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# Mitochondrial and nuclear DNA sequence data provide resolution to sister-group relationships within *Pteronotus* (Chiroptera: Mormoopidae)

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Whereas it is generally agreed that the Neotropical bat family Mormoopidae, as well as the two mormoopid genera (*Mormoops* and *Pteronotus*) are each monophyletic, relationships among the six extant species of *Pteronotus* remain unresolved. The purpose of this study was to evaluate phylogenetic relationships within *Pteronotus* using DNA sequence data from the mitochondrial ribosomal and cytochrome *b* genes and the nuclear Recombination Activating Gene-2 based on likelihood inferential techniques (maximum likelihood and Bayesian phylogenetics). Results of this study present, for the first time, a fully resolved and strongly supported phylogeny for all relationships within *Pteronotus*. These data strongly support: sister-group relationships between *davyi* and *gymnonotus* (subgenus *pteronotus*), between *macleayii* and *quadridens* (subgenus *chilonycteris*), and between the subgenera *pteronotus* and *chilonycteris*. *Pteronotus personatus* is sister to this clade and *P. parnellii* is the most basal lineage of *Pteronotus*. Although this is the first study to provide a fully-resolved and strongly supported hypothesis for the phylogenetic relationships among species of *Pteronotus*, future work must focus on phylogeographic surveys within each species because previous studies have suggested that *parnellii* and *personatus* may contain undescribed species.

*Key words*: *Mormoopidae*, *Pteronotus*, Bayesian phylogenetics, rDNA, RAG-2, cytochrome *b*

## INTRODUCTION

With recent controversies between morphological and molecular evidence many phylogenetic studies are faced with uncertainty of monophyly of the taxon understudy. For example, the validity of several traditional morphological groupings including Archonta, Ferrungulata, Glires, Paenungulata, Chiroptera, Megachiroptera, and Microchiroptera (Novacek and Wyss, 1986; Novacek, 1992) have recently been questioned based on molecular data (e.g., Nikaido *et al*., 2000; Teeling *et al*., 2000, 2002; Lin and Penny, 2001; Madsen *et al*., 2001; Murphy *et al*., 2001*a*, 2001*b*; Hulva and Horáček, 2002). This problem is not restricted to higher taxonomic levels as monophyly of several genera also has come under question. For example, within families of bats, the question of monophyly arises for *Eptesicus*, *Pipistrellus*, *Artibeus*, *Micronycteris*, *Tonatia*, and *Phyllostomus* (Van Den Bussche and Baker, 1993; Van Den Bussche *et al*., 1993, 1998; Volleth and Heller, 1994; Simmons and Voss, 1998; Baker *et al*., 2000; Volleth *et al*., 2001; Kearney *et al.,* 2002; Porter *et al*., In press). One taxon that

has sufficient morphological and chromosomal synapomorphies to make it highly unlikely it is diphyletic is the mormoopid genus *Pteronotus*.

Mormoopidae (Mammalia: Chiroptera) contains two genera (*Mormoops* and *Pteronotus*) and eight extant species (*M. blainvilli*, *M. megalophylla*, *P. davyi*, *P. gymnonotus*, *P. macleayii*, *P. parnellii*, *P. personatus*, and *P. quadridens*) restricted to the New World. Karyotypic studies document that all species of *Pteronotus* and *Mormoops* possess identical diploid and fundamental numbers and share a unique chromosomal fission that occurs in no other chiropteran family (Patton and Baker, 1978; Baker and Bickham, 1980; Sites *et al*., 1981). Chromosomal banding studies further support monophyly of *Pteronotus* based on a unique G-banded karyotype shared by all six species and monophyly of *Mormoops* due to a single difference in banding pattern between the karyotype of *Mormoops* and *Pteronotus* (Sites *et al*., 1981). Although no study has seriously questioned monophyly of *Pteronotus*, species-level relationships within the genus remain unresolved.

Smith (1972) proposed the first explicit phylogeny for relationships within Mormoopidae in which he partitioned species of *Pteronotus* into the subgenera *pteronotus* (*davyi* and *gymnonotus*), *chilonycteris* (*macleayii*, *quadridens*, *personatus*), and *phyllodia* (*parnellii*) (Fig. 1A). Although based on a phenetic analysis, his classification dramatically influenced the current thought on mormoopid evolution. To test the phylogenetic hypothesis of Smith (1972), Simmons and Conway (2001) performed a parsimony analysis of 209 morphological characters. Although they concluded that their results support Smith's (1972) subgenera, only the sister-group relationship of *davyi* and *gymnonotus* was strongly supported (Fig. 1B). Even though Simmons and Conway (2001) provide a cautionary statement regarding their results, they proposed taxonomic diagnoses of all mormoopid clades based on their shortest tree.

