

MOLECULAR SYSTEMATICS OF *NYCTINOMOPS* (CHIROPTERA: MOLOSSIDAE)

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ABSTRACT.—The genus *Nyctinomops* comprises 4 species of bats in the free-tailed family Molossidae: *Nyctinomops macrotis*, *Nyctinomops femorosaccus*, *Nyctinomops aurispinosus*, and *Nyctinomops laticaudatus*. The objective of this study was to use mitochondrial sequence data to test hypotheses of relationship within *Nyctinomops*. A total of 460 bases of cytochrome *b* and 686 bases of NADH dehydrogenase were sequenced and analyzed from 22 individuals by use of parsimony, maximum likelihood, and Bayesian methods. Sequences from other molossid genera (*Tadarida*, *Cynomops*, and *Otomops*) were used as outgroups. In addition, a series of *Nyctinomops* specimens from Peru was re-assessed using morphological and molecular data. Our results agree with previous studies using morphological similarity in the placement of the largest member of the genus, *N. macrotis*, as sister to the remaining species. Furthermore, both mitochondrial data sets recovered the following relationship for the other members of the genus: (*N. aurispinosus* (*N. laticaudatus*, *N. femorosaccus*)).

RESUMEN.—El género *Nyctinomops* contiene cuatro especies de murciélagos sin cola en la familia Molossidae: *Nyctinomops macrotis*, *Nyctinomops femorosaccus*, *Nyctinomops aurispinosus*, y *Nyctinomops laticaudatus*. El objetivo de este estudio era usar secuencias de datos mitocondriales para probar la hipótesis de parentesco dentro de *Nyctinomops*. Un total de 460 bases de citocromo *b* y 686 bases de NADH deshidrogenasa (ND1) fueron secuenciadas y analizadas en 22 individuos usando parsimonia, máxima verosimilitud y el método bayesiano. Secuencias de otros géneros de molósidos (*Tadarida*, *Cynomops* y *Otomops*) fueron usadas como grupos externos. Adicionalmente, una serie de especímenes de *Nyctinomops* del Perú fue re-evaluada usando datos morfológicos y moleculares. Nuestros resultados corroboran previos estudios usando similitud morfológica en la colocación del miembro más grande del género, *N. macrotis*, como grupo hermano de las especies restantes. Además, ambas bases de datos mitocondriales recuperaron la siguiente relación de parentesco para otros miembros del género: (*N. aurispinosus* (*N. laticaudatus*, *N. femorosaccus*)).

The family Molossidae is represented by approximately 16 genera and 100 species of free-tailed bats (Simmons 2005). The genus *Nyctinomops* contains 4 species found only in the New World: *Nyctinomops macrotis* (the big free-tailed bat), *Nyctinomops femorosaccus* (the pocketed free-tailed bat), *Nyctinomops laticaudatus* (the broad-eared bat), and *Nyctinomops aurispinosus* (Peale's free-tailed bat) (Simmons 2005). Historically, *Nyctinomops* has been treated as a subgenus within *Tadarida* and was presumed to have a close relationship to the only other New World species, *Tadarida brasiliensis* (Miller 1902). Elevation and current use of *Nyctinomops* was recommended by Freeman (1981), who conducted a morphometric analysis of the family Molossidae. Her study included a principal component analysis of 56 morphometric measurements, 12 qualitative multistate codes, and 8 ratios. All 4 species of *Nyctinomops* were found to cluster apart from *Tadarida* (Freeman 1981). Within

the genus, *N. macrotis* was least similar to the 3 other species. The remaining species formed a cluster with *N. aurispinosus* and *N. femorosaccus* being more similar than either was to *N. laticaudatus*.

The 4 species of *Nyctinomops* are discerned from *Tadarida* by a loss of incisor 3, a shortened second phalanx on digit IV, well-joined ears, narrower rostrum, and slightly narrower palatal emargination (Freeman 1981). The dental formula in *Nyctinomops* is $i\ 1/2$, $c\ 1/1$, $p\ 2/2$, $m\ 3/3$, total 30 (Jones and Arroyo-Cabrales 1990, Kimirai and Jones 1990, Milner et al. 1990, Avila-Flores et al. 2002).

While species in the genus *Nyctinomops* are similar morphologically, the most obvious differences are in size. *Nyctinomops macrotis*, the big free-tailed bat, is the largest member of the genus (Silva-Taboada 1979). First described as *Nyctinomus macrotis* by Gray in 1839 from a hollow tree in Cuba, the species has been renamed no less than 7 times since

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(Milner et al. 1990). Miller (1902) first used the current name combination. The distribution of *N. macrotis* is the largest of the genus, extending the farthest north, reaching into southern Colorado and Utah, with a southern extension into the northern quarter of Argentina (Milner et al. 1990).

