Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 47 (2008) 870–876
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Short Communication

Phylogenetic relationships of vampyressine bats and allies (Phyllostomidae: Stenodermatinae) based on DNA sequences of a nuclear intron (*TSHB-I2*)

Steven R. Hoofer *, William E. Flanary, Robert J. Bull, Robert J. Baker

Museum of Texas Tech University and Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA

Received 12 June 2007; revised 10 January 2008; accepted 23 January 2008
Available online 2 February 2008

1. Introduction

Vampyressine bats constitute a large and species rich group of fruit-eating specialists within the family Phyllostomidae (subfamily Stenodermatinae: subtribe Vampyressina—Baker et al., 2003). Approximately 29 species are currently recognized within seven genera (*Chiroderma*, *Mesophylla*, *Platyrrhinus*, *Uroderma*, *Vampyressa*, *Vampyriscus*, *Vampyrodes*). Traditionally, *Ectophylla alba* also has been recognized within Vampyressina (Lim, 1993; Wetterer et al., 2000). Systematic studies since the 1960s have produced two contrasting views of vampyressine relationships that are tied to inconsistencies between morphological and molecular data regarding affinities and rank status of some genera. For example, morphologically the monotypic genera *Ectophylla* (*E. alba*) and *Mesophylla* (*M. macconnelli*) are viewed as sister taxa recognized either generically (e.g., Koopman, 1994; Lim, 1993; Simmons, 2005) or congenerically (e.g., Koopman, 1985; Wetterer et al., 2000). In contrast, molecular evidence suggest a sister relationship between *Mesophylla* and *Vampyressa*, with *Ectophylla* placed singly in a subtribe (Ectophyllina) that is related to three other groups (Artibeina, Enchisthenina, Stenodermatina) more closely than the vampyressines (e.g., Greenbaum et al., 1975; Hoofer and Baker, 2006).

A diverse set of morphological features has been examined in >20 studies (reviewed in Lim, 1993; Owen, 1987; Wetterer et al., 2000), whereas molecular examinations are limited to a few studies of karyotypes (Baker et al., 1973; Gardner, 1977; Greenbaum et al., 1975) and a few of mitochondrial DNA sequences (Baker et al., 2003; Hoofer and Baker, 2006; Porter and Baker, 2004). Only one analysis of nuclear DNA sequences has been undertaken for vampyressine bats (Baker et al., 2000). However, that study was designed to explore higher-level diversification within Phyllostomidae through analysis of one exon sequence representing each genus in the family.

Given the disparity between morphological and molecular phylogenies, our purpose in this study was to examine vampyressine relationships through comprehensive taxon sampling and phylogenetic analysis of nuclear DNA sequence variation in intron 2 (*I2*) of the thyroid stimulating hormone gene, beta subunit (*TSHB-I2*). We chose this intron marker because previous studies of bats and other mammals have shown it to be useful for phylogeny reconstruction at low to high taxonomic levels (Eick et al., 2005; Matthee et al., 2001; Willows-Munro et al., 2005). These nuclear intron data should be independent from those examined previously and thusly help distinguish between morphological and molecular hypotheses of vampyressine relationship that have been in opposition for decades.

2. Methods and materials

2.1. Specimens examined

We generated complete *TSHB-I2* sequences for 33 individuals representing all putative vampyressine genera, including *Ectophylla*, as well as other stenodermatine genera. We obtained one *TSHB-I2* for *Artibeus jamaicensis* that was available in GenBank (Eick et al., 2005). We used sequences

* Corresponding author. Present address: Department of Ecology and Evolutionary Biology, The University of Kansas, Lawrence, KS 66045, USA.
E-mail addresses: srhoofer@hotmail.com, srhoofer@ku.edu (S.R. Hoofer).

1055-7903/$ - see front matter
doi:10.1016/j.ympev.2008.01.027

from *Rhinophylla* and *Sturnira* as outgroups for analyses 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; Hoofer and Baker, 2006; Lim, 1993; Porter and Baker, 2004; Wetterer et al., 2000).

