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Molecular systematics of Vampyressine bats (Phyllostomidae: Stenodermatinae) with comparison of direct and indirect surveys of mitochondrial DNA variation

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Abstract

Approximately 29 species in seven genera (*Chiroderma*, *Mesophylla*, *Platyrrhinus*, *Uroderma*, *Vampyressa*, *Vampyriscus*, and *Vampyrodes*) compose the Subtribe Vampyressina, a group of New World leaf-nosed bats (Phyllostomidae) specialized in fruit-eating. A recent study of restriction-site variability within the mitochondrial ND3–ND4 gene region contrasts with other molecular data, including sequence data from other mitochondrial genes, by suggesting that the monotypic genus *Ectophylla* (*E. alba*) also is member of the group and is related closely to *Mesophylla*. In this study, we address possible explanations for why the restriction-site data appear to contradict other molecular data by performing phylogenetic analysis of DNA sequence variation (direct survey) in the ND3–ND4 region and cytochrome *b* gene and by re-assessing ND3–ND4 restriction-site variability in the known sequences (indirect survey). Results from analysis of sequence data reject the *Ectophylla–Mesophylla* hypothesis ($P < 0.001$) and suggest four primary lineages within Vampyressina: (1) *Mesophylla–Vampyressa*; (2) *Chiroderma–Vampyriscus*; (3) *Platyrrhinus–Vampyrodes*; and (4) *Uroderma*. We also find no support for the *Ectophylla–Mesophylla* hypothesis in our re-analysis of ND3–ND4 restriction-site variability, and suggest the differences between molecular studies have a methodological basis.

Keywords: Cytochrome *b*; *Ectophylla*; *Mesophylla*; ND3–ND4; Phyllostomid bats; Restriction-sites; Vampyressines

1. Introduction

Vampyressine bats comprise approximately 29 species of the phyllostomid genera *Chiroderma*, *Mesophylla*, *Platyrrhinus*, *Uroderma*, *Vampyressa*, *Vampyriscus*, and *Vampyrodes* (subfamily Stenodermatinae: subtribe Vampyressina—Baker et al., 2003). Morphologically, *Ectophylla alba* shares close affinities with *Mesophylla macconnelli*, and also has been recognized as part of the vampyressines (Wetterer et al., 2000). Relationships among vampyressine bats continue to be debated and have proven difficult to resolve with either morphological or molecular data (e.g., Owen, 1987; Porter and Baker, 2004; Wetterer et al., 2000), perhaps reflecting a rapid and contemporaneous radiation among these fruit-eating specialists. Most of the debate seems rooted in "molecules versus morphology," and centers around two entangled issues about monophyly and rank status of *Vampyressa* and *Vampyriscus*, and their relationships with *Chiroderma*, *Ectophylla*, and *Mesophylla*. For example, nearly all morphological studies suggest a sister relationship between *Ectophylla* and *Mesophylla* (e.g., Lim, 1993; Wetterer et al., 2000), whereas nearly all molecular studies suggest a sister relationship between *Mesophylla* and *Vampyressa*, and a distant relationship for *Ectophylla* (e.g., Baker et al., 2003; Greenbaum et al., 1975).

We know of just three exceptions. One study of craniodental characters agrees with the molecular consensus suggesting a sister relationship between *Mesophylla* and

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Vampyressa (Starrett and Casebeer, 1968), whereas one study of restriction-site data agrees with the morphological consensus suggesting a sister relationship between *Ectophylla* and *Mesophylla* (Lim et al., 2003). A third study (Owen, 1987), of mensural and discrete-state morphological characters, does not agree with either consensus and suggests several novel relationships (e.g., *Mesophylla–Vampyriscus nymphaea* sister relationship). In this paper, we focus on the study of Lim et al. (2003) to help uncover possible explanations for why the restriction-site data appear to contradict all other molecular data, yet support previous morphological hypotheses.

Lim et al. (2003) examined mitochondrial DNA (mtDNA) variation indirectly by mapping restriction-sites within the ND3–ND4 region (approximately 2400 bp) from partial digests (Morales et al., 1993) of 13 unique enzymes. Their study included relatively few vampyressine taxa, and focused on a single species, *Vampyressa pusilla*. The primary conclusion Lim et al. (2003) made was recognizing the northern forms (<20° latitude south) of *V. pusilla* as a distinct species called *V. thyone*. They also draw conclusions about higher-level relationships within and among ingroup and outgroup genera (Fig. 1). For example, Lim et al. (2003) conclude that there are synapomorphies uniting *Ectophylla* and *Mesophylla*, and that the restriction-site data provide positive molecular evidence supporting the traditional morphological view of relationships.

Their molecular study therefore contrasts with much of what is known about vampyressine relationships based on essentially all other molecular data, including another mtDNA study focused on vampyressines. Porter and Baker (2004) examined mtDNA variation directly by analyzing sequences of the cytochrome *b* gene (1140 bp) in 30 specimens representing all genera of vampyressines, samples of

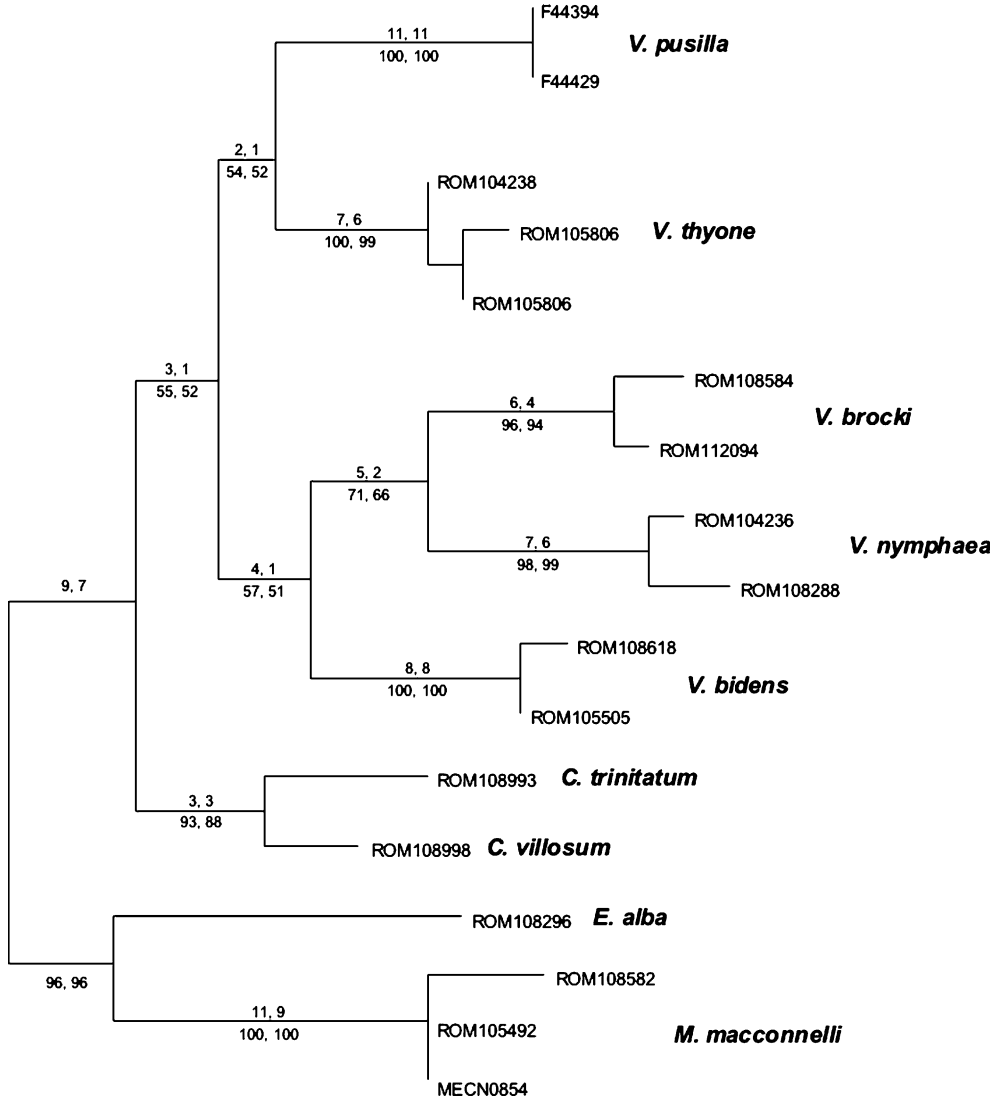

Fig. 1. Most-parsimonious phylogram reconstructed from Fig. 1 of Lim et al. (2003). Their results were based on parsimony analysis of restriction-site data in the ND3–ND4 gene region of *Vampyressa* (= "*V.*" using their nomenclature) with *Chiroderma*, *Ectophylla*, and *Mesophylla* as outgroups. Numbers above branches are branch lengths and decay vlaues, and those below are bootstrap and jackknife percentages from 1000 iterations. Branch lengths also correspond to number of character changes.

V. thyone and *V. pusilla* (as recognized by Lim et al., 2003), and several other stenodermatine genera (*Artibeus*, *Dermanura*, *Ectophylla*, *Enchisthenes*, and *Sturnira*). Their phylogenetic analyses support five major clades (Fig. 2): (1) *Platyrrhinus–Uroderma*; (2) *Vampyrodes*; (3) *Chiroderma*; (4) *V. pusilla–V. thyone–Mesophylla*; and (5) *Vampyriscus*. Overall, results from Porter and Baker (2004) and Lim et al. (2003) agree in recognizing *V. thyone* as a distinct species, but disagree in recognizing *Vampyriscus* (*bidens* and *brocki*) as a genus distinct from *Vampyressa* and in recognizing a close relationship between *Ectophylla* and *Mesophylla* or between *Ectophylla* and any other vampyressine.

