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ECHOLOCATION CALLS, DIET, AND PHYLOGENETIC RELATIONSHIPS OF STOLICZKA'S TRIDENT BAT, *ASELLISCUS STOLICZKANUS* (HIPPOSIDERIDAE)

GANG LI, BING LIANG, YINAN WANG, HUABIN ZHAO, KRISTOFER M. HELGEN, LIANGKONG LIN, GARETH JONES, AND SHUYI ZHANG*

Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuan Xilu, Haidian, Beijing, 100080, China (GL, BL, YW, HZ)
School of Life Science, East China Normal University, Shanghai, 200062, China (GL, HZ, SZ)
Division of Mammals, National Museum of Natural History, NHB 390 MRC 108, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012, USA (KMH)
South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia (KMH)
Laboratory of Wildlife Ecology, Department of Biology, Tunghai University, Taichung, Taiwan 407, Republic of China (LL)
School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, United Kingdom (GJ)

The comparative biology of the hipposiderid genus *Aselliscus* has been little studied. Here we report studies of echolocation, diet, and phylogeny of *Aselliscus stoliczkanus*. The phylogenetic relationships of *Aselliscus* were investigated based on sequence comparisons of mitochondrial cytochrome-*b* and nicotinamide adenine dinucleotide dehydrogenase subunit 1 genes. Dates of divergence within the hipposiderid radiation also were estimated. The echolocation call frequency of *A. stoliczkanus* is quite high, with the dominant constant frequency component at 119–120 kHz, and a terminal sweep down to 104.5 kHz. The call duration is about 5.4 ms. The diet of *A. stoliczkanus* is mainly composed of lepidopterans (78.5%), beetles (14.9%), and hemipteran insects (6.5%) in November. Our results indicate that *Aselliscus* is monophyletic and is correctly classified in the Hipposideridae, and the divergence time for *Aselliscus* was estimated at 22 million years ago.

Key words: *Aselliscus stoliczkanus, A. tricuspidatus*, Chiroptera, diet, echolocation, Hipposideridae, phylogeny

The family Hipposideridae comprises 9 currently recognized genera of extant leaf-nosed bats, distributed throughout the Old World from Africa to Australia and Melanesia (Simmons 2005). The hipposiderid genus *Aselliscus* is found from southern China to the Pacific archipelago of Vanuatu, and is represented by 2 small-bodied (forearm $<$ 45 mm), allopatric species. Stoliczka's trident bat (*Aselliscus stoliczkanus* (Dobson, 1871)) occurs in southeast Asia, including extreme southeastern China, Myanmar, Thailand, Laos, Vietnam, and the islands of Tioman and Penang fringing the Malay Peninsula (Bates et al. 2000; Corbet and Hill 1992). Temminck's trident bat bat (*Aselliscus tricuspidatus* (Temminck, 1834)) occurs in eastern Wallacea and throughout Melanesia, including the Moluccas, New Guinea, and associated islands, and in the Bismarck Archipelago, Solomon Islands, and Vanuatu (Corbet and Hill 1992; Flannery 1995a, 1995b; Schlitter et al. 1983). *Aselliscus* was originally erected by Tate (1941) to accommodate *A. tricuspidatus*, previously considered a morphologically unique member of the genus *Hipposideros* by Dobson (1871). Tate (1941) further suggested that *A. stoliczkanus* (previously classified in the genus *Asellia*) might also warrant inclusion in *Aselliscus*, a classification formalized by subsequent reviewers (e.g., Sanborn 1952). The 2 species of *Aselliscus* are morphologically highly distinctive, easily discriminated on the basis of external, cranial, and dental features (Corbet and Hill 1992). Interestingly, no species of *Aselliscus* is recorded from the wide intervening area between the geographic ranges of the 2 species (i.e., the Greater Sundas, Sulawesi, and Nusa Tenggara).

The relationships of hipposiderid genera have attracted considerable attention in recent literature (Bogdanowicz and Owen 1998; Jones et al. 2002; Wang et al. 2003) and the family has a rich fossil record from deposits in Queensland, Australia (Hand and Archer 2005; Hand and Kirsch 1998, 2003). Drawing from varying taxon sets and methodologies, various systematists have arrived at strongly divergent interpretations of relationships within the family, and the phylogenetic affinities of *Aselliscus* remain particularly poorly understood. In his description of the genus, Tate (1941) originally highlighted potential links

* *Correspondent: syzhang@bio.ecnu.edu.cn*

between *Aselliscus* and the hipposiderid genera *Anthops*, *Asellia*, *Triaenops*, and *Cloeotis*, all of which are characterized by a tridentate upper nose-leaf margin. In contrast, cladistic analyses by Hand and Kirsch (1998, 2003), drawing from craniodental characters, have suggested that *A. tricuspidatus* is a basal lineage within the family, perhaps sister to all other extant and fossil hipposiderids (their analyses did not include *A. stoliczkanus*). Based on early genetic comparisons, Pierson (1986) even raised the possibility that *Aselliscus* may be more closely allied to rhinolophids than to other hipposiderids. Further, cladistic analyses of discrete morphological characters have questioned whether the 2 species of *Aselliscus* truly comprise a monophyletic clade (Jones et al. 2002), or whether *Aselliscus* may be nested cladistically within the current taxonomic boundaries of *Hipposideros* (Wang et al. 2003).

