A NEW SPECIES FROM SOUTHWESTERN CHINA IN THE AFRO-PALEARCTIC LINEAGE OF THE HORSESHOE BATS (*RHINOLOPHUS*)

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A new species of horseshoe bat (Chiroptera: Rhinolophidae) is described from southwestern China. The presence of a wedge-shaped sella and pointed connecting process of the nose leaf aligns the new species to the *landeri* group in the Afro-Palearctic lineage of *Rhinolophus*. However, the new species is distinctly separable from these allopatrically distributed species by its noticeably larger body size. Other sympatric large-sized species of *Rhinolophus* have rounded connecting processes. Molecular systematic analyses based on mitochondrial cytochrome-*b* sequences confirmed the affinity of the new species to the Afro-Palearctic lineage, but in a clade most closely related to the *ferrumequinum*, *fumigatus*, and *maclaudi* groups. Of these species, only *R. ferrumequinum* ranges into Asia and overlaps in distribution with the new species. *R. ferrumequinum* is similar in general body size and external appearance; however, the new species is distinct in the characteristics of the nose leaf, skull, and baculum. The presence of a new species from southwestern China in the Afro-Palearctic lineage indicates a more complex historical biogeographic scenario within *Rhinolophus* than previously known. The difficulties found in allocating the new species to one of the phenetically described traditional species groups stress the convenience of using a phylogenetically based systematic organization of the genus *Rhinolophus*.

Key words: biogeography, Chiroptera, cytochrome *b*, molecular systematics, morphometrics, new species, Rhinolophidae, *Rhinolophus*, southwestern China, taxonomy

Horseshoe bats (*Rhinolophus*) comprise 77 species in the monogeneric family Rhinolophidae that are endemic to the Old World tropical and subtropical regions, and represent the 2nd most speciose genus within Chiroptera (Simmons 2005). Our understanding of rhinolophid systematics has greatly benefited from the monographic work of Csorba et al. (2003), who organized the genus into 15 supraspecific groups primarily based on the morphological classification of Andersen (1918) and the taxonomic proposals by Bogdanowicz (1992). However, a recent molecular phylogenetic analysis has hypoth-

esized several alternative higher-level relationships (Guillén-Servent et al. 2003), and species-level diversity is still underestimated, with new taxa recently described (Yoshiyuki and Lim 2005) or awaiting description (Francis et al. 2000; Struebig et al. 2005; Suyanto and Struebig 2007).

Rhinolophid bats are most diverse in the Indomalayan region, with 42 species in 9 species groups (*euryotis*, *ferrumequinum*, *hipposideros*, *megaphyllus*, *pearsonii*, *philippinensis*, *pusillus*, *rouxii*, and *trifoliatus*—Corbet and Hill 1992; Csorba et al. 2003). The Afro-tropical region is the next most speciose with 21 species in 6 species groups (*adami*, *capensis*, *ferrumequinum*, *fumigatus*, *landeri*, and *maclaudi*). The intervening temperate areas from the Mediterranean to the Indus Division in the southwestern Palearctic have a much less diverse rhinolophid fauna with 7 recognized species. The Australasian region, with 3 or 4 recognized species, is the poorest in rhinolophid diversity (Csorba et al. 2003; Simmons

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2005). The ecological and evolutionary processes that have promoted this uneven distribution of diversity remain unclear (Bogdanowicz 1992; Bogdanowicz and Owen 1992; Guillén-Servent et al. 2003).

During recent biodiversity surveys in southwestern China, we discovered a distinctive and hitherto undescribed species of *Rhinolophus*. Herein, we describe this new horseshoe bat, and compare it with sympatric species and others of similar body size using morphometric techniques. Molecular systematic analyses based on mitochondrial cytochrome-*b* nucleotide sequence data are used to review phylogenetic relationships of the new species. These phylogenetic relationships give new perspectives to the understanding of the historical biogeography of *Rhinolophus*. In addition, we give a preliminary evaluation of the conservation status of the new species.

MATERIALS AND METHODS

Fieldwork and museum collections.—The 1st study site was Wumulong, Yongde County, Yunnan Province, China, where a cave was surveyed during the daytime and a sample of bats was captured with a hand net in May 2005. The cave was in a gorge near farmland with tea plantations surrounded by subtropical montane mixed forest. The 2nd site was Kuankuoshui Nature Reserve in Suiyang County, Guizhou Province, China, which was surveyed for small mammals from 16 to 24 April 2006. The general habitat was subtropical lower montane mixed forest on karst formations; however, there were extensive agricultural fields primarily of tea plantations within the area. Mist nets and harp traps were set within the forest and at caves to capture bats.

