

SYSTEMATICS OF VAMPYRESSA AND RELATED GENERA OF PHYLLOSTOMID BATS AS DETERMINED BY CYTOCHROME-*B* SEQUENCES

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The phyllostomid bat genus *Vampyressa* often has been allied with *Uroderma*, *Chiroderma*, *Platyrrhinus*, *Vampyrodes*, and *Mesophylla*. However, the relationships among the genera have proven difficult to resolve, and a number of studies have suggested that *Vampyressa* is not monophyletic. *Mesophylla* includes a single species, which some taxonomists have placed in *Vampyressa* and others in the more distantly related genus *Ectophylla*. Our analysis of sequence data from the cytochrome-*b* gene (*Cytb*) gives strong support for *Mesophylla* being closely related to *Vampyressa*, rather than *Ectophylla*. Examination of the *Cytb* data indicates that *Vampyressa pusilla* and *Mesophylla* are members of a lineage distinct from *V. bidens* and *V. brocki*. Therefore, we recognize the genus *Vampyriscus* for *V. brocki* and *V. bidens*. Brazilian specimens of *V. pusilla* are strongly divergent from specimens from elsewhere in Latin America. The degree of genetic distance indicates species-level divergence between these taxa, and supports the recent recognition of *Vampyressa thyone* for the northern populations.

Key words: cytochrome-*b* gene, *Ectophylla*, *Mesophylla*, Phyllostomidae, systematics, *Vampyressa*, *Vampyriscus*

The New World leaf-nosed bats (family Phyllostomidae) are a diverse group that includes species specializing on a wide variety of food sources. Recent morphological (Wetterer et al. 2000) and molecular (Baker et al. 2000) studies have agreed on a monophyletic clade of about 18 genera (depending on nomenclature) of fruit-eating specialists among the phyllostomid bats. Within this group, Baker et al. (2000) recognized a clade consisting of the genera *Uroderma*, *Chiroderma*, *Platyrrhinus*, *Vampyrodes*, *Vampyressa*, and *Mesophylla*. The morphological study of Wetterer et al. (2000) recognized a similar clade, with the addition of *Ectophylla* as sister to *Mesophylla*.

Vampyressa includes between 5 and 7 species. *V. pusilla*, *V. melissa*, *V. nymphaea*, *V. brocki*, and *V. bidens* are all generally recognized as members of the genus. Lim et al. (2003) recently divided *V. pusilla* into 2 species, with the northern species being recognized as *V. thyone*. *Mesophylla macconnelli* is considered by some authors as a member of *Vampyressa*, but has sometimes been placed in *Ectophylla* (Goodwin and Greenhall 1962; Laurie 1955; Wetterer et al. 2000).

The purpose of this study was to use sequences of the mitochondrial cytochrome-*b* gene (*Cytb*) to determine relationships among the species of *Vampyressa* and related genera. We also examine geographic relationships within species. *Cytb* has proven useful in elucidating relationships within and between closely related genera of bats (Baker et al. 1994; Hoffmann and Baker 2001; Lewis-Oritt et al. 2001a, 2001b; Van Den Bussche and Baker 1993; Van Den Bussche et al. 1998; Wright et al. 1999).

MATERIALS AND METHODS

Specimens examined.—Specimens analyzed are listed in Appendix I, along with collecting localities, catalog number, and institution housing voucher specimens. We generated complete *Cytb* sequences from 24 individuals, which were combined with 6 additional sequences archived in GenBank, and originally determined by Baker et al. (1994), Van Den Bussche et al. (1998), and Wright et al. (1999).

The *Cytb* sequences of *Sturnira lilium* were used as an outgroup. Previous studies (Baker et al. 2000; Lim 1993; Wetterer et al. 2000) have agreed that *Sturnira* is an outgroup to the remainder of the taxa in the study. To determine the systematic position of *Ectophylla*, we included as ingroup species representatives of *Vampyressa*, *Mesophylla*, *Vampyrodes*, *Ectophylla*, *Platyrrhinus*, *Uroderma*, *Chiroderma*, *Artibeus*, *Dermanura*, and *Enchisthenes*.

Molecular methods.—DNA was isolated from frozen tissue by using the methods of Longmire et al. (1997). We performed polymerase chain reaction (PCR) with primers glo7L, glo6H (Hoffmann and Baker 2001), L14724, H15149, and H15915 (Irwin

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et al. 1991). The thermal profile for PCR included an initial 2-min denaturation at 95°C, followed by 35 cycles of 95°C denaturation for 30 s, 50°C annealing for 30 s, and 72°C extension for 2 min. The 35 cycles were followed by a final 8-min extension at 72°C. In some cases, the annealing temperature was reduced to 45°C if necessary to produce a product. The PCR product was purified by using a QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, California). Direct sequencing was performed by using Big Dye sequencing chemistry on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, California).

