2 *R. tumida* within that clade), 2 = *R. tumida* Atlantic Central American clade, 3 = *R. tumida* Atlantic Mexico clade, 4 = *R. velilla*, 5 = *R. tumida* Pacific clade, 6 = *R. genowaysi*, 7 = *R. io*, 8 = *R. parvula*, 9 = *R. mira*, 10 = *R. gracilis*, 11 = *Bauerus*, 12 = *Antrozous*, 13 = *Baeodon alleni*, 14 = *Plecotus*.

fluctuating likelihood scores. GARLI version 0.951 (http://www.zo.utexas.edu/faculty/antisense/garli/garli.html) was used to generate a maximum-likelihood tree, and bootstrap values were calculated using a genetic algorithm approach. No starting tree was specified in this analysis and the same model of evolution identified by Modeltest was used for both the Bayesian and GARLI analyses. Trees were visualized using TREEVIEW version 1.6.6 (Page 1996).

Karyotypic analysis.—Mitotic spreads stained with Giemsa from 1 specimen of *R. gracilis* (AK11059) were prepared in the field by L. A. Ruedas and J. C. Morales. Specimens of *Rhogeessa* from Ecuador also were karyotyped in the field (Baker et al. 2003) by members of the 2004 Sowell Expedition from Texas Tech University. Karyotypes from the latter were stained with 4,6-diamidino-2-phenylindole to produce banding patterns for analysis (Fig. 2). 4,6-Diamidino-2-phenylindole banding is equivalent to traditional G-banding because it stains AT regions (Ambros and Sumner 1987; Sumner 1990) and, therefore, karyotypes analyzed in this manner are directly comparable to those prepared by traditional G-banding methods. 4,6-Diamidino-2-phenylindole bands were not obtained from the sample of *R. gracilis* because of slight degradation of the karyotypes. Banded and nondifferentially stained karyotypes were photographed and arranged in pairs.

RESULTS

Phylogenetic analysis.—The phylogeny resulting from the *Cytb* sequence data (Fig. 3) lends support to the hypothesis of monophyly of the *R. tumida* complex (posterior probability =

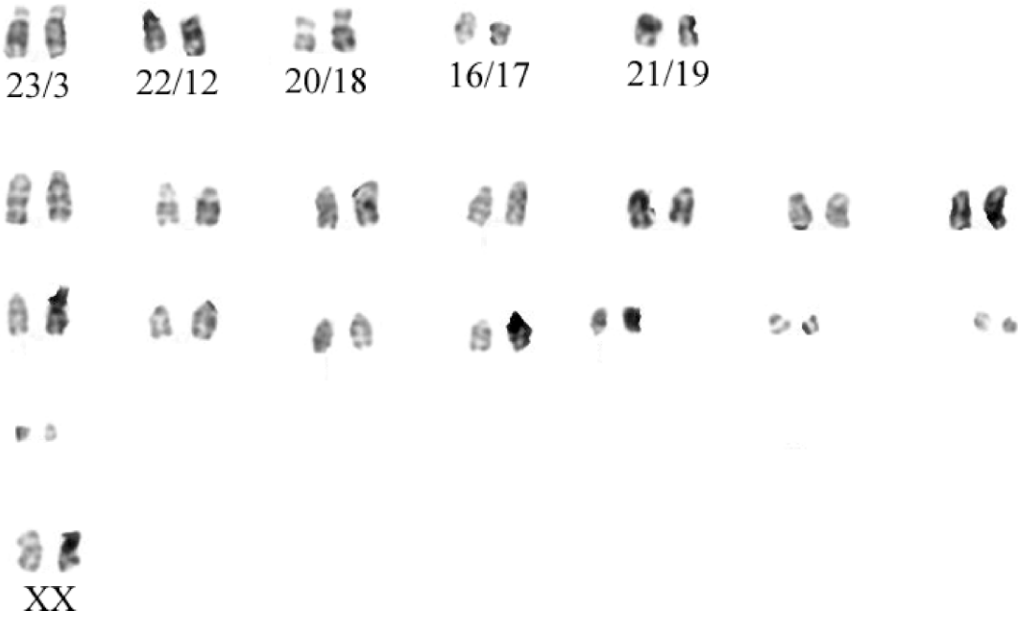

FIG. 2.—Karyotype of *Rhogeessa velilla* from Ecuador (2n = 42). Numbers below biarmed chromosomes represent arms involved in the centric fusions of those chromosomes (chromosomal nomenclature following Bickham [1979a and 1979b]).