Nyctinomops femorosaccus, the pocketed free-tailed bat, has the most-restricted distribution in the genus, living only in northwestern Mexico, Far West Texas, New Mexico, Arizona, and California (Hall 1981, Jones and Peters 1988). The first description of this species was *Nyctinomus femorosaccus* by Merriam in 1889, but subsequently Miller (1902) renamed it *Nyctinomops*. *Nyctinomops laticaudatus*, the broad-tailed bat, is the smallest species within the genus. It was first described as *Molossus laticaudatus* by Geoffroy St. Hilaire in 1805, but the taxonomy was revised by Miller (1902). *Nyctinomops laticaudatus* exhibits a number of karyotypes. This karyotypic variation, as well as variations in size of the bat, has resulted in the description of 5 subspecies: *N. l. laticaudatus*, *N. l. europs*, *N. l. ferrugineus*, *N. l. macarensis*, and *N. l. yucatanicus* (Koopman 1982). This species, as a whole, has a distribution that ranges from the Pacific and Caribbean coasts of Mexico south to northern Argentina (Avila-Flores et al. 2002).

Nyctinomops aurispinosus, Peale's free-tailed bat, was first described by Peale as *Nyctinomus* in 1848 after being captured on board the U.S.S. *Peacock* 160 km off the coast of Brazil (Jones and Arroyo-Cabrales 1990). As with the other members of this genus, Miller (1902) reclassified the species as a member of the genus *Nyctinomops*. The range of this species extends northward into southern Mexico just north of the Yucatan peninsula and southward into southern Brazil, Bolivia, and Paraguay (Jones and Arroyo-Cabrales 1990).

The genus *Nyctinomops* shows strict conservation in a diploid number of 48 (Warner et al. 1974). Three of the species, *N. aurispinosus*, *N. macrotis*, and *N. femorosaccus*, also share conserved fundamental numbers of 58 (Warner et al. 1974). *Nyctinomops laticaudatus* has a variable fundamental number ranging from 58 to 64 (Morielle-Versute et al. 1996).

Since Freeman's (1981) morphometric study, the monophyly of the genus *Nyctinomops* has not been questioned; however, there have

been no studies that explore the phylogenetic relationships among the 4 species. The objective of this project was to use 2 mitochondrial genes (cytochrome *b* [*cyt-b*] and NADH dehydrogenase subunit 1 [ND1]) to produce the first cladistic hypothesis for the relationships among *Nyctinomops* species. The molecular data were used to test the morphometric hypothesis of Freeman (1981), which placed the 4 species as follows: (*N. macrotis* (*N. laticaudatus* (*N. femorosaccus*, *N. aurispinosus*))).

METHODS

Taxonomic Sampling and Specimen Identification

Specimens of *Nyctinomops* for this study were obtained from tissue loans or from field collection (Appendix). In total, 5 *N. macrotis* from the USA (Texas), 1 *N. laticaudatus* from Mexico (Yucatán), 3 *N. femorosaccus* from the USA (Texas), and 8 *N. aurispinosus* from Mexico (Chihuahua, Tamaulipas) and Peru (Lambayeque) were included in the molecular analyses. Additional specimens of *Cynomops abrasus*, *Tadarida teniotis*, *Tadarida brasiliensis*, and *Otomops martiensseni* were included as outgroups based on results of Ammerman et al. (2012). Specimens of *Nyctinomops* were identified using keys in the published literature (Kumirai and Jones 1990, Gregorin and Taddei 2002, Eger 2007). A discrepancy in the identification of *N. laticaudatus* and *N. aurispinosus* collected in Peru was discovered as a result of the molecular analysis. To re-evaluate species identification, 9 specimens from Peru were examined morphologically and compared with 3 specimens from Mexico (MWSU 2126, MWSU 2332, CRD 4550) (Appendix). The following external and cranial measurements were recorded from tags or taken from prepared skins and skeletal material: total length, length of tail, length of hind foot, length of ear, length of tragus, length of forearm, greatest length of skull, maxillary tooththrow, and weight. Length of maxillary tooththrow and greatest length of skull are diagnostic measures used to discriminate among *Nyctinomops* species (Kumirai and Jones 1990, Gregorin and Taddei 2002). New morphological specimen identifications were interpreted with respect to the genetic identifications.

TABLE 1. Primers used for polymerase chain reaction (PCR) amplification of cytochrome *b* (*cyt-b*) and NADH dehydrogenase subunit 1 (ND1) in *Nyctinomops*.

