SYSTEMATICS OF THE GENERA *CAROLLIA* AND *RHINOPHYLLA* BASED ON THE CYTOCHROME-*B* GENE

Amanda J. Wright, Ronald A. Van Den Bussche, Burton K. Lim, Mark D. Engstrom, and Robert J. Baker

Department of Biology and the Museum, Texas Tech University, Lubbock, TX 79409 (AJW, RJB)
Department of Zoology, 430 Life Sciences West, Oklahoma State University, Stillwater, OK 74078 (RAVDB)
Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, Canada M5S 2C6 (BKL, MDE)
Present address of AJW: Department of Cell and Molecular Biology, Harvard University, Cambridge, MA 02138

Intrageneric relationships within *Carollia* and *Rhinophylla* (Phyllostomidae) are examined using the 1,140 base pairs of the mitochondrial cytochrome-*b* gene. We also examined the proposed sister relationship of *Carollia* and *Rhinophylla* by comparing the cytochrome-*b* gene from those two taxa to that of representatives of *Phyllonycteris, Lonchophylla, Uroderma, Artibeus, Dermanura, Enchisthenes,* and *Chiroderma*. Within *Carollia*, representatives of *C. brevicauda* and *C. perspicillata* are most closely related, whereas *C. subrufa* is more closely related to these two species than is *C. castanea*, which forms the basal divergence for the genus. Within *Rhinophylla*, data for relationships of species are equivocal and suggest a concomitant divergence for all three species. If the rate of evolution is the same for the cytochrome-*b* gene in the two genera, then species within *Rhinophylla* diverged earlier than did any of the species of *Carollia*. If those two genera shared a common ancestor to the exclusion of the remainder of the Phyllostomidae, common ancestry was of short duration. The alternative hypothesis, that *Carollia* and *Rhinophylla* are not sister taxa and shared morphological characteristics are convergent, remains viable based on our data.

Key words: *Carollia, Rhinophylla,* Phyllostomidae, cytochrome-*b*, mitochondrial DNA

This study is part of an effort to better understand systematic relationships among the diverse species of New World leaf-nosed bats, family Phyllostomidae. We investigated intrageneric and intergeneric relationships of *Carollia* and *Rhinophylla*. The four recognized species of *Carollia* are *C. brevicauda, C. castanea, C. subrufa,* and *C. perspicillata,* and the three recognized species of *Rhinophylla* are *R. pumilio, R. fischerae,* and *R. alethina*. Traditionally, *Carollia* and *Rhinophylla* were recognized as sister-taxa comprising the subfamily Carolliinae (Miller, 1907). More recently, Baker et al. (1989) placed *Carollia* and *Rhinophylla* in the tribe Stenodermatini, primarily on the basis of morphology of the female reproductive tract (Hood and Smith, 1982) and immunology (Honeycutt and Sarich, 1987). Analysis of mapped mtDNA restriction sites (Lim and Engstrom, 1998) failed to support the monophyly of the *Rhinophylla*–*Carollia* clade. Additionally, G-band studies of chromosomes did not provide support for this relationship (Baker et al., 1987), primarily because the karyotype of *Carollia* is so radically reorganized that it is difficult to identify any chromosomal rearrangements documenting relationships of this taxon to other genera of Stenodermatini or to other members of the family Phyllostomidae. The supposed sister-taxon relationship of these two genera is based primarily on tooth morphology, and

evidence supporting this proposed common ancestry from molecular data would strengthen confidence in this proposed clade.

Relationships of species within the genera *Carollia* and *Rhinophylla* also are unresolved. Although *Carollia* is one of the best studied genera of bats, there is little congruence among the phylogenetic hypotheses generated from morphology, karyology, and restriction-enzyme data concerning relationships of bats within the genus. Furthermore, because there are few specimens of *Rhinophylla alethina*, it has been included in few systematic studies.

To estimate systematic relationships of *Carollia* and *Rhinophylla*, we sequenced the mitochondrial cytochrome-*b* gene. This gene has proven useful in deriving phylogenetic hypotheses of relationships of species within other genera such as *Artibeus*, *Dermanura*, *Phyllostomus*, and *Chiroderma* (Baker et al., 1994; Van Den Bussche and Baker, 1993; Van Den Bussche et al., 1993, 1998). A robust phylogenetic hypothesis could be used to analyze in greater detail the wealth of ecological, behavioral, and biogeographical data that exists for *Carollia* (Fleming, 1988; Lim and Engstrom, 1998; Pine, 1972), provide insight into the placement of *R. alethina* within *Rhinophylla*, and provide additional information concerning validity of the proposed sister-taxon relationship between *Carollia* and *Rhinophylla*.