Two biological explanations for this discrepancy between mtDNA studies are (1) the ND3–ND4 gene region and cytochrome *b* gene have separate phylogenetic histories or (2) information gathered by direct and indirect surveys of mtDNA variation differ in phylogenetic signal. On the other hand, the discrepancy might be for reasons unrelated to biology such as taxonomic sampling, outgroup choice, or methods of data gathering and analysis. We address these alternative explanations in this study by inferring relationships of all putative vampyressine genera through phylogenetic analysis of DNA sequence variation in the ND3–ND4 region (2400 bp) and cytochrome *b* gene (1140 bp) and through re-assessing restriction-site variability from known DNA sequences. In addition, we provide the first estimate of relationship at the molecular level for *Vampyressa melissa*.

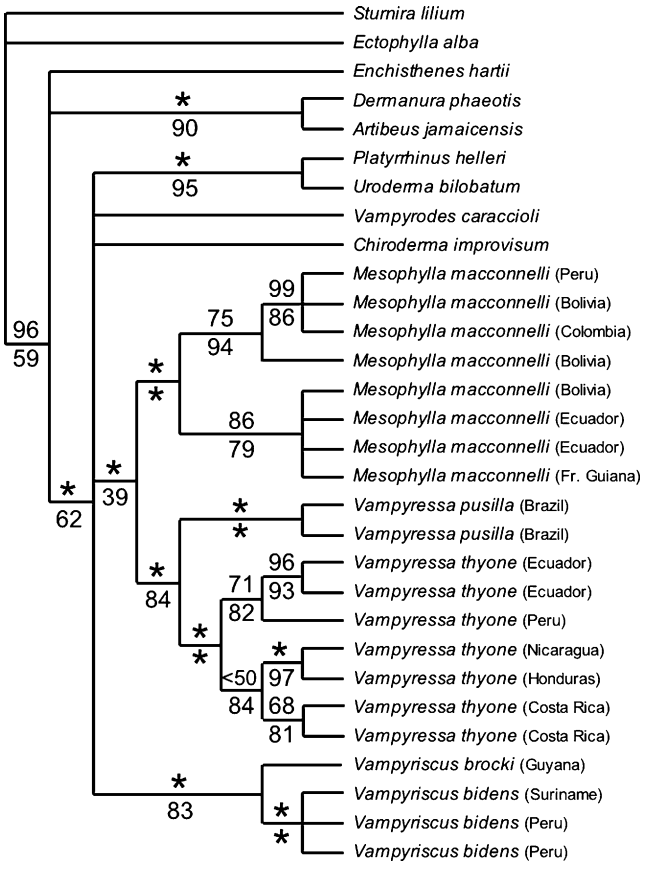

Fig. 2. Cladogram reconstructed from the supported branching order in Figs. 1–3 of Porter and Baker (2004). Their results were based on Parsimony, Bayesian, and Neighbor-Joining analyses of complete cytochrome *b* gene sequences with *Sturnira* as the outgroup. Asterisks above branches denote Bayesian posterior probabilities >0.96, whereas numbers below are parsimony bootstrap percentages. Only nodes supported by $P \geqslant 0.95$ or bootstrap percentage $\geqslant 50$, or both, are shown.

2. Methods and materials

2.1. Specimens examined

Specimens examined are listed in Appendix A, including information associated with museum vouchers. We generated complete ND3–ND4 sequences for 35 individuals and complete cytochrome *b* gene sequences for 30 individuals. We also retrieved 35 cytochrome *b* sequences archived in GenBank, which originally were generated by Baker et al. (1994), Hoffmann et al. (2003), Porter and Baker (2004), Van Den Bussche et al. (1998), and Wright et al. (1999). Lists of specimens examined including voucher information are accessible in each of those publications and Appendix A.

We used sequences from *Rhinophylla* and *Sturnira* as outgroups for analyses of both ND3–ND4 and cytochrome *b* data, as previous morphological and molecular studies agree that both taxa are outgroups to the remainder of taxa in this study (Baker et al., 2000, 2003; Lim, 1993; Porter and Baker, 2004; Wetterer et al., 2000). We inferred relationships among ingroup species representing all putative vampyressine genera, including *Ectophylla*, as well as other stenodermatines.

2.2. Molecular methods

We PCR amplified a mitochondrial DNA fragment approximately 2400 bp long encompassing the ND3, ND4L, and ND4 genes and the $tRNA^{Arg}$ that intervenes ND3 and ND4L by using primers 772 and 773 (Cronin et al., 1993) and two new, nested primers developed for phyllostomid bats, 772 *bat* and 773 *bat* (Table 1). Sometimes we used these primer pairs in a two-round, nested design to achieve optimal amplifications. We used primers 772 and 773 in the first round PCR using a 50 µl reaction, approximately 500 ng DNA, 0.26 µM each primer, 2.0 mM $MgCl_2$, 0.16 mM dNTPs, 1× final buffer concentration, and 0.75 U FailSafe PCR Enzyme Mix (Epicentre Biotechnologies, Madison, Wisconsin). We heated the reactants at 94 °C for 2′, then amplified for 35 cycles by denaturing at 94 °C for 40″, annealing at 45 °C for 2′, and extending at 72 °C for 3′; we included a final extension at 72 °C for 15′. If necessary, we used 1–2 µl of above PCR products and primers 772 *bat* and 773 *bat* in a second PCR using a 50 µl reaction, 0.26 µM each primer, 2.0 mM $MgCl_2$, 0.16 mM dNTPs, 1× final buffer concentration, and 1.2 U *Taq* DNA polymerase (Promega, Madison, Wisconsin). We heated the reactants at 95 °C for 2′, then amplified for 30 cycles by denaturing at 95 °C for 30″, annealing at 50 °C for 30″, and extending at 72 °C for 2′; we included a final extension at 72 °C for 15′.

Table 1
Forward (L) and reverse (U) primers for PCR amplification (denoted by *) and cycle sequencing of the mitochondrial ND3–ND4 region

Primer	Primer Sequence 5′–3′	Author
772*	TAA YTA GTA CAG YTG ACT TCA AA	Cronin et al. (1993)
773*	TTT TGG TTC CTA AGA CCA AYG GAT	Cronin et al. (1993)
772*bat**	GAC TTC AAA TCA RYT AGY TYC G	This study
773*bat**	GGC ATA GAR TTA GCA GTT CYT GC	This study
L450	ATA AAY ATA YTY HTA GC	This study
L451	AYA AGT GAT TTC GAC TCA C	This study
L452	ATT ATC TAA TGT CYC TYA C	This study
L730	TAG TAA TAG TMA TCH ACY ACY TAY GG	This study
L1400	AAR GCY CAY GTA GAA GCY CC	This study
Lend	WAA YYT WGC RCT YCC RCC	This study
Lend-2	TAY GAR CGA GTR CAY AGC CGA AC	This study
U500	TAG GAT TAT TGT TCG GCT	This study
U500-2	TAG GAT TAT TGT TCG GCT RTG	This study
U900	AAR TAR AGY CCT GCR TTY A	This study
U900-2	CGY TCT GCT TGR TTR CCY CAT CG	This study
Uend	ATA GGT RAG RGA CAT WAG A	This study
Uend-2	TTA YCA TAR TCT AGT GAG TCG	This study

We purified double-stranded PCR amplicons by using a QIAquick PCR Purification Kit (Qiagen, Chatsworth, California) and sequenced both strands by using Big-Dye version 3.1 chain terminators, followed by electrophoresis on a 3100-*Avant* Genetic Analyzer (Applied Biosystems, Foster City, California). We used appropriate external primers and a combination of several internal primers that we developed specifically for phyllostomid bats (Table 1) to sequence each strand entirely, and used AssemblyLIGN 1.0.9 software (Oxford Molecular Group PLC, 1998) to assemble resulting, overlapping fragments.

We amplified the entire cytochrome *b* gene (1140 bp) by PCR. We used external and internal primers and PCR conditions and thermal profile of Hoffmann and Baker (2001). We purified, sequenced, and assembled resulting fragments as described above.

2.3. Phylogenetic analysis

We performed multiple sequence alignments for both data sets in Clustal X software (Thompson et al., 1997) with default parameters for costs of opening and extending gaps. We viewed alignments in MacClade software (version 4.0; Maddison and Maddison, 2002) to ensure there were no insertions/deletions or stop codons in the protein coding portions and to inspect gap placement in the $tRNA^{Arg}$. We coded nucleotides as unordered, discrete characters, and multiple states as polymorphisms. In PAUP* software (test version 4.0b10; Swofford, 2002), we examined level of phylogenetic signal in each gene via the g_1-statistic (Hillis and Huelsenbeck, 1992) for 100,000 randomly drawn trees.

We inferred phylogenetic relationships by Bayesian analysis implemented in MrBayes 2.01 software (Huelsenbeck and Ronquist, 2001) and by maximum likelihood, minimum evolution, and parsimony analyses implemented in PAUP* software (test version 4.0b10; Swofford, 2002). We inferred relationships by analyzing complete sequences for the ND3–ND4 region and cytochrome *b* gene separately and in combination; all mitochondrial genes are linked and should have identical phylogenetic histories (Brown, 1985; Wiens, 1998). The general time reversible (GTR) model with allowance for gamma distribution of rate variation (Γ) and for proportion of invariant sites (I) best fit the ND3–ND4 and cytochrome *b* data, separately and combined, based on Hierarchical Likelihood Ratio Tests implemented in Modeltest 3.06 software (Posada and Crandall, 1998).

