Molecular Phylogenetics of Stenodermatini Bat Genera: Congruence of Data from Nuclear and Mitochondrial DNA

Ronald A. Van Den Bussche, Robert J. Baker, Holly A. Wichman, and Meredith J. Hamilton

Introduction

Although bats of the New World tribe Stenodermatini (family Phyllostomidae) have received much systematic attention, many of the phylogenetic relationships within this tribe are still problematic. This tribe, as now recognized, consists of approximately 18 genera and 71 species (Baker et al. 1989; Owen 1987, 1991) and is the most species-rich group within the morphologically diverse bat family Phyllostomidae. However, in many aspects it is the most homogeneous group within the family (Owen 1987). All bats of this tribe are either entirely or primarily frugivorous (Wilson 1973) and have evolved similar complexes of adaptations to this life-style, which complicates systematic studies using classical anatomical features.

Over the past 30 years, numerous systematic studies have been conducted to evaluate the phylogenetic affinities of the genus *Artibeus* (sensu Andersen 1908), yet no consistent phylogeny has emerged from these studies. For example, Baker (1973),...
who primarily used standard karyotypes and secondarily used morphological data, concluded that the genus *Artibeus* was split between two of the three basal stocks of this complex of frugivores, with *Enchisthenes* being derived from one of these basal stocks (fig. 1A). Smith (1976) aligned *Artibeus* and *Enchisthenes* with the short-faced bats (*Stenoderma, Pygoderma, Ariteus, Ardops, Centurio, Phyllops, Ametrida*, and *Sphaeronycteris*) on the basis of rostral length (fig. 1B). On the basis of a morphological assessment, Owen (1987) also concluded that the genus *Artibeus* consisted of two independent lineages (fig. 1C), including a large-sized and small-sized group of bats. On the basis of these results, Owen (1987) suggested limiting the genus *Artibeus* to the larger-sized group and resurrecting the name *Dermanura* for the smaller-sized taxa. With regard to the phylogenetic affinities of these two genera, his topology aligned *Artibeus* with *Uroderma* and *Ectophylla*, whereas the smaller *Dermanura* was aligned with the short-faced bats. More recently, Owen (1991) has concluded that *D. concolor* represents a lineage distinct from *Dermanura* and suggested recognizing this taxon as *Koopmania concolor*. This analysis also placed *Enchisthenes hartii* outside the *Dermanura* clade, yet he retained this taxon as *Dermanura hartii*. Finally, on the basis of ribosomal DNA (rDNA) restriction-site data, Van Den Bussche (1992) also found that *Artibeus* (sensu Andersen 1908) consisted of two independent lineages, supporting Owen’s (1987) establishment of the genus *Dermanura*. However, Van Den Bussche (1992) aligned *Artibeus* with *Chiroderma* and *Sturnira*, while the relationship of *Der-

![Diagram](https://via.placeholder.com/150)

**Fig. 1.**—Representation of character state evolution for: (A) chromosomes (Baker 1973, fig. 5); (B) morphology (Smith 1976, fig. 2); (C) morphology (Owen 1987, fig. 17; 1991, fig. 1); and (D) rDNA restriction sites (Van Den Bussche 1992; fig. 2).
manura was unresolved within the tribe (fig. 1D). In contrast to Owen’s (1987) placement of Enchisthenes within the genus Dermanura, restriction-site data of the rDNA gene complex found that Enchisthenes hartii represented a lineage distinct from Dermanura (Van Den Bussche 1992).

While most systematic studies have focused on the variation present in coding regions of the genome, there exist many other classes of DNA which may also provide phylogenetic information. One such component of the genome is satellite DNA. Although examples exist in which satellite DNA evolution does not follow a well-established phylogeny (Wichman et al. 1991), this component of the genome has provided phylogenetic information for many other taxa (Arnason et al. 1978; Bogenberger et al. 1987; Arnason and Widegren 1989; Tynan and Hoar 1989; Durfy and Willard 1990; Hamilton et al. 1990, 1992). Therefore, to determine whether satellite DNA will provide resolution of the phylogenetic relationships among Stenodermatini bats, we examined the phylogenetic distribution and chromosomal localization of an EcoRI-defined satellite DNA repeat.