Materials and Methods

DNA was extracted from heart, liver, kidney, or muscle tissue that had been frozen (Engstrom et al., 1999) or stored in lysis buffer following standard protocol (Longmire et al., 1997). The entire 1,140 base pair (bp) cytochrome-*b* gene was amplified via the polymerase chain reaction (Saiki et al., 1986, 1988) using primers located in the tRNA for threonine (H15915R—Irwin et al., 1991) and glutamic acid (MVZ05—Smith and Patton, 1991). Conditions for amplification and sequencing are described in Baker et al. (1994). PCR products were ligated and cloned using either p-GEM (Promega Corporation, Madison, WI) or TA cloning kits (Invitrogen, Inc., Carlsbad, CA). Double-stranded sequencing (Sanger et al., 1977) using the amplifying primers, as well as various internal primers (H15149; MVZ04—Smith and Patton, 1991; L15162, L15513—Irwin et. al., 1991; H15767, L15424), and a primer specific to *Carollia* and *Rhinophylla* (5′ RAAYGGRATTATRTCHGARTCYGATGG 3′) resulted in the sequence of the entire gene. For each specimen, a minimum of two clones was sequenced. TK numbers identify DNA samples from the frozen-tissue collection at Texas Tech University, whereas FN numbers identify DNA samples from the Royal Ontario Museum. In this list of specimens examined, geographic localities follow tissue-sample numbers, and gender is included in parentheses: *Carollia brevicauda*—TK 46009 (F), 46010 (F), Peru, Loreto, Quebrada Aguas Negras, Cocha Zoraida; TK 70412 (M), Peru, Cuzco, Convencion, Camisea, Armihuari; FN 31805 (F), Guatemala, El Petén, Poptún; *Carollia castanea*—TK 70672 (M), Peru, Cuzco, Convencion, Camisea, Armihuari; FN 38156 (M), Panama, Chiriqui, Ojo de Agua, 2 km N of Santa Clara; *Carollia perspicillata*—TK 17466 (F), Surinam, Nickerie, Kabalebo; TK 74345 (M), Peru, Cuzco, Convencion, Camisea, Armihuari; *Carollia subrufa*—TK 19550, (M), 19551, (F), Mexico, Jalisco, 22 km S Chamela; TK 15818 (F), El Salvador, Ahuachapan, El Refugio; *Lonchophylla thomasi*—TK 17177 (M), Surinam, Saramacca, Voltzberg; *Phyllonycteris aphylla*—TK 9280 (M), Jamaica, Westmoreland Parish, Bluefields; *Rhinophylla alethina*—FN 40073 (M), 40093 (M), Ecuador, Esmeraldas, Alto Tambo, 2 km S Alto Tambo; *Rhinophylla fischerae*—TK 46034 (M), Peru, Loreto, Quebrada Aquas Negras, Cocha Zoraida; *Rhinophylla pumilio*—TK 18825 (M), French Guiana, Paracou, Sinnamary; TK 46001 (M), 46026 (F), Peru, Loreto, Quebrada Aquas Negras, Cocha Zoraida; *Sturnira lilium*—TK 22651, Peru, Huanuco, Leoncia Prado, 1 km S Tingo Maria. Sequences are deposited in Genbank under accession numbers AF187017-AF187035. Other cytochrome-*b* sequences used in this study came from Genbank; *Uroderma bilobatum*—L28941, *Artibeus jamaicensis*—U66503, *Dermanura watsoni*—U66576, *Koopmania concolor*—L19515, *Enchisthenes hartii*—L19514, *Chiroderma villosum*—L28943.

The complete cytochrome-*b* sequence was aligned using MacVector (Eastman Kodak Company, Rochester, NY) or Sequencher (Gene Codes Corporation), and entered into MacClade

3.01 (Maddison and Maddison, 1992). Quantitative pairwise comparisons among all taxa were made for the cytochrome-*b* gene. Those comparisons included transition and transversion substitutions for the first, second, and third codon positions, and percentage of sequence divergence. Sequence-divergence values were corrected for multiple substitutions using the two-parameter model of Kimura (1980). To test if rates of nucleotide substitution of the cytochrome-*b* gene differed among stenodermatines and, specifically, within *Carollia* or *Rhinophylla* relative to the outgroups (*Lonchophylla* and *Phyllonycteris*), we used the relative rates test (Sarich and Wilson, 1967) and compared those values to a binomial distribution (Allard and Honeycutt, 1992; Mindell and Honeycutt, 1990). Using MacClade, nucleotide sequences were translated into amino acid residues, and a step matrix for protoparsimony analysis was created. Trees based on parsimony were constructed using test PAUP*4 (Swofford, 1998) using DNA sequence and amino acid residues as discrete, unordered characters. In addition to the protoparsimony analysis, phylogenetic relationships were evaluated by coding DNA sequences as discrete, unordered characters, employing various search algorithms (heuristic, branch-and-bound, and exhaustive, depending upon size of the dataset). Because several studies have shown that transitions and changes at third-codon positions tend to accumulate rapidly relative to transversions and changes at first- and second-codon positions (Brown et al., 1982; Honeycutt and Wheeler, 1990; Irwin et al., 1991), several approaches to character-weighting were used in addition to weighting all substitutions equally. Those approaches included first and second codon positions only, transversion parsimony analysis (weighting transversions over transitions by 1:0; 2:1; 3:1; 5:1; 10:1), and applying those weights in addition to weights of 2:1; 3:1, and 5:1 for first-and second-codon positions over third-codon positions.

To evaluate which datasets under the various weighting schemes contained significant levels of phylogenetic structure, distribution of 10,000 randomly generated or all possible trees was examined with the g_1 statistic (Hillis, 1991; Huelsenbeck, 1991) with the resulting g_1-values being compared with critical values (Hillis and Huelsenbeck, 1992). All datasets that contained significantly greater levels of phylogenetic signal than noise were used in subsequent phylogenetic analyses. The confidence or accuracy of each clade was evaluated through either heuristic or branch-and-bound bootstrap analysis with 500 iterations. To evaluate further the robustness of clades on the most parsimonious tree(s), a "Bremer support" analysis was performed (Bremer, 1988; Donoghue et al., 1992; Eernisse and Kluge, 1993; Kallersjo et al., 1992; Lundrigan and Tucker, 1994; Van Den Bussche et al., 1998).