Name	Sequence	Reference
(<i>Cyt-b</i>) Gludg	5'-TGA CTT GAA RAA CCA YCG TTG-3'	Palumbi 1996
(<i>Cyt-b</i>) CB6	5'-CTC CAG TCT TCG RCT TAC AAG-3'	Palumbi 1996
(<i>Cyt-b</i>) L15106	5'-TGA AAC ACA GGA ATT ATC CTC CTA-3'	Edwards et al. 1991
(<i>Cyt-b</i>) H15149	5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3'	Irwin et al. 1991
(<i>Cyt-b</i>) L14841	5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA AA-3'	Irwin et al. 1991
(<i>Cyt-b</i>) H15547	5'-GGC AAA TAG GAA ATA TCA TTC-3'	Edwards et al. 1991
(ND1) ER65	5'-CCT CGA TGT TGG ATC AGG-3'	Petit et al. 1999
(ND1) ER66	5'-GTA TGG GCC CGA TAG CTT-3'	Petit et al. 1999
(ND1) ER70	5'-CAG ACC GGC GTA ATC CAG GTC GGT T-3'	Petit et al. 1999
(ND1) ER89	5'-CTC TAT CAA AGT AAC TCT TTT ATC AGA-3'	Petit et al. 1999
(ND1) ER340	5'-AGG TTC AAY TCC TCT CTC TAA CA-3'	This study

DNA Amplification and Sequencing

Whole genomic DNA was extracted from frozen liver or liver in lysis buffer using a DNeasy Tissue Kit and manufacturer's protocol (Qiagen Inc., Valencia, CA). Two mitochondrial genes, *cyt-b* and ND1, were amplified using PCR (Palumbi 1996). We amplified the entire *cyt-b* gene (~1100 base pairs [bp]) using conserved vertebrate primers (Gludg, CB6; Table 1), with 4 additional primers used to generate the internal sequence (Table 1). In some cases (ASK415, 416, 8445, 8446, 8447, 8448), full gene fragments did not amplify. In those cases we used primers L14841 and H15547 to amplify an internal fragment of approximately 700 bp in length. The *cyt-b* was amplified in a total volume of 12.5 μ L including 3U Taq polymerase, 0.16 μ M each primer, 2 mM MgCl₂, 0.16 mM deoxynucleoside triphosphates, and 1 \times reaction buffer. The PCR amplification thermal profile started with 1 cycle at 94 °C for 3 min, 39 cycles at 94 °C, 48 °C, and 72 °C for 1 min each, and 1 final extension cycle at 72 °C for 3 min. The PCR amplification, reagent final volumes, and thermal profiles for the mitochondrial gene ND1 were congruent to that of *cyt-b*. Amplification involved primers ER65 and ER66, and 3 additional internal primers were used for sequencing (Table 1). In addition to the protein coding portion of the ND1 gene, a portion of upstream 16S rRNA, tRNA-leucine, and downstream tRNA-isoleucine were amplified. Prior to gene sequencing, the reaction products were cleaned using either the Wizard[®] SV Gel and PCR Clean-Up System (Promega Co., San Luis Obispo, CA), or ExoSAP-IT[®] (USB Co., Cleveland, OH). We sequenced using a quarter of the volume in the manufacturer's protocol

with a GenomeLab-DTCS Quick Start Kit on a Beckman Coulter CEQ 8000 Automated Sequencer (Beckman Coulter Inc., Fullerton, CA).

Data Analysis

Sequences were aligned using Sequencher[®] 4.8 (Gene Codes Corporation, Ann Arbor, MI) and refined by eye. After translation was confirmed, datasets were exported into PAUP version 4.0b10 (Swofford 2002) for phylogenetic analysis. Fifty-six maximum-likelihood models were analyzed in ModelTest 3.7 (Posada and Crandall 1998) to determine the appropriate model of gene evolution to use in the maximum-likelihood (ML) analysis (Felsenstein 1981). Following independent likelihood analyses for each gene, the resulting trees were compared by eye for congruence. Combining both mitochondrial data sets was not performed because 8 individual sequences would have to be clipped in order to achieve congruent specimen lists. Because of computing limitations, nodal support was evaluated by running 1000 parsimony bootstrap (BS) replicates instead of ML BS (Felsenstein 1985). In the heuristic parsimony BS, all sites were weighted equally and we used random sequence addition. Genetic distances were calculated as averages in PAUP using the Kimura 2-parameter (K2P; Kimura 1980) model of nucleotide substitution. K2P genetic distances recovered in the analyses of *cyt-b* were compared to those observed between sister taxa of bats by Bradley and Baker (2001) and Baker and Bradley (2006).

Bayesian analyses of *cyt-b* and ND1 data were performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Analyses consisted of 2 simultaneous runs, each with

4 Markov Chain Monte Carlo chains (1 heated and 3 cold) run for 2 million generations. Trees were sampled every 100 generations for a total of 40,000 trees sampled (pooled from the 2 independent runs). Stationarity was evaluated by the convergence of log likelihoods. The first 10% of trees were excluded and, therefore, only a total of 36,000 trees were evaluated in a 50% majority-rule consensus tree.