Data analysis.—Data contained no insertions or deletions, so sequence alignment was unequivocal and was performed by hand. We calculated Tamura–Nei genetic distances (Tamura and Nei 1993) for each pair of individuals with PAUP*, version 4.0b10 (Swofford 2001), and used the genetic distances to construct a neighbor-joining tree. To test data for presence of phylogenetic information, we used PAUP* to analyze 100,000 random trees and calculate a G_1 value. Nucleotides were coded as discrete unordered characters. For the maximum parsimony analysis, we used a heuristic search with tree-bisection–recombination branch-swapping with 20 random additions of the taxa. Bremer support values (Bremer 1988) were calculated for each node by using AutoDecay 3.0.3 (Eriksson 1997), and bootstrap analysis was performed with 1,000 iterations. Initial analyses were performed with equal weighting followed by various weighting schemes, including weighting transversions over transitions by 1:0, 2:1, 3:1, 5:1, and 10:1; and weighting 1st and 2nd position codons by 2:1, 3:1, and 5:1. For each analysis, we reanalyzed the data set constraining for monophyly of *Vampyressa* (both including and excluding *M. macconnelli*), and for an *Ectophylla*–*Mesophylla* clade. We compared the constrained trees with the most-parsimonious trees by using the Kishino–Hasegawa test (Kishino and Hasegawa 1989).

Bayesian analyses were performed with MrBayes, version 2.01 (Huelsenbeck and Ronquist 2001). The Bayesian analysis is based on a Markov chain Monte Carlo method of sampling trees from posterior probabilities (Mau and Newton 1997; Mau et al. 1999; Rannala and Yang 1996). Four chains were run simultaneously for 2,000,000 generations, with trees being sampled every 100 generations, to produce an initial sample of 20,000 trees. The maximum likelihood scores stabilized before the 1,000th tree, so the first 1,000 trees were discarded as “burn-in” and the posterior probability of the phylogeny was determined from the final 19,000 trees.

RESULTS

The *Cytb* sequences of 24 individuals have been deposited in GenBank under accession numbers AY157033–AY157055 and AY157584. Genetic distances are shown in Table 1. An analysis of 100,000 random trees produced a G_1 value of -0.568 , indicating that the data set contains potential phylogenetic signal.

Results of phylogenetic analysis.—The maximum parsimony analysis of the unweighted data resulted in 5 equally parsimonious trees (length = 1,412; consistency index = 0.453; retention index = 0.667; rescaled consistency index = 0.302), the strict consensus of which is shown in Fig. 1. Other weighting schemes resulted in maximum parsimony trees with topology either identical to the tree shown in Fig. 1, or in some cases, with only minor differences in the most weakly supported nodes.

The tree resulting from the Bayesian analysis is shown in Fig. 2. A neighbor-joining tree based on Tamura–Nei genetic

distances is shown in Fig. 3, with branch lengths indicating the degree of genetic distance along each branch.

Test of relationship between *Ectophylla* and *Mesophylla*.—The Kishino–Hasegawa test of the maximum parsimony trees produced from the unweighted data indicated that the unconstrained tree was significantly ($P < 0.05$) shorter than the shortest tree constrained for an *Ectophylla*–*Mesophylla* clade (length = 1,435). When using all weighting schemes, the unconstrained tree also was significantly shorter than the *Ectophylla*–*Mesophylla* tree. Some of the weighting schemes produced a maximum parsimony tree in which *Uroderma* and *Platyrrhinus* were sister to a clade consisting of both *Vampyressa* and *Mesophylla*.

Tests for *Vampyressa* monophyly.—The shortest maximum parsimony tree consistent with a monophyletic *Vampyressa* (with *Mesophylla* included) has a length of 1,413 in the unweighted analysis, only 1 step longer than the unconstrained tree. For all weighting schemes, the Kishino–Hasegawa test indicated no significant difference ($P > 0.05$) between the unconstrained tree and the shortest tree having a monophyletic *Vampyressa* with *Mesophylla* included.

The shortest tree consistent with a monophyletic *Vampyressa* (excluding *Mesophylla*) was 1,418 steps in the unweighted analysis, 6 steps longer than the unconstrained tree in which *Mesophylla* and *V. pusilla* are sister taxa. No significant difference ($P > 0.05$) was found between the constrained and unconstrained trees with unweighted data or with the transitions being downweighted. However, for all codon-weighting schemes, the Kishino–Hasegawa test indicated that the unconstrained tree was significantly shorter than any tree constrained for a monophyletic *Vampyressa* excluding *Mesophylla*.

DISCUSSION

Relationship of *Mesophylla* and *Vampyressa*.—*Mesophylla macconnelli* has been variously placed in *Vampyressa*, *Ectophylla*, or in its own genus. Starrett and Casebeer (1968) studied cranial and dental characteristics and concluded that *Mesophylla* was more closely related to *V. thyone* than to *Ectophylla*. Greenbaum et al. (1975) found that the karyotype of *Mesophylla* shared derived characteristics with that of *V. pusilla*, and concluded that *Ectophylla* was derived from a more primitive branch. Analysis of recombination activator gene-2 (*RAG2*) sequences led Baker et al. (2000) to conclude that *V. thyone* (*V. pusilla* in their nomenclature) is sister to *M. macconnelli*. Our analysis supports the conclusions of Starrett and Casebeer (1968), Greenbaum et al. (1975), and Baker et al. (2000) in that *M. macconnelli* is sister to *V. pusilla* and *V. thyone* to the exclusion of *Ectophylla*. This conclusion is strongly supported in the Bayesian analysis (Fig. 2), and is supported weakly in the maximum parsimony analysis (Fig. 1). The maximum parsimony analysis provides statistically significant evidence against an *Ectophylla*–*Mesophylla* relationship.