For Bayesian analysis, we ran 2×10^6 generations with 1 cold and 3 incrementally heated Markov chains, random starting trees for each chain, and trees sampled (saved) every 100 generations. We treated model parameters as unknown variables (with uniform priors) to be estimated in each Bayesian analysis (Leaché and Reeder, 2002). We ran sets of three independent analyses for each specified outgroup (*Rhinophylla*, *Sturnira*) with burn-in values (initial set of unstable generations to be ignored) based on empirical evaluation of likelihoods converging on stable values. We calculated a 50% majority-rule consensus tree from the sample of stabilized trees in PAUP* software (test version 4.0b10; Swofford, 2002) and obtained branch lengths via the "sumt" option in MrBayes software (Huelsenbeck and Ronquist, 2001). We assessed clade reliability via posterior probabilities and regarded values $\geqslant 0.95$ as significant.

For maximum likelihood and minimum evolution analyses, we used the GTR+Γ+I model and parameters (given by Modeltest), performed full heuristic searches with Neighbor Joining starting trees, tree-bisection-reconnection branch swapping, and allowance for negative branch lengths. For Parsimony analysis, we treated all characters and substitution types with equal probability and conducted full heuristic searches with 10 random additions, starting trees by simple addition, and tree-bisection-reconnection branch swapping. We assessed clade reliability via bootstrapping with 250 iterations for minimum evolution and parsimony analyses (Felsenstein, 1985). Due to computation time, we performed bootstrapping analysis under maximum likelihood for 100 iterations on just the combined dataset.

2.4. Hypothesis testing

We tested five genealogical hypotheses that have been proposed previously, but were absent in the 50% majority-rule consensus tree from Bayesian analysis: (1) monophyly of subtribe Ectophyllina (sensu Wetterer et al., 2000); (2) monophyly of genus *Ectophylla* (sensu Wetterer et al., 2000; = sister relationship for *Ectophylla* and *Mesophylla*); (3 and 4) monophyly of genus *Vampyressa* (sensu Koopman, 1994 and sensu Baker et al., 1989); (5) monophyly of genus *Mesophylla* (sensu Owen, 1987). To do this we searched for presence of genealogical hypotheses in the sample of

suboptimal trees from Bayesian analysis. Genealogical (null) hypotheses can be estimated at a statistical level because the frequency of trees in the sample agreeing with the null hypothesis equals the probability of the null hypothesis being correct. We calculated this frequency in PAUP* software (test version 4.0b10; Swofford, 2002) by applying constraint-based filter trees (Ihlen and Ekman, 2002).

2.5. Restriction-site mapping and analysis

We identified restriction enzyme motifs for each of the 13 enzymes employed by Lim et al. (2003) by searching our sequence alignment in a simple-text software program: 4 bp cutters—*Alu*I, *Bst*Z17I, *Dpn*II, *Hae*III, *Hha*I, *Hpa*II, *Nla*III, *Rsa*I, and *Taq*I; 6 bp cutters—*Aat*II, *Bst*UI, *Nde*I, and *Pst*I. We created a presence/absence matrix for identified cut-sites of each enzyme in MacClade 4 software (version 4.05; Maddison and Maddison, 2002), and subsequently concatenated them into one matrix. We analyzed these data with Parsimony criterion in PAUP* software (test version 4.0b10; Swofford, 2002) using two general approaches: (1) using the same taxonomic sampling, outgroup designations, and Parsimony methods as Lim et al. (2003); (2) using the taxonomic sampling and outgroup designations we employed above for our sequence data. This design should allow assessment of the relative merits of both partial endonuclease mapping and the role that taxonomic sampling and outgroup choice have when inferring genealogy and taxonomy of vampyressines.

3. Results

3.1. ND3–ND4 and cytochrome b

Complete sequence of the ND3, ND4L, and ND4 genes, and the intervening $tRNA^{Arg}$, is 2086 base pairs for all 34 sequences we generated (GenBank Accession Nos. DQ312362–DQ312395): 1–348, ND3; 349–416, $tRNA^{Arg}$; 417–713, ND4L; 707–2086, ND4. Complete sequence of the cytochrome *b* gene is 1140 bp for all 35 sequences we generated (GenBank Accession Nos. DQ312397–DQ312431) and the 31 we retrieved from GenBank. Sequence alignment was unequivocal even for $tRNA^{Arg}$. For the ND3–ND4 region, 806 of the 2086 characters (= sites) are parsimony informative, whereas 409 of the 1140 cytochrome *b* characters are parsimony informative. Nucleotide variation is distributed across codon positions within each of the four genes as expected for protein-coding genes (Table 2). Levels of phylogenetic signal are significant based on the g_1 statistic ($P < 0.01$–Hillis and Huelsenbeck, 1992) for each gene separately (ND3, −0.72; $tRNA^{Arg}$, −0.48; ND4L, −0.64; ND4, −0.85; cyt *b*, −0.532).

For both ND3–ND4 region and cytochrome *b* data sets, Bayesian likelihoods reached stationarity before 100,000 generations (i.e., burn-in = 1000), thinning our data to 9000 sample points. Topology and posterior probabilities for nodes and model parameters for all sets of runs (3 runs each) within data sets agreed regardless of choice of outgroup. Maximum likelihood analysis resulted in a single best tree for both ND3–ND4 (Ln1 = −16,272.69) and cytochrome *b* (Ln1 = −10,554.71) data sets. Minimum evolution analysis resulted in three least-evolved trees (score = 5.20) and 12 least evolved trees (score = 1.82) for ND3–ND4 and cytochrome *b* data sets, respectively. Parsimony analysis resulted in 10 most-parsimonious trees (length = 3510, CI = 0.38, RI = 0.66) and 56 most-parsimonious trees (length = 2218, CI = 0.32, RI = 0.75) for ND3–ND4 and cytochrome *b* data sets, respectively. Overall, there are some topological differences within and between data sets and between the four optimality criteria; however, none of the differences are supported. Statistically supported topologies (i.e., ⩾70% bootstrap value, ⩾0.95 Bayesian posterior probability) obtained from all optimality criteria agree within and between each data set (Figs. 3 and 4).

Table 2
Distribution of nucleotide variation across codon positions within each of the four protein-coding genes and within $tRNA^{Arg}$

	Length	Parsimony informative			
		1st position	2nd position	3rd position	Total
ND3	348 (1–348)	29 (23%)	7 (5%)	92 (72%)	128 (37%)
$tRNA^{Arg}$	68 (349–416)				10 (15%)
ND4L	297 (417–713)	24 (22%)	5 (4%)	81 (74%)	110 (37%)
ND4	1380 (707–2086)	135 (24%)	42 (8%)	381 (68%)	558 (40%)
Cyt *b*	1140	70 (17%)	20 (5%)	319 (78%)	409 (36%)

3.2. Combined ND3–ND4 and cytochrome b

We combined ND3–ND4 and cytochrome *b* sequences because there are no supported conflicts between them (Wiens, 1998). The combined dataset (3226 bp) includes the 32 specimens shared between data sets. It also includes two other species (*Chiroderma trinitatum* and *C. villosum*) that, in both cases, includes cytochrome *b* data from one specimen and ND3–ND4 data from another specimen. Bayesian likelihoods reached stationarity before 100,000 generations as above, and topology and posterior probabilities for nodes and model parameters for all sets of runs (three runs each) agreed regardless of substitution model or outgroup choice. Maximum Likelihood analysis resulted in a single best tree (Ln1 = −24,328.19), Minimum evolution analysis resulted in two least-evolved trees (score = 3.48), and Parsimony analysis resulted in two most-parsimonious trees (length = 5,182, CI = 0.38, RI = 0.65). Topologies and levels of nodal support obtained from all four optimality criteria are nearly identical (Fig. 5).

3.3. Hypothesis testing

All five null hypotheses are rejected at a probability below 0.001, indicating that, based on our separate ND3–