Outgroups that provided polarity for character-state changes in the various analyses were selected on the basis of previous systematic studies. *Lonchophylla* and *Phyllonycteris* were selected as outgroups for analyses concerning placement of *Carollia* and *Rhinophylla* in the tribe Stenodermatini, whereas *Chiroderma* and *Rhinophylla* served as outgroups for analyses concerning relationships within *Carollia*, and *Chiroderma* and *Carollia* served as outgroups for analyses of *Rhinophylla*.

RESULTS

Description of data.—Mean percentage of sequence divergence for all pairwise comparisons corrected for multiple substitutions revealed a wide range of nucleotide divergence values among representatives of stenodermatine genera ranging from 11.9% between *Artibeus* and *Koopmania* to 27.7% between *Sturnira* and *Rhinophylla* (Table 1). Mean sequence divergence within *Carollia* was 8.4%, with the greatest value of 14.2% between *C. castanea* and *C. brevicauda* and the lowest value of 4.1% between *C. perspicillata* and *C. brevicauda*. Mean percentage of sequence divergence within *Rhinophylla* was 13.9%, with the greatest value of 19.8% between *R. alethina* and *R. pumilio* and the lowest value of 16.1% between *R. fischerae* and *R. pumilio* (Table 1). Using *Lonchophylla* and *Phyllonycteris* as outgroups, tests for rate heterogeneity using pairwise comparisons of all taxa of Stenodermatini examined in this study were not significant at the 0.05 level. Six hundred forty-four bp (56.5%) of the 1,140 bp cytochrome-*b* gene were identical among all taxa examined. In *Carollia* and

the outgroups (*Rhinophylla pumilio* and *Chiroderma*), 798 bp (70.0%) were identical, and in *Rhinophylla* and the outgroups (*Carollia subrufa* and *Chiroderma*), 784 bp (68.8%) were identical. Among all taxa examined, 496 positions were variable. Among those, 102 were autapomorphous leaving 394 positions as potentially phylogenetically informative. Within species of *Carollia*, of the 342 variable positions, 119 were autapomorphic, leaving 223 positions as potentially phylogenetically informative. Of the 356 variable positions within *Rhinophylla*, 101 were autapomorphic, leaving 255 positions as potentially phylogenetically informative.

The spatial distribution of transitions and transversions resulting from a parsimony analysis of stenodermatines was as expected for a protein-coding gene. Overall, 69.3% of substitutions were transitions, and 30.7% were transversions (Table 2). In *Carollia* 66.7% were transitions and 33.3% were transversions, and within *Rhinophylla*, 67.3% were transitions and 32.7% were transversions. The distribution of transitions and transversions resulting from parsimony analysis as a function of codon position among all taxa of Stenodermatini examined, among *Carollia* and among *Rhinophylla* are given in Table 2.

Construction of trees for all taxa.—The topology in Fig. 1 is the result of an heuristic search using all variable positions but weighting the first- and second-codon positions three times that applied to third-codon positions and weighting transversion substitutions three times that of transition substitutions. This search resulted in a single most parsimonious tree of 3,312 steps and a highly significant g_1-statistic from 10,000 randomly drawn trees ($g_1 = -0.588$). Numbers in boxes along the internal lineages (Fig. 1) reflect the percentage of 500 bootstrap iterations that were detected for each clade with the branch-and-bound option (number in the upper portion of each box) and the number of additional steps from the most parsimonious tree required for a particular clade to collapse (Bremer support analysis). All other weighting schemes resulted in similar topologies concerning relationships of *Carollia*, *Rhinophylla*, and other genera of Stenodermatini. Difference among most parsimonious trees from the analyses with differential weights assigned to various types of character-state changes and codon positions concerned the placement of *Enchisthenes* and *Sturnira* and the level of resolution provided to terminal taxa.

Maximum-parsimony analysis (using all nucleotide substitutions with equal weight) to evaluate phylogenetic relationships within *Carollia* resulted in a single most parsimonious tree of 611 steps and a highly significant g_1-statistic of -0.932 ($P < 0.01$—Hillis and Huelsenbeck, 1992). The topology of that tree was identical to that presented for *Carollia* (Fig. 1). The single most parsimonious tree using only species of *Carollia*, plus *Rhinophylla* and *Chiroderma* as outgroups, had the same branching order as that presented in Fig. 1, but values for bootstrap and Bremer support analyses varied. Differential weighting schemes resulted in greater levels of support (compared with equal weighting) for each clade depicted in Fig. 1.

Maximum-parsimony analysis with equal weight applied to all codon positions and all substitutions resulted in a single most parsimonious tree of 583 steps and a highly significant g_1-statistic of -0.956 ($P < 0.01$—Hillis and Huelsenbeck, 1992). The topology of that tree was identical to that depicted in Fig. 1. Additionally, as in *Carollia*, the only differences between the single most parsimonious tree resulting from an exhaustive search of all species of *Rhinophylla* and the outgroups (*Chiroderma* and *Carollia*), as compared with that shown in Fig. 1, were slightly lower bootstrap and Bremer support values based on equal weights.

Discussion

Previous studies using the cytochrome-*b* gene to resolve systematic relationships

TABLE 1.—*Percentage of sequence divergence for all pairwise comparisons corrected for multiple substitution using the two-parameter model of Kimura (1980). C =* Carollia *and R. =* Rhinophylla.