Shortly after the publication by Simmons and Conway (2001), Lewis-Oritt *et al*. (2001) published results of their phylogenetic analysis of mormoopid relationships based on parsimony and distance-based analyses of DNA sequence data from the mitochondrial cytochrome *b* and nuclear Recombination Activating Gene-2 (RAG-2). Their combined tree revealed that *parnellii* represents the most basal lineage and the remaining five species comprised an unresolved trichotomy (Fig. 1C). They interpreted their results as supporting Smith's (1972) subgenera *phyllodia* (*parnellii*) and *pteronotus* (*davyi* and *gymnonotus*), however, because they detected a sister-group relationship between *macleayii* and *quadridens*, with *personatus* representing a separate lineage within this trichotomy, their data failed to support Smith's (1972) composition of *chilonycteris*.

In a subsequent attempt to provide resolution to the phylogenetic relationships within *Pteronotus*, Van Den Bussche *et al*. (2002) generated approximately 3 kilobases (kb) of mitochondrial ribosomal (mtrDNA) gene sequence data from representatives of each species of Mormoopidae and combined these data with the 209 morphological characters of Simmons and Conway (2001). Results of this combined morphological and mtrDNA sequence data support monophyly of Mormoopidae, *Mormoops*, *Pteronotus*, and sister-group relationships between *quadridens* and *macleayii* and between *davyi* and *gymnonotus* yet, there still remained an unresolved polytomy of ((*davyi*, *gymnonotus*), *parnellii*, *personatus*, (*macleayii*, *quadridens*)) (Fig. 1D).

Thus, there have been several recent attempts to provide resolution to the phylogenetic relationships among the six species