In the present paper we rely on sequence data from the mitochondrial cytochrome-*b* (*Cytb*) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*ND1*) genes, widely used in chiropteran phylogenetic studies (e.g., Baker et al. 1994; Hoofer and Van Den Bussche 2001; Hulva and Horacek 2002), to provide an independent test of previously proposed hypotheses regarding the phylogenetic relationship of these little-studied species of *Aselliscus*, with special reference to *A. stoliczkanus*. Drawing from our molecular data set, we also estimate selected dates of divergence in the hipposiderid radiation in order to better understand the evolutionary and biogeographic origins of *A. stoliczkanus*. We complement these genetic investigations into the diversification of *Aselliscus* with the 1st detailed discussion of the echolocation calls and diet of *A. stoliczkanus*. Echolocation call frequencies can be useful phylogenetic characters when used alongside genetic data in understanding the evolutionary history of hipposiderid bats (Guillén-Servent and Francis 2006; Thabah et al. 2006).

Materials and Methods

Sampling.—Individuals of *A. stoliczkanus* were captured by mistnetting at caves in Sichuan (29°34′N, 103°16′E), Guizhou (25°19′N, 105°05′E), and Yunnan (25°08′N, 102°38′E) provinces in mainland China, in November 2005. Bats were released after 3-mm punches were taken from the wing membrane. A JYT-1 balance (Shanghai Medical Laser Company, Shanghai, China) accurate to 0.1 g was used for weighing bats and vernier calipers accurate to 0.1 mm were used to obtain forearm lengths. Our tissues of *A. tricuspidatus* are from vouchered specimens (deposited at the Australian Museum in Sydney and the South Australian Museum in Adelaide) collected from a large cave roost in Vatthe Conservation Area, Espiritu Santo, Vanuatu (Helgen 2004). All animals were handled in accordance with guidelines for animal care and use established by the American Society of Mammalogists (Animal Care and Use Committee 1998).

Echolocation calls.—Echolocation calls of *A. stoliczkanus* were recorded in the hand, held approximately 30 cm from the microphone, using a Pettersson D980 bat detector (sampling rate 350 kHz; Pettersson Elektronik AB, Uppsala, Sweden). After downloading 10-times expanded calls onto a PC (soundcard sampling rate 44.1 kHz), the recordings were subsequently analyzed with the software package BatSound Pro, version 3.0 (Pettersson Elektronik AB), using 512 fast Fourier transform and 16-bit precision for the Hanning window (see Li et al. 2006; Zhao et al. 2003). The constant-frequency component of the call was measured from the power peak in the power spectrum. We analyzed 1 call per bat because we measured no intraindividual variation in the frequency of the constant-frequency component. We measured the frequency of most energy in the call, which, typical of hipposiderid bats, was in the 2nd harmonic. We recorded the calls of resting bats because hipposiderid bats can use Doppler shift compensation in flight, whereby they slightly reduce the frequency of calls as their flight speed increases (Hiryu et al. 2005). Recording the frequencies of handheld bats represents a standardized method of recording calls that are not subject to Doppler shift compensation, and is used routinely in analyses of variation in call frequencies of rhinolophoid bats (e.g., Li et al. 2006; Siemers et al. 2005). For logistical reasons we were unable to record the echolocation calls of *A. tricuspidatus*.

Dietary analysis.—Dietary analysis was undertaken by examining the remains of prey items in fecal pellets from *A. stoliczkanus*, following methods discussed by Kunz and Whitaker (1983) and Brack and LaVal (1985). Bats were captured after dawn and placed individually into clean cloth bags when they had finished foraging and returned to the roosting cave. Fecal pellets were recovered from the bags and air dried for subsequent laboratory analysis. Individual pellets were analyzed for insect remains by softening the samples in 70% ethanol and teasing them apart under a dissecting microscope, with all the droppings of an individual classified as 1 sample. Insect remains were identified taxonomically to ordinal level. Percentage volume occupied by each insect order was estimated visually to the nearest 5%, and frequency of occurrence of the different categories of prey was estimated for each fecal sample (Whitaker 1988; Zhang et al. 2005). A total of 100 pellets were analyzed from 8 individuals captured in Guizhou.