RESULTS

Analyses of Cytochrome *b*

After amplification and sequencing of *cyt-b*, only 460 nucleotide positions were shared across all taxa and could be aligned for phylogenetic analysis. Eighty-four of those 460 nucleotides were parsimony informative. Maximum likelihood (GTR+I) and Bayesian analyses each resulted in the same placement of *N. macrotis* as the oldest divergence in the genus (Bayesian Posterior Probability [BPP]: 1.0; Parsimony BS: 100) and monophyly of *N. femorosaccus* individuals (BPP: 0.92; BS: 93). The single *N. laticaudatus* individual was sister to specimens of *N. femorosaccus*. Specimens of *N. aurispinosus* form a monophyletic clade in all analyses with statistical support (BPP: 1.0; BS: 100), with additional support for the cluster containing all specimens from Peru to the exclusion of the single specimen from Mexico (BPP: 1.0; BS: 97) (Fig. 1). All 3 smaller species form a well-supported clade (BPP: 1.0; BS: 95). Additionally, when 2 partial *cyt-b* sequences labeled as *Nyctinomops* were included from GenBank (L19728, L19729), both clustered with specimens of *N. aurispinosus* (data not shown).

Average levels of *cyt-b* sequence divergence within species of *Nyctinomops* ranged from 0.30% (*N. femorosaccus*) to 1.33% (*N. aurispinosus*) (Table 2). Average levels of interspecific sequence divergence ranged between 1.69% (*N. femorosaccus* to *N. laticaudatus*) and 11.22% (*N. aurispinosus* to *N. macrotis*). Outgroup levels of divergence to all *Nyctinomops* specimens averaged 16.27%.

Analyses of NADH Dehydrogenase

Of the 686 bases sequenced and aligned, 126 were parsimony informative. Maximum likelihood (GTR+I) and Bayesian analyses resulted in several well-supported clades (Fig. 2). *Nyctinomops macrotis* individuals formed

a monophyletic clade basal to the other 3 species (BPP: 1.0; BS: 100). Specimens of *N. aurispinosus* from Peru (BPP: 0.97) form a monophyletic clade. *Nyctinomops femorosaccus* formed a sister relationship with *N. laticaudatus* (BPP: 1.0; BS: 98).

Average levels of ND1 sequence divergence within species of *Nyctinomops* ranged from 0.60% (*N. femorosaccus*) to 1.35% (*N. macrotis*) (Table 3). Average levels of interspecific sequence divergence ranged from 1.97% (*N. femorosaccus* to *N. laticaudatus*) to 11.05% (*N. femorosaccus* to *N. macrotis*). Outgroup levels of divergence to all specimens of *Nyctinomops* averaged 13.6%.

Confirmation of Species Identifications

All specimens from Peru (2 *N. laticaudatus* and 3 *N. aurispinosus*) clustered with the *N. aurispinosus* individual from Mexico (CRD 4450) in the *cyt-b* analysis (Fig. 1). Two additional *N. laticaudatus* specimens from Peru clustered with those of *N. aurispinosus* in the ND1 tree (Fig. 2). For this reason, a morphological assessment of these specimens was conducted. Nine specimens were identified as *Nyctinomops aurispinosus* based on 2 skull features: greatest length of skull and length of maxillary tooth row (Kumirai and Jones 1990, Gregorin and Taddei 2002). When specimens were keyed only with general measurements (total length, length of forearm, tail length, hind foot length, ear length), 4 were *N. laticaudatus* and 5 were *N. aurispinosus* (Table 4, specimens with LSU prefix). Additionally, the single specimen of *N. laticaudatus* (ASK 415) that clustered with *N. femorosaccus* in both mitochondrial data sets was measured to confirm identification. All measurements (data not shown) of this individual fell within those published for *N. laticaudatus* (Avila-Florez et al. 2002).

DISCUSSION

Based on our cladistic analyses of molecular data, the phenetic hypothesis for relationship of these species is rejected (Freeman 1981). The 2 analyses are congruent in the position of *N. macrotis* as the earliest divergence within the genus but differ with regard to the relationship of *N. femorosaccus*. Morphologically *N. femorosaccus* and *N. aurispinosus* are most similar, whereas our molecular results