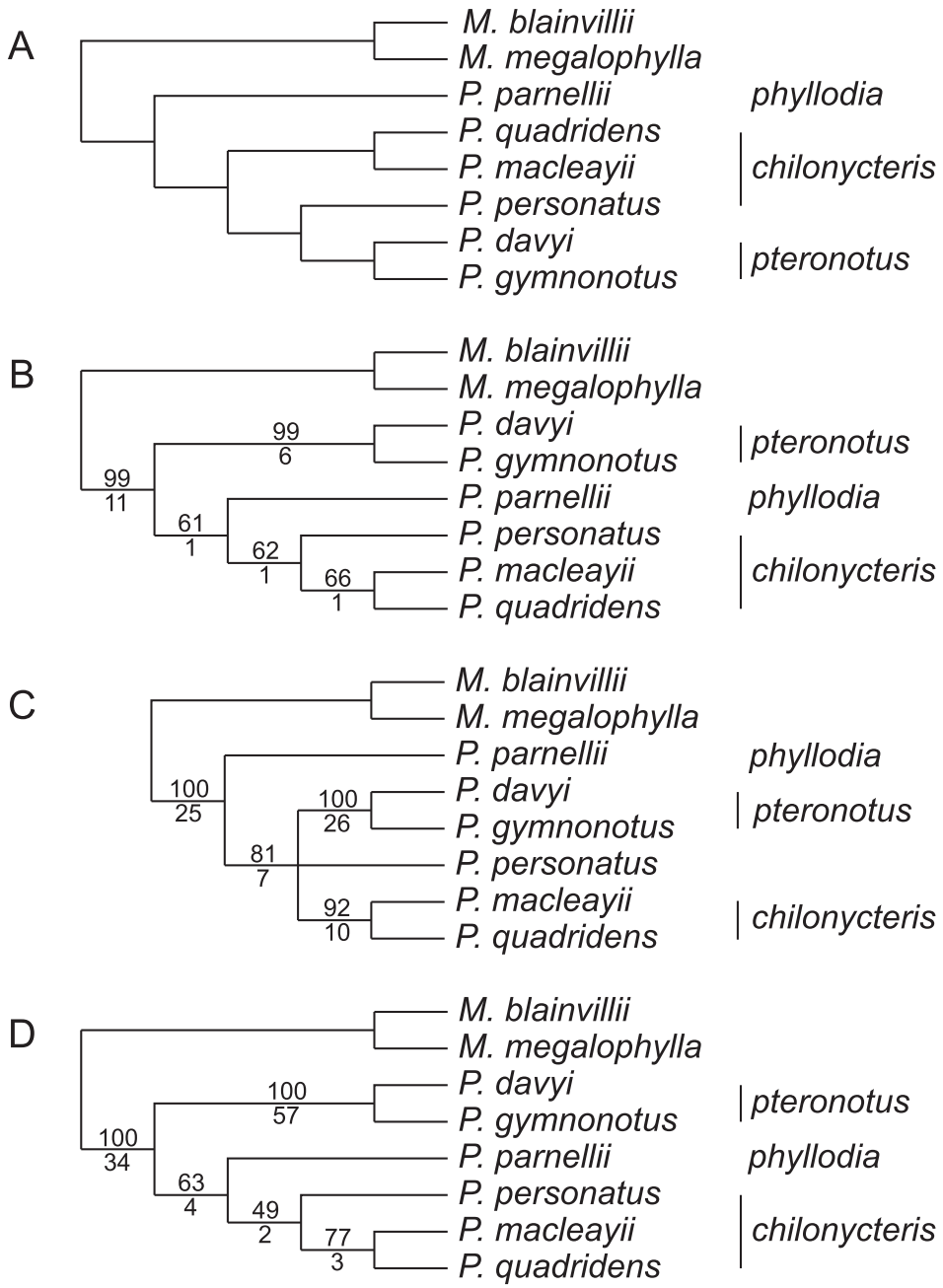


FIG. 1. (A). Redrawing of Smith's (1972) phenetic analysis depicting the phylogenetic relationships within Mormoopidae and his proposed subgeneric classification for species of *Pteronotus*. (B). Redrawing of Simmons and Conway (2001) parsimony analysis of 209 morphological characters and their proposed subgeneric groupings. (C). Redrawing of Lewis-Oritt *et al*. (2001) results of their combined cytochrome *b* and RAG-2 maximum parsimony analysis depicting the phylogenetic relationships within Mormoopidae and proposed subgeneric designations. (D). Redrawing of the phylogenetic relationships detected by Van Den Bussche *et al*. (2002) based on parsimony analysis of mitochondrial ribosomal sequences and the 209 morphological characters with subgeneric groupings. Abbreviations: *M*. = *Mormoops*, *P*. = *Pteronotus*. For trees B, C, and D, numbers above internal lineages are bootstrap percentages and numbers below are decay values

of *Pteronotus,* but with the exception of the sister-group relationship of *davyi* and *gymnonotus* and of *macleayii* and *quadridens*, no consensus for phylogenetic relationships among these six species has been reached (Fig. 1).

The lack of a well-resolved and strongly supported phylogeny for relationships within *Pteronotus* has hampered attempts to understand the biogeographic history of this group. Additionally, with the resurgence in re-coupling functional morphology and evolution (Adams and Pedersen, 2000), understanding the evolution of morphological and ecological traits unique to this taxon is difficult based on our current understanding of the phylogenetic relationships within this genus. This lack of phylogenetic resolution is due, at least in part, to fairly recent speciation in *Pteronotus* resulting in insufficient time for the development of a sufficient number of synapomorphies documenting sister-group relationships. Thus, we are faced with the difficulty of determining phylogenetic relationships based on few shared-derived characters. However, recent advances in statistical modeling of DNA sequence evolution provide alternative and more powerful approaches for elucidating phylogenetic relationships than parsimony or distance-based algorithms used in previous studies (Lewis-Oritt *et al*., 2001; Van Den Bussche *et al*., 2002). Moreover, it has been shown that a linear increase in phylogenetic resolution and possibly statistical support over the entire tree can best be obtained by increasing the amount of data examined per taxon (Goldman, 1998). Therefore, concatenating the three previously generated molecular datasets into a single 'meta-sequence' analysis and analyzing these data with likelihood-based inferential techniques that allow the use of more complex models of DNA sequence evolution should result in a more accurate and robust phylogenetic estimate with increased statistical confidence (Gaut and Lewis, 1995; Huelsenbeck, 1995; Whelan *et al*., 2001; Alfardo *et al*., 2003). Finally, because the genetic distance between *Mormoops* and *Pternotus* of 15% is nearly as great as the 16–20% detected among representatives of other noctilionoid families (Van Den Bussche *et al*., 2002: table 1), using representatives of Noctilionoidea as outgroups to polarize character-state changes within *Pteronotus* (e.g., Lewis-Oritt *et al*., 2001; Van Den Bussche *et al*., 2002), may have resulted in increased levels of homoplasy causing the lack of phylogenetic resolution for sister-group relationships within *Pternotus*. Because the monophyly of Mormoopidae, *Mormoops*, nor *Pternotus* has seriously been questioned, we used the two species of *Mormoops* as outgroups to polarize character-state changes within *Pteronotus*.

Therefore, to provide resolution to the phylogenetic relationships among species of *Pteronotus* we re-analyzed each of the three previously generated molecular data sets independently (Lewis-Oritt *et al*., 2001; Van Den Bussche *et al*., 2002) and also combined all three independently derived molecular data sets, polarized character-state changes using *M. blainvillii* and *M. megalophylla* as outgroups, and phylogenetically analyzed these data with appropriate models of DNA sequence evolution that extract from the data the maximum amount of information (Lewis, 2001; Whelan *et al*., 2001).