Molecular data collection.—We used DNeasy Tissue Kits (Qiagen, Shanghai, China) to isolate genomic DNA from wing membrane (*A. stoliczkanus*) and liver (*A. tricuspidatus*) tissue samples preserved in 95% ethanol. We amplified and sequenced complete *Cytb* (1,140 base pairs [bp]) and *ND1* (957 bp) gene sequences from samples of *Aselliscus*, and used newly sequenced or previously published sequences for both genes from *Coelops frithi* (from Taiwan), 4 species of Chinese *Hipposideros*, and 6 species of Chinese *Rhinolophus* in our phylogenetic comparisons. We also used some sequences we published previously and downloaded some relevant sequences from GenBank to carry out our phylogenetic investigations of *A. stoliczkanus* (Appendix I). A megadermatid (*Megaderma lyra)* and 2 pteropodids (*Pteropus scapulatus* and *Rousettus leschenaulti*) were employed as outgroups (Appendix I). *Cytb* sequences were amplified using the primers L14724 (5′-GGT CTT AGG CAA AAA ATT GGT GCA ACT C-3′—Kocher et al. 1989), Bat_Cytb_1 (5′-TAG AAT ATC AGC TTT GGG TG-3′—Li et al. 2006), and H15915R (5′-TCAGCTTTGGG

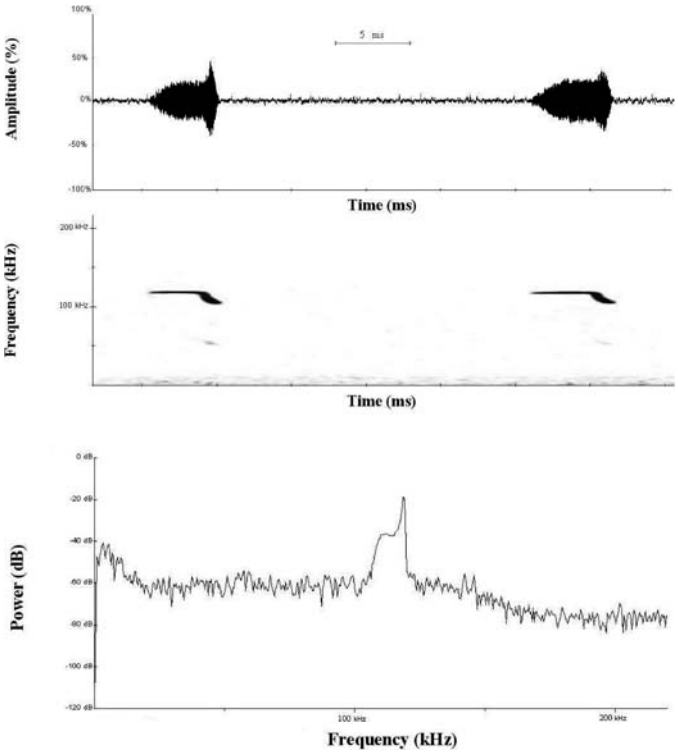

Fig. 1.—Oscillogram (top), sonogram (middle), and power spectrum (below) of typical calls from *Aselliscus stoliczkanus* recorded in Yunnan in November 2005.

TGTTGATGG-3′—Irwin et al. 1991). The primers for sequencing were similar to those used for amplifying.

Polymerase chain reaction conditions were as follows: 94°C (5 min); 35 cycles at 94°C (50 s), 50°C (40 s), and 72°C (80 s); 72°C (5 min). Primer pairs for *ND1* amplification and sequencing were L16S (5′-CCTCGATGTTGGATCAGG-3′) and HtMet (5′-GTATGGGCCCGATAGCTT-3′—Cao et al. 1998). The total volume of the polymerase chain reaction mixture was 50 μl, with reagents at a final concentration of 0.4 μM of each primer, 0.2 μM of each deoxynucleoside triphosphate, 1.5 μM $MgCl_2$, and 1 U of *Taq* DNA polymerase.

Phylogenetic analyses.—We used Modeltest, version 3.6 (Posada and Crandall 1998) to choose the best model of evolution for phylogenetic analyses. The program was used to determine the most appropriate substitution model for the *Cytb* and *ND1* sequence data, respectively, and the optimum maximum-likelihood parameters.

