SYSTEMATIC RELATIONSHIPS WITHIN CHIRODERMA
(CHIROPTERA: PHYLLOSTOMIDAE) BASED ON
CYTOCHROME B SEQUENCE VARIATION

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The Neotropical bat genus Chiroderma consists of five recognized species. This study uses
DNA-sequence variation of the mitochondrial cytochrome b gene to infer the phylogenetic
relationships within Chiroderma. Phylogenetic relationships deduced from these data by
parsimony analyses resulted in the discovery of a single most-parsimonious tree with C. salvini
diverging basal to the other four species of Chiroderma and sister-group relationships of C. villosum with C. improvisum and C. trinitatum with C. doriae. This is a relatively young group of species with approximate times of divergence ranging from 1.6
million years before present (mya) for the divergence of C. doriae from C. trinitatum to
4.6 mya for the divergence of C. salvini from the other four species of Chiroderma.

Key words: molecular phylogenetics, cytochrome b, Chiroderma, DNA sequence

Many phylogenetic studies of species relationships are faced with the uncertainty of
monophyly at the genus level. Therefore, these studies first must be concerned with
determining whether a particular collection of species represents a monophyletic group
and secondarily with resolving the relationships within that group. Within the major
bat families, this problem arises in numerous genera (e.g., Myotis, Eptesicus, Pipistrellus, Artibeus, and Phyllostomus). One
taxon that appears to have sufficient morphological synapomorphies to make it highly improbable that the genus is diphylectic is the phyllostomid genus Chiroderma
(Phyllostomidae, Phyllostominae, tribe Stenodermatini—Baker et al., 1989). The
unique configuration of the skull, including the absence of nasal bones, presence of unique incisors and wing structure, and enlarged eyes support the monophyly of this
genus (Goodwin, 1958; Miller, 1907; Owen, 1987). Additionally, each of the five
species within this genus is sufficiently dist-
tinct morphologically that no serious ques-
tions have been posed as to their validity.

Chiroderma consists of five species (villo-
sum, improvisum, trinitatum, salvini, dor-
iae) with the genus being distributed from
northwestern Mexico to southern Brazil
(Fig. 1; Hall, 1981; Koopman, 1982). In the
original description of C. improvisum, Bak-
er and Genoways (1976) noted that the two
largest species (improvisum and doriae)
were distributed at the periphery of the
range of the genus (Fig. 1c) and suggested
two possible explanations for these distrib-
utions: C. doriae and C. improvisum were
sister-taxon, and their large size relative to
other members of the genus evolved in their
common ancestor; these two species
evolved their large size independently, and
C. improvisum and C. villosum were sister-
taxa. Unfortunately, no published phyloge-
netic hypothesis for the relationships within
the genus exists that would provide the
proper evolutionary background to test the
alternative scenarios set forth by Baker and
Genoways (1976). Chromosomal data, which are available for the five species (Baker, 1973, 1979; Baker and Genoways, 1976; Baker and Hsu, 1970; Varella-Garcia and Taddei, 1989; Varella-Garcia et al., 1989), document a close phylogenetic relationship of these taxa yet are unable to resolve relationships within the genus. The most recent morphologic assessment of the genus (Owen, 1987) also documented its monophyletic nature, but did not resolve relationships among species.

We chose to examine the phylogenetic relationships of the species of *Chiroderma* and to interpret the current geographic distribution based on phylogenetic associations. Due to the low levels of variation found in traditional datasets, we chose to examine these relationships through DNA-sequence variation. DNA-sequence variation from the mitochondrial cytochrome *b* gene has provided phylogenetic resolution for mammalian taxa with divergence times ranging from ca. 4-44 million years (Irwin et al., 1991; Patton and Smith, 1992; Smith and Patton, 1991). More recently, it has been shown that the first 402 base pairs (bp) of cytochrome *b* provide resolution for addressing phylogenetic relationships among species (Van Den Bussche and Baker, 1993) and among genera (Van Den Bussche et al., 1993) of phyllostomid bats. Therefore, the cytochrome *b* gene should provide sufficient levels of DNA-sequence variation to address our proposed objectives.