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); 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.

Artibeus jamaicensis—GenBank Accession AJ865664 deposited by Eick et al. (2005). *Artibeus lituratus*—MEXICO. Chiapas: 9 km NW, 17.5 km SW Mapastepec, 15P-492279-1704747 (TK150506, TTU). *Chiroderma villosum*—ECUADOR. Esmeraldas: near main highway connecting Lita and San Lorenzo, about halfway between towns (TK135750, TTU). MEXICO. Chiapas: 3 km S, 25 km W Ocozocoautla, 15Q-450892-1829842 (TK150206, TTU). *Dermanura tolteca*—HONDURAS. Comayagua: Parque Nacional Cerrero Azul Meambar, 16-402621-1644465 (TK136035, TTU). *Ectophylla alba*—COSTA RICA. Limón: Barra Del Tortuguero; 7 km NNW Tortuguero, Caño Palma Biological Station, 10° 36′ N, 83° 32′ W (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*—FRENCH 13GUIANA. Paracou, near Sinnamary (TK18786, AMNH267281). PERU. Cusco: La Convencion, Camisea, Pagoreni (TK55316, USNM577952). *Platyrrhinus helleri*—BOLIVIA. Santa Cruz: Noel Kempff Mercado National Park, 23 km S Camp Los Fierros, 14° 37′ 45″ S, 60° 45′ 00″ W (NK22633, voucher location unknown; NK22677, voucher location unknown). *Rhinophylla pumilio*—FRENCH GUIANA. Paracou, near Sinnamary (TK18825, AMNH267158). SURINAME. Saramacca Co.; Taselberg, Arrowhead Basin, 3° 55′ N, 56° 10′ W (TK17728, CM76782). *Stenoderma rufum*—UNITED STATES: Puerto Rico. El Verde Field Station, near Caribbean National Forest, Route 186, and Río Grande (TK21797, TTU46377). *Sturnira lilium*—TRINIDAD AND TOBAGO. Tobgago: St. Patrick Co.; Grange (TK25163, TTU44085). *Uroderma bilobatum*—ECUADOR. Esmeraldas: E. San Lorenzo, La Guarapera banana farm and pasture (TK104630, TTU85402). *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). *Vampyressa thyone*—BOLIVIA. Pando: Independencia, 11.26° S, 67.34° W (NK13922, AMNH262524; NK14207, AMNH262550). COSTA RICA. Puntarenas: 2.1 mi. S, 1.1 mi. E San Vito, Las Cruces Tropical Botanical Garden (TK9020, TTU34408). ECUADOR. Pastaza: 18M 9830709N 830633E (TK104382, TTU85154). *Vampyriscus bidens*—PERU. Huanuco: Leoncio Prado; 6 km N Tingo Maria (TK22607, CM98808). Cusco: La Convencion, Camisea, Pagoreni (TK55322, USNM577948). *Vampyriscus brocki*—FRENCH GUIANA. Paracou, near Sinnamary (TK18823, AMNH267184). *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 (TK135843, TTU). *Vampyrodes caraccioli*—BOLIVIA. La Paz: 10 km S, 5 km W San Jose Camarusu Camp, 14° 18′ 33.5″ S, 68° 05′ 56.6″ W (NK37130, voucher location unknown).

2.2. Molecular methods

We PCR amplified a nuclear DNA fragment approximately 520 base pairs long encompassing the second intron within the *TSHB* gene by using chiropteran specific primers (THY F: 5′-GGG TAT GTA GTT CAT CTT ACT TC-3′; THY R: 5′-GGC ATC CTG GTA TTT CTA CAG TCT TG-3′; Eick et al., 2005). In a 35 μl PCR reaction, we added approximately 100 ng DNA, 0.3 μM each primer, 2.2 mM $MgCl_2$, 0.16 mM dNTPs, 1× final buffer concentration, and 0.75 U *Taq* DNA polymerase (Promega Corp., Madison, Wisconsin) or FailSafe PCR Enzyme Mix (Epicentre Biotechnologies, Madison, Wisconsin). We used the following thermal profile: 95 °C for 2 min initial denaturation, followed by 36 cycles of 95 °C for 35 s, 52 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 10 min.