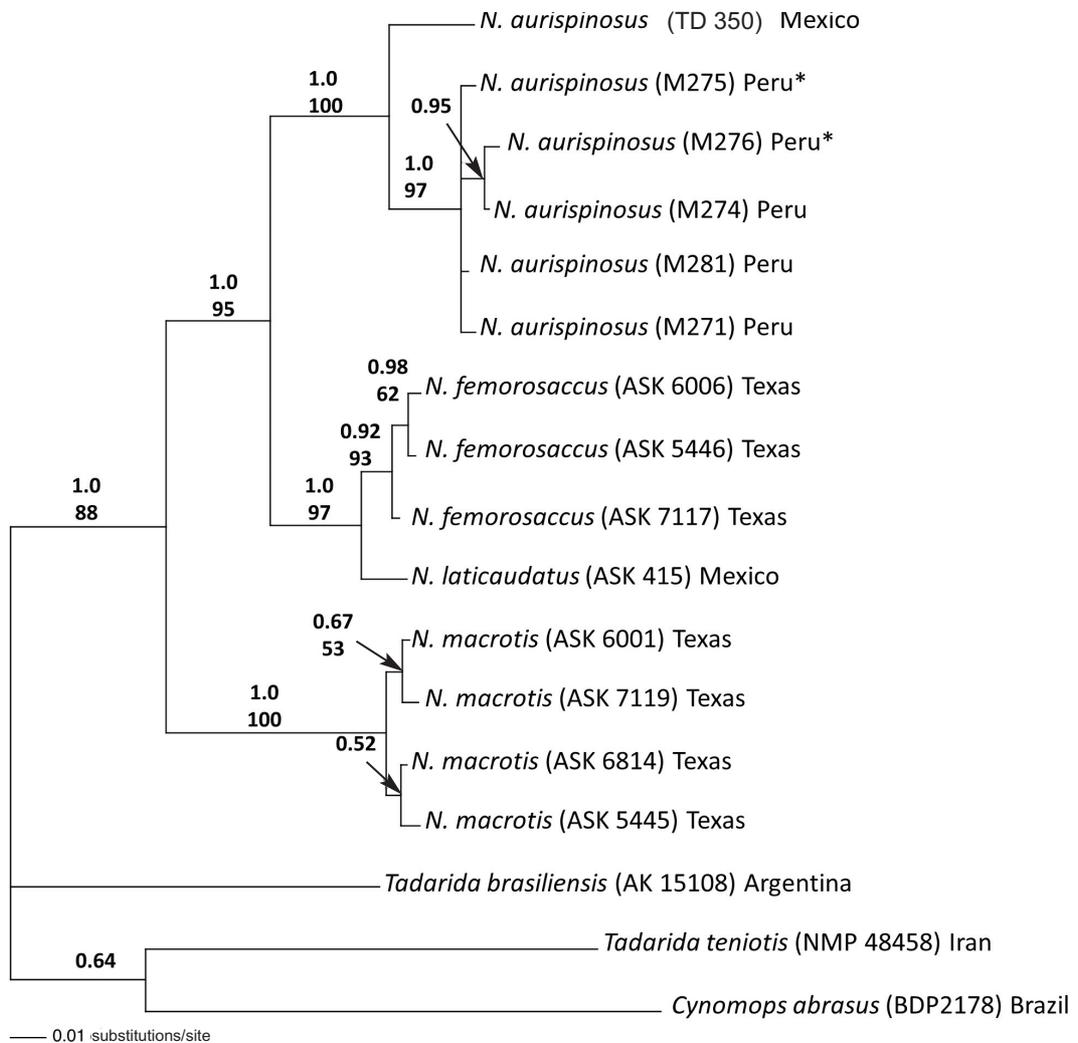


Fig 1. Likelihood analysis (GTR+I) of 460 bases of cytochrome *b* for molossid bats with Bayesian posterior probabilities (top) and parsimony bootstrap values (bottom) on the nodes. A total of 84 bases were parsimony informative. Specimens noted with an asterisk (*) were initially identified as *Nyctinomops laticaudatus* and were subsequently reidentified as *Nyctinomops aurispinosus*.

TABLE 2. Average cytochrome *b* sequence divergence (Kimura 2-parameter) within and between *Nyctinomops macrotis*, *N. aurispinosus*, *N. femorosaccus*, and *N. laticaudatus* expressed as a percentage. Intraspecific percentages are in bold.

	<i>N. macrotis</i> (n = 4)	<i>N. femorosaccus</i> (n = 3)	<i>N. aurispinosus</i> (n = 6)	<i>N. laticaudatus</i> (n = 1)
<i>N. macrotis</i>	0.41			
<i>N. femorosaccus</i>	8.90	0.30		
<i>N. aurispinosus</i>	11.22	6.01	1.33	
<i>N. laticaudatus</i>	8.70	1.69	5.54	0.00

support a sister relationship between *N. femorosaccus* and *N. laticaudatus*. However, this working hypothesis of relationship should

be examined more thoroughly when more specimens of *N. laticaudatus* are obtained. It appears that in this specific case, members of