Mitochondrial cytochrome b DNA sequence variation has been found to provide phylogenetic resolution for mammalian taxa with times of divergence ranging from approximately 4 to 44 Myr (Irwin et al. 1991; Smith and Patton 1991; Patton and Smith 1992). Within the family Phyllostomidae, cytochrome b has provided resolution of the phylogenetic relationships among species of the New World bat genus Phyllostomus (Van Den Bussche and Baker 1993), and, therefore, cytochrome b DNA sequence data should provide sufficient phylogenetic information for inferring phylogenetic relationships within this tribe.

This study will focus on the following: (1) validity of the genera Artibeus, Dermanura, and Koopmania, as established by Owen (1987, 1991); (2) the phylogenetic relationship of Enchisthenes hartii to Artibeus, Dermanura, and Koopmania; and (3) the relationships of Artibeus, Dermanura, Enchisthenes, and Koopmania to representatives of the other stenodermatine genera that have been proposed as close relatives. These relationships will be studied by examining the phylogenetic distribution, chromosomal location, and restriction fragment pattern of an EcoRI-defined satellite DNA repeat, as well as DNA sequence of the mitochondrial cytochrome b gene.

Material and Methods

Cloning of Satellite DNA

High-molecular-weight DNA was purified from heart, liver, kidney, or muscle, according to the method of Bingham et al. (1981). Highly repetitive sequences from Artibeus lituratus and Dermanura phaeotis were isolated by digestion of total genomic DNA with the restriction endonuclease EcoRI, which produced a ladder of fragments representing multiples of a monomer repeat detected on an ethidium bromide-stained agarose gel. This is a common feature of satellite DNA sequences (Bratlag 1980; Singer 1982). Digested DNA was electrophoresed on a 0.8% low-melting-point agarose gel. The approximately 900-bp fragment, which was common to both taxa and appeared to be the monomer repeat in A. lituratus, was cut from the gel and purified using Prep-A-Gene DNA binding matrix (Bio-Rad Laboratories, Richmond, CA). In addition to the 900-bp fragment, D. phaeotis also possessed 500-bp and 400-bp bands, which were also extracted from the gel and purified with Prep-A-Gene. Satellite DNA clones were constructed by ligating the isolated DNA into a linear plasmid (pBluescript; Stratagene, La Jolla, CA) followed by trans-
formation of the *Escherichia coli* host JM101 with recombinants identified on the basis of color selection. Repetitive clones were detected by hybridizing Southern transfers (Southern 1975) to radioactively labeled (Feinberg and Vogelstein 1984) genomic DNA from *A. lituratus*. Prior to hybridization with radioactive probes, the membranes were washed in prehybridization solution (4 × SSC (Sambrook et al. 1989, p. B.13), 1 × Denhardt’s solution (Sambrook et al. 1989, p. 9.49), 5% denatured salmon sperm DNA) at 60°C for 2 h. Labeled probes were denatured, combined with prehybridization solution (1 × 10^6 dpm/ml), and allowed to hybridize with the membranes for at least 12 h at 60°C. After hybridization, the membranes were washed (four times for 20 min each at 60°C in 2 × SSC, 0.1% SDS, 1 × Denhardt’s solution and then washed once at 60°C for 20 min in 2 × SSC) and exposed to X-ray film, using two intensifying screens, at −80°C. Clones that were highly repetitive in the genome were radioactively labeled and hybridized to a Southern blot of EcoRI-digested genomic DNA from *A. lituratus* and *D. phaeotis*, following the protocol outlined above. Hybridization to the ladder was accepted as indicative that the desired monomer had been cloned.