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 assembled resulting, overlapping fragments in AssemblyLIGN™ 1.0.9 software (Oxford Molecular Group PLC, Oxford, United Kingdom), and resolved base calling ambiguities on single strands in Sequencing Analysis 3.4.1 software (Applied Biosystems, Inc., Foster City, California) either by choosing the call on the cleanest strand or by using the appropriate standardized IUB ambiguity code if both strands showed the same ambiguity.

2.3. Phylogenetic analysis

We performed multiple sequence alignment in Clustal X software (Thompson et al., 1997) with default parameters

for costs of opening and extending gaps. We subsequently viewed the alignment in MacClade software (version 4.0; Maddison and Maddison, 2002), delimited ambiguously aligned sites following methods of Hoofer and Van Den Bussche (2003), and performed data analysis with and without those sites. We coded nucleotides as unordered, discrete characters, gaps as missing data, and multiple states as polymorphisms. In PAUP* software (test version 4.0b10; Swofford, 2002), we examined level of phylogenetic signal 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 software (version 3; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and by Maximum Likelihood and Parsimony analyses implemented in PAUP* software (test version 4.0b10; Swofford, 2002). Kimura's (1980; K80) model with allowance for gamma distribution of rate variation (Γ) best fit the data based on Hierarchical Likelihood Ratio Tests implemented in Modeltest 3.06 software (Posada and Crandall, 1998).

For Bayesian analysis, we ran two sets of two simultaneous runs of 2×10^6 generations with one cold and three 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 (Hoofer and Baker, 2006) and determined burn-in values (initial set of unstable generations to be ignored) based on empirical evaluation of likelihood scores, convergence statistics, and potential scale reduction factors (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). We obtained branch lengths via the "sumt" option and calculated a consensus tree (50% majority-rule) from the sample of stabilized trees. We assessed clade reliability via posterior probabilities and regarded values $\geqslant 0.95$ as significant.

For Maximum Likelihood analyses, we used the K80 + Γ model and parameters given by Modeltest (*ti*/*tv* ratio = 3.28, $\alpha = 0.58$), 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 Maximum Likelihood and Parsimony analyses (Felsenstein, 1985) and regarded values $\geqslant 70$ as support.

3. Results

3.1. TSHB-I2 data

Complete sequence of *TSHB-I2* averaged 399 base pairs for the 34 taxa examined (GenBank Accession Nos. EU371959–EU371991; AJ865664), ranging from 396 (*Sturnira*) to 402 (*V. melissa* TK82861), and corresponding in length and similarity to other *TSHB-I2* sequences in GenBank. Alignment of sequences resulted in 405 aligned sites and included ten insertion/deletion events. Nine of these involved from one to three base pairs, aligned unambiguously with regard to positional homology, and were phylogenetically informative (Table 1). The other insertion/deletion region (positions 201–210), partly including a guanosine repeat region of two to nine nucleotides, could not be aligned unambiguously and was deleted from subsequent analyses. After removing the ten ambiguous characters, 395 characters were available for analysis, of which 253 were constant and 64 were parsimony informative. Overall nucleotide frequencies varied slightly among genera, averaging 25.41% (A), 20.79% (C), 18.96% (G), and 34.84% (T). The transition to transversion ratio was 3.28 to 1. The number of heterozygous sites ranged from 0 (22 taxa) to 8 (*V. brocki*), with a mean of 1.41 per taxon. The g_1-statistic was skewed significantly left (-0.74; $P < 0.01$), indicating strong phylogenetic signal (Hillis and Huelsenbeck, 1992).

