SYSTEMATICS OF THE GENERA CAROLLIA AND RHINOPHYLLA
BASED ON THE CYTOCHROME-\(B\) GENE

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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 Phyllozycteris, Lonchophylla, Uro-
derma, Artibeus, Dermanura, Enchistesenes, and Chirottha. Within Carollia, representa-
atives 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 di-
verged 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 in-
vestigated intragenic 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.
fischeri, and R. aleithina. Traditionally, Carollia and Rhinophylla were recognized
as sister-taxon comprising the subfamily Carollinae (Miller, 1907). More recently, Ba-
ker et al. (1989) placed Carollia and Rhinophylla in the tribe Stenodermatini, primarily
on the basis of morphology of the fe-
male 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 karyo-
type of Carollia is so radically reorganized
that it is difficult to identify any chromo-
somal rearrangements documenting rela-
tionships of this taxon to other genera of
Stenodermatini or to other members of the
family Phyllostomidae. The supposed sis-
ter-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-taxa 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 rRNA of 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 amplying 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’AAAYGGRATATTGKACRTGYCATGG 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, Popotún; Carollia castanea—TK 70672 (M), Peru, Cuzco, Convencion, Camisea, Armihuari; FN 38156 (M), Panama, Chiriquí, Ojo de Agua, 2 km N of Santa Clara; Carollia perspicillata—TK 17466 (F), Surinam, Nickerie, Kabaleho; TK 74345 (M), Peru, Cuzco, Convencion, Camisea, Armihuari; Carollia subgularis—TK 19550 (M), 19551 (F), Mexico, Jalisco, 22 km S Chamael; TK 15818 (F), El Salvador, Ahuachapan, El Rufugio; Lonchophylla thomasi—TK 17177 (M), Surinam, Saramacca, Voltsberg; Phyllonycteris aphylla—TK 9280 (M), Jamaica, Westmorland Parish, Bluefields; Rhinophylla alethina—FN 40073 (M), 40093 (M), Ecuador, Esmeraldas, Alto Tambo, 2 km S Alto Tambo; Rhinophylla fischeri—TK 46034 (M), Peru, Loreto, Quebrada Aguas Negras, Cocha Zoraida; Rhinophylla pumilio—TK 18825 (M), French Guiana, Paracou, Sinnamary; TK 46001 (M), 46026 (F), Peru, Loreto, Quebrada Aguas Negras, Cocha Zoraida; Stenira lilium—TK 22651, Peru, Huancaco, 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—U06503, Dermanura watsoni—U066576, Koopmania concolor—L19515, Enchisthenes hattii—L19514, Chiroderma villanum—L289843.

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 Carrollia or Rhinophylla relative to the outgroups (Lonchophylla and Phyllonyceris), 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 taxonomic studies. Lonchophylla and Phyllonycteris were selected as outgroups for analyses concerning placement of Carrollia and Rhinophylla in the tribe Stenodermatini, whereas Chiroderma and Rhinophylla served as outgroups for analyses concerning relationships within Carrollia, and Chirotterina and Carrollia 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 Carrollia 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 Carrollia and
the outgroups (Rhinophylla pumilio and Chirolomera), 798 bp (70.0%) were identical, and in Rhinophylla and the outgroups (Carollia subrufa and Chirolomera), 784 bp (68.8%) were identical. Among all taxa examined, 496 positions were variable. Among those, 102 were autopomorphic leaving 394 positions as potentially phylogenetically informative. Within species of Carollia, of the 342 variable positions, 119 were autopomorphic, leaving 223 positions as potentially phylogenetically informative. Of the 356 variable positions within Rhinophylla, 101 were autopomorphic, 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 of $-0.956$ ($P < 0.01$—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 Chirolomera 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 $-0.932$ ($P < 0.01$—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 (Chirolomera 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

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 Erychisthenes and Sturnira and the level of resolution provided to terminal taxa.
**Table 1.** Percentage of sequence divergence for all pairwise comparisons corrected for multiple substitution using the two-parameter model of Kimura (1980). C = Carolia and R. = Rhinophylla.