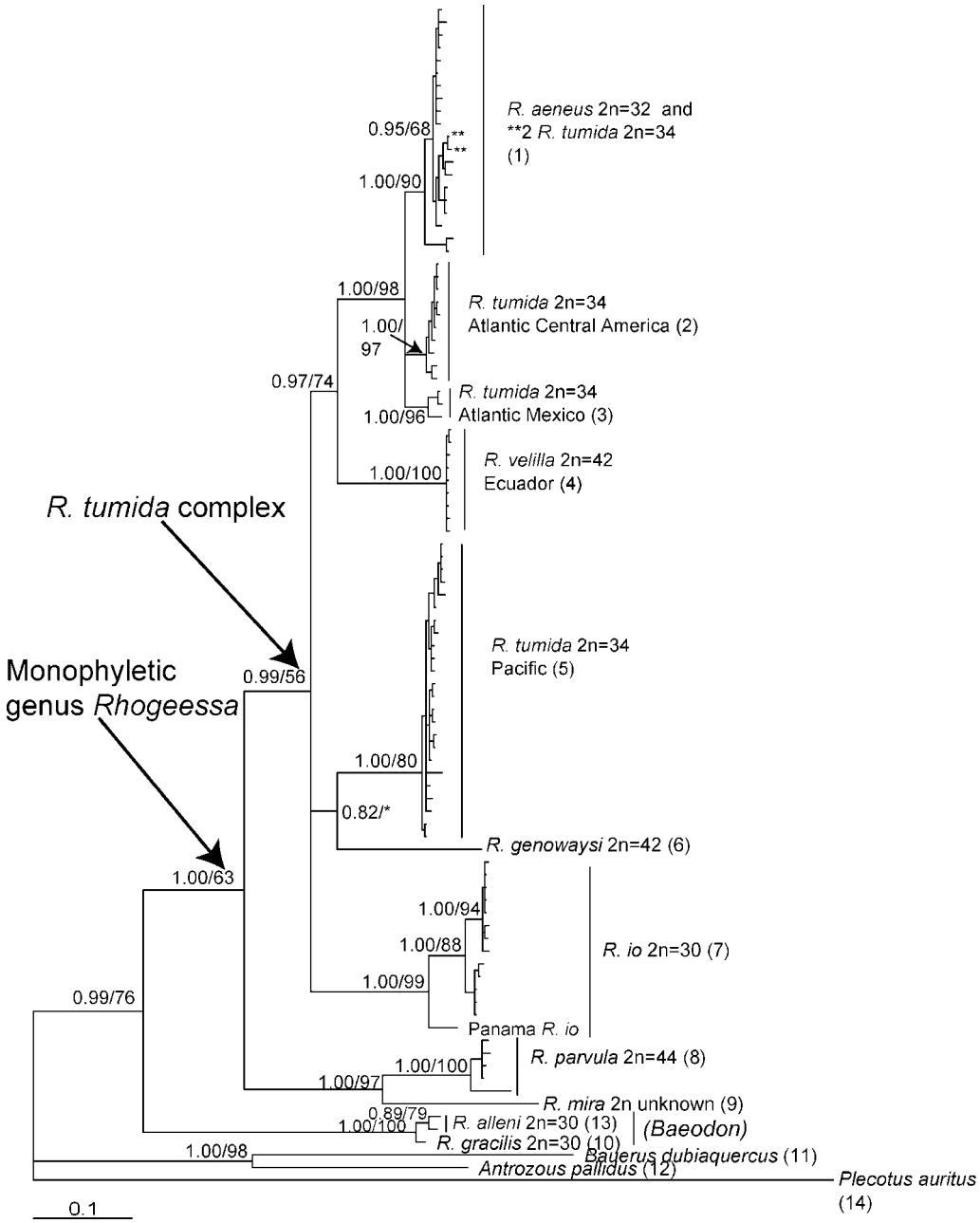

FIG. 3.—Phylogeny of *Rhogeessa* based on a Bayesian analysis of cytochrome-*b* sequences. Posterior probabilities of major clades are included, followed by maximum-likelihood bootstrap values. Asterisks indicate bootstrap values <50 for that clade. Numbers in parentheses indicate group numbers in Table 2. Collecting localities are listed in Appendix I.

0.99). Our results also show that *R. alleni* and *R. gracilis* form a clade sister to all other species of *Rhogeessa*. *R. parvula* and *R. mira* form a clade that is sister to the *R. tumida* complex. This observation is partially consistent with some of the findings of LaVal (1973), where certain morphological characters place *R. alleni* most basal followed by *R. gracilis*, with *R. mira* and *R. parvula* being closely related to one another. However, examination of our genetic data suggests that *R. alleni* and *R. gracilis* are more similar to one another (0.017 Kimura 2-parameter distance) than has been hypothesized based on morphological data (LaVal 1973).

The 2n = 34 karyotypic form, *R. tumida*, previously thought to be a single broadly distributed species, falls out as 4 separate lineages in Fig. 3. One lineage, composed of individuals from the Pacific versant of Mexico and Central America, is sister to *R. genowaysi*. Two lineages composed of individuals from the Atlantic versant of Mexico and Central America, respectively, form a polytomy with *R. aeneus*. Lastly, there are 2 individuals provisionally identified as *R. tumida*, 1 identified based on karyotype (2n = 34 confirmed from several different karyotypic spreads), the other was not karyotyped (both were collected in areas from which only *R. tumida* is known), which