In Situ Hybridization

Chromosomal material from *Chiroderma villosum, A. jamaicensis, D. azteca, D. tolteca, D. phaeotis, Uroderma, Platyrhinus, Sturnira, and Centurio* was prepared from bone marrow after incubation with Velban. In situ hybridization of the 900-bp fragment from *A. lituratus* and the 900-bp clone from *D. phaeotis* was performed according to the method of Hamilton et al. (1990). A minimum of 5 complete spreads and, in most cases, 10 or more spreads were analyzed for each individual examined.

Cytochrome *b*

The first 402 bp of cytochrome *b* from *Anoura caudifer, Sturnira tildae, Chiroderma trinitatum, Chiroderma salvini, Artibeus fuliginosus, Artibeus lituratus, D. manura cinerea, D. tolteca, D. azteca, D. phaeotis, Enchisthenes hartii, Centurio senex, Uroderma bilobatum, Platyrhinus helleri*, and two individuals of *Koopmania concolor* were amplified by the polymerase chain reaction (PCR; Saiki et al. 1988; White et al. 1989) using the primers MVZ05 (in the tRNA for glutamic acid) and MVZ04 (Smith and Patton 1991). Amplification of cytochrome *b* for direct sequence analysis essentially followed the procedure described by Knight et al. (1991). Seven microliters of the single-stranded PCR product was sequenced by the Sanger-dideoxy method (Sanger et al. 1977) using Sequenase II. The primer that was limited in the asymmetrical PCR was used for sequencing. To score sequences close to the primers, both strands were amplified and sequenced for all individuals. DNA for most taxa was amplified and sequenced at least twice.

Phylogenetic Analysis

DNA sequence data were used as discrete, unordered phylogenetic characters, and trees were constructed using version 3.0s of PAUP (Swofford 1991) with the polarity of character-state changes established by using *Anoura* (a member of the tribe Glossophagini) as the outgroup. A heuristic search was used to find the shortest tree, with the reliability of branches on the most parsimonious tree being estimated using a bootstrap analysis with 100 iterations (Felsenstein 1985). The initial parsimony analyses were unweighted and used all varied positions. Because a transition bias has been noted for vertebrate mitochondrial DNA (Brown et al. 1982; Higuchi et al. 1984,
1987; Irwin et al. 1991; Smith and Patton 1991), various weighting schemes, which place greater weight on transversion substitutions relative to transition substitutions (1:0, 3:1, 5:1, 10:1, 15:1), were employed. Finally, to evaluate whether these data contained phylogenetic information, the Random Tree option of PAUP was used to evaluate the distribution of tree lengths from 10,000 random trees, and the gl statistic (Hillis 1991; Huelsenbeck 1991; Hillis and Huelsenbeck 1992) was used for both the unweighted and weighted data sets.

Results

Organization and Distribution of Satellite DNA

The 900-bp EcoRI fragment from Artibeus lituratus was hybridized to EcoRI-digested genomic DNA from several taxa of phyllostomine bats. A similar pattern was found only in the genera Artibeus, Dermanura, and Koopmania (fig. 2). An identical pattern was produced by using the approximately 900-bp EcoRI fragment from Dermanura. When either the 500-bp or 400-bp EcoRI fragment from D. phaeotis was used as a probe, the same pattern was observed, except that the 400-bp and 500-bp fragments did not cross-hybridize. In addition, this EcoRI-defined satellite DNA was not present in any of the other 35 species of bats or flying lemur examined (table 1).

Representative results of the chromosomal localization of the satellite DNA repeat are shown in figure 3. With the exception of the largest pair of autosomes in the diploid complement of Artibeus jamaicensis, the probe constructed from the 900-bp repeat isolated from Artibeus lituratus hybridized to all centromeric regions of all chromosomes from Artibeus jamaicensis, D. azteca, D. phaeotis, and D. tolteca. This probe failed to hybridize to chromosomes from Chiroderma, Uroderma, Platyrrhinus, and Sturnira.