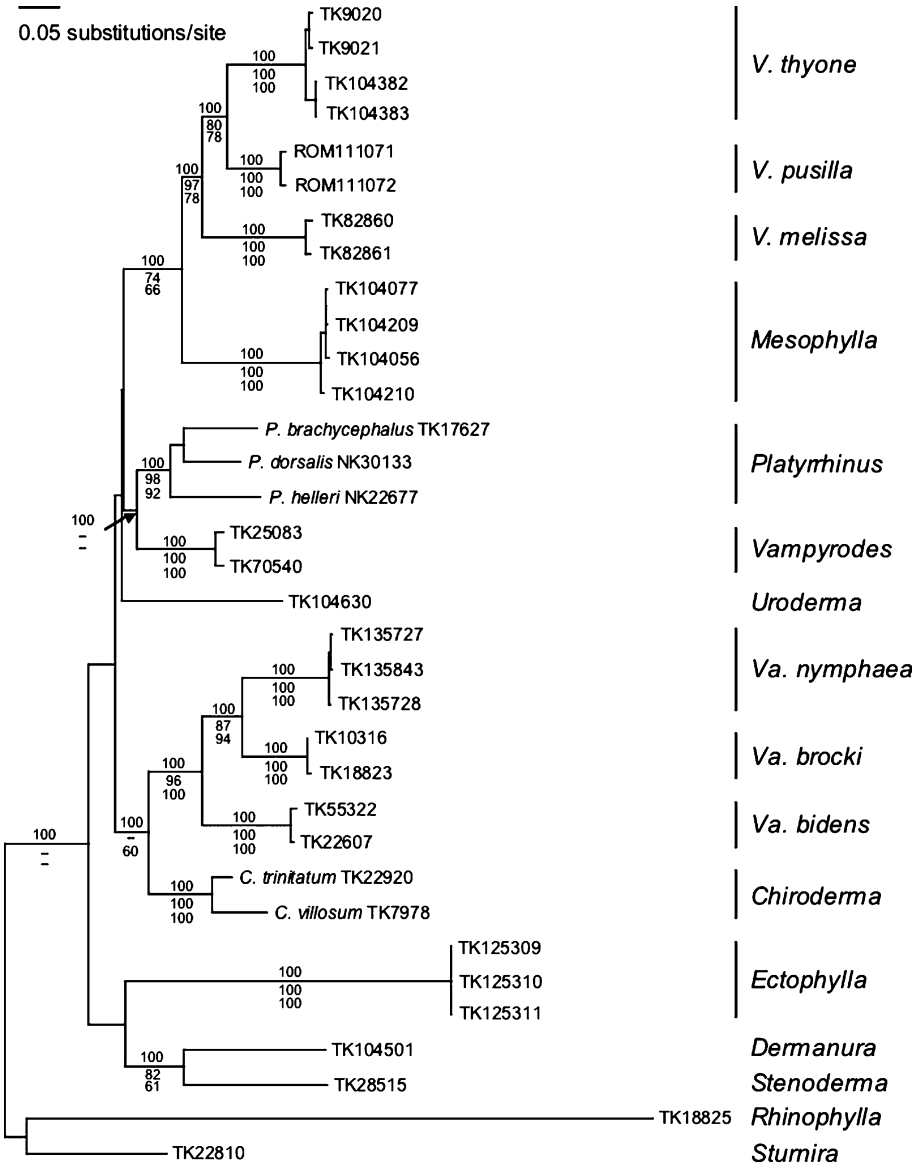

Fig. 3. Maximum likelihood phylogram (Ln l = −16,272.69) from analysis of complete sequences of the ND3–ND4 gene region (2086 bp) using best-fit model (GTR+Γ+I; $r_{AC} = 0.96$, $r_{AG} = 18.44$, $r_{AT} = 0.59$, $r_{CG} = 0.59$, $r_{CT} = 14.61$, $\pi A = 0.36$, $\pi C = 0.31$, $\pi G = 0.06$, $\pi T = 0.27$, $\alpha = 0.79$, $P_{inv} = 0.40$). We designated *Rhinophylla* and *Sturnira* as outgroups. Numbers above branches are Bayesian posterior probabilities, whereas those below are bootstrap percentages from minimum evolution and parsimony, respectively. Values are shown only for nodes supported by $P \geqslant 0.95$ or bootstrap percentage $\geqslant 50$, or both. "*V.*" = *Vampyressa*, "*Va.*" = *Vampyriscus*.

ND4 and cytochrome *b* sequence data, the subtribe Ectophyllina (sensu Wetterer et al., 2000), genus *Ectophylla* (sensu Wetterer et al., 2000), genus *Vampyressa* (sensu Koopman, 1994 or sensu Baker et al., 1989), and genus *Mesophylla* (sensu Owen, 1987) each are not monophyletic.

3.4. Restriction-site mapping and analysis

We identified 165 unique cut sites (133 parsimony informative) in the ND3–ND4 sequence alignment of 34 specimens (Appendix A) via simple text searches of cut-sites for 13 restriction enzymes, whereas we identified 124 cut sites (110 parsimony informative) when searching only taxa sampled by Lim et al. (2003). Following are numbers of cut sites per enzyme, with numbers for taxa sampled by Lim et al. (2003) shown in parentheses: 0 (0) *Aat*II, 42 (29) *Alu*I, 3 (1) *Bst*UI, 2 (2) *Bst*Z17I, 21 (14) *Dpn*II, 23 (18) *Hae*III, 7 (4) *Hha*I, 4 (3) *Hpa*II, 3 (1) *Nde*I, 29 (25) *Nla*III, 1 (1) *Pst*I, 17 (15) *Rsa*I, and 13 (11) *Taq*I. g_1 statistic of −0.866 and −0.991 for overall and truncated taxon sets, respectively, are skewed significantly left ($P < 0.01$), indicating strong phylogenetic signal (Hillis and Huelsenbeck, 1992). Parsimony analysis of all 34 taxa resulted in 19 most-parsimonious trees (length = 363; CI = 0.45; RI = 0.68). Parsimony analysis using taxonomic sampling scheme and outgroup choice of Lim et al. (2003) resulted in 12 most-parsimonious trees (length = 185; CI = 0.65; RI = 0.83). Fifty-percent majority-rule consensus for both analyses produced moderate resolution of relationships, but most relationships received no support from bootstrap analysis (Fig. 6). These

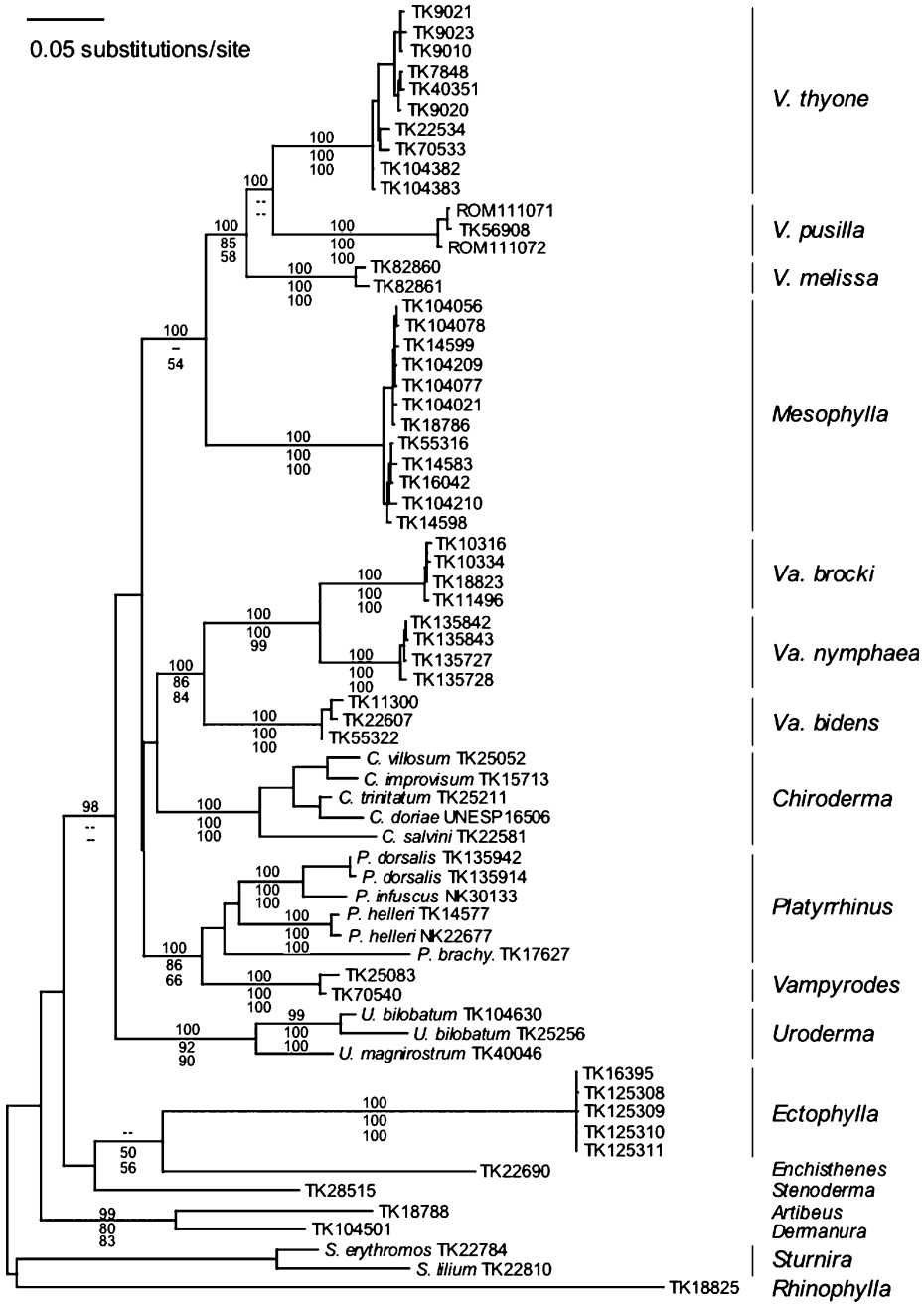

Fig. 4. Maximum likelihood phylogram (Ln l = −10,554.71) from analysis of complete cytochrome *b* sequences (1140 bp) using best-fit model (GTR+Γ+I; $r_{AC} = 1.29$, $r_{AG} = 15.99$, $r_{AT} = 1.00$, $r_{CG} = 1.10$, $r_{CT} = 22.08$, $\pi A = 0.33$, $\pi C = 0.34$, $\pi G = 0.08$, $\pi T = 0.24$, $\alpha = 1.09$, $P_{inv} = 0.54$). We designated *Rhinophylla* and *Sturnira* as outgroups. Numbers above branches are Bayesian posterior probabilities, whereas those below are bootstrap percentages from minimum evolution and parsimony, respectively. Values are shown only for nodes supported by $P \geqslant 0.95$ or bootstrap percentage $\geqslant 50$, or both. "*V.*" = *Vampyressa*, "*Va.*" = *Vampyriscus*.

data support (bootstrap value $\geqslant 70\%$) clades corresponding to species, for which we sampled >1 individual, but none of the relationships above the species level.