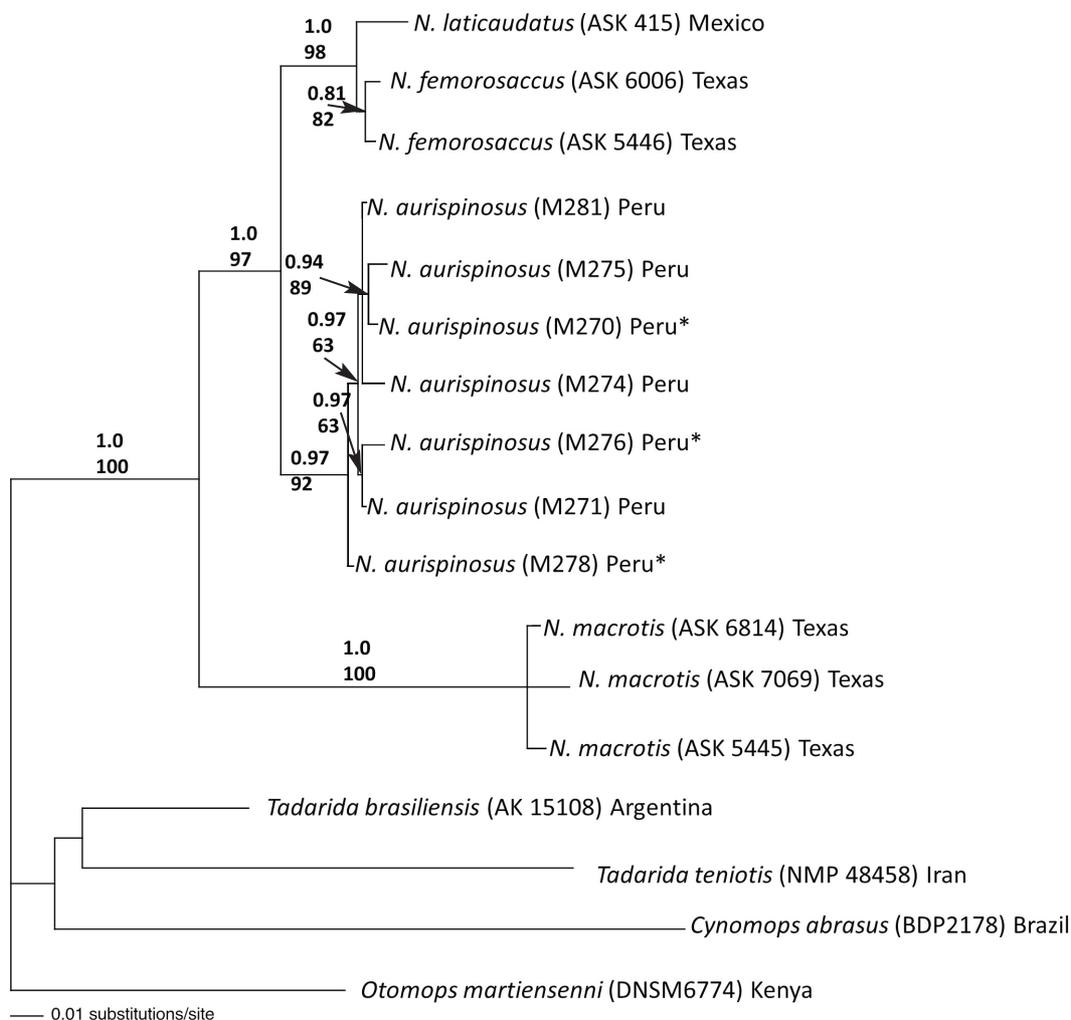


Fig 2. Likelihood analysis (GTR+I) of 686 bases of ND1 for molossid bats with Bayesian posterior probabilities (top) and bootstrap values (bottom) on the nodes. A total of 126 bases were parsimony informative. Specimens noted with an asterisk (*) were initially identified as *Nyctinomops laticaudatus* and were subsequently re-identified as *Nyctinomops aurispinosus*.

TABLE 3. Average NADH dehydrogenase sequence divergence (GTR+I) within and between *Nyctinomops macrotis*, *N. aurispinosus*, *N. femorosaccus*, and *N. laticaudatus* expressed as a percentage. Intraspecific percentages are in bold.

	<i>N. macrotis</i> (n = 3)	<i>N. femorosaccus</i> (n = 3)	<i>N. aurispinosus</i> (n = 7)	<i>N. laticaudatus</i> (n = 1)
<i>N. macrotis</i>	1.35			
<i>N. femorosaccus</i>	11.05	0.60		
<i>N. aurispinosus</i>	10.65	5.16	0.74	
<i>N. laticaudatus</i>	10.92	1.97	5.47	0

the genus evolved toward reduced body size. *Nyctinomops macrotis* is the largest species overall, *N. aurispinosus* is medium-sized, and the remaining 2 species are the smallest and

most-recently diverged taxa. Although contrary to Cope's rule of general phyletic increase in body size, this result is consistent with general trends seen in small mammals (Alroy 1998).

TABLE 4. Nine morphological measurements of 12 specimens that were presumed to be *Nyctinomops aurispinosus*. Tot = total length, TL = tail length, HF = hind foot length, ER = ear length, Tr = tragus length, FA = forearm length, GLS = greatest length of skull, LMT = length of maxillary tooththrow, WT = weight. Dashes (—) indicate measurements not provided by specimen tags. All measurements except weight are given in millimeters (mm).