fall within the *R. aeneus* (2n = 32) clade. The Pacific and Atlantic lineages of *R. tumida* differ by about 10% Kimura 2-parameter distance (Table 2). The 2 Atlantic lineages differ by 2.5%, whereas *R. aeneus* differs from the 2 individuals of *R. tumida* within that clade by about 1%.

The 2n = 42 karyotypic forms (*R. genowaysi* from Chiapas, Mexico, and the western Ecuadorian population of putative *R. io*, labeled as *R. velilla* in Fig. 3) appear as separate genetic lineages on the tree. The Ecuadorian form is sister to the Atlantic *tumida–R. aeneus* clade, whereas *R. genowaysi* is sister to the Pacific *R. tumida* clade.

Karyotypic analysis.—The putative specimens of *R. io* from western Ecuador possess 2n = 42 (Fig. 2). Differentially stained preparations using 4,6-diamidino-2-phenylindole banding allowed us to determine the arms of the biarmed chromosomes with a high degree of certainty. These bats possess the 5 plesiomorphic biarmed chromosomes common to the entire *R. tumida* complex (Bickham and Baker 1977): fusions of chromosomes 23/3, 22/12, 20/18, 16/17, and 21/19 (chromosomal nomenclature following Bickham [1979a, 1979b]). Thus, the 2n = 42 karyotype of the Ecuadorian population of *R. io* is identical to the 2n = 42 karyotype of *R. genowaysi*, and the 2 species do not differ by monobrachial rearrangements with respect to the other species in the *R. tumida* complex.

The standard karyotype for *R. gracilis* had a diploid number of 2n = 30, but we were unable to obtain adequate differentially stained preparations from this specimen.