4. Discussion

4.1. Vampyressa pusilla and V. thyone

Our phylogenetic analyses of ND3–ND4 sequences and cytochrome *b* sequences affirm Lim et al. (2003) in distinguishing Brazilian specimens of *V. pusilla* from those of *V. thyone*. Tamura-Nei distances from ND3–ND4 sequences are <2 and <1% within *V. pusilla* and *V. thyone*, respectively, whereas the mean distance between them is 11.8%. Our cytochrome *b* distances mirror these values as well as those in Porter and Baker (2004; i.e., 11.6%). This level of cytochrome *b* divergence is indicative of species-level divergence in mammals (Bradley and Baker, 2001). Although there are little or no ND3–ND4 sequence data available for bats or other mammals to facilitate meaningful

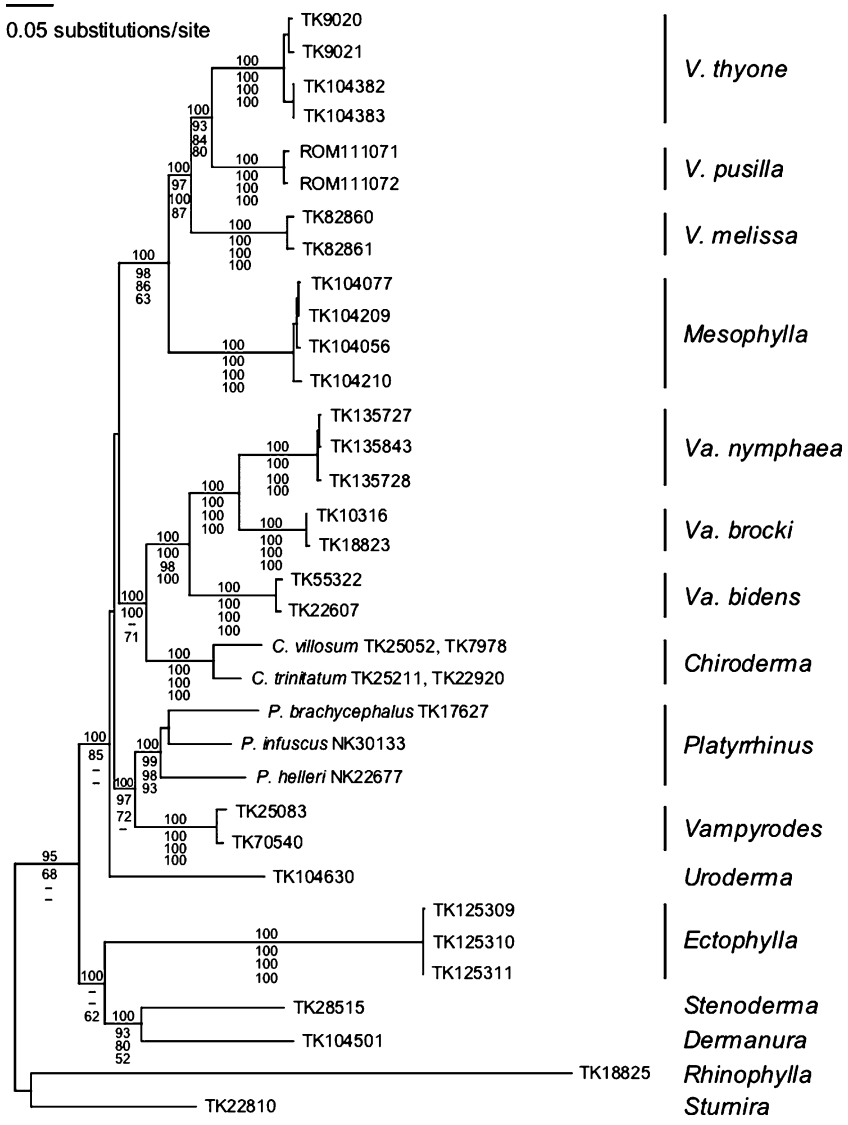

Fig. 5. Maximum likelihood phylogram (Lnl = −24,328.19) from analysis of combined ND3–ND4 and cytochrome *b* sequences (3226 bp) using best-fit model (GTR+Γ+I; $r_{AC} = 1.64$, $r_{AG} = 18.48$, $r_{AT} = 1.00$, $r_{CG} = 0.46$, $r_{CT} = 23.39$, $\pi A = 0.34$, $\pi C = 0.32$, $\pi G = 0.08$, $\pi T = 0.26$, $\alpha = 1.42$, $P_{inv} = 0.54$). We designated *Rhinophylla* and *Sturnira* as outgroups. Numbers above branches are Bayesian posterior probabilities, whereas those below are bootstrap percentages from maximum likelihood, minimum evolution, and parsimony, respectively. Values are shown only for nodes supported by $P \geqslant 0.95$ or bootstrap percentage $\geqslant 50$, or both. "*V.*" = *Vampyressa*, "*Va.*" = *Vampyriscus*.

comparisons of species-level divergences, the nearly identical distance within and between *V. pusilla* and *V. thyone* based on both of our mitochondrial data sets serves as additional positive evidence for recognizing *V. thyone*.

We also examined one specimen (TK 56908, TTU 94775) from Paraguay (cytochrome *b* only) that is clearly identified as *V. pusilla* in our analysis. To our knowledge, this is only the third specimen known from Paraguay and the only specimen from that country that has been examined at the molecular level; both specimens examined in the molecular analyses of Lim et al. (2003) and Porter and Baker (2004) are from Brazil. Lim et al. (2003) measured cranial measurements on two specimens of *V. pusilla* from the Department of Paraguarí in southern Paraguay that, based on their distribution map and species accounts, represent the only published records of *V. pusilla* in Paraguay. Apparently, few specimens of *V. pusilla* have been collected from scattered localities in the Atlantic Forest Region of extreme northeastern Argentina (Barquez et al., 1999), southern Paraguay, and southeastern Brazil (Taddei, 1979). Our specimen from Paraguay was collected in the Department of Canindeyu, approximately 200 km northeast of Paraguarí, and represents the northernmost locality of record for *V. pusilla* in Paraguay. The Department of Canindeyu is well within the Atlantic Forest Region of Paraguay, the known habitat of *V. pusilla*, southward and eastward of the dry Chaco forest and wetlands of the Pantanal, the presumed ecological barriers between *V. pusilla* and *V. thyone*. It is also noteworthy that one other specimen from Paraguay, identified as *V. pusilla* based on

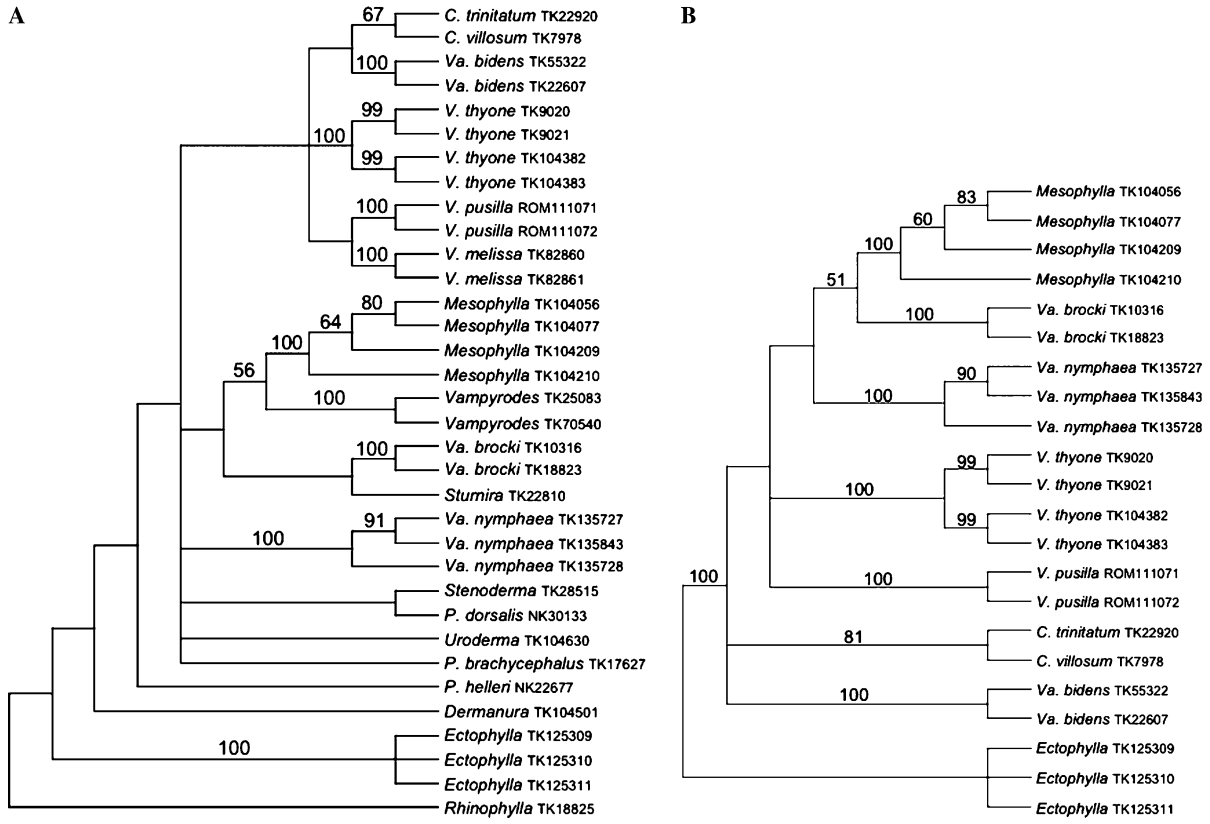

Fig. 6. Fifty-percent majority-rule trees from parsimony analysis of restriction-site variability in our ND3–ND4 sequence alignment. (A) using all taxa examined in this study, with *Rhinophylla* and *Sturnira* designated as outgroups; (B) using the taxonomic sampling scheme and outgroup choice (*Chiroderma*, *Ectophylla*, *Mesophylla*) of Lim et al. (2003). Bootstrap values ⩾ 50% are shown. "*C.*" = *Chiroderma*; "*P.*" = *Platyrrhinus*; "*V.*" = *Vampyressa*, "*Va.*" = *Vampyriscus*.

morphological comparisons, is housed in the Museum of Texas Tech University (TTU 96554). This specimen was collected in the Department of San Pedro, which borders Canindeyu to the west. Unfortunately, no tissue samples were collected from this specimen.