Cytochrome b DNA Sequence Variation

Two hundred and fifty-nine base pairs (64.4%) of the 402 bp of cytochrome b examined were identical between the outgroup (Anoura) and all Stenodermatini bats included in this study. Of the 143 variable sites, 25 were autapomorphous, and 118 potentially contained phylogenetic information. Of the 143 variable sites, 26 (18.2%) were variable at the first position, 9 (6.3%) were variable at the second position, and 108 (75.5%) were variable at the third position. Table 2 shows the percent sequence divergence for all pairwise comparisons, correcting for multiple substitutions, by using the method of Kimura (1980).

Phylogenetic analyses of the cytochrome b data were performed to evaluate the relationships among these nine Stenodermatini genera. The topology presented in figure 4 is the result of a bootstrap analysis of 100 iterations in which transversion substitutions were assigned a weight 10 times greater than transition substitutions. This analysis resulted in a single most parsimonious tree of 1,338 mutational events. The gl statistic of 10,000 random trees (−0.375) indicates that the distribution is highly skewed to the left, which suggests with high probability that the correct topology is either the most parsimonious tree or a few steps longer (Hillis 1991; Huelsenbeck 1991; Hillis and Huelsenbeck 1992). Irrespective of whether transversions were assigned a greater weight than transitions or of the magnitude of the weight assigned to transversions, the resulting trees always reflected a closer relationship among representatives of Artibeus, Dermanura, and Koopmania to each other than to any other Stenoder-
matini bat examined in this study. For all weighting schemes, examination of trees longer than the most parsimonious tree revealed the same close relationship for the representatives of *Artibeus*, *Dermanura*, and *Koopmania*. The differences among the less parsimonious trees concerned the relationships of all other stenodermatine taxa examined and not the relationships among *Artibeus*, *Dermanura*, and *Koopmania*.
Table 1
Representatives of the Orders Dermoptera and Chiroptera That Were Used in Southern Blot Analyses to Examine the Phylogenetic Distribution of the EcoRI-defined Satellite DNA Repeat

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<td>Tribe Stenodermatini—<em>Carollia, Uroderma, Vampyrophes, Platyrhinus, Vampyressa, Mesophylla, Sturnira, Enchisthenes, Ardops, Ariteus, Stenoderma, Centurio, Pygoderma, Chiroderma</em></td>
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<tr>
<td><em>Artibeus, Koopmania, Dermanura</em></td>
<td>Present</td>
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Discussion

The New World bat tribe Stenodermatini is an evolutionarily complex group that has received considerable systematic attention with very diverse data sets, including allozymes (Straney et al. 1979), immunology (Honeycutt and Sarich 1987), karyology (Baker 1967, 1979; Baker et al. 1979), skeletal morphology (Walton and Walton 1968; Slaughter 1970; Owen 1987), uterine histomorphology (Hood and Smith 1982), and rDNA restriction-site data (Van Den Bussche 1991, 1992). However, organizing the results of these studies into unambiguous resolution of the relationships of taxa within this tribe has proved difficult. As explained below, results from two independent molecular data sets (nuclear satellite DNA and mitochondrial cytochrome b DNA sequence variation) support the same phylogenetic relationships among three problematic Stenodermatini genera (*Artibeus, Dermanura, and Koopmania*).

Satellite DNA Organization

The EcoRI-defined satellite DNA is restricted to the Stenodermatini genera *Artibeus, Dermanura*, and *Koopmania*. In these genera, the approximately 900-bp monomer is defined by the restriction endonuclease EcoRI and is not found in any other phyllostomid bat examined or in representatives of eight additional families of
bats or Dermoptera (table 1). In an overview, these results are most compatible with the systematic hypotheses that *Artibeus*, *Dermanura*, and *Koopmania* are more closely related to each other than to any other Stenodermatini taxon examined and that *Enchisthenes* is excluded from this monophyletic group (fig. 2). The organization of
Table 2
Pairwise Comparisons of Sequence Divergence Corrected for Multiple Substitutions (Kimura 1980)