	C. brevicauda	*C. brevicauda*	*C. brevicauda*	*C. brevicauda*	*C. castanea*	*C. castanea*	*C. subrufa*	*C. subrufa*	*C. perspicillata*	*C. perspicillata*	*R. alethina*
C. brevicauda											
C. brevicauda	5.0										
C. brevicauda	4.7	1.3	—								
C. brevicauda	4.9	1.0	1.2	—							
C. castanea	13.3	13.6	13.4	13.9	—						
C. castanea	12.8	13.4	13.5	14.2	8.7	—					
C. subrufa	7.4	7.7	7.9	8.1	13.8	13.8	—				
C. subrufa	7.1	7.6	7.9	8.2	13.4	13.3	1.7	—			
C. perspicillata	4.8	4.3	4.2	4.3	14.7	12.8	8.0	8.6	—		
C. perspicillata	4.4	4.2	4.1	4.1	13.8	12.8	8.4	8.2	2.3	—	
R. alethina	20.8	20.4	21.3	20.9	21.2	20.4	22.5	22.9	21.0	20.4	
R. alethina	20.2	20.0	20.9	20.5	20.6	20.3	22.1	22.5	20.7	20.6	1.1
R. pumilio	19.1	18.6	19.6	19.1	22.6	21.5	20.3	20.4	19.8	19.9	19.8
R. pumilio	19.9	19.3	20.1	19.7	22.3	21.6	21.2	21.4	20.5	20.1	19.1
R. pumilio	20.0	19.5	20.2	19.7	22.2	21.5	21.1	21.5	20.5	19.9	18.8
R. fischerae	20.1	19.3	20.1	19.9	22.0	20.7	21.0	20.5	20.3	20.0	17.8
Sturnira	26.4	26.7	27.2	26.7	27.1	26.6	26.0	25.9	26.3	25.6	26.4
Uroderma	20.3	20.2	20.7	20.2	22.9	20.9	21.4	21.7	21.0	20.8	23.7
Chiroderma	18.4	17.3	17.7	17.9	18.8	16.9	19.5	19.5	18.6	18.6	20.4
Artibeus	19.6	19.3	20.0	19.7	20.5	19.9	20.0	20.0	20.0	20.3	23.1
Koopmania	16.8	17.4	17.6	17.5	21.0	19.4	17.8	18.7	18.5	18.7	20.1
Dermanura	17.8	16.6	17.5	17.2	19.8	19.0	18.2	18.2	17.6	17.8	21.0
Enchisthenes	19.2	19.9	20.2	20.0	21.4	21.0	20.0	20.1	20.4	19.9	20.1
Phyllonycteris	20.9	20.2	20.6	20.6	20.5	19.9	21.6	21.2	21.7	21.3	21.6
Lonchophylla	18.8	18.8	19.7	19.5	20.4	20.3	20.2	20.7	19.6	20.0	22.8

within the Phyllostomidae indicate that the rate of nucleotide substitution in this gene is most appropriate for resolution of intrageneric relationships or relationships of closely related species. Therefore, we will examine the systematic implications to the intrageneric relationships of *Carollia* and *Rhinophylla* first.

Intrageneric relationships of Carollia.—Members of the genus *Carollia* are among the most intensively studied species of the Phyllostomidae (Fleming, 1988). Nonetheless, aspects of the systematics of this genus remain unresolved. Reasons for this lack of systematic resolution are complex and include failure to examine all four recognized species, problems with identification of species and geographical variation, or lack of resolution among the characters examined (Baker and Bleier, 1971; McLellan, 1984; Owen, 1987; Owen et al., 1984; Pine, 1972; Stock, 1975).

We found *C. perspicillata* and representatives of *C. brevicauda* from Peru as sister, with a single *C. brevicauda* from Guatemala, *C. subrufa*, and *C. castanea*, as successively more basal lineages (Fig. 1). The close genetic relationship between the Peruvian specimens of *C. brevicauda* and *C. perspicillata* is compatible with the difficulty in distinguishing cranial morphometrics between the two (McLellan, 1984). Stock's (1975) examination of C-bands also found that *C. brevicauda* and *C. perspicillata* were more similar to each other in abundance and position of heterochromatin than either is to *C. castanea*. Karyotypes of phyllostomids generally are characterized by reduced amounts of heterochromatin, and the addition of C-band-positive mate-

TABLE 1.—*Extended.*

R. alethina	*R. pumilio*	*R. pumilio*	*R. pumilio*	*R. fischerae*	*Sturnira*	*Uroderma*	*Chiroderma*	*Artibeus*	*Koopmania*	*Dermanura*	*Enchisthenes*	*Phyllonycteris*	*Lonchophylla*
19.5	—												
18.9	3.6	—											
18.7	3.5	0.6	—										
17.6	16.9	16.3	16.1	—									
27.1	27.3	27.7	27.7	26.6	—								
23.6	17.3	18.9	19.2	19.7	26.8	.							
20.0	18.6	19.3	19.6	20.0	25.3	14.6							
22.5	19.9	21.0	21.4	21.0	26.1	17.4	15.7	—					
19.8	19.9	19.9	20.0	17.8	24.9	17.6	15.0	11.9	—				
20.6	20.0	20.9	20.8	19.6	26.1	17.3	14.3	15.1	13.6	—			
20.4	21.6	21.0	21.3	22.1	26.7	18.8	17.0	17.5	15.4	17.4	—		
21.9	21.3	21.9	21.8	22.7	28.6	20.0	20.3	20.3	21.0	21.5	21.0	—	
22.7	20.6	21.1	21.3	22.1	26.4	18.9	18.4	20.4	18.9	18.8	19.8	18.9	—