Relationship of *Vampyressa bidens* and *Vampyressa brocki*.—Our analysis of *Cytb* sequences indicates a sister-group relationship between *V. brocki* and *V. bidens*. This

TABLE 1.—Tamura–Nei genetic distances for cytochrome-*b* gene sequences of 30 bat specimens. Distances are expressed as percentage sequence divergence.

Specimen	1	2	3	4	5	6	7	8	9	10	11
1 <i>Sturnira lilium</i> TK22631	—										
2 <i>Ectophylla alba</i> TK16395	25.35	—									
3 <i>Enchisthenes hartii</i> TK22690	27.12	18.40	—								
4 <i>Dermanura phaeotis</i> TK104501	26.76	17.82	18.17	—							
5 <i>Artibeus jamaicensis</i> TK18788	26.58	18.86	18.32	14.34	—						
6 <i>Chiroderma improvisum</i> TK15713	24.96	18.47	17.55	14.85	16.23	—					
7 <i>Vampyroides caraccioli</i> TK25083	27.19	20.34	17.42	16.75	16.56	13.86	—				
8 <i>Platyrrhinus helleri</i> TK17627	26.16	17.84	17.53	16.20	16.78	13.36	13.24	—			
9 <i>Uroderma bilobatum</i> TK25256	27.06	19.77	19.71	18.32	18.06	15.09	16.22	11.92	—		
10 <i>Mesophylla macconnelli</i> TK55316 Peru	26.26	19.05	18.26	17.09	17.52	14.74	14.85	16.77	16.11	—	
11 <i>M. macconnelli</i> TK14583 Bolivia	25.88	19.02	18.29	16.96	17.75	14.41	14.63	16.27	16.14	0.44	—
12 <i>M. macconnelli</i> TK14598 Bolivia	26.26	18.75	17.75	16.86	17.48	14.26	15.14	16.41	15.76	0.71	0.98
13 <i>M. macconnelli</i> TK14599 Bolivia	26.31	19.47	17.78	17.79	17.52	14.32	14.82	16.07	15.91	1.70	1.80
14 <i>M. macconnelli</i> TK16042 Colombia	26.00	19.02	18.02	16.86	17.48	14.51	14.88	16.54	15.88	0.18	0.44
15 <i>M. macconnelli</i> TK104209 Ecuador	26.04	19.33	17.64	17.25	17.24	14.18	14.94	15.81	16.16	1.70	1.79
16 <i>M. macconnelli</i> TK104077 Ecuador	26.20	19.23	17.54	17.42	17.15	14.35	14.85	15.98	15.94	1.61	1.70
17 <i>M. macconnelli</i> TK18786 French Guiana	26.46	19.19	17.91	17.26	17.25	14.44	15.21	16.07	16.17	1.52	1.61
18 <i>Vampyriscus brocki</i> TK11496 Guyana	28.84	19.26	20.11	17.64	16.76	15.84	16.81	16.67	17.67	15.12	14.99
19 <i>Vampyriscus bidens</i> TK11300 Suriname	25.23	18.21	18.03	15.35	18.06	13.14	14.29	14.58	16.85	15.13	15.26
20 <i>Vampyriscus bidens</i> TK55322 Peru	25.94	17.72	17.54	14.99	17.57	13.77	13.83	14.5	16.38	15.55	15.52
21 <i>Vampyriscus bidens</i> TK22607 Peru	26.37	18.07	17.27	15.22	17.64	13.39	13.90	14.84	16.45	15.26	15.13
22 <i>Vampyressa pusilla</i> TK11494 Brazil	27.52	19.17	18.40	18.38	18.51	15.82	15.84	16.71	17.97	14.88	14.91
23 <i>Vampyressa pusilla</i> TK11495 Brazil	27.93	19.34	18.37	18.49	18.35	16.06	15.30	16.82	17.95	14.72	14.75
24 <i>Vampyressa thyone</i> TK104382 Ecuador	26.49	17.79	19.02	16.62	18.24	13.59	13.44	15.29	15.46	13.78	13.56
25 <i>Vampyressa thyone</i> TK104383 Ecuador	26.63	17.92	19.16	16.49	18.37	13.71	13.56	15.17	15.59	13.91	13.68
26 <i>Vampyressa thyone</i> TK70533 Peru	26.78	17.85	19.60	16.62	18.69	13.84	13.23	15.04	15.92	13.68	13.21
27 <i>Vampyressa thyone</i> TK9023 Costa Rica	26.20	18.09	18.89	16.59	17.74	14.11	13.36	15.71	15.49	13.78	13.56
28 <i>Vampyressa thyone</i> TK9010 Costa Rica	26.06	18.09	19.03	16.53	18.01	14.11	13.36	15.45	15.44	13.99	13.76
29 <i>Vampyressa thyone</i> TK7848 Nicaragua	25.63	18.09	18.79	16.48	17.96	14.04	13.54	15.38	15.65	13.49	13.27
30 <i>Vampyressa thyone</i> TK40351 Honduras	26.13	18.01	19.11	16.79	18.41	14.02	13.85	15.81	15.96	13.92	13.70