Research on wild mammals followed the guidelines of the American Society of Mammalogists (Gannon et al. 2007) and was approved by the Royal Ontario Museum Animal Use and Care Committee and the Yunnan Provincial Forestry Department. Specimens of *Rhinolophus* were examined in the major Chinese mammal collections, including the Kunming Institute of Zoology (KIZ), Institute of Zoology in Beijing, Guangdong Entomological Institute in Guangzhou, China West Normal University in Nanchong, and Guangzhou University in Guangzhou.

Morphometric data.—In addition to the 5 collected specimens of the new species described below, other specimens of sympatric species of *Rhinolophus* in most species groups (Csorba et al. 2003) and all the main phylogenetic lineages (Guillén-Servent et al. 2003) were examined and used in the morphometric analyses (Appendix I). These included specimens collected from Yunnan and Guizhou provinces in China, and deposited in KIZ (Chinese Academy of Sciences) and Royal Ontario Museum (ROM); and specimens from Afrotropical, Mediterranean, and South East Asian regions deposited in the Estación Biológica de Doñana (EBD; Spanish Council for Scientific Research, Sevilla, Spain), and the Instituto de Ecología A.C. (IEX, Mexican National Council for Science and Technology, Xalapa, Mexico). Standard cranial and external measurements (in mm) were taken with electronic calipers as described in Csorba et al. (2003). For those specimens not collected by us, data for body mass (g), length of head and body, tail length, and ear length were taken from the field tags. The specimens examined were adults, as indicated by ossification of the arthroses of the wing fingers. There were no obvious differences between males and females, and therefore data for both sexes were combined.

The external measurements recorded (in mm, unless stated otherwise) were: mass (in g) of body (M), length of head and body (HB), length of forearm (FA), length of metacarpal of 3rd digit (M-III), length of 1st phalanx of 3rd digit (P1-III), length of 2nd phalanx of 3rd digit (P2-III), length of metacarpal of 4th digit (M-IV), length of 1st phalanx of 4th digit (P1-IV), length of 2nd phalanx of 4th digit (P2-IV), length of metacarpal of 5th digit (M-V), length of 1st phalanx of 5th digit (P1-V), length of 2nd phalanx of 5th digit (P2-V), length of tibia (TB), length of tail (TL), length of ear (E), and width of horseshoe (HW). The craniodental measurements were: length of skull (SL), mastoid width (MW), zygomatic width (ZW), interorbital width (IOW), length of palate (PL), length of upper toothrow (CM3U), rostral width (M3M3WU), length of lower toothrow (CM3L), and length of mandible (ML).

Multivariate analysis.—We used principal component analyses to summarize the morphometric variation and explore the overall phenetic similarity of the new species to other species of *Rhinolophus*. In order to facilitate the interpretation of the patterns, the analyses were performed independently for the wing and the skull variables. We used arithmetic means for each variable within species as input data. The mean values were logarithmically (log_{10}) transformed for a multivariate normal distribution of the species in morphospace. Vectors in this logarithmic morphospace correspond to products and ratios of variables, which may be interpreted as size and shape parameters. We used covariance matrix scaling for preserving the original position of the species in morphological space without distortion, which allows for comparison with other studies (Ricklefs and Miles 1994). We calculated euclidean distances on component morphospace as overall indices of morphological similarity. Minimum spanning trees were calculated on the euclidean distances in the morphospace defined by components 2–4 for each analysis. Principal component analyses were run with the R version 2.3.1 statistical package (R Development Core Team 2006), and the euclidean distances and minimum spanning trees were calculated using Ntsyspc 2.1 (Rohlf 2000).

Molecular data.—We obtained nucleotide data from 14 species of *Rhinolophus* for the mitochondrial cytochrome-*b* gene (*Cytb*), including 2 specimens of the new species (the holotype from Yunnan and 1 of the paratypes from Guizhou). In addition, we included sequences from 8 species in the genus available from GenBank. The data set included 26 specimens representing all 6 major lineages identified by Guillén-Servent et al. (2003). Because the morphology and the preliminary molecular analyses indicated an allegiance of the bat with the Afro-Palearctic lineage, we generated comparative data for the largest sample of species in this lineage that we could obtain,

including most species groups recognized by Csorba et al. (2003). Only the *adami* group, which includes only 2 very rare taxa, was missing. Because the new species was morphologically similar to *R. ferrumequinum* in many aspects, we included samples from western and eastern populations of that species (Rossiter et al. 2007). We used GenBank sequences of 4 taxa in 3 genera of the sister family Hipposideridae (*Aselliscus stoliczkanus*, *Coelops frithii*, *Hipposideros bicolor*, and *H. pratti*) as outgroups.