Specimen	Sex	Tot ^a	TL	HF	ER	Tr	FA ^a	GLS ^a	LMT ^a	WT	Original identification
LSU 25021	M	114.0	42.0	9.0	23.0	7.0	46.6	19.5	7.7	14 g	<i>N. aurispinosus</i>
LSU 25015	F	104.0	41.0	8.0	24.0	9.0	48.9	18.7	8.8	11 g	<i>N. laticaudatus</i>
LSU 25014	F	107.0	43.0	8.0	24.0	7.0	47.7	19.1	7.7	12 g	<i>N. aurispinosus</i>
LSU 25016	F	104.0	44.0	8.0	22.0	7.0	46.5	18.7	7.4	11 g	<i>N. laticaudatus</i>
LSU 25010	F	103.0	38.0	8.0	23.0	7.0	45.8	18.9	7.2	11 g	<i>N. laticaudatus</i>
LSU 25011	F	105.0	42.0	8.0	23.0	8.0	47.7	18.9	7.7	11 g	<i>N. aurispinosus</i>
LSU 25012	F	102.0	55.0	10.0	23.0	7.0	46.0	18.6	8.5	10 g	<i>N. aurispinosus</i>
LSU 25013	F	110.0	43.0	8.0	23.0	8.0	45.4	19.8	8.1	16 g	<i>N. aurispinosus</i>
LSU 25018	F	102.0	40.0	7.0	22.0	7.0	45.6	18.8	7.5	11 g	<i>N. laticaudatus</i>
MWSU2126	M	120.0	45.0	10.0	—	—	47.0	20.9	7.5	—	<i>N. aurispinosus</i>
MWSU2332	F	116.0	50.0	10.0	—	—	46.9	20.2	7.7	—	<i>N. aurispinosus</i>
CRD 4550	F	115.0	47.0	11.0	21.0	—	46.9	19.8	7.2	13.0 g	<i>N. aurispinosus</i>

^aAverage reported in Table 3.

Both *cyt-b* and ND1 data sets were consistent concerning relative sequence divergence values and statistical support of tree topology. Congruence between these 2 data sets provides additional confidence with the relationships they recovered (Hillis 1995, Miyamoto and Fitch 1995). In the case of *N. femorosaccus* and *N. laticaudatus*, relatively small *cyt-b* and ND1 divergences (2.29% and 1.97%, respectively) are consistent with the degree of morphological separation observed (Freeman 1981). According to Bradley and Baker (2001) these values are also within the expected range of *cyt-b* divergence between 2 sister species.

Both mitochondrial data sets and morphological characters contributed to the reidentification of 4 specimens from Peru originally identified as *N. laticaudatus*. We conclude that all specimens in the study from Peru, including those from GenBank (L19728, L19729), are *N. aurispinosus*. Our own measurements and those in published accounts provide strong evidence for historical misidentification. In this study, both greatest length of skull (GLS) and length of maxillary tooththrow (LMT) contributed to the molecular evidence that these bats are *N. aurispinosus* (Table 5). The average GLS for specimens from Peru was 19.3 mm, lower than the average published for *N. aurispinosus* (Kumirai and Jones 1990, Gregorin and Taddei 2002). Conversely, the 19.3-mm average is one of the highest published for *N. laticaudatus* in the same studies. The specimens in question had an average LMT of 7.75 mm, intermediate between the 2 species but much closer to *N. aurispinosus*. Specimens of *N. aurispinosus* (MWSU 2126, MWSU 2336, and CRD 4550) and *N. laticaudatus* (ASK 415, ASK 416) from Mexico do follow the general morphology described by Jones and Arroyo-Cabrales (1990) and Avila-Flores et al. (2002). Two of the same specimens of *N. aurispinosus* from MWSU (2126 and 2336) had a GLS average >20.0 mm, which is expected for *N. aurispinosus*, but an LMT intermediate to that expected for *N. laticaudatus* and *N. aurispinosus* (Table 5). The third specimen of *N. aurispinosus* (CRD 4550) from Mexico was smaller in both measures than published reports (Kumirai and Jones 1990, Gregorin and Taddei 2002). Sexual dimorphism has been documented in the family (Freeman 1981, Gregorin and Taddei 2002) but is not sufficient to explain this discrepancy with identification.

TABLE 5. Average measurements from Table 2 of 8 specimens from Peru, and 3 from Mexico, reidentified as *Nyctinomops aurispinosus*, compared to 2 published keys (Kimurai and Jones 1990, Gregorin and Taddei 2002). FA = forearm length, GLS = greatest skull length, LMT = length of maxillary tooththrow, Tot = total length. All measurements are given in millimeters (mm).