TABLE 2.—*Spatial distribution of nucleotide variation based on the most-parsimonious arrangement of nucleotide substitutions on the phylogenetic tree for all taxa examined within* Carollia *and within* Rhinophylla, *reflecting the number and percentage of transitions and transversions (in parentheses) at the three codon positions.*

	First	Second	Third	Total
All taxa				
Transitions	194 (10.8)	55 (3.1)	998 (55.5)	1,247 (69.4)
Transversions	57 (3.2)	32 (1.8)	463 (25.7)	552 (30.7)
Total	251 (14.0)	87 (4.9)	1,461 (81.2)	1,799
Carollia				
Transitions	29 (20.6)	7 (5.0)	58 (41.1)	94 (66.7)
Transversions	3 (2.1)	0 (0.0)	44 (31.2)	47 (33.3)
Total	32 (22.7)	7 (5.0)	102 (72.3)	141
Rhinophylla				
Transitions	38 (12.1)	7 (2.2)	167 (53.0)	212 (67.3)
Transversions	8 (2.5)	5 (1.6)	90 (28.6)	103 (32.7)
Total	46 (14.6)	12 (3.8)	257 (81.6)	315

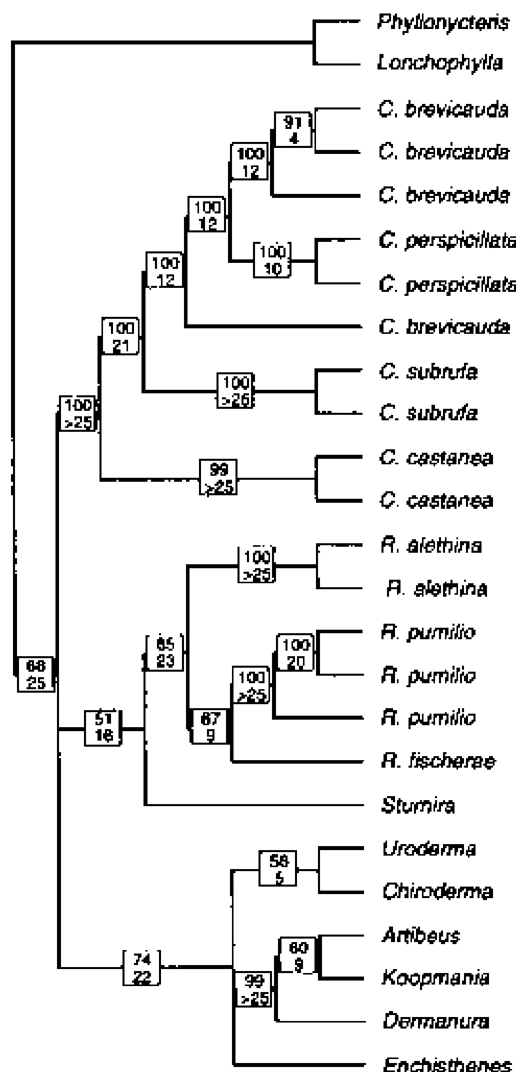

FIG. 1.—Topology of the single, most parsimonious tree of 3,312 steps from an heuristic search algorithm and 500 bootstrap iterations depicting the phylogenetic relationships among the taxa of Stenodermatini examined. This tree was constructed using the weighting scheme of 1:3 transitions : transversions and 1:3 third codon position : first or second codon position for each change in the nucleotide sequence. Numbers above each lineage indicate bootstrap values; numbers below each lineage indicate results of the Bremer decay analysis. This tree has a g_1-statistic of −0.588. C. = *Carollia*; R. = *Rhinophylla*.

rial probably represents the derived condition. Our phylogenetic hypothesis can explain the addition of heterochromatin as a single event in the common ancestor of *C. brevicauda* and *C. perspicillata*.

Methods used to set confidence levels on clades in phylogenetic trees have been the source of considerable debate (Bremer, 1988, 1994; Donoghue et al., 1992; Felsenstein, 1985; Felsenstein and Kishino, 1993; Hillis and Bull, 1993; Penny and Hendy, 1985, 1986). Although there is little agreement as to the best method to estimate the robustness of clades, the combination of bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988, 1994) has been shown to be a useful approach for examining overall stability and reliability of clades (Simmons, 1998; Van Den Bussche et al., 1998). However, care should be taken in interpreting Bremer support values applied to trees generated from DNA substitutions. When all nucleotides are weighted equally, each step indicates a change in a single base pair of the gene. However, in our weighted scheme (Fig. 1), transitions can generate a score of one (third-codon position transitions) or three (first- and second-codon position transitions), and transversions can generate a score of three (third-codon position transversions) or nine (first- and second-codon position transversions). Therefore, scores can be altered highly by the weighting schemes and may or may not be reflective of single events. For example, a score of three may be generated by three transitions at the third-codon position or a transversion at the third-codon position or a transition at the first- or second-codon positions.