3.2. Phylogenetic analyses

Two simultaneous Bayesian analyses yielded identical topologies and nearly identical posterior probabilities for nodes and model parameters. Additional sets of Bayesian runs agreed. Maximum likelihood analysis resulted in a single best tree (Lnl = -1524.61; Fig. 1). Parsimony analysis resulted in 218 most-parsimonious trees (length = 204, CI = 0.84, RI = 0.88) that differed primarily in alternative arrangements among terminal, closely related taxa for which there was no supported resolution. Overall, statistically supported topologies (i.e., $\geqslant$70% bootstrap value, $\geqslant 0.95$ Bayesian posterior probability) obtained from the three optimality criteria agreed, supporting monophyly of all genera (for which >1 individual was sampled) except *Chiroderma*, and supporting a sister relationship between *Mesophylla* and *Vampyressa* and a clade containing *Artibeus*, *Dermanura*, *Ectophylla*, *Enchisthenes*, and *Stenoderma* (Fig. 1).

4. Discussion

A prevalent view of vampyressine systematics recognizes a sister taxon relationship for the monotypic genera *Ectophylla* (*E. alba*) and *Mesophylla* (*M. macconnelli*) and another sister taxon relationship for *Vampyressa* (*V. melissa*, *V. pusilla*, *V. thyone*) and *Vampyriscus* (*V. bidens*, *V. brocki*, *V. nymphaea*). Stemming from a consensus among morphological studies (reviewed in Wetterer et al., 2000), this view has been widely accepted among mammalogists (e.g., Corbet and Hill, 1991; Koopman, 1985, 1994; Simmons, 2005). Although most of this consensus manifests from different authors recognizing strong similarity among skin and skeletal features, three studies employed

Table 1
Portion of *TSHB-I2* sequence alignment showing nine insertion/deletion events that were aligned unambiguously with regard to positional homology

Taxon	Alignment position												
	53	82	83	84	120	132	156	169	205	206	292	293	351
Rhinophylla pumilio TK17728		C	T	T	C	A	G	C	T	G	T	T	C
Rhinophylla pumilio TK18825		C	T	T	C	A	G	C	T	G	T	T	C
Sturnira lilium TK25163	C	C	T	T	C	A	G	C			T	T	C
Enchisthenes hartii TK22690	C	C	T	T			G	C	T	G			C
Stenoderma rufum TK21797	C	C	T	T			G	C	T	G	C	T	C
Artibeus jamaicensis AJ865663	C	C	T	T			G	C	T	G	T	T	C
Artibeus lituratus TK150506	C	C	T	T			G	C	T	G	T	T	C
Dermanura tolteca TK136035	C	C	T	T			G	C	T	G	T	T	C
Ectophylla alba TK125308	C	C	T	T			G	C	T	G	T	T	C
Ectophylla alba TK125309	C	C	T	T			G	C	T	G	T	T	C
Ectophylla alba TK125310	C	C	T	T			G	C	T	G	T	T	C
Ectophylla alba TK125311	C	C	T	T			G	C	T	G	T	T	C
Platyrrhinus helleri NK22633	C	C	T	T				C	T	G	T	T	C
Platyrrhinus helleri NK22677	C	C	T	T				C	T	G	T	T	C
Uroderma magnirostrum TK46006	C	C	T	T				C	T	G	T	T	
Uroderma bilobatum TK104630	C	C	T	T				C	T	G	T	T	
Chiroderma villosum TK135750	C	C	T	T				C	T	G	T	T	C
Chiroderma villosum TK150206	C	C	T	T				C	T	G	T	T	C
Mesophylla macconnelli TK55316	C	C	T	T				C	T	G	T	T	C
Mesophylla macconnelli TK18786	C	C	T	T				C	T	G	T	T	C
Vampyriscus brocki TK18823	C	C	T	T				C	T	G	T	T	C
Vampyriscus bidens TK55322	C	C	T	T				C	T	G	T	T	C
Vampyriscus bidens TK22607	C	C	T	T				C	T	G	T	T	C
Vampyriscus nymphaea TK135727	C	C	T	T				C	T	G	T	T	C
Vampyriscus nymphaea TK135728	C	C	T	T				C	T	G	T	T	C
Vampyriscus nymphaea TK135843	C	C	T	T				C	T	G	T	T	C
Vampyressa pusilla ROM111071	C	C	T	T					T	G	T	T	C
Vampyressa thyone TK104382	C							C	T	G	T	T	C
Vampyressa thyone NK14207	C							C	T	G	T	T	C
Vampyressa thyone NK13922	C							C	T	G	T	T	C
Vampyressa melissa TK82860	C	C	T	T				C	T	G	T	T	C
Vampyressa melissa TK82861	C	C	T	T				C	T	G	T	T	C
Vampyrodes caraccioli NK37130	C	C	T	T				C	G	G	T	T	C