relationship is strongly supported in the maximum parsimony analysis (Fig. 1), and receives very strong support in the Bayesian analysis. Wetterer et al. (2000) and Baker et al. (2000) did not include *V. brocki* in their studies, but Davis (1975) placed the 2 species together in subgenus *Vampyriscus*. Nowak (1999) placed *V. brocki* with *V. nymphaea* in subgenus *Metavampyressa*, with *V. bidens* being placed in *Vampyriscus*. Owen's (1988) morphological study concluded that *V. pusilla*, *V. brocki*, and *V. bidens* form a distinct clade only distantly related to the other species of *Vampyressa*. Our study contradicts Owen's (1988) results, as we find that *V. pusilla* is allied with *M. macconnelli* rather than with *V. brocki* and *V. bidens*. We have been unable to obtain samples of *V. nymphaea*; therefore, we cannot comment on its possible relationship with *V. brocki*.

Intergeneric relationships.—Several studies (Baker et al. 2000; Owen 1988; Wetterer et al. 2000) suggest that *Vampyressa* is not monophyletic. The tree of Wetterer et al. (2000) placed *V. pusilla* as sister to an *Ectophylla*–*Mesophylla* clade, with *V. nymphaea* and *V. bidens* being derived from more primitive nodes. However, this arrangement is supported by extremely low bootstrap values (23–53%) and by decay values of 1. In the analysis of Wetterer et al. (2000), the *Vampyressa*–*Ectophylla*–*Mesophylla* clade is supported by a moderate (70%) bootstrap value. However, because of the weak support for the internal nodes, the analysis of Wetterer

et al. (2000) is best understood as providing little or no resolution for the relationships among *Ectophylla*, *Mesophylla*, and the 3 *Vampyressa* species examined in their study. An analysis of *RAG2* sequences (Baker et al. 2000) provided little resolution for relationships among these taxa, but did provide moderate support for *V. thyone* as sister to *Mesophylla*, with *V. bidens* being derived from an earlier branch.

The Bayesian analysis of the *Cytb* data discounts the possibility of a monophyletic *Vampyressa* that excludes *M. macconnelli*. The maximum parsimony analysis also suggests a relationship of *Mesophylla*, *V. pusilla*, and *V. thyone*, and when data are weighted by codon position, the unconstrained tree is significantly better than the tree constrained for a monophyletic *Vampyressa* that excludes *M. macconnelli*.

Our analyses support the following 5 major clades in the ingroup: *Platyrrhinus*–*Uroderma*; *Vampyroides*; *Chiroderma*; *V. pusilla*–*V. thyone*–*M. macconnelli*; and *V. brocki*–*V. bidens*. However, the relationship among these clades remains largely unresolved, as has been the case in recent morphological (Wetterer et al. 2000) and molecular (Baker et al. 2000) studies. The maximum parsimony (Fig. 1), Bayesian (Fig. 2), and neighbor-joining (Fig. 3) analyses all differ in the relationship of *V. brocki* and *V. bidens* to the remaining *Vampyressa*. None of the analyses of *Cytb* data indicate that *V. bidens* and *V. brocki* are monophyletic with *M. macconnelli*, *V. thyone*, and *V. pusilla*, although it takes only 1 additional step to move

TABLE 1.—Extended.

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
—																	
1.52	—																
0.53	1.70	—															
1.51	0.53	1.70	—														
1.43	0.44	1.61	0.44	—													
1.34	0.53	1.52	0.53	0.44	—												
14.89	15.14	15.10	15.40	15.29	15.14	—											
15.10	14.67	15.10	14.41	14.57	14.41	12.96	—										
15.52	15.19	15.52	14.92	15.09	14.67	12.59	1.34	—									
15.23	15.06	15.23	14.79	14.96	14.54	12.71	1.25	0.98									
14.65	14.17	14.65	14.16	14.32	14.17	17.11	16.14	15.42	16.53	—							
14.50	14.02	14.50	14.01	13.92	14.02	17.35	16.38	15.65	16.77	0.89	—						
13.31	13.33	13.56	13.58	13.48	13.58	15.44	15.59	14.87	15.46	11.77	11.52	—					
13.43	13.46	13.68	13.70	13.61	13.70	15.57	15.47	14.75	15.34	11.89	11.64	0.09	—				
13.21	13.23	13.46	13.48	13.38	13.48	15.80	16.16	15.44	16.03	11.66	11.41	1.07	1.15	—			
13.31	13.34	13.56	13.33	13.24	13.58	15.91	15.49	14.77	15.36	11.32	10.84	2.06	2.15	2.43	—		
13.51	13.54	13.76	13.53	13.68	13.78	15.66	15.59	14.88	15.47	11.19	10.74	1.88	1.97	2.24	0.35	—	
13.03	13.05	13.27	13.05	13.19	13.29	16.14	15.42	14.71	15.29	11.83	11.58	1.88	1.97	2.43	1.06	0.88	—
13.46	13.48	13.70	13.47	13.62	13.72	16.33	15.60	14.89	15.48	12.25	12.00	2.06	2.15	2.61	1.24	1.06	0.53

V. bidens and *V. brocki* into a clade with the other *Vampyressa*. Previous studies (Baker et al. 2000; Wetterer et al. 2000) also differ in the exact arrangement, but all agree that *V. bidens* and *V. pusilla* derive from separate clades.