Discussion

Phylogenetic relationships.—Our phylogenetic analysis supports the monophyly of the *R. tumida* complex as a whole. However, it shows that populations presently considered to be *R. tumida* do not comprise a monophyletic lineage, although evidence to date suggests that they all possess 2n = 34 karyotypes with the same set of chromosomal fusions. The 2 lineages with 2n = 42 karyotypes (*R. genowaysi* and the putative western Ecuadorian *R. io*) appear to be separate species. Within the *R. tumida* complex, there are 3 major clades: 1 contains *R. aeneus*, several *R. tumida* lineages with 2n = 34 karyotypes, and the Ecuadorian 2n = 42 population; the 2nd contains Pacific *R. tumida* (2n = 34) and *R. genowaysi* (2n = 42); and the 3rd is *R. io* with 2n = 30. The first 2 of these 3 clades possess populations with identical 2n = 42 and identical 2n = 34 karyotypes. The observation that 2n = 34 forms and 2n = 42 forms do not represent monophyletic groups could be explained in 2 ways: the karyotypes have converged on these diploid numbers; or the ancestral population to the *R. tumida* complex contained both the 2n = 34 and 2n = 42 karyotypes, which have become fixed in separate mitochondrial lineages by lineage sorting (Avise 2000) or random genetic drift. A population containing 2n = 34 and 2n = 42 karyotypes would have to contain many different intermediate karyotypes as well, assuming that there is complete interbreeding between karyotypic forms. The chance that the same 2 karyotypes would randomly become fixed more than once is likely miniscule, making the 1st scenario more probable than the 2nd.

The major unexpected result from our phylogeny is that putative *R. tumida* occurs in 4 different clades on the tree. Under the model of speciation by monobrachial centric fusions, we would expect all populations of 2n = 34 to be able to interbreed and thus appear as a single monophyletic lineage, which they are not based on this mtDNA data set. In the Atlantic clade, 2 individuals of *R. tumida* fall within a clade of *R. aeneus*. These 2 samples of *R. tumida* are from the province of Atlantida in Honduras and Izabal in Guatemala. Other individuals from these same localities fall within the Atlantic Central American *R. tumida* clade. Samples of *R. aeneus* come from Belize, the Yucatan region of Mexico, and the Petén region of Guatemala (Fig. 1). Three possible explanations can account for the observed relationship between *R. aeneus* and the 2 individuals of *R. tumida* within the *R. aeneus* clade: there has been incomplete lineage sorting for the *Cytb* gene in this group; there has been hybridization between *R. aeneus* and *R. tumida* in this region; or *R. aeneus* is a mixture of 2n = 32 and 2n = 34 karyotypes. *R. aeneus* and *R. tumida* differ by monobrachial rearrangements in their karyotypes (Bickham and Baker 1977), so under the hypothesis of speciation by monobrachial centric fusions we would expect them to be reproductively isolated. However, hybridization seems a likely alternative in this case because of the geographic proximity of the samples and the fact that other individuals from those localities group with other *R. tumida* from Central America. If this is true, it represents the 1st report of hybridization among species of *Rhogeessa* sharing monobrachial differences. Nuclear biparentally inherited markers must be examined to test the hypothesis of hybridization between these species. Based on *Cytb* sequence and karyotype alone, it is not absolutely certain that these individuals are hybrids. We can rule out the possibility of them being F_1 hybrids because they lack a diploid number intermediate between the 2n = 32 and 2n = 34 karyotypes (the assumed 2 parental lineages). However, if they are hybrids from anything greater than an F_1 generation, we would most likely be unable to distinguish them karyotypically from the parental species to which they backcrossed. Therefore, nuclear sequencing must be performed to determine with greater certainty whether these individuals are of hybrid origin.

The 3rd possible explanation of the relationships observed in the *R. aeneus* clade, that *R. aeneus* is simply a population of mixed karyotypes including both 2n = 32 and 2n = 34, seems unlikely based on the fact that no intermediate karyotypes between the 2 forms have ever been observed. One would assume under this scenario that interbreeding between the 2 forms would be common if they are a single species. In that case, we would expect to see intermediate karyotypes in the population.