Six events involved one nucleotide, two events involved two nucleotides (positions 205–206 and 292–293), and one involved three nucleotides (positions 82–84).

explicit cladistic analyses on morphological data (Lim, 1993; Owen, 1987; Wetterer et al., 2000). The relative merits and weaknesses of these studies and incongruencies among them have been reviewed (Baker et al., 2003; Hoofer and Baker, 2006; Lim, 1993; Wetterer et al., 2000), demonstrating the lack of consensus for both the *Ectophylla–Mesophylla* relationship and *Vampyressa–Vampyriscus* relationship when morphological datasets are subjected to explicit cladistic analysis.

An alternative view recognizes a sister taxon relationship for *Mesophylla* and *Vampyressa* and a sister taxon relationship for *Chiroderma* and *Vampyriscus*, and that *Ectophylla* is related to other stenodermatine genera more closely than to any of the vampyressines. This view stems from a consensus among earlier studies examining karyotypes (Baker et al., 1973; Gardner, 1977; Greenbaum et al., 1975;) and more recent studies of mitochondrial DNA sequences (Baker et al., 2003; Hoofer and Baker, 2006; Porter and Baker, 2004). These relationships have been accepted in only one formal classification (Baker et al., 2003).

Our results from phylogenetic analysis of nuclear *TSHB-I2* sequences strongly support four relationships that are key to distinguish between these contrasting views: (1) monophyly of *Vampyressa* (*melissa*, *pusilla*, *thyone*); (2) monophyly of *Vampyriscus* (*bidens*, *brocki*, *nymphaea*); (3) sister taxon relationship between *Mesophylla* and *Vampyressa*; and (4) clade containing *Ectophylla* and other non-vampyressine genera (*Artibeus*, *Dermanura*, *Enchisthenes*, *Stenoderma*). Taken together, these results are in strong agreement with previous molecular studies as reflected in the Baker et al. (2003) classification, and reject morphological hypotheses of *Ectophylla–Mesophylla* and *Vampyressa–Vampyriscus*. Thus, according to the molecular hypotheses, the morphological characteristics (gaps between mandibular cheekteeth, yellow thumbs, absence of facial stripes, color of noseleaf, shape of incisor, papillae on pharyngeal tongue) regarded as derived characters shared by *Ectophylla* and *Mesophylla* (Lim, 1993; Wetterer et al., 2000) are the result of parallelisms or convergence, not shared ancestry (reviewed in Hoofer and Baker, 2006). Moreover, the taxonomy in the Baker et al. (2003)