MrBayes 3.1 (Huelsenbeck and Ronquist 2001) and PAUP, version 4.0b (Swofford 2003) were employed to construct the phylogenetic trees. In the control block of each program, the setting for the codon substitution model and maximum-likelihood parameters followed the results of MODELTEST 3.6. The general time reversible model GTR+Γ+I was selected as the most appropriate model of nucleotide substitution ($\pi_A = 0.350$, $\pi_C = 0.393$, $\pi_G = 0.073$, $\pi_T = 0.185$; $r_{AC} = 0.400$, $r_{AG} = 12.885$, $r_{AT} = 0.556$, $r_{CG} = 0.250$, $r_{CT} = 8.730$; $I = 0.512$; $\alpha = 0.833$). In the Bayesian analyses, 6 Markov chains with 1 million generations were used for simulation. After 400,000 generations, the trees were sampled. Other sets were analyzed according to the options for vertebrate mitochondrial sequences. PAUP, version 4.0b, program used heuristic searches and tree-bisection-reconnection branch swapping options. In PAUP, version 4.0b, we also generated bootstrap values (2,000 replicates) with neighbor-joining and maximum-parsimony methods to test robustness of the tree topologies. We used MEGA3 (Kumar et al. 2004) to calculate the genetic distances of the different taxa using the Kimura 2-parameter model.

Table 1.—Diet composition of *Aselliscus stoliczkanus*. Data represent percent volume (%) of total diet represented by each insect group ($n = 100$ fecal samples).

Insect orders	Current study	Feng (2001)
Lepidoptera	78	43
Coleoptera	15	29
Hemiptera	6	
Odonata	<1	
Diptera		14
Trichoptera		5
Hymenoptera		2
Unidentified		7

Divergence estimates.—We combined our molecular sequence data with information from the fossil record to estimate divergence times within the genus *Aselliscus* and among other rhinolophoid bats represented in our data set. We utilized the software packages PAML 3.14 (Yang 1997), ESTBRANCHES, and MULTIDIVITIME (Kishino et al. 2001; Thorne and Kishino 2002; Thorne et al. 1998) for these analyses. For our divergence estimates, we followed Teeling et al. (2005) in using 2 fossil constraints. First, the basal date of divergence for Rhinolophoidea (Hipposideridae and Rhinolophidae plus Megadermatidae) is held to be no older than 55 million years (Paleocene–Eocene boundary) because no rhinolophoid fossils are known before the middle Eocene (McKenna and Bell 1997; Simmons and Geisler 1998). Second, the date of rhinolophid–hipposiderid divergence is taken to have occurred not less than 37 million years ago (mya) because fossils referable to both Hipposideridae and Rhinolophidae are known from the middle Eocene (Hand and Archer 2005; Hand and Kirsch 2003; McKenna and Bell 1997; Simmons and Geisler 1998). The split of Megadermatidae from other Rhinolophoidea (Hipposideridae and Rhinolophidae) was placed at 50 mya, and the split between Hipposideridae and Rhinolophidae at 40 mya (Teeling et al. 2005).

Results

Echolocation calls and diet.—The echolocation call frequency of *A. stoliczkanus* from Sichuan and Guizhou is high, with a constant-frequency component at 120.3 ± 0.3 kHz ($n = 10$), and a terminal frequency-modulated sweep down to 104.5 ± 2.1 kHz. The call duration is 5.4 ± 0.3 ms. The echolocation call frequency of bats from Yunnan is a little lower (1 individual called with the constant-frequency component at 118.4 kHz and a 2nd calledat 119.3 kHz; Fig. 1).