Measurements	<i>Nyctinomops laticaudatus</i>		Specimen average (n = 12)	<i>Nyctinomops aurispinosus</i>	
	Kimurai and Jones (1990)	Gregorin and Taddei (2002)		Kimurai and Jones (1990)	Gregorin and Taddei (2002)
FA	41.0–45.0	42.3–47.3	46.8	>47.0	50.0–52.2
GLS	~18.2	17.7–19.2	19.3	≥20.0	20.1–21.6
LMT	<7.0	—	7.75	≥7.9	—
Tot	102.0	—	108.5	109.0–112.0	—

According to the morphological assessment and results of our mitochondrial analyses, *N. aurispinosus* individuals from Peru and Mexico are smaller than those from farther south in their distribution (e.g., Brazil) (Table 3; Kumirai and Jones 1990, Gregorin and Taddei 2002). Eger (2007) also examined *N. aurispinosus* specimens from Peru and found that they were smaller than what was to be expected from published literature (Jones and Arroyo-Cabrales 1990, Avila-Flores et al. 2002).

The 3 smaller species of *Nyctinomops* only occur in sympatry in a very small area on the Pacific coast of central Mexico. *Nyctinomops laticaudatus* and *N. aurispinosus* share the majority of their distribution, causing them to be potentially captured together; even though they are not most similar in size within the genus, they are close enough to be misidentified. Examination of existing specimens and sampling of both species from as many sites as possible should be conducted in the future to evaluate the morphological variation within each species and to assess the subspecies designations for *N. laticaudatus*.

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APPENDIX.—Species; locality; catalog number; analysis type(s) used: cytochrome *b* (C), ND1 (N), morphometric analysis (MM); and GenBank accession number (GQ, JF) for specimens used in the analyses. AK (tissue number) = Texas A&M University; ASK (tissue number) and ASNHC (catalog number) = Angelo State Natural History Collection, Angelo State University; LSUMZ = Louisiana State University Museum of Zoology (catalog number), TD (tissue number) and CRD (catalog number) = Colección Regional Durango, Instituto Politécnico Nacional, Mexico; BDP (tissue number) = Bruce D. Patterson; NMP (catalog number) = National Museum of Prague; MWSU (catalog number) = Midwestern State University.

Nyctinomops macrotis.—United States: Texas: Brewster Co.; Terlingua, Terlingua Creek, ASNHC 11533 (ASK 5445) (C, N) (GQ424044, JF509377); Big Bend National Park, Rio Grande River Mile 736.2, ASNHC 11588 (ASK 6001) (C) (GQ424037); 02 Ranch, Terlingua Creek 13R 620729 3305220, ASNHC 12971 (ASK 6814) (C, N) (GQ424043, JF509371); Big Bend National Park, Hot Springs, 29.1776°N, 102.99783°W, ASNHC 13293 (ASK 7069) (N) (JF509372); 02 Ranch, ASNHC 13294 (ASK 7119) (C, MM) (GQ424045).

Nyctinomops femorosaccus.—United States: Texas: Brewster Co.; Terlingua, Terlingua Creek, ASNHC 11528 (ASK 5446) (C, N) (GQ424042, JF509368); Terlingua, Terlingua Creek 13R 0636529 3247884, ASNHC 13290 (ASK 7117) (C) (GQ424041); United States: Texas: Terrell Co.; Rio Grande River Mile 710.5, ASNHC 11587 (ASK 6006) (C, N) (GQ424039, JF509362).

Nyctinomops laticaudatus.—Mexico: Yucatan; Ruinas de Uxmal, ASNHC 3820 (ASK 415) (C, N, MM) (GQ424040, JF509361); ASNHC 3821 (ASK 416) (MM).

Nyctinomops aurispinosus.—Peru: Lambayeque Department; 12 km N Olmos, 150 m elev. LSUMZ 25010 (M270) (N, MM) (JF509369); LSUMZ 25011 (M271) (C, N, MM) (GQ424035, JF509367); LSUMZ 25012 (M272) (MM); LSUMZ 25013 (M273) (MM); LSUMZ 25014 (M274) (C, N, MM) (GQ424033, JF509376); LSUMZ 25015 (M275) (C, N, MM) (GQ424030, JF509365); LSUMZ 25016 (M276) (C, N, MM) (GQ424032, JF509364); LSUMZ 25018 (M278) (N, MM) (JF509366); LSUMZ 25021 (M281) (C, N, MM) (GQ424034, JF509363); Mexico: Chihuahua, Municipio Morelos, San Miguel on Rio San Miguel, 438 m, 26°46.66' N, 107°41.25' W, CRD 4550 (TD350) (C, MM) (GQ424029); Mexico: Tamaulipas: 10 km N Antigua Morelos MWSU 2126 (MM), MWSU 2332 (MM).

Cynomops abrasus.—Brazil: Cachoeira Nazare, Rio Ji-Rondonia BDP 2178 (C, N) (GQ424038, JF509375).

Otomops martiensseni.—South Africa: DNSM 6774 (N) (JF509374).

Tadarida brasiliensis.—Argentina: Catamarca, Capayan AK 15108 (C, N) (GQ424031, JF509370).

Tadarida teniotis.—Iran: C Zagros Mountains: Firuzabad, cave in a valley, 28°56' N 52°32' E (NMP 48458) (C, N) (GQ424036, JF509373).