Phylogenetic relationships within *Carollia*, based on DNA-sequence data of the cytochrome-*b* gene, are robust in terms of both bootstrap and Bremer support values (Fig. 1). The strongest evidence in our most parsimonious tree indicates that *C. castanea* is basal to the remainder of the species of *Carollia*. Four other clades in Fig. 1 are supported by bootstrap values of 100%, and

high Bremer support values. Relative to the currently recognized taxonomy, the distribution of specimens identified as *C. brevicauda* is problematic. The single *C. brevicauda* from northern Guatemala clearly fits within the *Carollia* clade. However, it does not group with the other *C. brevicauda* or within any of the other species of *Carollia*. High bootstrap values (100%) and moderate Bremer decay values (12 steps) are comparable with values that separate other species of *Carollia* from each other. The fact that the cytochrome-*b* gene from this individual does not cluster with any other recognized species makes it less probable that this individual is simply a case of misidentification of another species. Its position in the tree and percentage of sequence divergence may indicate an as yet unrecognized species of *Carollia* or at least significant levels of geographic variation. We interpret these results as suggesting that, as presently recognized, *C. brevicauda* consists of two biological species. However, if we are correct, to determine the range of each species will require examination of geographic variation of this gene or perhaps other characters across the recognized geographic range of *C. brevicauda*. Without this dataset, it seems inappropriate to change the existing taxonomy.

Our study is the first to evaluate intrageneric relationships of *Carollia* using DNA-sequence data. However, Lim and Engstrom (1998) also have used molecular methods (mitochondrial restriction-enzyme sites) to evaluate intrageneric relationships of *Carollia*. Mapping restriction sites provides an advantage over DNA sequencing in the feasibility of examining larger numbers of individuals to better estimate geographic and phylogenetic variation. However, the ability to detect phylogenetically informative characters is substantially reduced with restriction-site data compared with DNA sequencing. Lim and Engstrom (1998) proposed that *C. perspicillata* and *C. subrufa* are sister-taxa, that these taxa were sister to *C. brevicauda*, and that *C. castanea* represents the basal divergence. Our data are compatible with the hypothesis that *C. castanea* represents the basal lineage within *Carollia*, but not with their proposed relationships among *C. perspicillata*, *C. subrufa*, and *C. brevicauda*. Bremer support analysis indicated a low level of support for three of the six lineages reported by Lim and Engstrom (1998). The largest level of support is the branch uniting *C. brevicauda* as the sister taxon to the clade containing *C. perspicillata* and *C. subrufa*, and this clade collapses with the addition of two steps to the most parsimonious arrangement. Lim and Engstrom (1998) detected only 123 phylogenetically informative sites among 75 unique haplotypes and 145 individuals. Therefore, one possible reason for the discrepancy between the phylogenetic hypothesis of Lim and Engstrom (1998) and our study is the level of resolution provided by a reduced number of informative sites. A second explanation concerns geographic variation. Lim and Engstrom (1998) documented significant levels of geographic variation within all four species of *Carollia*. Because our study was based on a small number of individuals per species, it is possible that our study has been affected by mtDNA lineage sorting and our gene trees do not accurately represent species trees. In any case, we agree with Lim and Engstrom (1998) that relationships within *Carollia* are evolutionarily more complex than indicated by their relatively conservative morphology.

Intrageneric relationships of Rhinophylla.—Phylogenetic relationships among species of *Rhinophylla* are poorly known compared with *Carollia* because members of this genus are less well represented in museum collections and the genus is restricted to South America where there have been fewer faunal studies. *Rhinophylla pumilio* and *R. fischerae* are similar in size and appearance, whereas *R. alethina* is considerably larger, has a distinctive pelage, and relatively well-haired interfemoral membrane. All three species are morphologically dis-

tinct. Analysis of cytochrome-*b* data results in a cladogram placing *R. pumilio* and *R. fischerae* as sister taxa relative to *R. alethina*. The bootstrap value (67%) and the Bremer support value (9) for this *pumilio-fischerae* sister relationship are weaker than any that support clades for species relationships of *Carollia*, and this intrageneric relationship should be viewed with caution.

Percentage of sequence divergence shows that the three species of *Rhinophylla* are quite different from each other relative to intrageneric relationships among other phyllostomids. The lowest percentage of sequence divergence is 16.0% between a specimen of *R. fischerae* and one of *R. pumilio*, the highest is 19.8% between a specimen of *R. alethina* and one of *R. pumilio*. In comparison, the highest percentage of sequence divergence seen among *Carollia* is 13.6% between *C. castanea* and *C. subrufa* and the lowest is 4.3% between *C. perspicillata* and *C. brevicauda*. Applying the commonly used molecular clock for the rate of evolution of mtDNA at 2% sequence divergence/1.0 $\times$ 10^6 years (Brown et al., 1979; Irwin et al., 1991; Shields and Wilson, 1987; Smith and Patton, 1991; Sudman and Hafner, 1992) provides a rough estimate for the first divergence within *Rhinophylla* at ca. 8–10 million years and for *Carollia* as $\leq$ 7 million years ago. An alternative way to examine these data is to compare substitutions resulting in the branching pattern of the topology of Fig. 1 for each of the three-codon positions and documenting if these changes are transitions or transversions (Table 2). None of the changes in lineages of *Carollia* are due to second-codon transversions, but five of the changes in the lineages of *Rhinophylla* are due to second-codon position transversions. These observations, along with the percentage of sequence divergence values, corrected for multiple substitutions, lend additional support to the proposition that either speciation events in *Rhinophylla* were earlier than in *Carollia* or that there is heterogeneity in rates of nucleotide substitutions between these genera. However, the latter explanation is less probable because we did not detect significant rate heterogeneity among pairwise comparisons of the taxa of Stenodermatini relative to our outgroups based on relative-rates tests. Therefore, available DNA-sequence data are compatible with the hypothesis that speciation events in *Rhinophylla* occurred prior to those in *Carollia*.