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<th>Chiroderma</th>
<th>C. trinitatum</th>
<th>Sturnira</th>
<th>Artebius fuliginosus</th>
<th>Artebius lituratus</th>
<th>Dermanura azteca</th>
<th>D. tolteca</th>
<th>D. phaeotis</th>
<th>D. cinerea</th>
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<th>Koopmania</th>
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<td>28.6</td>
<td>30.0</td>
<td>31.7</td>
<td>30.8</td>
<td>31.0</td>
<td>35.8</td>
<td>...</td>
</tr>
</tbody>
</table>

Note.—Data above the diagonal are the percent sequence divergence for all pairwise comparisons; and data below the diagonal are the percent of the variation due to transversion substitutions.
this satellite DNA in the three species of *Dermanura* can be distinguished from that found in *Artibeus* and *Koopmania* by the presence of approximately 500-bp and 400-bp components identified by an additional *EcoRI* site.

The hybridization data demonstrate that these smaller DNA fragments belong to the same satellite family because they cross-hybridize with the 900-bp satellite DNA from all three genera. These observations suggest that the 500-bp and 400-bp repeats in *Dermanura* represent a subfamily of the 900-bp monomer with an internal *EcoRI* site. If this additional *EcoRI* site has evolved since the divergence from *Artibeus* and *Koopmania*, then this is a synapomorphy uniting species of *Dermanura*. 
The absence of this satellite DNA on the largest pair of chromosomes in *A. jamaicensis* may reflect a loss due to reorganization during chromosomal evolution (fig. 3). This largest chromosome pair in the *Artibeus* karyotype is the result of a fusion between chromosomes 3 and 18 from that proposed as primitive for the family (Baker 1979). The loss of heterochromatin resulting from a chromosomal rearrangement also has been observed in an *Equus hemionus* individual in which a fusion metacentric chromosome, t(23;24), lacks the intensity of heterochromatin staining that can be found on either acrocentric chromosome 23 or 24 (Ryder 1978).

**Cytochrome b**

Recently, advances based on computer simulations (Hillis 1991; Huelsenbeck 1991; Hillis and Huelsenbeck 1992) and comparisons of phylogenetic trees from various algorithms to known phylogenies (Hillis et al. 1992) have been made in determining whether data sets are providing phylogenetic information or whether the phylogenetic trees produced are the result of random noise. Such studies allow more confidence to be placed on phylogenetic trees, by examining the distribution of tree lengths generated from a large number of random trees produced from the data set and by placing 95% and 99% confidence intervals on the resultant topologies, using the $g_1$ statistic.

Sequence data from the cytochrome *b* gene show that *Dermanura, Artibeus,* and *Koopmania* share a closer relationship with each other than to any other *Stenodermatini* genus (fig. 4). Cytochrome *b* and satellite DNA data both indicate that *Artibeus, Dermanura,* and *Koopmania* are monophyletic, after diverging from the remainder of the Stenodermatini genera examined. In a study of the cranial and skeletal morphology of stenodermatine bats, Owen (1987, 1991) generated systematic hypotheses to explain the evolution of these taxa. In several of his analyses, the last common ancestor for *Dermanura* and *Artibeus* would have included the common ancestor for several other genera. This hypothesized paraphyletic nature of the genus *Artibeus* (sensu Andersen 1908) led Owen (1987) to recognize two genera (*Artibeus* and *Dermanura*). Subsequently, problems in aligning *D. concolor* with other species of *Dermanura* were interpreted as a basis for the description of the genus *Koopmania* (Owen 1991). Our results indicate that these three taxa (*Artibeus, Dermanura,* and *Koopmania; sensu Owen 1987, 1991*) did share a common ancestor after diverging from the remainder of the Stenodermatini genera. If our data are correct, the need to recognize distinct genera to avoid paraphyly does not exist.