## Materials and Methods

Sequences for the mitochondrial 12S rRNA, $tRNA^{Val}$, 16S rRNA, cytochrome *b*, and the nuclear RAG-2 gene, respectively, were obtained from GenBank for the following taxa: *Mormoops blainvilli* (AF407172, AF338685, AY028169), *M. megalophylla* (AF407173, AF338688, AF338702), *Pteronotus davyi* (AF407176, AF338671, AF338692), *P. gymnonotus* (AF407177, AF338674, AF338694), *P. macleayii* (AF407178, AF338683, AF338700), *P. parnellii* (AF407180, AF330807, AF330817),

*P. personatus* (AF407182, AF338680, AF338699), and *P. quadridens* (AF407179, AF338681, AF338695). Sequences of the three regions were not available from the same individual for *M. megalophylla* or *P. gymnonotus* thus we concatenated sequences from two individuals for each of these taxa. For each of the three data sets (mtrDNA, cytochrome *b*, RAG-2), sequences were aligned using default options in CLUSTAL X (Thompson *et al*., 1997) and imported into MacClade (Maddison and Maddison, 1992) for visual inspection. For mtrDNA, those regions potentially violating the assumption of positional homology were excluded from all phylogenetic analyses. All nucleotides were coded as one of four discrete character states (A, T, G, C) and gaps in the mtrDNA alignment were coded as missing data. For phylogenetic analysis, character-state changes were polarized by designating *M. blainvillii* and *M. megalophylla* as outgroups.

## *mtrDNA*

Alignment of mtrDNA sequences was accomplished using default parameters in CLUSTALX with the resulting alignment refined based on published secondary structural models (Anderson *et al*., 1982; De Rijk *et al*., 1994; Springer and Douzery, 1996) and, with few exceptions, alignment gaps occurred in loop regions. The most appropriate model of DNA sequence evolution and model parameters (as determined via modeltest; Posada and Crandall, 1998) for the mtrDNA data were: GTR + Γ + I; base frequencies = 0.3582, 0.2232, 0.1858, 0.2328; R-Matrix = 9.4469, 26.0791, 8.0184, 0.1923, 86.1086; shape parameter ($\alpha$) of the gamma distribution = 0.5274, and proportion of variable sites = 0.5740. These model parameters were used in a maximum likelihood analysis using PAUP* (Swofford, 2000). The starting tree was obtained via random addition with the Branch-and-Bound option and tree-bisection-reconnection (TBR) branch-swapping. Reliability of clades on the optimal tree was evaluated through bootstrap analysis with 500 iterations.

As a second measure of reliability of clades, we performed Bayesian phylogenetic analyses (MRBAYES; Hulsenbeck and Ronquist, 2001). For Bayesian analysis, we used the GTR + Γ + I model of sequence evolution as determined via Modeltest, however, we did not define values for model parameters *a priori*, but treated them as unknown variables with uniform priors to be estimated in each analysis. Bayesian analysis was conducted with random starting trees without constraints, four simultaneous Markov chains were run for 1,000,000 generations, trees were sampled every 10 generations, and temp was set to 0.02. Resulting burnin values (the point at which model parameters and tree score reach stationarity) were empirically determined based upon evaluation of likelihood scores. Finally, three independent runs of MRBAYES were conducted to ensure that final trees converged upon the same topology.

## *Cytochrome b*

Alignment of cytochrome *b* sequences was performed using CLUSTALX and these data were analyzed using maximum likelihood and Bayesian phylogenetics as described above. For maximum likelihood analysis, Modeltest choose the GTR + Γ + I model of sequence evolution with the following parameters as most appropriately fitting our data: base frequencies = 0.3238, 0.3751, 0.1151, 0.1860; R-Matrix = 57129.13, 447322.03, 94336.63, 23868.34, 1507731.38; $\alpha$ = 1.1971, and proportion of invariant sites = 0.5881. Bayesian analysis for the cytochrome *b* data were performed as described above with the exception that we used site-specific rate model by partitioning the data into each of the three positions of the codon (Huelsenbeck and Ronquist, 2001).

## *RAG-2*

Alignment of RAG-2 sequences was performed using CLUSTALX and these data were analyzed using maximum likelihood and Bayesian phylogenetics as described above. For maximum likelihood analysis, Modeltest choose the HKY + Γ model of sequence evolution with the following parameters as most appropriately fitting our data: base frequencies = 0.2970, 0.2265, 0.2168, 0.2597; transition: transversion ratio = 4.4820, and $\alpha$ = 0.0152. Bayesian analysis for the cytochrome *b* data were performed as described above with the exception that we used site-specific rate model by partitioning the data into each of the three positions of the codon (Huelsenbeck and Ronquist, 2001).