Two ideas emerge as a consensus from these studies. First, the difficulty in resolving the relationships among the 5 major clades suggests that these lineages may have differentiated within a relatively short time. Second, no conclusive evidence is available from any analysis linking *V. bidens* and *V. brocki* as sister taxa to *V. pusilla*, *V. thyone*, or *M. macconnelli*. On the other hand, our data do not rule out that relationship. However, based on our analysis, we recognize *Mesophylla* as a valid genus, and raise *Vampyriscus* to the genus level with *Vampyriscus bidens* and *Vampyriscus brocki* as members of the genus. Our *Cytb* analysis did not include data from *V. melissa* or *V. nymphaea*, so we cannot shed any additional light on their phylogeny. However, we follow previous classifications (Davis 1975; Nowak 1999) in placing *V. melissa* in *Vampyressa* and *V. nymphaea* in *Vampyriscus*.

Species content and phylogeography of Vampyressa.—Lim et al. (2003) recently recognized *V. thyone* as a species distinct from *V. pusilla*. Our study includes 2 of the same Brazilian specimens of *V. pusilla* that were examined by Lim et al. (2003). The Brazilian specimens have a Tamura–Nei distance from each other of only 0.89%, whereas they differ

from *V. thyone* by a mean of 11.55% (Table 1). This level of genetic distance in *Cytb* invariably indicates species-level divergence among mammals (Bradley and Baker 2001). Therefore, examination of the *Cytb* data indicates that the Brazilian specimens represent a distinct species of *Vampyressa*.

Our sample of *V. thyone* is divided by neighbor-joining and maximum parsimony analyses into distinct South American and Central American clades. However, the Bayesian analysis provides weak support for the Costa Rican specimens as sister to the remaining bats from South and Central America. The mean Tamura–Nei genetic distance (2.15%; Table 1) between the South and Central American clades is within the range typical of within-population variation, although a distance of 2.15% is at the low end of the range where speciation is known to occur (Bradley and Baker 2001). More study is warranted to determine if speciation has occurred between these 2 clades.

Phylogeography of Mesophylla macconnelli.—Specimens of *M. macconnelli* are divided in all analyses (Figs. 1–3) into 2 distinct groups. Mean Tamura–Nei genetic distance between these groups is 1.51%, whereas mean distances within the groups is 0.46% (Table 1). Three specimens collected from a single locality in Bolivia include 1 (TK14599) that joins specimens from French Guiana and Ecuador, and 2 that join specimens from Peru and Colombia. The fact that specimens from a single locality are found in 2 distinct lineages might

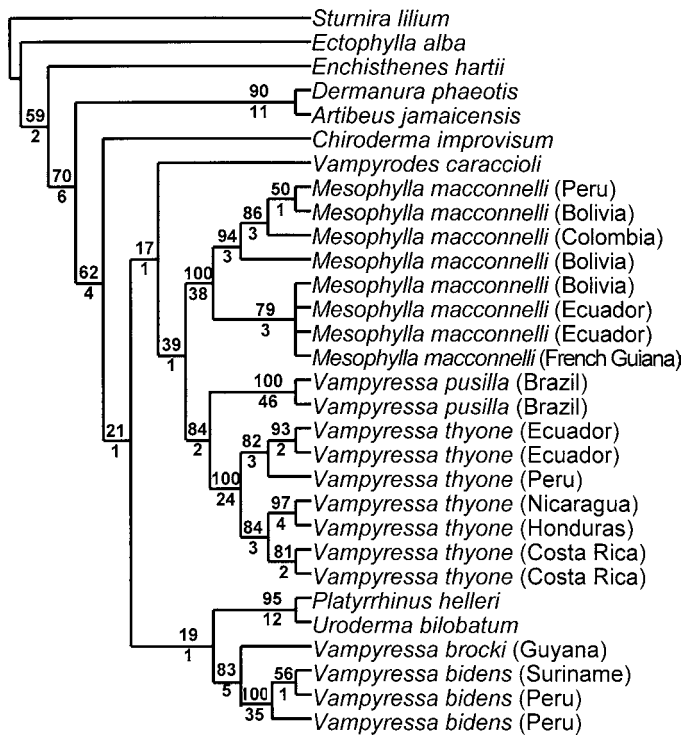


FIG. 1.—Strict consensus of 5 most-parsimonious trees based on analysis of cytochrome-*b* gene sequences. Numbers above each node represent bootstrap percentages; numbers below the nodes represent Bremer support values.