**Materials and Methods**

DNA was extracted from frozen heart, liver, kidney, or muscle tissue following standard protocols (Bingham et al., 1981; Strauss, 1987). The cytochrome *b* gene was amplified using the polymerase chain reaction (PCR; Saiki et al., 1986, 1988) utilizing primers located in the *tRNAs* for glutamic acid (MVZ05—Smith and Patton, 1991) and threonine (H15915R—Irwin et al., 1991). Products from PCR were subsequently ligated and cloned using the TA cloning kit (Invitrogen, Inc.). DNA sequence of the entire 1,140-bp cytochrome *b* gene was accomplished through double-stranded sequencing.
(Sanger et al., 1977) using the primers located in the tRNAs plus four additional internal primers. Sequences of all primers came from the published literature (Irwin et al., 1991; Smith and Patton, 1991). Primer name indicates the DNA strand (H = heavy or L = light) and the position of the 3' end of the oligonucleotide sequence according to the numbering system for the human mitochondrial genome (Anderson et al., 1981): L14724 [MVZ05], H15149 [MVZ04] (Smith and Patton, 1991), L15162, L15513, and H15915R (Irwin et al., 1991). Multiple clones or PCR products were sequenced for all individuals. Sequences have been deposited in GenBank under accession numbers ???.

Cytochrome b DNA sequences were entered into GeneWorks (IntelliGenetics, 1991) for alignment with the published human sequence (Anderson et al., 1981). Sequence data were entered as discrete, non-ordered phylogenetic characters (G, A, T, C), and trees were constructed using version 3.0s of PAUP (Phylogenetic Analysis Using Parsimony—Swofford, 1990). Polarity of character state changes was established using Uroderma bilobatum and Platyrhinus helleri as outgroups.

The exhaustive search option of PAUP was used to find the shortest tree. To estimate the reliability of branches on the most parsimonious tree, a bootstrap analysis (Felsenstein, 1985) was performed using the branch-and-bound option with 1,000 iterations. Finally, to evaluate whether these data contained significant phylogenetic information, the $g_i$ statistic was used to measure skewness of tree-length distribution of all possible trees, and this value was compared to critical values for tree-length distributions (Hillis, 1991; Hillis and Huelsenbeck, 1992; Huelsenbeck, 1991).

Tissues were obtained from the frozen tissue collection at The Museum, Texas Tech University. TK numbers cross reference laboratory records and tissues to voucher specimens deposited in The Museum, Texas Tech University. Uroderma bilobatum (TK 25256): Trinidad: St. George Co., SIMLA Research Center, 4 miles N Arimas Platyrhinus helleri (TK 17627): Surinam: Mavowijne, Perica. Chiroderma salvini (TK 22581): Panama: Darién, 6 km SE Cana. C. villosum (TK 25052) Trinidad: St. George Co., SIMLA Research Center. C. improvisum (TK 15713): Montserrat: St. Anthony, on Belham River, ½ mile from mouth. C. trinitatum (TK 25211): Trinidad: St. George Co., SIMLA Research Center. C. doriae (UNESP 16506) Brazil: São Paulo, Pindorama. The voucher specimen of C. doriae is deposited in the Laboratory of Chiropterology, Universidade Estadual Paulista (UNESP), São José do Rio Preto, São Paulo, Brazil.

RESULTS

Eight hundred ninety-one (78.16%) of the 1,140-bp cytochrome b gene were identical among all Chiroderma species and the outgroups. Of the 249 variable sites, 72 were autapomorphous, and 117 potentially contained phylogenetic information. Of the 249 variable positions, 33 (13.25%) were variable at the first position, 21 (8.43%) second-position sites were variable, and 195 (78.31%) were variable at the third position. Table 1 shows the percent sequence divergence for all pairwise comparisons (correcting for multiple substitutions using the method of Kimura, 1980) above the diagonal, and the transition: transversion ratio for all pairwise comparisons below the diagonal. As would be expected for a functional protein-coding gene, the transition: transversion ratio decreases with increasing percent sequence difference. Considering just the five species of Chiroderma, the percent sequence divergence among pairwise comparisons ranges from 3.20% (C. trinitatum versus C. doriae) to 9.70% (C. salvini versus C. doriae), with a mean of 6.99% sequence divergence. For these same taxa, the transition: transversion ratio ranges from 4.8 (C. villosum versus C. salvini) to 13.7 (C. improvisum versus C. villosum), with a mean transition: transversion ratio of 7.34 within Chiroderma.