4.2. Vampyressa melissa

Vampyressa melissa is a rare monotypic species known previously by eight published specimens (including the type) from the eastern slope of the Andes Mountains in Peru (Emmons and Pacheco, 1997; Gardner, 1976; Koopman, 1978; Pacheco et al., 1993; Thomas, 1926) and one specimen from the western slope of the Cordillera Oriental in Colombia (Lemke et al., 1982). Some additional specimens of *V. melissa* probably exist in systematic collections of the western hemisphere. For example, Owen's (1987) specimens examined lists 10 *V. melissa* from Peru that are housed in mammal collections at the American Museum of Natural History (AMNH; 1), Louisiana State University, Museum of Natural Science (LSUMZ; 7), and University of California, Berkeley, Museum of Vertebrate Zoology (MVZ; 2). We also find through informal online queries that 13 specimens, all from Peru, are housed in LSUMZ; six of these were not reported in Gardner (1976) or Owen (1987). The specimens we examined were two in a series of *V. melissa* collected in Cusco, Peru in 1993 by B. Patterson and S. Solari. Detailed descriptions for the collecting localities can be found in Patterson et al. (in press).

The present study, the first to examine *V. melissa* at the molecular level, affirms previous studies supporting a sister relationship between *V. melissa* and *V. pusilla* (the latter species now divided into *pusilla* and *thyone*), to the exclusion of other yellow-eared bats of the genus *Vampyriscus* (Davis, 1975; Gardner, 1977; Peterson, 1968). Our separate and combined analyses of ND3–ND4 and cytochrome *b* sequences strongly support *V. melissa* as the basal lineage of genus *Vampyressa*, from which diverged the common ancestor of *V. pusilla* and *V. thyone* (Figs. 3–5). Furthermore, our analyses provide no support for Owen's (1987) classification distinguishing *V. melissa* from *Vampyressa* and other vampyressine genera in a new unnamed genus and unnamed subtribe.

4.3. Vampyressa–Vampyriscus

Rank status of *Vampyriscus* (*bidens*, *brocki*, and *nymphaea*) and whether or not its species and those of *Vampyressa* (*melissa*, *pusilla*, and *thyone*) shared a most recent common ancestry has been debated extensively. Several studies of morphological (Goodwin, 1963; Owen, 1987, 1988; Wetterer et al., 2000) and molecular (e.g., Baker et al., 1973, 2003; Porter and Baker, 2004) data suggest that

Vampyressa and *Vampyriscus* together do not form a natural assemblage. However, most major classificatory syntheses, past and present, recognize them as such by relegating *Vampyriscus* subgeneric rank within *Vampyressa* (e.g., Corbet and Hill, 1991; Jones and Carter, 1976; Koopman, 1993, 1994; McKenna and Bell, 1997; Simmons, 2005).

Our analyses support a clade including *V. melissa*, *V. pusilla*, and *V. thyone* (*Vampyressa*) and another including *V. nymphaea*, *V. brocki*, and *V. bidens* (*Vampyriscus*), and affirm previous morphological and molecular studies (see above) suggesting Vampyressa (sensu Simmons, 2005) is not monophyletic (Figs. 3–5). Separate and combined analyses of ND3–ND4 and cytochrome *b* sequences support a sister relationship between *Mesophylla* and *Vampyressa*, a relationship documented repeatedly with morphological (Starrett and Casebeer, 1968), karyological (Baker et al., 1973; Gardner, 1977; Greenbaum et al., 1975), mtDNA sequence (Baker et al., 2003; Porter and Baker, 2004), and nuclear DNA sequence (Baker et al., 2000, 2003) data. Our analyses also support a sister relationship between *Chiroderma* and *Vampyriscus*, a relationship already documented by mitochondrial and nuclear DNA sequence data (Baker et al., 2000, 2003; Porter and Baker, 2004). These sets of relationships form the objective justification in the Baker et al. (2003) classification for recognizing *Vampyriscus* as a valid genus distinct from *Vampyressa*, rather than the alternative of relegating *Chiroderma*, *Mesophylla*, and *Vampyriscus* as subgenera of *Vampyressa*.

Furthermore, mean percent distance (Tamura-Nei) between *Vampyressa* and *Vampyriscus* for both ND3–ND4 (19.0%) and cytochrome *b* (15.6%) data sets is slightly less than the mean distance for all intergeneric comparisons (19.4%, ND3–ND4; 16.2%, cytochrome *b*), yet slightly greater than distances between several well-accepted genera (e.g., *Uroderma* versus *Chiroderma*, *Platyrrhinus*, *Stenoderma*, and *Vampyrodes*). Thus, from both cladogenic and anagenic perspectives, our results coupled with previous morphological and molecular data support generic distinction between *Vampyressa* and *Vampyriscus*.

Within *Vampyriscus*, our separate and combined analyses support a sister relationship between *V. brocki* and *V. nymphaea*, with *V. bidens* representing the basal lineage of the genus (Figs. 3–6). These relationships agree with most studies of morphology, karyotypes, and DNA sequences (Davis, 1975; Gardner, 1977; Peterson, 1968) although do contradict the morphological studies of Owen (1987) and Goodwin (1963).

4.4. Ectophylla–Mesophylla

Affinities of the monotypic genera *Ectophylla* (*E. alba*) and *Mesophylla* (*M. macconnelli*) have been the source of debate since the 1960s, which mostly is the result of incongruence between morphological and other types of data ("molecules versus morphology"). The consensus from morphological studies is that *Ectophylla* and *Mesophylla* are sister taxa. Systematists have recognized the relationship differently, with *Mesophylla* sometimes accorded subgeneric rank within *Ectophylla* (e.g., Anderson et al., 1982; Goodwin and Greenhall, 1962; Jones and Carter, 1976; Koopman and Jones, 1970; Laurie, 1955; Simmons and Voss, 1998; Wetterer et al., 2000) and sometimes accorded generic rank sister to *Ectophylla* (e.g., Hall, 1981; Koopman, 1994; Lim, 1993; Smith, 1976; Simmons, 2005). Most of these authors recognized the close relationship based on classical interpretations of skin and skeletal similarities, whereas Lim (1993) and Wetterer et al. (2000) did so based on explicit cladistic analyses.

In contrast, a sister relationship between *Mesophylla* and *Vampyressa* is suggested in studies of craniodental characters (Starrett and Casebeer, 1968); karyotypes (Baker et al., 1973; Gardner, 1977; Greenbaum et al., 1975); mtDNA sequences (Baker et al., 2003; Porter and Baker, 2004); and nuclear DNA sequences (Baker et al., 2000, 2003). Our separate and combined analyses of ND3–ND4 and cytochrome *b* sequences affirm these previous studies supporting a sister relationship between *Mesophylla* and *Vampyressa* (sensu stricto). Our analyses further suggest that the genera *Chiroderma*, *Platyrrhinus*, *Uroderma*, *Vampyressa*, *Vampyriscus*, and *Vampyrodes* all are related to *Mesophylla* more closely than to *Ectophylla*, and reject the morphological hypothesis of *Ectophylla–Mesophylla* at the $P < 0.001$ level.

To our knowledge, the study of restriction-sites by Lim et al. (2003) is the only molecular study supporting the classical *Ectophylla–Mesophylla* hypothesis. However, we find no support for it in our re-analysis of restriction-site data obtained from known ND3–ND4 sequences (Fig. 6). For this and other reasons (see section below for further discussion), we do not view results from Lim et al. (2003) as evidence for the *Ectophylla–Mesophylla* relationship.

Furthermore, the morphological evidence supporting the *Ectophylla–Mesophylla* relationship is limited. Lim (1993) found two synapomorphies uniting *Ectophylla* and *Mesophylla*, gaps between the mandibular cheekteeth and yellow thumbs, and Wetterer et al. (2000) found four, absence of facial stripes, color of noseleaf, shape of first incisor, and distribution of papillae on pharyngeal tongue. In our view, the phyletic utility of some or all of these characters is questionable, especially dental characteristics, which have long-been perceived as adaptive and unreliable phyletic criteria for higher level relationships (Hill and Topál, 1973; Topál, 1970; Van Valen, 1979). Dental features also have been cited in other vampyressine studies as support for a *Mesophylla–Vampyressa* thyone sister relationship or a distant relationship between *Ectophylla* and *Mesophylla* (Owen, 1987; Starrett and Casebeer, 1968). Furthermore, phylogenetic signal provided by these synapomorphies is weak or effectively masked in cladistic analyses including several other morphological characters. For example, vampyressine relationships in Wetterer et al. (2000) either were unresolved or supported weakly based on bootstrapping and decay analysis; the *Ectophylla–Mesophylla* relationship received a bootstrap value of 53% and

decay value of 1 in their overall parsimony analysis of all characters, and was unresolved (i.e., <50% bootstrap values) in each of the separate parsimony analyses of pelage and integument characters, craniodental characters, and tongue characters.