## *Combined Analysis*

Because our ultimate objective was to perform a phylogenetic analysis combining all three data sets, it was necessary to evaluate the degree of taxonomic congruence among these data sets. Traditionally, such tests of taxonomic congruence have been accomplished by performing independent phylogenetic analyses and then testing for congruence among resulting phylogenies using the incongruence-length-difference test (ILD — Mickevich and Farris, 1981; Farris *et al*., 1994). However, the ILD test has

recently come under criticism as a poor measure of congruence, especially when data sets are of different sizes (Yoder *et al*., 2001; Dowton and Austin, 2002). Therefore, we used a more straightforward approach to assess data combinability in which results from independent analyses are first examined for clades that may be in strong conflict among competing trees (Weins, 1998; Leaché and Reeder, 2002; Hoofer *et al*., In press). If many such strongly supported conflicts exist, then the resulting combined analysis should reflect unresolved relationships for such taxa in the combined analysis, due to different underlying phylogenetic histories. For our analyses, we considered only those clades receiving bootstrap support of ≥ 70% and Bayesian posterior probabilities of ≥ 0.95 to be strongly supported. An advantage of this approach is that it assumes that weakly supported conflict may be due to stochastic error and does not preclude data set combination when only a few clades exhibit strongly supported conflict among independent data (Weins, 1998; Leaché and Reeder, 2002; Hoofer *et al*., In press). Using this approach, the tree or trees from the combined analysis are considered the best estimate of species phylogeny.

Modeltest choose the GTR + Γ + I model of sequence evolution with the following parameters as best explaining our data: base frequencies = 0.3291, 0.2514, 0.1840, 0.2355; R-Matrix = 9.2660, 21.4954, 5.0508, 1.6526, 63.5032; $\alpha = 0.5271$, and proportion of invariant sites = 0.5556. Maximum likelihood and Bayesian analyses of the combined data were performed as described above with the exception that we used site-specific rate model by partitioning the data into each of the three positions of the codon for each of the protein coding genes and using a seventh partition to represent the mtrDNA data (Huelsenbeck and Ronquist, 2001).

## Results

### *mtrDNA*

Alignment of 12S rRNA, $tRNA^{Val}$, and 16S rRNA genes resulted in 2,643 aligned positions of which 225 were excluded from phylogenetic analysis due to potentially violating positional homology. Of the remaining 2,418 aligned positions, 436 were variable. Maximum likelihood analysis resulted in an optimal tree of -6554.21 that strongly supported sister-group relationships of *macleayii* and *quadridens* as well as *davyi* and *gymnonotus* (Fig. 2A). Bayesian phylogenetic analyses were in agreement between the three independent runs and burnin values for each run were 3,500 trees, resulting in 96,500 trees for phylogenetic analysis. Results of Bayesian analysis produced a topology identical to the topology resulting from the maximum likelihood analysis and corroborated results from maximum likelihood analysis by supporting the same sister-group relationships with posterior probabilities ≥ 0.98 (Fig. 2A).

### *Cytochrome b*

Alignment of cytochrome *b* resulted in 1,140 aligned positions of which 358 were variable. Of these variable sites, 41, 21, and 296 occurred at first, second, and third positions of the codon, respectively. Maximum likelihood analysis resulted in an optimal tree of -4525.04 depicting sister-group relationships between *macleayii* and *quadridens*, between *davyi* and *gymnonotus*, and between *parnellii* and *personatus* however, only the sister-group relationships between *davyi* and *gymnonotus* and between *macleayii* and *quadridens* received bootstrap support of about 70% or greater (Fig. 2B). Results of three Bayesian analyses were in excellent agreement and the burnin value of 5,000 resulted in 95,000 trees for the evaluation of clade composition. Topology of the Bayesian tree was identical to the topology produced by maximum likelihood analysis and the sister-group relationships between *davyi* and *gymnonotus* and between *macleayii* and *quadridens* were each supported with a posterior probability = 1.00 (Fig. 2B).

### *RAG-2*

Alignment of RAG-2 sequences resulted in 1,362 aligned positions of which 112 were variable. Of the 112 variable sites, 16,