Phylogenetic analyses of the cytochrome b gene were performed to evaluate relationships among the five species. The topology presented in Fig. 2 is the result of an unweighted parsimony analysis in which all variable positions were used. The exhaustive search resulted in a single most parsimonious tree of 292 steps with a consistency index of 0.771. The $g_i$ statistic calculated
Table 1.—Percent sequence divergence (Kimura, 1980) above the diagonal and transition:transversion ratio below diagonal for all pairwise comparisons of the seven taxa in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Uroderma</th>
<th>Platyrhinus</th>
<th>C. salvid</th>
<th>C. villosum</th>
<th>C. trinitatum</th>
<th>C. improvisum</th>
<th>C. doriae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uroderma</td>
<td></td>
<td>11.6</td>
<td>13.9</td>
<td>14.8</td>
<td>15.3</td>
<td>14.1</td>
<td>13.4</td>
</tr>
<tr>
<td>Platyrhinus</td>
<td>2.2</td>
<td></td>
<td>4.4</td>
<td>9.3</td>
<td>4.1</td>
<td>5.7</td>
<td>7.0</td>
</tr>
<tr>
<td>C. salvid</td>
<td></td>
<td>2.8</td>
<td>15.2</td>
<td>13.8</td>
<td>9.0</td>
<td>8.7</td>
<td>9.7</td>
</tr>
<tr>
<td>C. villosum</td>
<td>2.8</td>
<td>2.3</td>
<td></td>
<td>4.8</td>
<td>13.7</td>
<td>5.5</td>
<td>7.7</td>
</tr>
<tr>
<td>C. trinitatum</td>
<td>3.1</td>
<td>2.4</td>
<td>5.3</td>
<td></td>
<td>6.8</td>
<td>7.6</td>
<td>3.2</td>
</tr>
<tr>
<td>C. doriae</td>
<td></td>
<td>2.8</td>
<td>2.5</td>
<td>6.2</td>
<td>5.8</td>
<td>7.1</td>
<td>10.7</td>
</tr>
</tbody>
</table>

from all possible trees was highly significant \((-1.296)\), indicating that the distribution of tree lengths was highly skewed to the left. This suggests a high probability of the correct topology being either the most parsimonious tree or a tree only a few steps longer (Hillis, 1991; Hillis and Huelsenbeck, 1992; Huelsenbeck, 1991). A bootstrap analysis with 1,000 iterations also resulted in a single most parsimonious tree, which was identical to that obtained from the exhaustive search. Numbers in rectangles along internal branches in Fig. 2 indicate the percentage of the 1,000 bootstrap iterations in which each clade was detected, whereas numbers in circles are the assigned branch lengths from PAUP. A high degree of confidence can be placed in the branching order of this topology because all clades were detected in \(>97\%\) of the bootstrap iterations (Fig. 2). Smith (1989) advocated the examination of near-most (1%) parsimonious trees as a method to investigate the cladistic structure of sequence data. Examination of trees longer than the most parsimonious tree (Fig. 2) revealed that no trees were found within 1% of the length of the most parsimonious tree. The next most parsimonious arrangement consisted of a single tree of 300 steps or 2.8% longer than the most parsimonious tree. We do not consider this to be an acceptable alternative topology to that presented in Fig. 2 because too many additional assumptions would be required to explain the distribution of character-states along this tree as compared to that shown in Fig. 2.

**Fig. 2.**—Topology of the single most parsimonious tree of 292 steps \((CI = 0.771)\) resulting from an exhaustive search, hypothesizing phylogenetic relationships within the genus *Chiroderma*. The \(g\), statistic reflecting tree-length distribution from these data is \(-1.296\). Numbers in rectangles along internal branches reflect the percentage of 1,000 bootstrap iterations using the branch-and-bound option in which each clade was detected. Numbers in circles reflect the assigned branch lengths for each lineage.

**DISCUSSION**

Although many different character sets have been utilized over the years to determine the interspecific relationships within *Chiroderma*, providing resolution within this genus has proven difficult. Not only do DNA-sequence data from the cytochrome \(b\) gene provide resolution of relationships among all five species, the consistency of the data suggest a high probability for the relationships depicted by the maternal lineages (Fig. 2) being accurate. Relative to
the two alternative hypotheses previously suggested for the relationship of *C. improvisum* (Baker and Genoways, 1976), these data suggest that large size has evolved independently in *C. improvisum* and *C. doriae* and that *C. improvisum* is best explained as a geographic isolate of the common ancestor of *C. improvisum* and *C. villosum*. In the original description of *improvisum*, Baker and Genoways (1976) suggested that the only possible conspecific taxon for *C. improvisum* was *C. doriae*. Cytochrome *b* data further document the specific distinctness of *C. improvisum* from other recognized species.