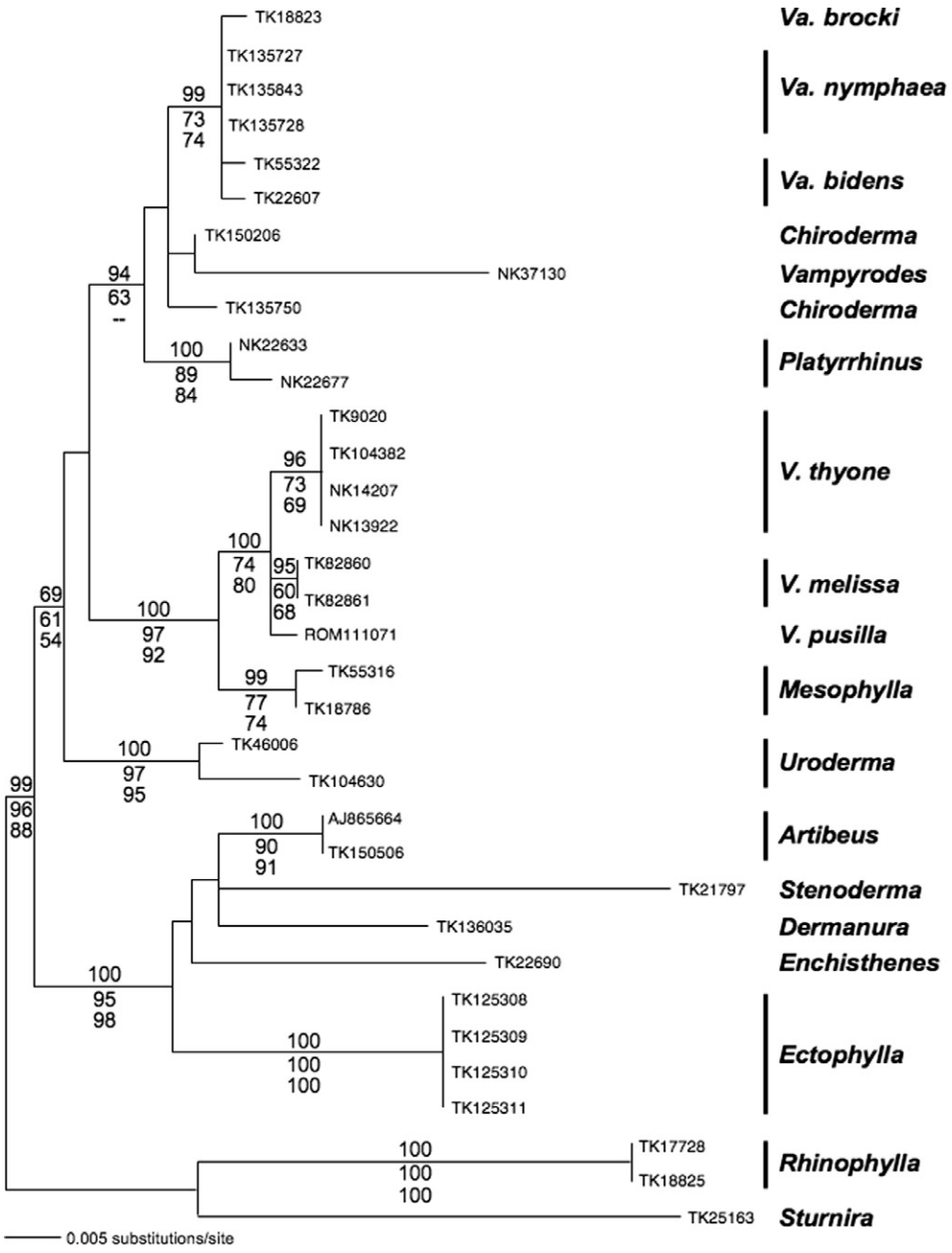

Fig. 1. Maximum likelihood phylogram (Lnl = −16,272.69) from analysis of *TSHB-I2* (395 base pairs) with best-fit model (K80 + Γ; ti/tv ratio = 3.28, $\alpha = 0.58$). We designated *Rhinophylla* and *Sturnira* as outgroups. Numbers above branches are Bayesian posterior probabilities, whereas those below are bootstrap percentages from Parsimony and Maximum Likelihood, respectively. Values are shown only for nodes supported by $P \geqslant 0.95$ or bootstrap percentage $\geqslant 50$, or both. "*V.*" = *Vampyressa*, "*Va.*" = *Vampyriscus*.

classification better reflects actual phyletic relationships rather than adaptive similarity.