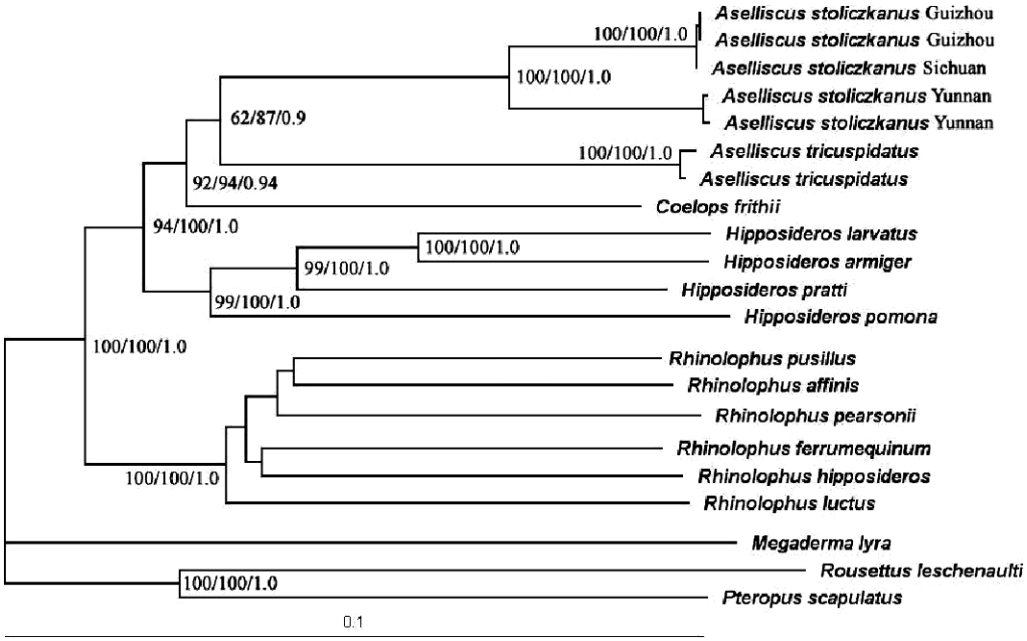

FIG. 2.—Maximum likelihood tree (ln-likelihood = −15,283 [likelihood value]) based on the combined mitochondiral DNA analysis (*Cytb* plus *ND1*) for *Aselliscus*, other hipposiderids (*Coelops* and *Hipposideros*), rhinolophids (*Rhinolophus*), and 3 outgroups (*Megaderma*, *Pteropus*, and *Rousettus*). Numbers at each node are neighbor-joining bootstrap values, maximum-parsimony bootstrap values, and posterior probability values given by Bayesian analyses, respectively. The bar at the bottom of the phylogenetic tree is a scale bar representing substitutions per site.

The diet of *A. stoliczkanus* is mainly composed of lepidopterans, beetles, and hemipterans (Table 1). Lepidopterans were the most abundant food items in the samples (79% of the diet in volume), followed by coleopterans (15%), hemipterans (7%), and odonates (<1%).

Phylogeny.—Based on the combined sequences, the neighbor-joining, maximum-parsimony, and Bayesian phylogenies were identical in topology but slightly different in branch support (Fig. 2). Within the bounds of our taxon sampling, the different reconstruction methods each supported several main phylogenetic conclusions: each tree supported the generic monophyly of *Aselliscus*, grouping *A. stoliczkanus* and *A. tricuspidatus* as sister lineages; all trees clustered *Aselliscus* and *Coelops* into a single lineage with high bootstrap test values (92 for neighbor-joining and 94 for maximum-parsimony) and a posterior probability value of 0.94; and Hipposideridae and Rhinolophidae constituted monophyletic sister clades. Relationships among different species of *Rhinolophus* included in our study were not always well resolved and are not considered further here. Table 2 presents the genetic distances among species sequenced in this study. Sequence diverge values at the 2 genes are broadly similar. Three individuals of *A. stoliczkanus* from Guizhou and Sichuan provinces of China have 5–6% sequence divergence compared with the 2 bats that were sampled in Yunnan Province. Sequence divergence between *A. stoliczkanus* and *A. tricuspidatus* is between 14% and 16%. The table also showed comparatively large divergence values among *Aselliscus* and other genera in the Rhinolophoidea, *Hipposideros*, and *Rhinolophus* at both genes.

Molecular dating.—We estimate the earliest divergences represented in our sampling of hipposiderids at 30 mya, whereas the deepest split within the family Rhinolophidae is estimated at 20 mya (Fig. 3). Interspecific splits within *Hipposideros* range from 6 to 20.5 mya. We estimate that *Coelops* diverged from ancestral *Aselliscus* at approximately 22 mya, and that within *Aselliscus*, the split between *A. tricuspidatus* and *A. stoliczkanus* dates to approximately 20 mya.

DISCUSSION

Echolocation and diet.—*Aselliscus* fly at low speeds and are very small-bodied, roosting in caves and foraging in cluttered microhabitats (Feng 2001; Lekagul and McNeely 1977; McKean 1972). Their low wing loading (as in most rhinolophid and hipposiderid species) lends flexibility when hunting for prey in a complicated environment. According to our results, the echolocation calls of *A. stoliczkanus* are of the typical hipposiderid constant-frequency–frequency-modulated type, characterized by high frequency and short call duration.

Bats of the families Hipposideridae and Rhinolophidae generally forage in forested areas, catching insects either aerially or by gleaning off foliage or the ground in these narrowed, cluttered environments (Bogdanowicz et al. 1999; Denzinger et al. 2004; Schnitzler and Kalko 1998). Previous investigations into the diets of hipposiderids and rhinolophids have demonstrated that moths and beetles dominate in the diets of these bats, and are selected in larger proportions than available in the local environment (Bowie et al. 1999; Churchill 1994; Goiti et al. 2004; Jones 1990; Jones et al. 1993; Pavey and Burwell 2000, 2004). Our results show that *A. stoliczkanus* primarily consumes lepidopterans and coleopterans. Research carried out at the same sites where we collected *A. stoliczkanus*