4.5. Platyrrhinus–Vampyrodes and Uroderma

We examined three of the 10 recognized species of *Platyrrhinus* (*brachycephalus*, *dorsalis*, and *helleri*), both recognized species of *Uroderma* (*bilobatum*, *magnirostrum*), and *Vampyrodes caraccioli*. Our separate and combined analyses of ND3–ND4 and cytochrome *b* sequences support monophyly of each genus for which we sampled >1 individual; although, monophyly of *Platyrrhinus* is weakly supported by cytochrome *b* data. All analyses also support a sister relationship between *Platyrrhinus* and *Vampyrodes* (Figs. 3–5), affirming numerous studies of morphological and molecular data (Baker, 1979; Baker et al., 1982, 2000, 2003; Jones et al., 2002; Koopman, 1994; Lim, 1993; Smith, 1976; Velazco, 2005; Wetterer et al., 2000). Our separate and combined analyses also support a position for *Uroderma* within the Vampyressina clade, although there is no supported resolution for its relationship among the other three vampyressine clades (Figs. 3–5). Some studies suggest *Uroderma* is closely related to *Artibeus* (Lim, 1993; Owen, 1987) or the clade of *Platyrrhinus–Vampyrodes* (Jones et al., 2002; Wetterer et al., 2000), and other studies leave its relationship unresolved among vampyressines (Baker et al., 2000, 2003). Thus, there is no consensus for the relationship of *Uroderma*, and our study only reaffirms its position within the Vampyressina clade.

Porter and Baker's (2004) study was somewhat unique regarding these three genera. Their analyses supported a sister relationship between *Platyrrhinus* and *Uroderma* and left the position of *Vampyrodes* unresolved relative to the rest of the vampyressines. Thus, our study directly contradicts that of Porter and Baker (2004) for relationships of *Platyrrhinus*, *Uroderma*, and *Vampyrodes*. However, we suspect that the *Platyrrhinus–Uroderma* relationship in Porter and Baker's (2004) analysis may have been spurious, resulting from inadequate sampling of taxa within those genera and *Vampyrodes*; they sampled one individual representing one species within each of the three genera. Their sampling produced relatively long branch lengths (their Fig. 3), a situation that can lead to decreased efficiency of phylogeny estimation. Whereas likelihood-based methods (e.g., GTR+Γ+I) typically help to overcome problems associated with long branches, it is better to break up potentially long branches by adding closely related taxa (Graybeal, 1998; Hillis, 1998; Poe, 1998; Swofford et al., 1996). The fact that our analysis of cytochrome *b* sequences supports a *Platyrrhinus–Vampyrodes* relationship may be because we examined several more taxa, including multiple individuals of several species within *Platyrrhinus*, *Uroderma*, and *Vampyrodes*. Furthermore, the *Platyrrhinus–Uroderma* relationship is not supported in analyses of ND3–ND4 and combined ND3–ND4+ cytochrome *b* data (Figs. 3–5). Our analysis of cytochrome *b* data agrees closely with many former studies of morphological and molecular data with regard to a sister relationship between *Platyrrhinus–Vampyrodes* (discussed above), and we believe our analysis supersedes that of Porter and Baker (2004).

4.6. Direct versus indirect surveys of variation in ND3–ND4 region

Part of our motivation in this study was to help assess why the restriction-site study of Lim et al. (2003) contradicts the molecular consensus of vampyressine relationships, yet matches previous morphological hypotheses (e.g., *Ectophylla–Mesophylla* sister relationship). One contributing factor is the potential problem associated with using a morphological phylogeny as a taxonomic framework for molecular sampling of a polyphyletic group. We have reanalyzed the presence/absence data published in Lim et al. (2003) and obtain a tree nearly identical to theirs (see our Fig. 1). Thus, the discrepancy may have a biological basis or methodological basis, or both. Below we discuss some of the possibilities.

First, particular mitochondrial genes (e.g., ND3–ND4 versus cytochrome *b*) could have different phylogenetic histories. Our study of ND3–ND4 sequence variation (direct survey) clearly indicates that this is not the case, and joins a growing list of studies documenting congruent phylogenetic signals (=histories) among different mitochondrial (e.g., cytochrome *b*, 12S rRNA, and 16S rRNA) and nuclear (e.g., *RAG2*) markers not only in vampyressines but in other groups of bats as well (e.g., Baker et al., 2000, 2003; Hoofer et al., 2003; Hoofer and Van Den Bussche, 2001, 2003; Lewis-Oritt et al., 2001; Van Den Bussche and Weyandt, 2003; Van Den Bussche et al., 2003). Also, all mitochondrial genes are linked and should have identical phylogenetic histories (Brown, 1985; Wiens, 1998).

Second, information gathered through direct (sequences) and indirect (restriction-sites) surveys of ND3–ND4 variation might differ in phylogenetic signal. Our study indicates an obvious difference in signals, one that relates to resolving power or the level of divergence at which variation in the data provide resolution. Using data for the 13 enzymes, all relationships above the species level receive no support from bootstrapping analysis (i.e., $\leqslant$70%), which also was the case for most relationships in Lim et al. (2003). Additional data using more restriction enzymes might help to alleviate this problem by providing greater resolution to intergeneric relationships. However, this kind of difference is not equivalent to "conflicting phylogenetic signal," where discordant results are statistically supported in each data set. Thus, the restriction-site data that we gathered do not support or contradict our results from analysis of sequence data or the restriction-site results in Lim et al. (2003), regardless of taxonomic sampling or outgroup choice (Figs. 1, 3–5, and 6), and are not explained most-parsimoniously by a common ancestry for *Ectophylla* and *Mesophylla* to

the exclusion of the remainder of the vampyressines. In fact, an *Ectophylla–Mesophylla* relationship receives <5% bootstrap support in restriction-site analyses using both taxonomic sampling schemes (Fig. 6). We interpret this as meaning it is less likely that the discrepancy is due to differences between sequence data and restriction-site data, but more likely due to differences between studies.

An alternative explanation for the discrepancy could have a methodological basis. Whereas Lim et al. (2003) identified a total of 94 cut-sites, we identified 124 in our sequence alignment (using their taxonomic sampling), and the source for disparate phylogenetic results appears to be variation in just five of the enzymes. In the Lim et al. (2003) data set, nine cut-sites in *Alu*I (2), *Dpn*II (1), *Hae*III (3), *Nde*I (1), and *Taq*I (2) unite *Chiroderma*, *Vampyressa*, and *Vampyriscus* (i.e., to the exclusion of *Ectophylla* and *Mesophylla*). We identify only one such cut-site (HaeIII; also present in *V. thyone*) in the ND3–ND4 sequence alignment. We simply are unable to identify all of the restriction-sites reported by Lim et al. (2003), despite the fact we identified as many or more sites for all 13 enzymes, a total of 30 more sites.

We suggest that there are three alternatives to account for the discrepancy: (1) misidentification of specimens, particularly *Ectophylla* and *Mesophylla*; (2) our methods used in generating sequence data; or (3) methods used by Lim et al. (2003) for partial endonuclease digestion. Although we cannot exclude any of these alternatives with certainty, we have addressed the first two by re-sequencing the ND3–ND4 region in several specimens and by double-checking identifications for nearly all specimens examined, including *Ectophylla* and *Mesophylla*. Regarding the third alternative, we see no reason to believe that, besides knowing the actual sequence, any method should have 100% efficiency in identifying all cut-sites present in a DNA molecule. For example, partial or complete restriction digest procedures tend to underestimate the number of cut sites if they are located in close, physical proximity, perhaps within 50–100 bp. Although this situation sometimes can contribute to misinterpretation of homology, we still would expect that most of the inefficiency inherent to the method would be randomized and unbiased with respect to gains or losses in phylogenetic signal. If this expectation is false, then based on our study, the partial digestion method of Morales et al. (1993) represents a biased or inaccurate estimate of restriction-site data. If the expectation is true, then the discrepancy between our restriction-site results and those obtained by Lim et al. (2003) is an artifact of the specific procedures employed in their study.

One possibility involves the purity of the DNA being digested (i.e., PCR amplified ND3–ND4 fragment). In this study, we were unable to amplify a high quality and high quantity product consistently, across all taxa examined, with primers 772 and 773 (Cronin et al., 1993) used by Lim et al. (2003), which made it necessary for us to design new primers for PCR. If amplicons digested in the Lim et al. (2003) study contained some additional non-specific DNA fragments, then their data probably would include restriction-sites from DNA fragments other than the ND3–ND4 region. A piece of missing data critical to distinguish among these alternatives is the actual ND3–ND4 sequence for the particular specimens of *Ectophylla* and *Mesophylla* examined in Lim et al. (2003).

Finally, we also note concerns we have about the scope of the Lim et al. (2003) study in terms of ingroup versus outgroup taxonomic sampling. Their experimental design (and title of the paper) focused on relationships within and between *Vampyressa* and *Vampyriscus* (=ingroup), with *Chiroderma*, *Ectophylla*, and *Mesophylla* reported as the designated outgroups. However, they devote a good portion of their discussion to broader issues extending beyond inferences of ingroup relationships. In short, Lim et al. (2003, p.23) interpret their results as positive evidence supporting the classical *Ectophylla–Mesophylla* relationship, concluding they "are sister taxa well supported by several morphological and molecular synapomorphies." We find no evidence for this statement. Even if their restriction-site data are accurate, the characters "supporting" the *Ectophylla–Mesophylla* relationship (Fig. 1) are best interpreted, in their study, as shared primitive characters (symplesiomorphies).