Molecular laboratory methods.—Total genomic DNA was extracted from 20–40 mg of tissue preserved at −80°C, in 96% ethanol, lysis buffer (Longmire et al. 1997) or dimethylsulf-oxide buffer (Seutin et al. 1991), with a salting-out protocol as implemented in the Puregene kit (Gentra Systems, Inc., Minneapolis, Minnesota) according to the directions of the manufacturer, and using proteinase K overnight digestion. Other samples were extracted according to standard phenol–chloroform extraction protocols (Hillis et al. 1996) after proteinase K digestion in cetyltrimethylammonium bromide digestion buffer (G. Seutin, pers. comm.). Skin samples from museum specimens were extracted by using standard phenol–chloroform protocols (Maniatis et al. 1982) with proteinase K digestion.

The complete *Cytb* and short flanking sequences were amplified by polymerase chain reactions using primers L14724ag (5′-ATGATATGAAAAACCATCGTTG-3′) and H15915ag (5′-TTTCCNTTTCTGGTTTACAAGAC-3′) in a reaction cocktail as detailed in Guillén-Servent and Francis (2006). Polymerase chain reaction conditions were an initial cycle with a 45-s denaturation at 94°C, 30-s annealing at 50°C, and 70-s extension at 72°C followed by 39 cycles with the denaturation phase shortened to 30 s, and a final extension of 4 min at 72°C. Products were prepared for sequencing by running them in a 1.2% agarose gel stained with ethidium bromide. Bands containing target products were excised from the gel and the agarose was removed by using a Geneclean-III kit (BIO 101, Inc., Vista, California) following the manufacturer's instructions. Both strands of the polymerase chain reaction products were amplified in sequencing reactions with BigDye version 1.2 chain terminators (Applied Biosystems, Inc., Foster City, California) according to the manufacturer's instructions. The sequencing products were electrophoresed on an ABI 310 capillary sequencer (Applied Biosystems, Inc.) after isopropanol precipitation and denaturing in 20 μl of deionized formamide. Chromatograms were visualized and sequences aligned by eye with BioEdit version 7.0.1 software (Hall 1999) and inspected for misreads. Reads were clear at least to 650 base pairs (bp) in all sequences used in the analyses and an approximately 200-bp clean overlap between the light and the heavy strands was usually obtained.

Phylogenetic inference.—Nucleotides were coded as unordered, discrete characters (G, A, T, and C) in a phylogenetic analysis using the parsimony criterion. In order to correct for transitional bias, the phylogeny was inferred under a transversion-weighted parsimony criterion, whereby transversions were weighted 6 times over transitions (the ratio was calculated empirically by maximum likelihood with PAUP* onto the best maximum-parsimony tree, specifying a substitution model with gamma rates and a proportion of invariant sites [Swofford 2002]). Best phylogenetic hypotheses were found with PAUP* by using 100 heuristic searches with random addition of sequences, and branch and bound swapping. Confidence in the resulting topology was assessed by a bootstrap analysis with 1,000 replications.

Phylogenetic relationships also were inferred by Bayesian analysis (Felsenstein 2004; Huelsenbeck et al. 2001; Rannala and Yang 1996) as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). MrBayes allows application of different models of evolution and estimation of specific parameter values for multiple data partitions. Because mode of evolution can differ among genes and different positions in coding genes, which may make a single model approach inappropriate (Brandley et al. 2005), we partitioned *Cytb* into sets corresponding to the 3 codon positions, and analyzed them in a combined analysis. The appropriate model for each partition was determined by using the Akaike information criterion for model selection, as implemented in MrModeltest version 2.2 (a variant of Posada and Crandall's [1998, 2001] Modeltest version 3.6 [Nylander 2004]). The tree used for calculation of likelihoods under the different models was the shortest obtained under weighted parsimony criterion (transversions weighted 6 times over transitions).

A Hasegawa–Kishino–Yano model (Hasegawa et al. 1985) with a proportion of invariant sites (I) and gamma-distributed rate variation among the remaining sites (Γ—Yang 1996) was the best fit for the 1st codon position (HKY85+I+Γ). A general time-reversible (GTR—Tavaré 1986)+I+Γ model was the best fit for both the 2nd and 3rd position sites. In all 3 cases, unequal base frequencies fitted the data better than equal frequencies. Four rate categories were specified for the discrete approximation to gamma-distributed rates among sites.