TABLE 2.—Sequence divergence matrix based on complete mitochondrial *Cytb* (1,140 bp, above the diagonal) and *ND1* (957 bp, below the diagonal) gene sequences for 2 species of *Aselliscus* and partial species of *Hipposideros*, *Rhinolophus*, and outgroup (*Megaderma lyra*, *Rousettus leschenaulti*, and *Pteropus scapulatus*). MEGA3 was used to calculate the genetic distances based on the Kimura 2-parameter model.[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1		0	0	0.06	0.062	0.16	0.163	0.139	0.202	0.194	0.199	0.197	0.189	0.196	0.193	0.213	0.238	0.2	0.256	0.286	0.267
2	0		0	0.06	0.062	0.16	0.163	0.139	0.202	0.194	0.199	0.197	0.189	0.196	0.193	0.213	0.238	0.2	0.256	0.286	0.267
3	0	0		0.06	0.062	0.16	0.163	0.139	0.202	0.194	0.199	0.197	0.189	0.196	0.193	0.213	0.238	0.2	0.256	0.286	0.267
4	0.056	0.056	0.056		0.002	0.159	0.162	0.151	0.204	0.187	0.189	0.184	0.195	0.199	0.185	0.211	0.237	0.199	0.245	0.264	0.251
5	0.056	0.056	0.056	0		0.159	0.162	0.151	0.204	0.187	0.187	0.184	0.195	0.199	0.188	0.208	0.234	0.199	0.245	0.264	0.248
6	0.142	0.142	0.142	0.151	0.151		0.002	0.156	0.198	0.196	0.183	0.203	0.204	0.211	0.21	0.22	0.219	0.223	0.256	0.287	0.258
7	0.142	0.142	0.142	0.15	0.15	0.004		0.157	0.199	0.198	0.185	0.204	0.207	0.214	0.213	0.22	0.222	0.223	0.257	0.288	0.261
8	0.16	0.16	0.16	0.161	0.161	0.158	0.158		0.174	0.173	0.169	0.187	0.183	0.201	0.179	0.193	0.192	0.18	0.246	0.265	0.234
9	0.182	0.182	0.182	0.184	0.184	0.164	0.164	0.172		0.088	0.133	0.18	0.197	0.204	0.19	0.21	0.208	0.19	0.26	0.289	0.272
10	0.194	0.194	0.194	0.187	0.187	0.177	0.176	0.171	0.093		0.129	0.149	0.204	0.208	0.207	0.212	0.218	0.207	0.252	0.283	0.264
11	0.171	0.171	0.171	0.173	0.173	0.151	0.151	0.172	0.122	0.115		0.164	0.196	0.208	0.181	0.205	0.2	0.189	0.234	0.262	0.245
12	0.174	0.174	0.174	0.187	0.187	0.161	0.161	0.164	0.156	0.172	0.155		0.212	0.206	0.209	0.224	0.233	0.218	0.254	0.285	0.278
13	0.171	0.171	0.171	0.19	0.19	0.174	0.17	0.172	0.19	0.19	0.167	0.186		0.145	0.112	0.142	0.162	0.151	0.247	0.268	0.251
14	0.196	0.196	0.196	0.205	0.205	0.183	0.178	0.189	0.197	0.199	0.189	0.202	0.111		0.149	0.159	0.181	0.158	0.269	0.264	0.255
15	0.199	0.199	0.199	0.198	0.198	0.187	0.185	0.179	0.187	0.183	0.194	0.189	0.121	0.121		0.149	0.167	0.135	0.257	0.253	0.244
16	0.18	0.18	0.18	0.189	0.189	0.185	0.18	0.177	0.187	0.183	0.184	0.2	0.117	0.123	0.121		0.164	0.137	0.267	0.236	0.246
17	0.176	0.176	0.176	0.185	0.185	0.174	0.174	0.185	0.19	0.196	0.2	0.184	0.132	0.13	0.135	0.126		0.168	0.259	0.247	0.261
18	0.206	0.206	0.206	0.205	0.205	0.181	0.179	0.178	0.191	0.19	0.191	0.187	0.136	0.131	0.14	0.125	0.129		0.265	0.251	0.234
19	0.23	0.23	0.23	0.226	0.226	0.22	0.216	0.236	0.233	0.233	0.217	0.236	0.201	0.213	0.209	0.22	0.218	0.215		0.281	0.265
20	0.248	0.248	0.248	0.252	0.252	0.248	0.246	0.223	0.241	0.24	0.221	0.246	0.243	0.235	0.243	0.244	0.244	0.252	0.235		0.205
21	0.224	0.224	0.224	0.235	0.235	0.21	0.208	0.217	0.204	0.21	0.195	0.215	0.203	0.216	0.216	0.215	0.225	0.227	0.236	0.175	

[a] 1, *Aselliscus stoliczkanus* Guizhou; 2, *A. stoliczkanus* Guizhou; 3, *A. stoliczkanus* Sichuan; 4, *A. stoliczkanus* Yunnan; 5, *A. stoliczkanus* Yunnan; 6, *A. tricuspidatus*; 7, *A. tricuspidatus*; 8, *Hipposideros larvatus*; 9, *H. armiger*; 10, *H. larvatus*; 11, *H. pratti*; 12, *H. pomona*; 13, *Rhinolophus pusillus*; 14, *R. pearsonii*; 15, *R. affinis*; 16, *R. ferrumequinum*; 17, *R. luctus*; 18, *R. hipposideros*; 19, *Megaderma lyra*; 20, *Rousettus leschenaulti*; 21, *Pteropus scapulatus*.