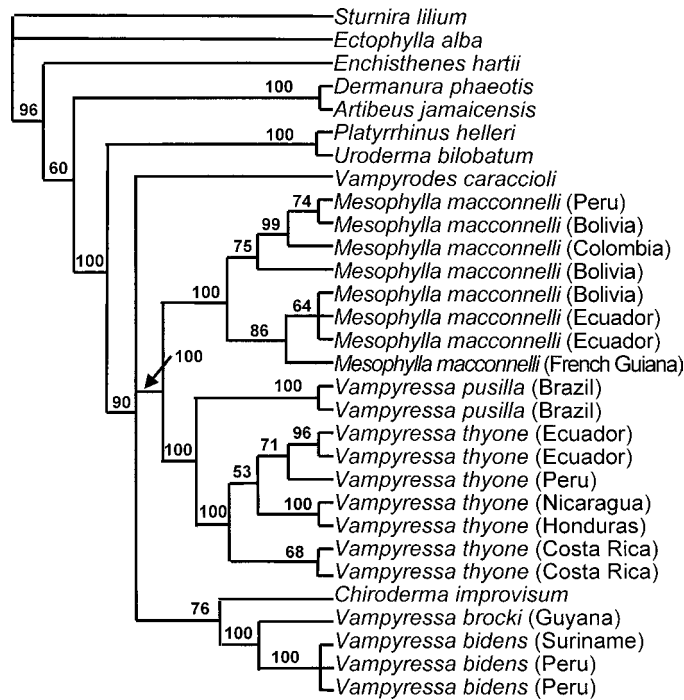


FIG. 2.—Tree based on Bayesian analysis of cytochrome-*b* gene sequences. Numbers at each node represent percentage of trees supporting the node.

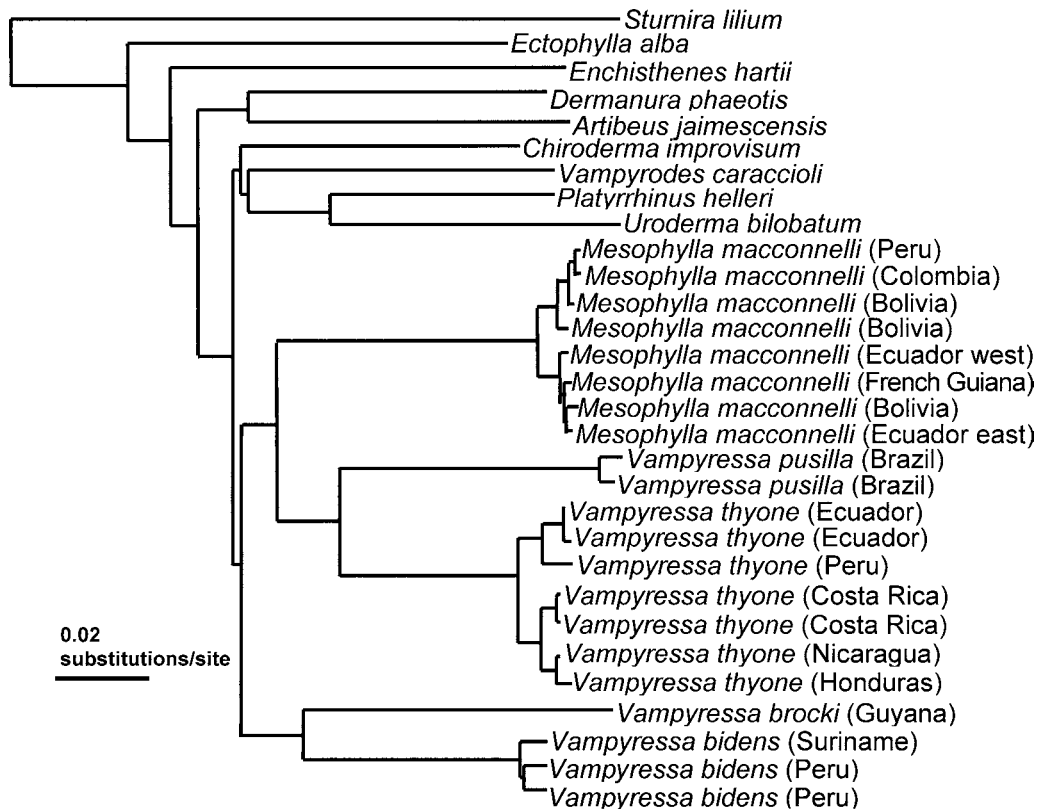


FIG. 3.—Neighbor-joining tree based on analysis of Tamura-Nei genetic distances.

suggest that the lineages represent separate species. However, the genetic distance (1.51%) between the 2 groups is within the range typical of intrapopulation variation (Bradley and Baker 2001). The genetic variation within our samples of *M. macconnelli* probably does not reflect any noteworthy geographic differentiation within the species.