Information on the approximate age of the genus is useful in light of status of *R. pumilio* as an example of karyotypic megaevolution. Karyotypic megaevolution is a model of chromosomal evolution proposed by Baker and Bickham (1980), in which the karyotype undergoes a radical reorganization in what is proposed to be a short period of time. Baker and Bickham (1980) suggested that *R. pumilio* underwent karyotypic megaevolution after diverging from *R. fischerae*. Data from the cytochrome-*b* gene suggest that these two lineages have been separated from each other for a relatively long time (relative to other congeners within this family) and that there have been several million years (8–10) available for extensive autapomorphic chromosomal rearrangements to evolve that distinguish *R. pumilio* from the rest of the phyllostomids. Nonetheless, within this time frame, chromosomal evolution within *R. pumilio* has been several times greater than that typical for most other congeners within the family.

Sister-taxon grouping of Carollia and Rhinophylla.—Certain aspects of the cytochrome-*b* gene sequence (third-codon position and transitions) do not appear appropriate for evaluating this relationship because of the amount of homoplasy in these characters (Brown, 1983; Brown et al., 1982; Hogan et al., 1997; Honeycutt and Wheeler, 1990; Irwin et al., 1991). However, information on the order of amino acid residues and changes at the first- and second-codon positions should provide some insight in evaluating this relationship. Analyses of the entire nucleotide dataset and of transversions only produce a tree in which

Carollia and *Rhinophylla* are sister taxa, but all other analyses of the data, including those used to generate Fig. 1, result in trees with *Rhinophylla* allied to other members of the Stenodermatini to the exclusion of *Carollia*. Two possible explanations of these data remain viable. First, *Rhinophylla* and *Carollia* are sister taxa, but they shared a short period of common ancestry after diverging from the remainder of the Stenodermatini, and the cytochrome-*b* gene is inappropriate for examining such a distant relationship. Second, either *Rhinophylla* or *Carollia* diverged basally from the remainder of the Stenodermatini (Baker at al., 1989) and the dental features used to justify the taxon Carolliinae (Miller, 1907) are convergent. These two possible explanations are compatible with the data of Lim and Engstrom (1998) who found little evidence to support a sister-group relationship between *Carollia* and *Rhinophylla*.

Acknowledgments

Don E. Wilson provided assistance in identification of specimens and support for collecting trips. J. Arroyo-Cabrales, C. Ascorra, S. Solari, and D. E. Wilson assisted in collecting specimens. We thank L. Wiggins for assistance in preparation of the manuscript and for reviewing numerous early drafts. C. Krajewski and R. Pine provided a valuable review. Tissues were supplied by the vital-tissue collections of Texas Tech University and the Royal Ontario Museum. Support for this research was provided (in part) by grants from the Howard Hughes Medical Institute through the Biological Sciences Undergraduate Education Program at Texas Tech University and by the National Science Foundation grant BSR-91-07-143 to R. J. Baker.

Literature Cited

Allard, M. W., and R. L. Honeycutt. 1992. Nucleotide sequence variation in the mitochondrial 12S rRNA gene and the phylogeny of African mole-rats (Rodentia: Bathyergidae). Molecular Biology and Evolution, 9:27–40.

Baker, R. J., and J. W. Bickham. 1980. Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. Systematic Zoology, 9:239–253.

Baker, R. J., and W. J. Bleier. 1971. Karyotypes of bats of the subfamily Carolliinae (Mammalia; Phyllostomatidae) and their evolutionary implications. Experimentia, 7:220–222.

Baker, R. J., C. S. Hood, and R. L. Honeycutt. 1989. Phylogenetic relationships and classification of the higher categories of the New World bat family Phyllostomidae. Systematic Zoology, 3:228–238.

Baker, R. J., M. B. Qumsiyeh, and C. S. Hood. 1987. Role of chromosomal banding patterns in understanding mammalian evolution. Pages 67–96, *in* Current mammalogy (H. H. Genoways, ed.). Plenum Press Corporation, New York.

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.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution, 42:795–803.

———. 1994. Branch support and tree stability. Cladistics, 10:295–304.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pp. 62–88, *in* Evolution and genes in proteins (M. Nei and R. K. Kohen, eds.). Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Brown, W. M., M. George, Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences, 76:1967–1971.

Brown, W. M., E. M. Prager, A. Wang, and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. Journal of Molecular Evolution, 18:225–239.

Donoghue, M. J., R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of dipscales based on *rbc*L sequences. Annals of the Missouri Botanical Garden, 79:333–345.

Eernisse, D. J., and A. G. Kluge. 1993. Taxonomic congruence versus total evidence, and the phylogeny of amniotes inferred from fossils, molecules, and morphology. Molecular Biology and Evolution, 10: 1170–1195.

Engstrom, M. D., R. W. Murphy, and O. Haddrath 1999. Sampling vertebrate collections for molecular research: practice and policies. Pp. 315–330, *in* Managing the modern herbarium (D. A. Metzger and S. C. Byers, eds.). Society for the Preservation of Natural History Collections. Washington, D.C.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783–791.

Felsenstein, J., and H. Kishino. 1993. Is there something wrong with the bootstrap analysis? A reply to Hillis and Bull. Systematic Biology, 42:193–200.

Fleming, T. H. 1988. The short-tailed fruit bat, a study in plant-animal interactions. The University of Chicago Press, Chicago, Illinois.

Hillis, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. Pp. 278–294, *in* Phylogenetic analysis of DNA sequences (M. M. Miyamoto and J. Cracraft, eds.). Oxford University Press, New York.

Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confi-

dence in phylogenetic anlaysis. Systematic Biology, 42:182–192.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analysis. Journal of Heredity, 83:189–195.