revealed that the percentage of Coleoptera in the diets of sympatric *Hipposideros armiger*, *H. pratti*, and *H. larvatus* was 42%, 43%, and 38%, respectively, and for Lepidoptera, 22%, 26%, and 31%, respectively (Feng 2001). Our results on *A. stoliczkanus* obtained in November 2005 are quite different from those reported by Feng (2001) obtained in June 2000 at the same site, although the relative rankings of Lepidoptera and Coleoptera were the same. We suspect that annual or seasonal variation in the availability of various insects might explain these differences.

Because we did not intensively study the relative proportion of biomass of different insect orders at this site, it is impossible to determine whether selection of certain insects by these hipposiderids was disproportionate to their occurrence in the landscape as a whole. Because diets of insectivorous bats can be highly plastic, varying with local environment, seasonality, and food resource availability (Kunz 1982), it is not surprising that the diet of *A. stoliczkanus* recorded in our study is somewhat different from that recorded by Feng (2001). Our results are consistent with the limited findings of Nabhitabhata

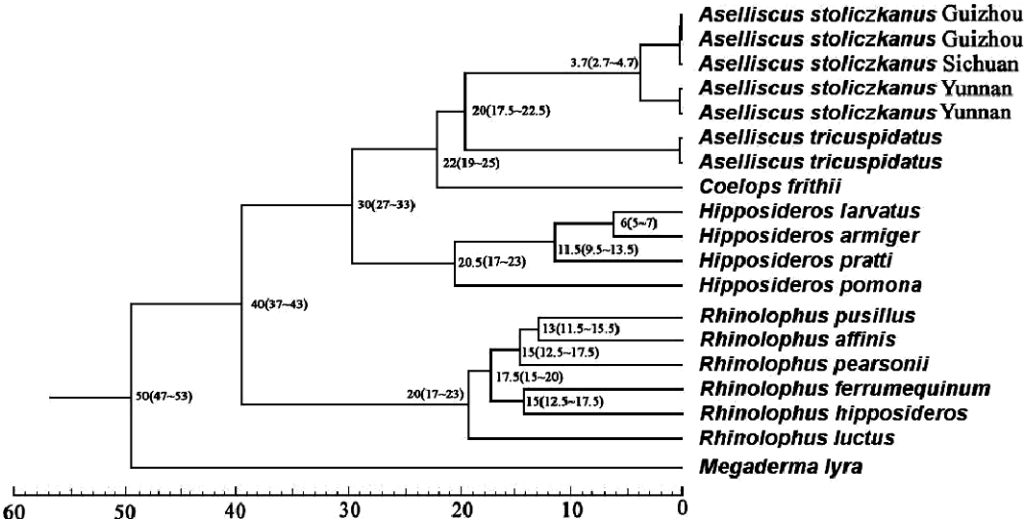

FIG. 3.—Estimated timescale (in millions of years; means with ranges showing standard errors) for diversification of selected rhinolophoid taxa based on our combined mitochondrial DNA analysis with the imposition of 2 fossil constraints (see text). The x axis represents millions of years ago (mya).

(1986), who found moth remains in the stomachs of all 3 bats examined in Thailand, and Diptera in 1 stomach. No comparative data are yet available regarding the diet of *A. tricuspidatus* (Bonaccorso 1998).

Phylogenetic affinities and divergence date estimates.—Sequence divergence values between *A. stoliczkanus* sampled in Guizhou and Sichuan versus Yunnan provinces was relatively high (5–6%). However, these values were considerably lower than the divergence between *A. stoliczkanus* and *A. tricuspidatus*. In combination with the absence of echolocation call frequency differences among Chinese populations, the sequence divergence estimates suggest that Chinese *A. stoliczkanus* may represent geographic races, rather than distinct species, given that cryptic species of hipposiderid bats usually diverge in call frequency (Guillén-Servent and Francis 2006; Thabah et al. 2006).

Each of our trees (maximum-likelihood and Bayesian) supported the sister-relationship of *Aselliscus* and *Coelops*, suggesting that *Coelops* is more closely related to *Aselliscus* than to *Hipposideros*. Recently, the supertrees of Jones et al. (2002) questioned the monophyly of *A. stoliczkanus* and *A. tricuspidatus*, but our trees showed the 2 species of *Aselliscus* truly comprised a monophyletic group. Our results also reject the idea that *Aselliscus* is a basal lineage within Hipposideridae (Hand and Kirsch 1998, 2003), that it may be linked phylogenetically with rhinolophids (Pierson 1986), or that it is nested within *Hipposideros* (Wang et al. 2003).