*Enchisthenes hartii* is a problematic taxon that appears unique in many different data sets (Baker et al. 1979; Koop and Baker 1983; Owen 1987, 1991; Van Den Bussche 1992). Although numerous studies have demonstrated the uniqueness of *Enchisthenes hartii*, the problem has been that from a cladistic standpoint, there are no character states that document that *Enchisthenes hartii* did not share a common ancestor with *Dermanura* after separating from all other stenodermatine genera. This question is resolved by both satellite DNA and cytochrome *b* data sets. *Artibeus, Dermanura,* and *Koopmania* share a centromeric satellite DNA repeat defined by a 900-bp EcoRI site. *Dermanura,* the taxon most likely to be associated with *Enchisthenes,* contains the additional inferred EcoRI site within the 900-bp monomer. *Enchisthenes* shares none of these synapomorphies. The cytochrome *b* data place *Artibeus, Dermanura,* and *Koopmania* as monophyletic. As with the satellite data set, *Enchisthenes* does not share the level of sequence identity that forms the basis of the *Artibeus/Koopmania/Dermanura* complex. The fact that both nuclear and mitochondrial data
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Document this nesting of *Artibeus, Dermanura,* and *Koopmania* to the exclusion of *Enchisthenes* provides the critical evidence that the *Enchisthenes* lineage was distinct from its proposed congener. *Enchisthenes* is best characterized as a genus with unique karyology (Baker et al. 1979), allozymes (Koop and Baker 1983), morphology (Owen 1987, 1991), and rDNA restriction-site data (Van Den Bussche 1992), as well as the molecular data described herein.

Although the use of satellite DNA for systematic studies has failed to provide phylogenetic information for some taxa, this study can be added to those in which satellite DNA provides valuable phylogenetic information, especially when used in conjunction with independent data sets or when compared with a well-documented phylogeny (Peacock et al. 1981; Bogenberger et al. 1987; Hamilton et al. 1990, 1992). Although for a few of these taxa discrepancies exist between the phylogenetic relationships reported in this study and other previously published phylogenies, there is support, for many of these relationships, based on data sets as diverse as morphology, karyology, and rDNA restriction-site data. Whereas some of these discrepancies may reflect differences in the choice of species representative of the taxa examined, satellite DNA and cytochrome *b* data have added to our understanding of the phylogenetic history of this evolutionarily complex group of bats.

**Sequence Availability**

These sequences have been deposited in GenBank under accession numbers L19505–L19520.

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**Appendix**

Tissues from the following specimens were obtained from the frozen tissue collection at The Museum, Texas Tech University and the Museum of Southwestern Biology, University of New Mexico. TK and NK numbers cross-reference laboratory records and tissues to voucher specimens deposited in The Museum, Texas Tech University and the Museum of Southwestern Biology, University of New Mexico, respectively.

**Order Dermoptera:** *Cynocephalus volans* (TK 21407)—Thailand: Surat Thani Province, Tha Chang District, 15 km N, 23 km W Ban Muruan.

**Order Chiroptera: Family Pteropodidae:** *Rousettus* sp. (TK 27199)—Kenya: Western Province, Kakamega District, 6 km S, 6 km W Kakamega. *Nyctimene* sp. (TK 20095)—Papua New Guinea: East New Britain Province, Gela Gela Plantation. *Macroglossus* (TK 20305)—Papua New Guinea: Central Province, Lakoke Quarantine Station, 9 km NE Port Moresby. *Family Megadermatidae:* *Megaderma lyra* (TK 21288)—Thailand: Uthi Thani Province, Lansak District, Huai Kha Khang Wildlife Sanctuary, Tam Khe Nok, 3.6 km N, 2.6 km W sanctuary headquarters. *Family Nycteridae:* *Nycteres graudis* (TK 21558)—Gabon: Estuarie Province, 2 km SE Cape

**LITERATURE CITED**


bat genus *Phyllostomus* based on cytochrome b DNA sequence variation. J. Mammal. 74: 793–802.


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