Our analysis included 1 individual from Darien, Panama, that was most closely related to our samples of *R. io* from Venezuela and Trinidad. This specimen showed about 4.5% sequence divergence from other individuals of *R. io* (Kimura 2-parameter distance; Table 2). This could be sufficient

divergence to indicate that there may be a distinct species in the southern part of Central America, where our sampling is sparse. The relationship of *R. io* in Panama to individuals in South America should be examined in further detail with additional sampling.

Our samples of *R. gracilis* and *R. alleni* are very closely related to one another genetically. LaVal (1973) previously hypothesized based on morphology that *R. alleni* should be the most distant *Rhogeessa*, followed by *R. gracilis* and then the remaining taxa. Based on its very different morphology, he placed *R. alleni* in the subgenus *Baeodon*, whereas the remaining species (including *R. gracilis*) were all put in the subgenus *Rhogeessa*. Thus, our genetic data give an unexpectedly close relationship between *R. alleni* and *R. gracilis* that is not predicted by morphology.

We included the same individuals of *R. alleni* used by Hoofer and Van Den Bussche (2003—TK45023) and Volleth and Heller (1994—SMF77908; also reported in Volleth et al. [2006]), both of which are sister to our sample of *R. gracilis* (AK11059). Within the clade of *R. gracilis–R. alleni*, samples differ from one another by only an average of 1.5% divergence (Kimura 2-parameter distance). The sample of *R. alleni* reported in Volleth and Heller (1994) and Volleth et al. (2006) is the only individual of that species for which a karyotype has been reported. Our sample of *R. gracilis* (AK11059; 2n = 30) matches that karyotype as far as can be determined. This contradicts previous findings by Baker and Patton (1967), who reported a karyotype of 2n = 44 for *R. gracilis*, although later (LaVal 1973:3) these specimens were reported as being ''almost certainly *R. parvula*.'' Therefore, our results are likely the 1st confirmed karyotype of *R. gracilis*. We compared the voucher of *R. gracilis* (AK11059) to *R. alleni* not included in this study and confirmed its identification. As of the publication of this paper, we have been unable to locate 1 of the vouchers of *R. alleni* and have located, but not verified, the identity of the 2nd voucher. Because our confirmed specimen of *R. gracilis* matches the karyotype of a supposed specimen of *R. alleni* (and not the previously reported karyotype of *R. gracilis*, although the previous karyotype was likely from a misidentified *R. parvula*) and because it is extremely similar genetically to the samples of *R. alleni*, we are still somewhat in doubt of the accuracy of the identification of the samples of *R. alleni*. We are currently in the process of obtaining additional, confirmed, individuals of *R. alleni* to include in future studies of *Rhogeessa*.

Our phylogenetic analyses of *Cytb* sequences cannot be used, alone, to accept or reject the chromosomal speciation hypothesis because of the limited power of mtDNA to test for gene flow between species. However, these results do show that if speciation has occurred via this mechanism in *Rhogeessa*, it is unlikely to be the only speciation mechanism at work in this group. The chromosomal speciation model predicts monophyly of the 2n = 34 chromosomal form, whereas our analyses suggest that populations with 2n = 34 are structured more based on geography. The mountain ranges throughout the central parts of Mexico and Central America could be a potential source of genetic isolation between these clades, which is independent of karyotypic isolation. This and other geographic speciation hypotheses can be explored if our reported phylogenetic relationships are verified with nuclear sequence data.

Taxonomy.—The current precedent for the taxonomic status of *R. alleni* comes from Hoofer and Van Den Bussche (2003), who placed this species in the genus *Baeodon* based primarily on genetic distance from the remaining species of *Rhogeessa* they examined. Our study also finds *R. alleni* to be very distant from all species of *Rhogeessa* except its sister taxon, *R. gracilis*. If we follow current precedent and classify *R. alleni* as a member of the genus *Baeodon* without including *R. gracilis* in that genus as well, *Rhogeessa* would be paraphyletic. Therefore, there are 2 options for classifying *R. alleni*: move it back into the genus *Rhogeessa* or leave it as *B. alleni* and move *R. gracilis* into the genus *Baeodon* as well. We tentatively support the latter option, but strongly recommend further study on the relationship of these 2 species. Our support for this option is based not only on their genetic distance from the remaining species of *Rhogeessa*, but also on the fact that both *R. alleni* and *R. gracilis* are highly divergent morphologically from other *Rhogeessa* (LaVal 1973).