HOGAN, K. M., S. K. DAVIS, AND I. F. GREENBAUM. 1997. Mitochondrial DNA analysis of the systematic relationships within the *Peromyscus maniculatus* species group. Journal of Mammalogy, 78:733–743.

HONEYCUTT, R. L., AND V. M. SARICH. 1987. Albumin evolution and subfamilial relationships among New World leaf-nosed bats (family Phyllostomideae). Journal of Mammalogy, 68:505–517.

HONEYCUTT, R. L., AND W. C. WHEELER. 1990. Mitochondrial DNA: variation in humans and higher primates. Pp. 91–129, *in* DNA systematics: humans and higher primates (S. K. Dutta and W. P. Winter, eds.). CRC Press, Inc., Boca Raton, Florida, 3:1–205.

HOOD, C. S., AND J. D. SMITH. 1982. Cladistical analysis of female reproductive histomorphology in phyllostomoid bats. Systematic Zoology, 31:241–251.

HUELSENBECK, J. P. 1991. Tree-length distribution skewness: an indicator of phylogenetic information. Systematic Zoology, 40:257–270.

IRWIN, D. M., T. D. KOCKER, AND A. C. WILSON. 1991. Evolution of the cytochrome-*b* gene in mammals. Journal of Molecular Evolution, 32:128–144.

KALLERSJO, M., J. S. FARRIS, A. G. KLUGE, AND C. BULT. 1992. Skewness and permutations. Cladistics, 8:275–287.

KIMURA, M. 1980. A simple method for estimating rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16:11–120.

LIM, B. K., AND M. D. ENGSTROM. 1998. Phylogeny of Neotropical short-tailed fruit bats, *Carollia* spp.: phylogenetic analysis of restriction site variation in mtDNA. Pp. 43–58, *in* Bat biology and conservation (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington, D.C.

LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of lysis buffer in DNA isolation and its implications for museum collections. Occasional Papers, The Museum, Texas Tech University, 163:1–4.

LUNDRIGAN, B. L., AND P. K. TUCKER. 1994. Tracing paternal ancestry in mice, using the Y-linked, sex-determining locus, *Sry*. Molecular Biology and Evolution, 11:483–492.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Version 3.0. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

MCLELLAN, L. J. 1984. A morphometric analysis of *Carollia* (Chiroptera, Phyllostomidae). American Museum Novitates, 2791:1–35.

MILLER, G. S., JR. 1907. The families and genera of bats. Bulletin of the United States National Museum, 57:1–282.

MINDELL, D. P., AND R. L. HONEYCUTT. 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. Annual Review of Ecology and Systematics, 21:541–566.

OWEN, J. G., D. J. SCHMIDLY, AND W. B. DAVIS. 1984. A morphometric analysis of three species of *Carollia* (Chiroptera, Glossophaginae) from Middle America. Mammalia, 48:85–93.

OWEN, R. D. 1987. Phylogenetic analyses of the bat subfamily Stenodermatinae (Mammalia: Chiroptera). Special Publications, The Museum, Texas Tech University, 26:1–65.

PENNY, D., AND M. HENDY. 1985. Testing methods of evolutionary tree construction. Cladistics, 1:266–278.

———. 1986. Estimating the reliability of evolutionary trees. Molecular Biology and Evolution, 3:403–417.

PINE, R. H. 1972. The bats of the genus *Carollia*. Technical Monograph, The Texas Agricultural Experiment Station, Texas A&M University, 8:1–125.

SAIKI, R. K., T. L. BUGAWAN, G. T. HORN, K. B. MULLIS, AND H. A. ERLICH. 1986. Analysis of enzymatically amplified beta-globin HLA-DQalpha DNA with allele-specific oligonucleotide probes. Nature, 324:163–166.

SAIKI, R. K., ET AL. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science, 239:487–491.

SANGER, F. S., NICKLON, AND A. R. COULSON. 1977. DNA sequencing with chain terminating inhibitors. Proceedings of the National Academy of Sciences, 74:5463–5467.

SARICH, V. M., AND A. C. WILSON. 1967. Immunological time scale for hominid evolution. Science, 158: 1200–1202.

SHIELDS, G. F., AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. Journal of Molecular Evolution, 24:212–217.

SIMMONS, N. B. 1998. A reappraisal of interfamilial relationships of bats. Pp. 3–26, *in* Bat biology and conservation (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington, D.C.

SMITH, M. F., AND J. L. PATTON. 1991. Variation in the mitochondrial cytochrome-b sequences in natural populations of South American akodontine rodents (Muridae: Sigmodontinae). Molecular Biology and Evolution, 8:85–103.

STOCK, A. D. 1975. Chromosome banding pattern, homology and its phylogenetic implications in bat genera *Carollia* and Choeroniscus. Cytogenetics and Cell Genetics, 14:34–41.

SUDMAN, P. D., AND M. S. HAFNER. 1992. Phylogenetic relationships among Middle American pocket gophers (genus *Orthogeomys*) based on mitochondrial DNA sequence. Molecular Phylogenetics and Evolution, 1:17–25.

SWOFFORD, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

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.

Van Den Bussche, R. A., R. J. Baker, H. A. Wichman, and M. J. Hamilton. 1993. Molecular phylogenetics of Stenodermatini bat genera: congruence of data from nuclear and mitochondrial DNA. Molecular Biology and Evolution, 10:944–959.

Submitted 10 June 1998. Accepted 30 January 1999.

Associate Editor was Troy L. Best.