Two other higher-level relationships depicted in best trees from each optimality criterion but weakly supported also affirm results from previous molecular studies (Fig. 1). These relationships include monophyly of Vampyressina (sensu Baker et al., 2003) and a clade containing *Chiroderma*, *Platyrrhinus*, *Vampyriscus*, and *Vampyrodes*. Also noteworthy is the lack of support for monophyly of three taxa, *Chiroderma*, *Vampyrsicus bidens*, and *V. nymphaeae*. However, these results should not be interpreted as contradictory to monophyly of each of these taxa, rather a lack of resolving power of the *TSHB-I2* data. In this study, many relationships are resolved (i.e., with concomitant statistical support) with these data, and several relationships are unresolved, at various taxonomic levels. In fact, the magnitude of supported resolution overall, but especially at the species level, is somewhat unexpected given that it is based on only 392 base pairs of nuclear DNA sequence.

Provisional statements of homology within a sequence alignment are of special concern when aligning non-coding DNA sequences that possibly contain insertion/deletions (Giribet and Wheeler, 1999). It can be problematic and possibly influence phylogenetic inference (reviewed in Hoofer and Van Den Bussche, 2003). In this study, however, *TSHB-I2* sequence alignment was not complicated by insertion/deletions events, and none of the ten insertion/deletion events that we observed were homoplastic. Although results were essentially identical between analyses including and excluding the insertion/deletion regions, in some cases insertion/deletion events were phylogenetically informative at various taxonomic levels (Table 1). For example, a one base-pair insertion/deletion (position 156) unites *Chiroderma*, *Mesophylla*, *Platyrrhinus*, *Uroderma*, *Vampyressa*, *Vampyriscus*, and *Vampyrodes* to the exclusion of *Artibeus*, *Dermanura*, *Ectophylla*, *Enchisthenes*, *Rhinophylla*, *Stenoderma*, and *Sturnira*, thereby supporting monophyly of Vampyressina (sensu Baker et al., 2003). Additionally, a three base-pair deletion (position 82–84) defines *V. thyone*, and a one base-pair deletion (position 169) defines *V. pusilla*. These features document a nuclear DNA distinction between *V. pusilla* and *V. thyone*, the latter being elevated to species status just recently (from subspecific status within *V. pusilla*) based on morphological, chromosomal, and mitochondrial DNA differences (Lim et al., 2003).

In conclusion, the present study offers an explicit and thorough assessment of the two contrasting views regarding relationships of vampyressine bats that is independent from previous examinations of morphological, karyotypic, and mitochondrial data. Phylogenetic analysis of the 395 base pairs intron provided high statistical support to four key relationships that in combination affirm the molecular view of vampyressine relationships and classification of Baker et al. (2003). These results are encouraging toward the goal of recovering a fully resolved phylogeny for vampyressine bats on the basis of digenomic congruence.

Acknowledgments

We thank the following persons and institutions for their generosity in loaning tissue samples and assistance in locating voucher specimens: B. Patterson, B. Stanley, and J. Phelps of the Field Museum of Natural History; T. Yates and C. Parmenter of the Museum of Southwestern Biology, University of New Mexico; M. Engstrom and B. Lim of the Royal Ontario Museum; H. Garner and K. MacDonald of the Museum of Texas Tech University; D. Wilson, J. Mead, L. Gordon, and D. Schmidt of the United States National Museum of Natural History. We thank the numerous individuals who collected specimens over the years, and S. Solari of Texas Tech University for valuable assistance with species identification. Furthermore, the two rare samples of *Vampyressa melissa* were collected in Peru by B. Patterson and S. Solari during expeditions organized by Field Museum and Universidad de San Marcos and funded by FMNH, MUSM, and the NSF (DEB-9870191). Aspects of data analysis in this study benefited from two anonymous reviewers. Funding for this project came from the Natural Science Research Laboratory and Biological Database Initiative of Texas Tech University and personal donations from J. Sowell.

References

Baker, R.J., Genoways, H.H., Bleier, W.J., Warner, J.W., 1973. Cytotypes and morphometrics of two phyllostomid bats, *Micronycteris hirsuta* and *Vampyressa pusilla*. Occas. Pap., Mus. Texas Tech Univ. 17, 1–10.

Baker, R.J., Porter, C.A., Patton, J.C., Van Den Bussche, R.A., 2000. Systematics of bats of the family Phyllostomidae based on *RAG2* DNA sequences. Occas. Pap., Mus. Texas Tech Univ. 202, 1–16, i.