Populations currently recognized as *R. tumida* likely comprise at least 2 species and possibly 3. The Pacific *R. tumida* clade is genetically distinct from the Atlantic *R. tumida* clades. The Central American and Mexican Atlantic *R. tumida* clades are less distinct but might nonetheless represent different species. We tentatively support the hypothesis of 3 different species of *R. tumida* based on a genetic species concept (see Baker and Bradley [2006] for a review of applying this species concept to mammals). The main criterion for supporting a genetic species concept is the lack of evidence for interbreeding between the 3 different lineages of *R. tumida*. It could be argued that the Atlantic *R. tumida* clades and *R. aeneus* should belong to the same species based on low genetic distances between them. Although the genetic distance between these clades is low, we do not support a species concept based on genetic distance alone. We also do not currently support this hypothesis based on the karyotypic differences between the clades. We are currently investigating *R. tumida* in more detail with nuclear DNA sequences. Other than the mtDNA sequences presented here, there are no morphological, karyological, or other molecular data available to justify any changes to this species at this time.

The putative samples of *R. io* from western Ecuador and *R. genowaysi* from Chiapas, Mexico, share identical 2n = 42 karyotypes but are genetically distinct and geographically separated. Based on this evidence, we do not believe them to be conspecific. The Ecuadorian samples also are distinct from *R. io* (the geographically nearest *Rhogeessa* species), both genetically and karyotypically. The name *R. velilla* is available for the Ecuadorian samples. The status of *R. velilla* has changed several times since its initial description (Thomas 1903). Goodwin (1958) treated it as a subspecies of *R. parvula*, whereas LaVal (1973) considered it as a member of *R. tumida*. Genoways and Baker (1996), when elevating *R. io* to specific status, noted that specimens from Ecuador have a baculum

morphology more similar to that of *R. minutilla* than to that of *R. io*; however, they did not have enough data to place these specimens into either species with certainty. We are unaware of any karyotypic data existing for *R. minutilla* that may show additional similarity to specimens from Ecuador. However, because of their geographic distance from any known samples of *R. minutilla*, we believe that the Ecuadorian samples should be elevated to species level as *R. velilla*. This taxonomic change is reflected in the synonymy below.

Rhogeessa velilla *Thomas, 1903*
Ecuadorian Little Yellow Bat

Rhogeessa velilla Thomas, 1903:383. Type locality "Puná, Puná Island, Gulf of Guyaquil, [Guayas Province,] Ecuador." Holotype: adult male, British Museum of Natural History number 99.8.1.5; fluid specimen.

Rhogeessa parvula velilla: Goodwin (1958:8). Name combination.

Rhogeessa (*Rhogeessa*) *tumida*: LaVal, 1973:29. Part: specimens from Puná Island, Ecuador, only.

Rhogeessa io: Genoways and Baker, 1996:84. Part: specimens from Puná Island, Ecuador, only.

Geographic range.—Known from type locality and Guayas Province on the mainland of Ecuador.

Description.—According to Thomas (1903), *R. velilla* is similar to *R. io* in size, color, and proportions, except that *R. velilla* lacks the marked "helmet" formed by prominent sagittal and occipital crests. The baculum of *R. velilla* is similar to that of *R. minutilla* (Genoways and Baker 1996). The karyotype of *R. velilla* is 2n = 42, differing from any species to which it was previously allocated.