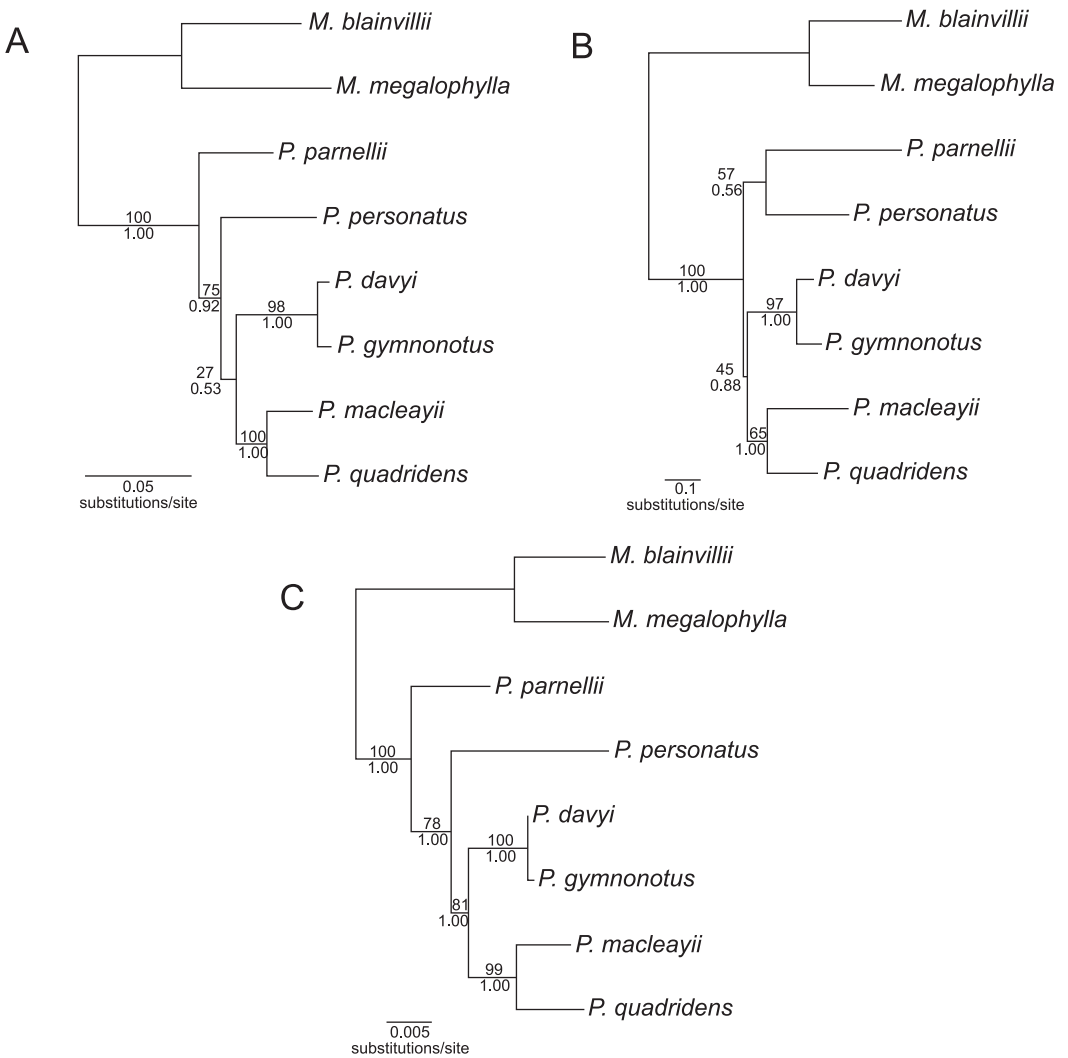


FIG. 2. Optimal topologies resulting from maximum-likelihood phylogenetic analysis of (A) mitochondrial rDNA sequences (ln = -6554.21), (B) mitochondrial cytochrome *b* DNA sequences (ln = -4525.04); and, (C) nuclear Recombination Activating Gene-2 DNA sequences (ln = -2664.88). Numbers above each node document the percentage of 500 bootstrap iterations that each node was detected; numbers below each node represent Bayesian posterior probabilities. Abbreviations: *M*. = *Mormoops*, *P*. = *Pteronotus*

5, and 91 were at first, second, and third positions of the codon, respectively. Maximum likelihood analysis resulted in an optimal tree of -2664.88 which was fully resolved for all relationships within *Pteronotus* and all relationships on the shortest tree were supported by bootstrap values ≥ 78% (Fig. 2C). Bayesian parameters were in excellent agreement among all three independent runs and burnin values were empirically determined to be 3,100 trees, resulting in 96,900 trees for evaluation of phylogenetic relationships and clade composition. Resulting topology from Bayesian analysis was identical to that produced by maximum likelihood analysis and all clades were supported by posterior probabilities of 1.00 (Fig. 2C).

*Combined Analysis*

Because no strong conflict exists among topologies resulting from the independent analyses (Fig. 2), we concatenated all three alignments into a single file. Maximum likelihood analysis resulted in an optimal tree of -14020.37 with a topology identical to that produced by the RAG-2 data and bootstrap analysis detected strong support for most clades (Fig. 3). Bayesian analysis with site-specific rate variation was in excellent agreement among the three runs and stationarity was reached (burnin value) at 3,700 trees, resulting in phylogenetic relationships and clade composition to be evaluated based on 96,300 trees. The topology resulting from the Bayesian analysis was identical to that produced based on maximum likelihood and all clades were supported with posterior probabilities of 1.00 (Fig. 3).