4.7. Taxonomic conclusions

Several alternative relationships have been proposed for vampyressine bats (Baker et al., 1989, 2003; Koopman, 1994; Lim, 1993; Lim et al., 2003; Owen, 1987; Simmons, 2005; Wetterer et al., 2000). The present study represents a robust and taxonomically thorough assessment of higher-level vampyressine relationships that supports recognizing seven genera (and approximately 29 species) within Subtribe Vampyressina (sensu Baker et al., 2003): *Chiroderma* (five species; sensu Simmons, 2005); *Mesophylla* (one species; *macconnelli*); *Platyrrhinus* (14 species; sensu Velazco, 2005); *Uroderma* (two species; sensu Simmons, 2005); *Vampyressa* (three species; *melissa, pusilla, thyone*); *Vampyriscus* (three species; *bidens, brocki, nymphaea*); *Vampyrodes* (one species; *caraccioli*). Our results further suggest three sets of sister relationships among genera and four primary lineages within the subtribe: (1) *Mesophylla–Vampyressa*; (2) *Chiroderma–Vampyriscus*; (3) *Platyrrhinus–Vampyrodes*; and (4) *Uroderma*.

A logical alternative classification could be to recognize only four genera within Vampyressina thereby recognizing each of the four primary lineages with formal taxonomic rank. This would require relegating *Mesophylla*, *Vampyriscus*, and *Vampyrodes* subgeneric rank within *Vampyressa*, *Chiroderma*, and *Platyrrhinus*, respectively. We do not prefer this alternative, however, primarily because it ignores the genetic and morphological distinctiveness among them (e.g., Gardner, 1977; Greenbaum et al., 1975; Lim, 1993; Owen, 1987; Wetterer et al., 2000). Although we do not propose any names at this time, unranked names can be assigned to each of the four primary clades within Vampyressina in lieu

of formal ranked names, facilitating phylogenetic classification (de Quieroz and Gauthier, 1990, 1992, 1994).

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Appendix A

A.1. Specimens examined

Voucher specimens are housed in the following institutions: American Museum of Natural History (AMNH); Carnegie Museum of Natural History (CM); Field Museum of Natural History (FMNH); Laboratory of Chiropterology, Universidad Estadual Paulista, São Paulo, Brazil (UNESP); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM); Museum of Texas Tech University (TTU); Royal Ontario Museum (ROM); United States National Museum of Natural History (USNM). Two numbers separated by a comma identify each specimen: tissue number, museum catalog number (e.g., TK82860, FMNH174909). Museum catalog numbers are missing for vouchers that are housed but not yet cataloged or the number is unknown. Asterisks denote specimens for which cytochrome *b* sequences were obtained from GenBank.

Artibeus jamaicensis.— FRENCH GUIANA. Paracou, near Sinnamary (TK18788*, AMNH267202). *Chiroderma doriae*.—BRAZIL. São Paulo: Pindorama (UNESP16506*, UNESP16506). *Chiroderma improvisum*.—MONTSERRAT. St. Anthony: 0.5 mi. (0.8 km) above mouth Belham River (TK15713*, TTU31403). *Chiroderma salvini*.—PANAMA. Darién: 6 km S Cana (TK22581*, TTU). *Chiroderma trinitatum*.—PERU. Huanuco: Leoncio Prado; 9 km S, 2 km E Tingo Maria (TK22920, CM98745). TRINIDAD AND TOBAGO. Trinidad: St. George Co.; 4 mi. N Arima, Simla (TK25211, TTU44082). *Chiroderma villosum*.—COSTA RICA. San José: 12.3 mi. SSE San Isidro (TK7978, TTU34311). TRINIDAD AND TOBAGO. Trinidad: St. George Co.; 4 mi. N Arima, Simla (TK25052, CM97374). *Dermanura rosenbergi*.—ECUADOR. Esmeraldas: S San Lorenzo, La Chiquita Experimental Station, 18N 74893N 0136902E (TK104501*, TTU85273). *Ectophylla alba*.—COSTA RICA. Limón: Barra Del Tortuguero; 7 km NNW Tortuguero, Caño Palma Biological Station, 10°36′N, 83°32′W (TK16395*, ROM108296; TK125309, USNM568513; TK125310, USNM568511; TK125311, USNM568512). PANAMA. Bocas Del Toro: Isla Popa, S shore, 1 km E Sumwood Channel (TK125308, USNM579079). *Enchisthenes hartii*.—PERU. Huanuco: Leoncio Prado; 11 km N, 6 km S Tingo Maria (TK22690*, CM98710). *Mesophylla macconnelli*.—BOLIVIA. La Paz: 1 mi. W Puerto Linares (TK14583*, TTU34881; TK14598*, TTU34883; TK14599*, TTU34884). COLOMBIA. Meta: Villavicencio, El Hachon, Centro Agricola SENA (TK16042*, TTU). ECUADOR. Tungurahua: La Estancia, 18M 983999N 809292E (TK104209*, TTU84981). Pastaza: 5 km E Puyo, Safari Hosteria Park (TK104021, TTU84793; TK104210, TTU84982). Pastaza: Puyo, Finca El Pigual, 18M 9836288N 0166599E (TK104056, TTU84828; TK104077*, TTU84849; TK104078, TTU84850). FRENCH GUIANA. Paracou, near Sinnamary (TK18786*, AMNH267281). PERU. Cusco: La Convencion, Camisea, Pagoreni (TK55316*, USNM577952). *Platyrrhinus brachycephalus*.—SURINAME. Marowijne: Perica (TK17627*, CM77660). ECUADOR. Esmeraldas: Comuna San Francisco de Bogota between Lita and San Lorenzo (TK135914, TTU; TK135942, TTU). *Platyrrhinus helleri*.—BOLIVIA. La Paz: 1 mi. W Puerto Linares (TK14577, TTU34923). *Platyrrhinus infuscus*.—BOLIVIA. Cochabamba: 12.5 mi. SW Villa Tunari, 17°03′51″S, 65°28′33″W (NK30133, AMNH264997). Santa Cruz: 23 km S Campamento Los Fierros, National Park Noel Kempff Mercado, 14°37′45″S, 60°45′00″W (NK22677, MSB). *Rhinophylla pumilio*.—FRENCH GUIANA. Paracou, near Sinnamary (TK18825, AMNH267158). *Stenoderma rufum*.—PUERTO RICO. El Verde Field Station, near Caribbean National Forest, Route 186, and Río Grande (TK28515, TTU). *Sturnira erythromos*.—PERU. Huanuco: Leoncio Prado; 9 km S, 2 km E Tingo Maria (TK22784, CM98769). *Sturnira lilium*.—PERU. Huanuco: Leoncio Prado; 9 km S, 2 km E Tingo Maria (TK22810, CM98764). *Uroderma bilobatum*.—ECUADOR. Esmeraldas: E. San Lorenzo, La Guarapera banana farm and pasture (TK104630, TTU85402). TRINIDAD AND TOBAGO. Trinidad: St. Georgre Co.; 4 mi. N Arima, Simla Research Center (TK25256*, TTU44100). *Uroderma magnirostrum*.—EL SALVADOR. San Miguel: Hacienda Lechero, El Cañal (TK40046, TTU62670). *Vampyressa melissa*.—PERU. Cusco: 15 km SW Pillcopata, Paucartambo, Consuelo, 13°01′25″S, 71°29′31″W (TK82860, FMNH174909; TK82861, FMNH174910). *Vampyressa pusilla*.—BRAZIL. São Paulo: Caetetus Ecological Station (TK11494*, ROM111071; TK11495*, ROM111072). PARAGUAY. Canindeyu: 3.5 km W Puerto Horqueta, Reserva Natural del Bosque M'Baracayu (TK56908, TTU94775). *Vampyressa*

thyone.—COSTA RICA. Puntarenas: 2.1 mi. S, 1.1 mi. E San Vito, Las Cruces Tropical Botanical Garden (TK9010*, TTU34398; TK9020, TTU34408; TK9021, TTU34409; TK9023*, TTU34411). ECUADOR. Pastaza: 18M 9830709N 830633E (TK104382*, TTU85154). Pastaza: Madre Tierra, 18M 9828271N 830135E (TK104383*, TTU85153). HONDURAS. Atlantida: Lancetilla (TK40351*, TTU61144). NICARAGUA. Zelaya: 3 km NW Rama (TK7848*, TTU30639). PANAMA. Darién: Cana (TK22534, TTU39140). PERU. Cusco: La Convencion, Camisea, Pagoreni (TK70533*, USNM577938). *Vampyriscus bidens*.—PERU. Huanuco: Leoncio Prado; 6 km N Tingo Maria (TK22607*, CM98808). Cusco: La Convencion, Camisea, Pagoreni (TK55322*, USNM577948). SURINAME. Brokopondo: 1.5 km W Rudi, Kappelvliegveld (TK11300*, CM63869). *Vampyriscus brocki*.—FRENCH GUIANA. Paracou, near Sinnamary (TK18823, AMNH267184). GUYANA. Potaro-Siparuni: Iwokrama Forest, Three Mile Camp (TK11496*, ROM112094). SURINAME. Nickerie: Grassalco, 4°46′N, 56°46′W (TK10334, CM63873). Nickerie: Grassalco, 4°41′N, 56°07′W (TK10316, CM63871). *Vampyriscus nymphaea*.—ECUADOR. Esmeraldas: near main highway connecting Lita and San Lorenzo, about halfway between towns (TK135727, TTU; TK135728, TTU). Esmeraldas: Terrenos aledaños de la Comuna San Francisco de Bogota (TK135842, TTU; TK135843, TTU). *Vampyrodes caraccioli*.—PERU. Cusco: La Convencion, Camisea, Pagoreni (TK70540, USNM582872). TRINIDAD AND TOBAGO. Trinidad: St. George Co.; 4 mi. N Arima, Simla Research Center (TK25083*, CM94707).

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