In support of traditional taxonomic arrangements (e.g., Koopman 1994) and more recent molecular assessments, examination of our data supports the hypothesis that rhinolophids and hipposiderids are monophyletic sister lineages (Hutcheon et al. 1998; Jones et al. 2002; Levasseur et al. 2003; Springer et al. 2003; Teeling et al. 2002, 2003). Our molecular divergence estimates indicate that *Aselliscus* is an old genus. The split between *A. stoliczkanus* and *A. tricuspidatus* is estimated at 20 mya, which would indicate that the 2 species diverged from each other in the early Miocene. The well-supported topology of our phylogenetic trees, with *Aselliscus* as sister to *Coelops* (a generic lineage endemic to eastern Asia and the Sunda Shelf), strongly indicates a mainland Asian origin for *Aselliscus*. We suggest that the split between *A. stoliczkanus* (today endemic to eastern Asia and Indochina) and *A. tricuspidatus* (with distribution centered on New Guinea) ultimately reflects a dispersal event from Asia to emergent areas of Melanesia, perhaps (if our molecular dating is accurate) in the early Miocene, when New Guinea is thought to have comprised a series of small, discrete islands separated from Australia (Aplin et al. 1993; Flannery 1995a). Whatever its precise biogeographic history, *A. tricuspidatus* is quite likely to be the most ancient endemic rhinolophoid lineage present in Melanesia today, along with the endemic nominal hipposiderid genus *Anthops* (Flannery 1995a).

Our molecular sampling included 4 species of *Hipposideros* that are often classified in different ''species-groups'' within the genus (Hill 1963; Koopman 1994). Our estimates suggest that divergences within the genus date back 20 million years—a similar time frame for divergences within *Aselliscus*. The oldest known fossil occurrence of *Hipposideros* is from the Oligocene of Africa, and fossils attributed to *Hipposideros* are recorded from the Miocene of South Africa and are abundant in the Miocene record of Riversleigh, Australia (Hand and Archer 2005; Hand and Kirsch 1998). Accordingly, our molecular divergence estimates within the genus are compatible with current knowledge regarding the fossil record. Similarly, *Rhinolophus* species included in our sampling are classified in several different species-group within *Rhinolophus*; of these, *R. luctus* is generally classified in the *trifoliatus* group, considered by some reviewers to be among the most plesiomorphic lineages of horseshoe bats (Bogdanowicz 1992; Guillén-Servent et al. 2003), an interpretation consistent with our results, which indicate *luctus* to be the most basal lineage sampled. Our results also suggest that the basal split in *Rhinolophus* occurred in the same time range as the origin and initial diversification of *Hipposideros* and *Aselliscus*—that is, about 20 mya, a date likewise consistent with fossil data and previous biogeographic interpretations (Bates et al. 2004; Guillén-Servent et al. 2003).

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APPENDIX I

Collection localities of the bats analyzed, with the corresponding GenBank accession numbers.

Species	Collection locality	Accession no.	
		Cytb	*ND1*
Aselliscus stoliczkanus	Yunnan, China	DQ888668	DQ888648
A. stoliczkanus	Yunnan, China	DQ888670	DQ888660
A. stoliczkanus	Sichuan, China	DQ888673	DQ888654
A. stoliczkanus	Guizhou, China	DQ888676	DQ888665
A. stoliczkanus	Guizhou, China	DQ888677	DQ888667
A. tricuspidatus	Espiritu Santo, Vanuatu	DQ888675	DQ888652
A. tricuspidatus	Espiritu Santo, Vanuatu	DQ888679	DQ888657
Coelops frithii	Dayuanshan, Kenting, Taiwan	DQ888674	DQ888666
Hipposideros armiger	Guizhou, China	DQ297585	DQ888663
H. larvatus	Guangdong, China	DQ888672	DQ888653
H. pratti	Guangxi, China	DQ297584	DQ888651
H. pomona	Yunnan, China	DQ888671	DQ888662
Rhinolophus pearsonii	Sichuan, China	DQ297587	DQ888664
R. affinis	Guizhou, China	DQ297582	DQ888661
R. hipposideros	Upper Langford, United Kingdom	DQ297586	DQ888658
R. ferrumequinum	Yunnan, China	DQ297575	DQ888656
R. pusillus	Hubei, China	DQ297583	DQ888655
R. luctus	Hubei, China	DQ297596	DQ888659
Megaderma lyra	Yunnan, China	DQ888678	DQ888650
Rousettus leschenaulti	Yunnan,China	DQ888669	DQ888649
Pteropus scapulatus	Australia	NC_002619	NC_002619