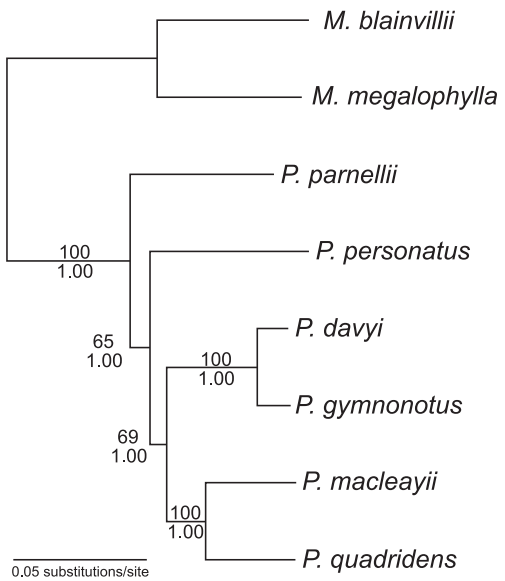


FIG. 3. Optimal topology resulting from maximum-likelihood phylogenetic analyses of the concatenated mtrDNA, cytochrome *b*, and nuclear RAG-2 DNA sequences documenting phylogenetic relationships among species of *Pteronotus* (ln = -14020.37). Numbers above each node document percentage of 500 bootstrap iterations that each clade was detected; numbers below each node represent Bayesian posterior probabilities. *M.* = *Mormoops*, *P.* = *Pteronotus*

## DISCUSSION

Although many different character sets have been used in attempts to elucidate interspecific relationships within *Pteronotus*, providing unambiguous resolution to the phylogenetic relationships within this genus has been difficult. Chromosomal data suggest that the six species of *Pteronotus* form a natural assemblage but provide no resolution to the phylogenetic relationships within this genus (Patton and Baker, 1978; Baker and Bickham, 1980; Sites *et al*., 1981). More recently, morphological, molecular, and combined morphological and molecular data have been used to assess phylogenetic relationships within *Pteronotus* but these data have also provided little phylogenetic resolution (Lewis-Oritt *et al*., 2001; Simmons and Conway, 2001; Van Den Bussche *et al*., 2002). The only areas of agreement among recent phylogenetic studies of *Pteronotus* are the sister-group relationships of *davyi* with *gymnonotus* and *macleayii* with *quadridens* (Lewis-Oritt *et al*., 2001; Simmons and Conway, 2001; Van Den Bussche *et al*., 2002).

Based on analysis of the three independent data sets using complex models of DNA sequence evolution coupled with likelihood based inferences and Bayesian phylogenetics, each tree strongly supports sister-group relationships between *davyi* and *gymnonotus* and between *macleayii* and *quadridens* (Fig. 2). However, the relationship of these two sister-groups to each other and to *parnellii* and *personatus* remains problematic (Fig. 2). Although these relationships remain unresolved among independent data sets, the resulting phylogenies are not in conflict because with the exception of the sister-group relationships of *macleayii* and

*quadridens* and *davyi* and *gymnonotus*, only the RAG-2 data provide statistically significant support for any other clades (Fig. 2). Therefore, we concatenated all three data sets into a single file for a 'total molecular evidence' assessment of the phylogenetic relationships within *Pteronotus*.

The use of statistically appropriate models of DNA evolution and tree-searching (Lewis, 2001; Whelan *et al*., 2001) from approximately 4.9 kb of mitochondrial and nuclear DNA sequence data, coupled with the use of closely related taxa to polarize character-state changes, resulted in a well-resolved and strongly supported phylogeny for relationships within *Pteronotus* (Fig. 3). Although two clades did not meet our established criteria for strong support (bootstrap support ≥ 70% and Bayesian posterior probabilities ≥ 0.95) by having bootstrap support of 65% and 69%, we still consider these clades to be strongly supported for the following reasons. First, Alfaro *et al*. (2003) conducted simulation studies on the behavior of bootstrap and Bayesian posterior probabilities under 18 scenarios involving different tree topologies and rates of evolution and concluded that "an internode having a very high posterior probability but a moderate bootstrap value should be interpreted as an internode that has a high probability of being correct, conditional on the data that has been collected so far (and model of evolution)". Second, although Alfaro *et al*. (2003) found that Bayesian analyses did assign high posterior probabilities for short internodes more often than bootstrapping, the overall rate of assigning high support to such incorrect nodes was extremely low. Because the topology presented in figure 3 is the same as obtained using maximum likelihood analyses under complex models of DNA sequence evolution that did not account for codon positions as well as Bayesian analyses that not only included complex models of sequence evolution but also partitioned the data into positions of the codon (for cytochrome *b* and RAG-2) as well as positions of the codon and mtrDNA for the combined analyses, lends additional support for the relationships depicted in figure 3 and argues against the possibility that the high posterior support is due to problems associated with the Bayesian approach. Thus, the more complex nature of these models allows us to place greater support in the combined tree for posterior probabilities over maximum likelihood bootstrap support. Moreover, Alfaro *et al*. (2003) found that bootstrap analyses had a very difficult time supporting the correct phylogeny when there existed very few synapomorphies as is the case in this study. Finally, as pointed out by Alfaro *et al*. (2003) bootstrap support is best interpreted as reflecting the sensitivity of observed results to the sampling error associated with collecting characters whereas Bayesian posterior probabilities represent a more accurate confidence measure, under the conditions of the probabilistic model of DNA sequence evolution and the observed data. Although we feel comfortable in placing greater weight in the results from the Bayesian analyses, it is necessary to remember that any phylogenetic tree represents a hypothesis that requires further testing with new data.