Base frequencies, rates in the substitution matrix, shape of the gamma distribution, and proportion of invariable sites were treated as unknown variables with uniform priors to be estimated in the analysis. Parameters, except topology and relative branch lengths, were unlinked such that each partition had its own set of independent estimates, which allowed the 3 partitions to evolve under different rates. We ran 2 simultaneous Bayesian analyses consisting of 4×10^6 generations with 1 cold and 3 incrementally heated Markov chains (with default heating values) with random starting trees. Trees were sampled every 100 generations. Burn-in values were determined by monitoring convergence on plots of log-likelihood values and by checking the average standard deviation of split frequencies between the 2 runs. Results were summarized as the majority rule consensus of all trees obtained in the 2 runs after the burn-in period, and posterior probabilities of nodes were regarded as estimators of confidence (e.g., Alfaro et al. 2003). *C. frithii* was designated as the outgroup for rooting the resulting trees.

We employed a Bayesian approach to test the hypothesis of monophyly of the new species with the species in the Afro-Palearctic lineage by building a 99.9% credible set of unique

TABLE 1.—Mean (1st row) and range (2nd row) of the external and cranial measurements for *Rhinolophus xinanzhongguoensis*, sp. nov., and samples of 16 other species of *Rhinolophus*. The name below the species is the species group used by Csorba et al. (2003) and, in parentheses, the lineage described by Guillén-Servent et al. (2003). We have not assigned the new species to any of the traditional species groups, because both morphologically and phylogenetically it is located in an intermediate position. Acronyms for the variables are explained in the "Materials and Methods." All measurements are in millimeters except for body mass in grams. Sample size is indicated in parentheses after the species name, and after the mean where some specimens could not be measured for that variable. Museum numbers, sex, country, and province of collection of the specimens are listed in Appendix I. The last 2 columns contain the euclidean distances of *R. xinanzhongguoensis* to the other species in the morphospace defined by principal components 2 and 3 for the analyses for the wing (EUWC23) and skull (EUSC23) variables, respectively.