Acknowledgments

We thank the Natural Science Research Laboratories at Texas Tech University, Carnegie Museum, Texas Cooperative Wildlife Collection (Texas A&M University), M. Engstrom and the Royal Ontario Museum, M. Volleth, and the Angelo State Natural History Collection for supplying frozen tissue for the molecular portion of this study. S. Pérez of the Natural History Museum, San Carlos University in Guatemala City, was instrumental in helping us collect individuals from Motagua Valley in Guatemala and loaned us tissue samples from Petén. We also thank members of the Sowell Expedition to Ecuador (Texas Tech University) and V. Swier for help in obtaining karyotypes of *R. velilla* and J. C. Morales and L. Ruedas for the karyotype of *R. gracilis*. M. Volleth and R. J. Baker reviewed earlier versions of the manuscript and provided helpful comments. This paper is part of the doctoral dissertation of ABB. ABB thanks her advisory committee (D. M. Hillis, R. J. Baker, D. C. Cannatella, J. J. Bull, and B. B. Simpson) for help and support. T. J. McCarthy provided many helpful comments regarding the biology of *Rhogeessa*, and R. J. Baker was especially helpful in the conception of this study. Funding was provided by National Science Foundation grant EF0629849 to JWB.

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Submitted 7 May 2007. Accepted 22 October 2007.

Associate Editor was Mark S. Hafner.

APPENDIX I

Specimens examined.—TK (tissue) and TTU (skin) = Natural Science Research Laboratories, Texas Tech University; AK (tissue) and TCWC (skin) = Texas Cooperative Wildlife Collection, Texas A&M University; ASNHC = Angelo State Natural History Collection, Angelo State University; SGP = Sergio G. Perez collection number; FN = Royal Ontario Museum; SP = Carnegie Museum; SMF = Senckenberg Museum, Frankfurt am Main.

Species	Museum no.	Locality	GenBank accession no.
Baeodon alleni	TK45023	Michoacan, Mexico	EF222375
	SMF77908	Puebla, Mexico	EF222412
Rhogeessa aeneus	TK20704/TTU40003	Belize District, Belize	EF222329
	TK20706/TTU40010	Belize District, Belize	EF222361
	TK20707/TTU40005	Belize District, Belize	EF222363
	TK20710/TTU40009	Belize District, Belize	EF222395
	TK20712/TTU40012	Belize District, Belize	EF222364
	AK7771	Orange Walk, Belize	EF222325
	FN30223	Campeche, Mexico	EF222334
	FN30224	Campeche, Mexico	EF222328
	FN30225	Campeche, Mexico	EF222327
	FN30226	Campeche, Mexico	EF222408
	FN30462	Yucatan, Mexico	EF222405
	FN30463	Yucatan, Mexico	EF222406
	FN30464	Yucatan, Mexico	EF222331
	FN30677	Campeche, Mexico	EF222333
	FN30678	Campeche, Mexico	EF222337
	ASNHC1414	Campeche, Mexico	EF222359
	SGP1030	Peten, Guatemala	EF222418
	SGP1140	Peten, Guatemala	EF222419
Rhogeessa genowaysi	TK20597/TTU36171	Chiapas, Mexico	EF222326
Rhogeessa gracilis	AK11059/TCWC58228	Oaxaca, Mexico	EF222360
Rhogeessa io	TK15163/TTU33400	Guarico, Venezuela	EF222410
	TK15164	Guarico, Venezuela	EF222384
	TK15179	Guarico, Venezuela	EF222391
	TK15209	Guarico, Venezuela	EF222392
	TK15286/TTU33402	Guatopo, Venezuela	EF222358
	TK19004	Bolivar, Venezuela	EF222393
	TK19005	Bolivar, Venezuela	EF222394
	TK19043	Bolivar, Venezuela	EF222347
	TK19450	Barinas, Venezuela	EF222404
	TK19458	Barinas, Venezuela	EF222348
	TK19459	Barinas, Venezuela	EF222330
	TK22536/TTU39147	Darien, Panama	EF222369
	TK25079	Trinidad Nariva, Trinidad	EF222379
	TK19519	Barinas, Venezuela	EF222407
Rhogeessa mira	TK45014	Michoacan, Mexico	EF222336
Rhogeessa parvula	TK4765/TTU46788	Guerrero, Mexico	EF222353
	TK14502/TTU35588	Sinaloa, Mexico	EF222344
	TK14504/TTU46783	Sinaloa, Mexico	EF222357
	TK20651/TTU36632	Sonora, Mexico	EF222355
	TK20653/TTU36633	Sonora, Mexico	EF222346
Rhogeessa tumida	AK7136/TCWC49808	Atlantida, Honduras	EF222370
	AK7137	Atlantida, Honduras	EF222371
	TK20516/TTU36168	Oaxaca, Mexico	EF222349
	TK20594/TTU36161	Chiapas, Mexico	EF222338
	TK20596/TTU36164	Chiapas, Mexico	EF222356
	TK27068/TTU44867	Tamaulipas, Mexico	EF222345
	TK34866/TTU60986	San Salvador, El Salvador	EF222380
	TK34867/TTU60987	San Salvador, El Salvador	EF222353
	TK34902/TTU60985	La Paz, El Salvador	EF222385
	TK34980	La Paz, El Salvador	EF222390
	TK40186/TTU61231	Valle, Honduras	EF22350
	TK40345/TTU61229	Atlantida, Honduras	EF222377
	TK40360/TTU61230	Atlantida, Honduras	EF222378
	TK101020/TTU83681	Valle, Honduras	EF222351
	TK101021/TTU83682	Valle, Honduras	EF222352
	TK101044/TTU83705	Valle, Honduras	EF222367
	TK101052/TTU83713	Valle, Honduras	EF222368