In agreement with the conclusion of Smith (1972) and Lewis-Oritt *et al*. (2001), results of our combined analyses place *parnellii* as the most basal and most divergent lineage of *Pternotus*. The basal placement of *parnellii* relative to other species of *Pternotus* is also consistent with previous karyological and morphological studies (Smith, 1972; Patton and Baker, 1978) and has considerable implications regarding the evolution of echolocation in mormoopids (Novick, 1963; Vater, 1998, 2000). With the exception of *P. parnellii*, mormoopid bats produce frequency-modulated (FM)

echolocation signals that provide a measurement of target distance and detailed information about target texture (Vater, 2000). In contrast, the echolocation of *parnellii* comprises a combination of long constant-frequency (CF) and FM signals (Vater, 2000). This form of echolocation (referred to as CF-FM) is also found in species of the Old World genera *Rhinolophus* and *Hipposideros*. Bats with CF-FM echolocation are characterized by Doppler-shift compensation, made possible through the evolution of unique specializations in organs necessary for sending and receiving such high frequency sound. It has been suggested that this type of echolocation evolved to allow hunting insects in densely cluttered spaces and is optimized to small periodic changes in both the frequency and amplitude of echoes from wing beats of prey insects (Vater, 2000). The presence of CF-FM echolocation in both rhinolophoids and *P. parnellii* is believed to be due to convergent evolution. The fact that our tree places *parnellii* as the most basal lineage of *Pteronotus* is interpreted as indicating that the common ancestor of Mormoopidae possessed the more typical CF echolocation and the evolution of CF-FM echolocation in *parnellii*, along with the morphological adaptations of the cochlea associated with this form of echolocation evolved after *parnellii* diverged from the remainder of the mormoopids.

Also in agreement with previous studies (Smith, 1972; Lewis-Oritt *et al*., 2001; Simmons and Conway, 2001; Van Den Bussche *et al*., 2002), *davyi* and *gymnonotus* as well as *macleayii* and *quadridens* represent natural assemblages and their placement in the subgenera *pternotus* and *chilonycteris*, respectively, are well supported (Figs. 2 and 3). Aside from these commonalities, all other relationships proposed in our study are discordant with other phylogenetic hypotheses for species of *Pteronotus* (Fig. 1). Smith (1972) and Simmons and Conway (2001) included *personatus* within the subgenus *chilonycteris* whereas Lewis-Oritt *et al*. (2001) proposed that *personatus* represented an undescribed subgenus. Our data are in agreement with the conclusion of Lewis-Oritt *et al*. (2001), as *personatus* does not appear to be more closely related to *macleayii* and *quadridens* (*chilonycteris*), as was proposed by Smith (1972), Simmons and Conway (2001) and Van Den Bussche *et al*. (2002) or to the subgenus *pteronotus* and does not form a sister-group relationship with *parnellii*. Finally, our data strongly support a sister-group relationship between the subgenera *pteronotus* (sensu Smith, 1972) and *chilonycteris* (sensu Lewis-Oritt *et al*., 2001).

Smith (1972) proposed that the center of origin of mormoopid radiation was either southern Middle America or northwestern South America and that the Greater Antilles were probably invaded from the Middle America region. Results of our phylogenetic analysis are compatible with this interpretation as *parnellii*, our most basal lineage, is broadly distributed, occurring from the Greater Antilles and tropical Mexico to northeastern Brazil (Koopman, 1993, 1994; Emmons, 1997; Reid, 1997), and that *personatus*, our next most basal lineage, is broadly distributed in Central and South America (Bowles *et al*., 1979; Koopman, 1993, 1994; Emmons, 1997; Reid, 1997). Regarding the zoogeographic history of the remaining four species (*davyi*, *gymnonotus*, *macleayii*, *quadridens*), our phylogeny is interpreted as indicating a slightly different biogeographic scenario than proposed by Smith (1972). However, because we included only a single exemplar of each taxon, coupled with the high degree of genetic variation previously detected in *parnellii* (Lewis-Oritt *et al*., 2001; Van Den Bussche *et al*., 2002) and *personatus* (Lewis-Oritt *et al*., 2001), any biogeographic scenario to

explain current distributions of species of *Pteronotus* throughout Mexico, Central and South America, and the Antilles must await more thorough phylogenetic and phylogeographic studies. It will be necessary to elucidate whether *parnellii* and *personatus* comprise as of yet undescribed cryptic species, as well as to conduct more taxon-dense sampling of all species of *Pternotous* to obtain a better estimate of intra-specific geographic variation.

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