RESUMEN

El género de murciélagos filostómidos *Vampyressa* ha sido generalmente agrupado junto a los géneros *Chiroderma*, *Platyrrhinus*, *Vampyrodes*, y *Mesophylla*. Sin embargo, las relaciones filogenéticas entre estos géneros han sido difíciles de resolver, y hay varios estudios que sugieren que *Vampyressa* no es un grupo monofilético. El género *Mesophylla* incluye a una sola especie, incluida en *Vampyressa* por algunos taxónomos, mientras que otros la colocan en el más distante género *Ectophylla*. El análisis de las secuencias del gen del citocromo-*b* mitocondrial apoyan fuertemente una relación cercana de *Mesophylla* con *Vampyressa* y no con *Ectophylla*. El citocromo-*b* indica que *V. pusilla* y *Mesophylla* son miembros de un linaje distinto al que agrupa a *V. bidens* junto a *V. brocki*. Basados en estos resultados proponemos el reconocimiento del género *Vampyriscus*, donde se incluye a *V. brocki* y a *V. bidens*. Los especímenes de *V. pusilla* provenientes de Brasil son claramente divergentes de los representantes del resto de América Latina. En este caso, el valor de distancia genética es comparable a análisis entre distintas especies dentro de estos 2 grupos, y apoya el reconocimiento de *V. thyone* para las poblaciones del norte.

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LITERATURE CITED

- BAKER, R. J., C. A. PORTER, J. C. PATTON, AND R. A. VAN DEN BUSSCHE. 2000. Systematics of bats of the family Phyllostomidae based on RAG2 DNA sequences. Occasional Papers, Museum of Texas Tech University 202:i + 1–16.
- BAKER, R. J., V. A. TADDEI, J. L. HUDGEONS, AND R. A. VAN DEN BUSSCHE. 1994. Systematic relationships within *Chiroderma* (Chiroptera: Phyllostomidae) based on cytochrome-*b* sequence variation. Journal of Mammalogy 75:321–327.
- BRADLEY, R. D., AND R. J. BAKER. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.
- BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803.
- DAVIS, W. B. 1975. Individual and sexual variation in *Vampyressa bidens*. Journal of Mammalogy 56:262–265.
- ERIKSSON, T. 1997. Autodecay, version 3.03. Botaniska Institutionen, Stockholm University, Stockholm, Sweden.
- GOODWIN, G. G., AND A. M. GREENHALL. 1962. Two new bats from Trinidad, with comments on the status of the genus *Mesophylla*. American Museum Novitates 2080:1–18.
- GREENBAUM, I. F., R. J. BAKER, AND D. E. WILSON. 1975. Evolutionary implications of the karyotypes of the stenodermine genera *Ardops*, *Ariteus*, *Phyllops*, and *Ectophylla*. Bulletin of the Southern California Academy of Sciences 74:156–159.
- HOFFMANN, F. G., AND R. J. BAKER. 2001. Systematics of bats of the genus *Glossophaga* (Chiroptera: Phyllostomidae) and phylogeography in *G. soricina* based on the cytochrome-*b* gene. Journal of Mammalogy 82:1092–1101.
- HUELSENBECK, J. B., AND F. R. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.
- IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome-*b* gene of mammals. Journal of Molecular Evolution 32:128–144.
- KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29:170–179.
- LAURIE, E. M. O. 1955. Notes on some mammals from Ecuador. Annals and Magazine of Natural History, Series 12 8:268–276.
- LEWIS-ORITTI, N., C. A. PORTER, AND R. J. BAKER. 2001a. Molecular systematics of the family Mormoopidae (Chiroptera) based on cytochrome *b* and recombination activating gene 2 sequences. Molecular Phylogenetics and Evolution 20:426–436.
- LEWIS-ORITTI, N., R. A. VAN DEN BUSSCHE, AND R. J. BAKER. 2001b. Molecular evidence for evolution of piscivory in *Noctilio* (Chiroptera: Noctilionidae). Journal of Mammalogy 82:748–759.
- LIM, B. K. 1993. Cladistic reappraisal of neotropical stenodermatine bat phylogeny. Cladistics 9:147–165.
- LIM, B. K., W. A. PEDRO, AND F. C. PASSOS. 2003. Differentiation and species status of the neotropical yellow-eared bats *Vampyressa pusilla* and *V. thyone* (Phyllostomidae) with a molecular phylogeny and review of the genus. Acta Chiropterologica 5:15–29.
- LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. Occasional Papers, Museum of Texas Tech University 163:1–3.
- MAU, B., AND M. NEWTON. 1997. Phylogenetic inference for binary data on dendrograms using Markov chain Monte Carlo. Journal of Computational and Graphical Statistics 6:122–131.
- MAU, B., M. NEWTON, AND B. LARGET. 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. Biometrics 55:1–12.
- NOWAK, R. M. 1999. Walker’s mammals of the world. 6th ed. Johns Hopkins University Press, Baltimore, Maryland.
- OWEN, R. D. 1988. Phenetic analyses of the bat subfamily Stenodermatinae (Chiroptera: Phyllostomidae). Journal of Mammalogy 69:795–810.
- RANNALA, B., AND Z. H. YANG. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43:304–311.
- STARRETT, A., AND R. S. CASEBEER. 1968. Records of bats from Costa Rica. Contributions in Science, Los Angeles County Museum 148:1–21.
- SWOFFORD, D. L. 2001. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinaur Associates Inc., Publishers, Sunderland, Massachusetts.
- TAMURA, K., AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA

- in humans and chimpanzees. *Molecular Biology and Evolution* 10:512–526.
- VAN DEN BUSSCHE, R. A., AND R. J. BAKER. 1993. Molecular phylogenetics of the New World bat genus *Phyllostomus* based on cytochrome-*b* DNA sequence variation. *Journal of Mammalogy* 74:793–802.
- VAN DEN BUSSCHE, R. A., J. L. HUDGEONS, AND R. J. BAKER. 1998. Phylogenetic accuracy, stability, and congruence: relationships within and among the New World bat genera *Artibeus*, *Dermanura*, and *Koopmania*. Pp. 59–71 in *Bat biology and conservation* (T. H. Kunz and P. A. Racey, eds.) Smithsonian Institution Press, Washington, D.C.
- WETTERER, A. L., M. V. ROCKMAN, AND N. B. SIMMONS. 2000. Phylogeny of phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. *Bulletin of the American Museum of Natural History* 248: 1–200.
- WRIGHT, A. J., R. A. VAN DEN BUSSCHE, B. K. LIM, M. D. ENGSTROM, AND R. J. BAKER. 1999. Systematics of the genera *Carollia* and *Rhinophylla* based on the cytochrome-*b* gene. *Journal of Mammalogy* 80:1202–1213.

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APPENDIX I

Specimens examined.—Voucher specimens are deposited in the following institutions: American Museum of Natural History (AMNH); Carnegie Museum of Natural History (CM); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM); Royal Ontario Museum (ROM); Texas Tech University (TTU); United States National Museum, Smithsonian Institution (USNM). Specimens identified only with a museum acronym have not yet been cataloged or the catalog number is unknown. Each specimen is identified both by a Texas Tech University tissue (TK) number and by the museum acronym and catalogue number. Numbers separated by commas refer to the same specimen. Asterisks indicate specimens for which sequence data were taken from GenBank.

Artibeus jamaicensis.—FRENCH GUIANA. Paracau, near Sinnamary (TK18788, AMNH267202*).

Chiroderma improvisum.—MONTSERRAT. St. Anthony: 0.5 mi (0.8 km) above mouth Belham River (TK15713, TTU31403*).

Dermanura phaeotis.—ECUADOR. Esmeraldas Province: La Chiquita Experimental Station, S of San Lorenzo, UTM 748935E 0136902N (TK104501, TTU85273*).

Ectophylla alba.—COSTA RICA. Cano Palma Biological Station, 7 km NNW Tortuguero (TK16395, ROM108296).

Enchisthenes hartii.—PERU. Huanuco: 11 km N, 6 km S Tingo Maria, 9°55'N, 55°11'W (TK22690; CM98710*).

Mesophylla macconnelli.—BOLIVIA. La Paz: 1 mi (1.6 km) W Puerto Linares (TK14583, TTU34881; TK14598, TTU34883; TK14599, TTU23884). COLOMBIA. Meta, Villavicencio, El Hachon, Centro Agrícola SENA (TK16042). ECUADOR. Tungurahua Province: La Estancia, UTM 809292E 983999N (TK104209, TTU84981). Pastaza District Puyo, Finca El Pigual, UTM 0166599E 9836288N (TK104077, TTU84849). FRENCH GUIANA. Paracau, near Sinnamary (TK18786, AMNH267281). PERU. Cusco: La Convencion, Camisea, Pagorini (TK55316, USNM).

Platyrrhinus helleri.—SURINAME. Marowijne: Perica (TK17627, CM77660*).

Sturnira lilium.—PERU. Huanuco Dept.: Leoncia Prado, 1 km S Tingo Maria (TK22631, TTU46161*).

Uroderma bilobatum.—TRINIDAD AND TOBAGO. Trinidad: St. George: Simla Research Center, 4 mi (6.4 km) N Arima (TK25256, TTU44100*).

Vampyressa bidens.—PERU. (TK22607). Cusco: La Convencion, Camisea, Pagorini (TK55322, MUSM). SURINAME. Brokopondo: 1.5 km W Rudi, Kappelvliedveld (TK11300, CM63869).

Vampyressa brocki.—GUYANA. Potaro-Siparuni: Three Mile Camp, Iwokrama Forest (TK11496, ROM112094).

Vampyressa pusilla.—BRAZIL. São Paulo: Caetetus Ecological Station (TK11494, ROM111071; TK11495, ROM111072).

Vampyressa thyone.—COSTA RICA. Puntarenas, 2.1 mi S, 1.1 mi E (3.4 km S, 1.8 km E) San Vito, Las Cruces Tropical Botanical Garden (TK9023, TTU34411; TK9010, TTU34398). ECUADOR. Pastaza Dist. UTM 830633E 9830709N (TK104382, TTU85154). Madre Tierra UTM 83135E 9828271N (TK104383, TTU85153). HONDURAS. Atlántida: Lancitilla (TK40351, TTU 61144). NICARAGUA. Zelaya: 3.0 km NW Rama (TK7848, TTU30639). PERU. Cusco: La Convencion, Camisea, Pagorini (TK70533, MUSM).

Vampyroides caraccioli.—TRINIDAD AND TOBAGO. Trinidad: St. George: Simla Research Center, 4 mi (6.4 km) N Arima (TK25083, CM94707).