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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 Carolia and Rhinophylla first.

**Intrageneric relationships of Carolia.—** Members of the genus Carolia 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 mater-
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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 Carolia and within Rhinophylla, reflecting the number and percentage of transitions and transversions (in parentheses) at the three codon positions.

<table>
<thead>
<tr>
<th></th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Total</th>
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<tr>
<td><strong>All taxa</strong></td>
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<td></td>
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<tr>
<td>Transitions</td>
<td>194 (10.8)</td>
<td>55 (3.1)</td>
<td>998 (55.5)</td>
<td>1,247 (69.4)</td>
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<td>Transversions</td>
<td>57 (3.2)</td>
<td>32 (1.8)</td>
<td>463 (25.7)</td>
<td>552 (30.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>251 (14.0)</td>
<td>87 (4.9)</td>
<td>1,461 (81.2)</td>
<td>1,799</td>
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<tr>
<td><strong>Carolia</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Transitions</td>
<td>29 (20.6)</td>
<td>7 (5.0)</td>
<td>58 (41.1)</td>
<td>94 (66.7)</td>
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<tr>
<td>Transversions</td>
<td>3 (2.1)</td>
<td>0 (0.0)</td>
<td>44 (31.2)</td>
<td>47 (33.3)</td>
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<tr>
<td><strong>Total</strong></td>
<td>32 (22.7)</td>
<td>7 (5.0)</td>
<td>102 (72.3)</td>
<td>141</td>
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<tr>
<td><strong>Rhinophylla</strong></td>
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<tr>
<td>Transitions</td>
<td>38 (12.1)</td>
<td>7 (2.2)</td>
<td>167 (53.0)</td>
<td>212 (67.3)</td>
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<tr>
<td>Transversions</td>
<td>8 (2.5)</td>
<td>5 (1.6)</td>
<td>90 (28.6)</td>
<td>103 (32.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>46 (14.6)</td>
<td>12 (3.8)</td>
<td>257 (81.6)</td>
<td>315</td>
</tr>
</tbody>
</table>
Phyllanctens
Lonchophylla
C. brevicauda
C. brevicauda
C. brevicauda
C. perspicillata
C. perspicillata
C. brevicauda
C. subrufa
C. subrufa
C. castanea
C. castanea
R. aethina
R. aethina
R. pumilio
R. pumilio
R. pumilio
R. fischerae
Stumira
Uroderma
Chiorderma
Artibeus
Koopmania
Demanura
Enchisthenes

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 gi-statistic of -0.588. C. = Carolia; R. = Rhinophylla.

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

Percentage of sequence divergence shows that the three species of \textit{Rhinophylla} are quite different from each other relative to intrageneric relationships among other phylllostomids. The lowest percentage of sequence divergence is 16.0\% between a specimen of \textit{R. fischeriae} and one of \textit{R. pumilio}, the highest is 19.8\% between a specimen of \textit{R. alethina} and one of \textit{R. pumilio}. In comparison, the highest percentage of sequence divergence seen among \textit{Carollia} is 13.6\% between \textit{C. castanea} and \textit{C. subrufa} and the lowest is 4.3\% between \textit{C. perspicillata} and \textit{C. brevicauda}. Applying the commonly used molecular clock for the rate of evolution of mtDNA at 2\% sequence divergence/1.0 \times 10^8 years (Brown et al., 1979; Irwin et al., 1991; Shields and Wilson, 1987; Smith and Patton, 1991; Sudman and Haftner, 1992) provides a rough estimate for the first divergence within \textit{Rhinophylla} at ca. 8–10 million years and for \textit{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 \textit{Carollia} are due to second-codon transversions, but five of the changes in the lineages of \textit{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 \textit{Rhinophylla} were earlier than in \textit{Carollia} or that there is heterogeneity in rates of nucleotide substitu-

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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 Carolliliinae (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.

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Literature Cited


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