Magnitude of molecular evolution has been used to estimate the age at which lineages diverged (Brown et al., 1979; Irwin et al., 1991; Shields and Wilson, 1987; Smith and Patton, 1991; Sudman and Hafner, 1992). Brown et al. (1979) and Shields and Wilson (1987) suggested that vertebrate mitochondrial DNA evolves at a rate of ca. 2%/million years. Using this value as a rough estimate of rate of divergence, these cytochrome *b* data indicate that the ancestors of *Uroderma* and *Chiroderma* diverged from each other 7.2 million years before present (mya) and that *C. salvinii* diverged from the remainder of the genus 4.6 mya. The *doriae-trinitatum* clade is estimated to have diverged from the *villosum-improvisum* clade ca. 2.6 mya. *C. villosum* diverged from *C. improvisum* 2.1 mya, and *C. trinitatum* and *C. doriae* diverged from each other 1.6 mya. The most striking of these estimates of time of divergence is the *trinitatum-doriae* date because of the magnitude of difference in their relative body sizes.

Based on DNA-sequence variation from the entire cytochrome *b* gene, Sudman and Hafner (1992), using 2% divergence/million years as an estimate for rate of nucleotide substitutions, concluded that divergence estimates within the gopher subgenus *Macrogeomys* were on the order of 1.9–2.9 mya. Even though we are comparing species occupying the fossorial habitus (*Macrogeomys*) to those occupying a volant habitus (*Chiroderma*), we note that the estimated times of divergence are similar between species of *Macrogeomys* and *Chiroderma*. As additional studies of this gene complex are made, it will be of interest to see how common this 2–3-million-year-old radiation of species within extant genera is.

Data from starch-gel electrophoresis used in systematic studies of phyllostomid genera indicate that the Stenodermatini genera are more similar to each other than are species within genera of the Phyllostomini (Arnold et al., 1983; Baker et al., 1981; Koop and Baker, 1983; Straney et al., 1979). A possible explanation of this pattern is that the Stenodermatini genera diverged more recently from a common ancestor than did comparable taxonomic units in the Phyllostomini. The maternally inherited mitochondrial genome is an appropriate independent test of hypotheses based on nuclear allozyme variation. Van Den Bussche and Baker (1993) sequenced a smaller portion of the cytochrome *b* gene in species of *Phyllostomus* and calculated divergence times ranging from 3.5 to 6.7 mya using uncorrected divergence values (Kimura, 1980). When the 10 pairwise comparisons in *Phyllostomus* are corrected for multiple substitutions using the formula of Kimura (1980), all but one of the divergences appears older than the most ancient divergence found in *Chiroderma*. The youngest divergence in *Phyllostomus* is approximately equal in age to the divergence of *C. salvinii* from the remainder of the *Chiroderma*. We suggest that this might indicate that the Stenodermatini niche (obligate frugivores) may have been invaded more recently than the more omnivorous niche of the Phyllostomini.

If the relationships for these five taxa are as depicted in Fig. 2, would these relationships have been predicted from current geographic distributions? *C. improvisum* is equally distant geographically from populations of *C. villosum* and *C. trinitatum*, which occur on the island of Trinidad. Morphologically, *C. villosum* is more similar to *C. improvisum* than to *C. trinitatum*. More-
over, *C. improvisum* certainly is not geographically associated with *C. doriae*, and it may be significant that *C. salvini* is not established on the island of Trinidad. The geographic distribution of *C. villosum* and *C. improvisum* is compatible with the cytochrome *b* based phylogeny (Fig. 2). From a geographic standpoint, it is not obvious that *C. trinitatum* and *C. doriae* are sister-taxa. *C. villosum* is distributed sympatrically with *C. doriae* in the Minas Gerais and São Paulo regions of Brazil, whereas *C. trinitatum* currently is known to be distributed as far south as Bolivia in northcentral South America (Koopman, 1982; Taddei, 1979, 1980). *C. salvini*'s basal position to the other four species of *Chiroderma*, along with its restricted geographic range in Middle America and western South America, may be explained by *C. salvini* being isolated in Middle America from South American ancestral stock that gave rise to *C. villosum, C. improvisum, C. trinitatum*, and *C. doriae*. Allopatric speciation appears to be the best speciation model to explain the patterns observed in this genus.

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**LITERATURE CITED**


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