Baker, R.J., Hoofer, S.R., Porter, C.A., Van Den Bussche, R.A., 2003. Diversification among New World leaf-nosed bats: an evolutionary hypothesis and classification inferred from digenomic congruence of DNA sequence. Occas. Pap., Mus. Texas Tech Univ. 230, 1–32, i.

Corbet, G.B., Hill, J.E. 1991. A world list of mammalian species, third ed. Nat. Hist. Mus. Pub., Oxford Univ. Press, New York, pp. 243.

Eick, G.N., Jacobs, D.S., Matthee, C.A., 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). Mol. Biol. Evol. 22, 1869–1886.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gardner, A.L., 1977. Chromosomal variation in *Vampyressa* and a review of chromosomal evolution in the Phyllostomidae (Chiroptera). Syst. Zool. 26, 300–318.

Giribet, G., Wheeler, W.C., 1999. On gaps. Mol. Phylogenet. Evol. 13, 132–143.

Greenbaum, I.F., Baker, R.J., Wilson, D.E., 1975. Evolutionary implications of the karyotypes of the stenodermine genera *Ardops*, *Phyllops*, and *Ectophylla*. Bul. South. Cal. Acad. Sci. 74, 156–159.

Hillis, D.M., Huelsenbeck, J.P., 1992. Signal, noise, and reliability in molecular phylogenetic analysis. J. Hered. 83, 189–195.

Hoofer, S.R., Baker, R.J., 2006. Molecular systematics of vampyressine bats (Phyllostomidae: Stenodermatinae) with comparison of direct and indirect surveys of mitochondrial DNA variation. Mol. Phylogenet. Evol. 39, 424–438.

Hoofer, S.R., Van Den Bussche, R.A., 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chirop. 5 (supplement), 1–63.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

Koopman, K.F., 1985. A synopsis of the families of bats, Part VII. Bat Res. News 25, 25–27 (dated 1984 but issued in 1985).

Koopman, K.F., 1994. Chiroptera: Systematics. Handbook of Zoology, vol. 8, Part 60: Mammalia. Walter de Gruyter, Berlin, Germany, 224 pp.

Lim, B.K., 1993. Cladistic reappraisal of Neotropical stenodermatine bat phylogeny. Cladistics 9, 147–165.

Lim, B.K., Pedro, W.A., Passos, F.C., 2003. Differentiation and species status of the Neotropical yellow-eared bats *Vampyressa pusilla* and *V. thyone* (Phyllostomidae) with a molecular phylogeny and review of the genus. Acta Chirop. 5, 15–29.

Maddison, D.R., Maddison, W.P., 2002. MacClade 4 (version 4.05). Sinauer Associates, Sunderland, Massachusetts.

Matthee, C.A., Burzlaff, J.D., Taylor, J.F., Davis, S.K., 2001. Mining the mammalian genome for artiodactyls systematics. Syst. Biol. 50, 367–390.

Owen, R.D., 1987. Phylogenetic analyses of the bat subfamily Stenodermatinae (Mammalia: Chiroptera). Spec. Pub., Mus. Texas Tech Univ. 26, 1–65.

Porter, C.A., Baker, R.J., 2004. Systematics of *Vampyressa* and related genera of phyllostomid bats as determined by cytochrome-*b* sequences. J. Mamm. 85, 126–132.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Simmons, N.B., 2005. Order Chiroptera. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference, third ed. Johns Hopkins University Press, Baltimore, Maryland, pp. 312–529.

Swofford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Wetterer, A.L., Rockman, M.V., Simmons, N.B., 2000. Phylogeny of phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. Bul. Amer. Mus. Nat. Hist. 248, 1–200.

Willows-Munro, S., Robinson, T.J., Matthee, C.A., 2005. Utility of nuclear DNA intron markers at lower taxonomic levels: phylogenetic resolution among the nine *Tragelaphus* spp. Mol. Phylogenet. Evol. 35, 624–636.