APPENDIX I.—Continued.

Species	Museum no.	Locality	GenBank accession no.
	TK101266/TK83927	Valle, Honduras	EF222409
	TK101367/TTU84027	Comayagua, Honduras	EF222383
	TK101370/TTU84030	Comayagua, Honduras	EF222411
	AK1638	Tamaulipas, Mexico	EF222360
	AK7022/TCWC47833	Guanacaste, Costa Rica	EF222335
	AK9585/TCWC49791	Valle, Honduras	EF222326
	AK9587/TCWC49793	Valle, Honduras	EF222372
	AK9615/TCWC49797	Valle, Honduras	EF222373
	AK9617/TCWC49799	Valle, Honduras	EF222373
	SP12543	Izabal, Guatemala	EF222396
	SP12544	Izabal, Guatemala	EF222397
	SP12606	Izabal, Guatemala	EF222398
	SP12615	Izabal, Guatemala	EF222399
	SP12617	Izabal, Guatemala	EF222400
	SP12650	Izabal, Guatemala	EF222401
	SP12771	Zacapa, Guatemala	EF222402
	SP12772	Zacapa, Guatemala	EF222403
	AK25022	El Progreso, Guatemala	EF222416
	AK25023	El Progreso, Guatemala	EF222413
	AK25024	El Progreso, Guatemala	EF222414
	AK25065	Izabal, Guatemala	EF222417
	AK25093	Izabal, Guatemala	EF222415
Rhogeessa velilla	TK134692/TTU103525	Guayas, Ecuador	EF222341
	TK134693/TTU103526	Guayas, Ecuador	EF222342
	TK134792/TTU103254	Guayas, Ecuador	EF222339
	TK134868/TTU103292	Guayas, Ecuador	EF222366
	TK134869/TTU103341	Guayas, Ecuador	EF222365
	TK134870/TTU103293	Guayas, Ecuador	EF222386
	TK134871/TTU103294	Guayas, Ecuador	EF222387
	TK134872/TTU103295	Guayas, Ecuador	EF222388
	TK135175/TTU102429	Guayas, Ecuador	EF222389
Antrozous pallidus	AK21090		EF222382
Bauerus dubiaquercus	SP12598		EF222381
Plecotus auritus			AY665169