Species	M	HB	FA	M-III	P1-III	P2-III	M-IV	P1-IV	P2-IV	M-V	P1-V	P2-V	TB
R. acuminatus (2)	13.1	53.5	47.3	35.2	14.5	21.6	36.3	9.6	12.9	36.5	10.7	14.0	22.3
pusillus (Rhynophyllotis)	12.4–13.7	53.0–54.0	47.2–47.3	34.0–36.4	14.0–15.0	21.5–21.7	35.6–37.0	9.2–10.0	12.8–13.0	35.4–37.6	10.0–11.4	13.4–14.5	20.2–24.3
R. affinis (3)	15.5	58.3	52.4	38.5	15.1	27.5	39.4	10.3	16.9	41.5	12.5	13.5	23.5
megaphyllus (Coelophyllus)	14.5–16.0	56.0–60.0	51.7–53.3	36.7–39.7	15.0–15.2	24.3–29.1	37.4–40.9	10.1–10.6	14.6–18.0	39.5–42.6	11.8–12.9	12.9–14.2	22.6–24.8
R. alcyone (2)	16.3	66.5	52.5	36.6	16.6	30.5	41.0	8.1	19.9	40.3	11.5	18.4	23.5
landeri (Rhinolophus)	16.0–16.5	61.0–66.0	52.4–52.6	36.3–36.8	15.9–17.3	30.0–31.0	40.8–41.1	7.7–8.2	19.2–20.5	40.3–40.3	11.3–11.7	18.3–18.4	23.3–23.6
R. blasii (1)	11.0	50.0	49.0	32.0	16.0	35.0	34.0	9.2	15.6	35.8	12.0	13.5	21.1
landeri (Rhinolophus)	—	—	—	—	—	—	—	—	—	—	—	—	—
R. xinanzhongguoensis, sp. nov. (5)	22.8 (4)	62.6	59.8	41.7	19.3	33.9	44.2	11.2	21.2	44.5	14.7	16.5	24.9
incertae sedis (Rhinolophus)	20.0–26.0	59.0–70.0	58.7–60.4	40.7–42.2	18.9–19.8	31.9–34.9	42.5–45.8	10.8–11.5	20.6–21.7	43.5–45.5	14.4–15.0	16.0–17.3	23.2–25.9
R. euryale (2)	9.9	50.0	47.8	33.3	14.3	29.8	35.9	6.9	19.3	35.8	10.5	12.9	20.4
euryale (Rhinolophus)	9.3–10.5	50.0–50.0	46.8–48.8	33.0–33.6	14.3–14.3	29.5–30.0	35.0–36.7	6.7–7.0	19.0–19.5	35.1–36.5	10.3–10.7	12.8–13.0	19.5–21.3
R. ferrumequinum (5)	19.8	65.0	60.7	40.9	21.5	34.2	45.1	12.6	21.6	46.6	15.3	18.2	26.1
ferrumequinum (Rhinolophus)	16.0–22.0	61.0–71.0	58.9–63.7	39.5–42.0	20.0–23.0	32.3–35.3	43.7–45.8	11.6–13.3	20.8–22.5	45.0–47.7	14.8–15.8	17.1–20.0	25.1–27.5
R. fumigatus (1)	14.0	55.0	53.7	36.0	18.6	31.0	40.3	10.7	20.7	42.0	12.2	17.1	21.9
fumigatus (Rhinolophus)	—	—	—	—	—	—	—	—	—	—	—	—	—
R. landeri (2)	8.2	48.5	44.0	29.8	13.5	24.5	34.4	6.8	15.3	33.5	9.3	14.4	18.1
landeri (Rhinolophus)	8.0–8.3	48.0–49.0	43.0–44.9	29.8–29.8	13.0–13.9	24.0–25.0	33.7–35.1	6.1–7.4	15.2–15.4	33.0–34.0	8.9–9.7	14.1–14.7	17.6–18.5
R. luctus (2)	39.5	90.5	73.5	48.5	28.0	39.7	57.7	15.6	24.9	58.7	16.7	28.1	37.3
trifoliatus (Aquias)	39.0–39.9	90.0–91.0	71.1–76.0	47.8–49.1	26.5–29.4	36.4–43.0	56.2–59.1	14.8–16.3	23.7–26.0	58.0–59.3	15.7–17.8	26.5–29.6	37.0–37.5
R. mehelyi (2)	—	52.5	50.5	36.5	14.1	29.1	38.0	7.8	19.1	39.2	11.4	12.5	20.7
euryale (Rhinolophus)	—	52.0–53.0	50.4–50.6	36.1–36.8	13.6–14.5	28.0–30.1	37.0–38.9	7.6–8.0	18.4–19.8	38.1–40.2	11.3–11.5	12.2–12.8	20.6–20.7
R. pearsonii (6)	20.5	61.8	58.0	39.1	19.6	28.0	42.1	12.8	18.3	44.7	13.9	19.1	29.1
pearsonii (Coelophyllus)	19.0–22.0	52.0–65.0	56.3–60.2	38.0–40.7	19.1–20.8	26.7–29.3	40.3–43.2	12.3–13.9	16.9–19.8	43.5–46.2	13.5–14.2	17.8–20.8	28.3–31.7
R. simulator (1)	8.4	50.0	48.5	32.9	16.2	29.0	37.1	9.0	11.0	37.5	11.1	14.0	19.3
capensis (Rhinolophus)	—	—	—	—	—	—	—	—	—	—	—	—	—
R. sinicus (3)	12.7	54.0	47.5	34.2	15.2	25.1	35.6	11.7	14.7	36.3	12.8	12.1	19.9
rouxi (Indorrhinolophus)	12.0–14.0	53.0–54.0	48.0–48.3	32.1–35.5	14.8–15.6	24.0–25.9	33.2–37.0	11.2–12.1	14.3–15.1	34.0–37.7	12.8–12.9	11.4–12.6	18.3–20.7
R. thomasi (6)	14.5	56.8	50.6	36.9	16.6	27.1	37.5	12.0	16.4	39.1	13.8	11.8	20.8
rouxi (Indorrhinolophus)	10.0–17.0	47.0–61.0	49.2–51.5	36.0–38.1	16.0–17.2	26.1–28.2	36.2–38.9	11.5–12.3	15.0–17.3	37.7–40.5	13.4–14.1	10.3–13.1	20.0–21.8
R. yunanensis (2)	18.5	62.5	56.1	37.5	19.1	27.5	41.7	12.1	17.3	44.3	13.2	19.5	28.6
pearsonii (Coelophyllus)	17.0–20.0	60.0–65.0	55.7–56.5	27.3–37.7	19.0–19.1	25.9–29.2	40.9–42.5	11.9–12.3	16.9–17.7	44.0–44.6	13.0–13.3	18.8–20.2	27.9–29.3
R. trifoliatus (2)	11.4	55.3	49.5	31.9	18.8	28.6	37.4	11.5	16.3	39.4	12.1	17.6	24.1
trifoliatus (Aquias)	10.8–12.0	54.0–56.5	47.2–51.8	29.9–33.9	17.4–20.1	27.4–29.8	35.5–39.2	11.3–11.7	16.0–16.5	38.5–40.2	11.5–12.6	16.4–18.8	21.8–26.3

TABLE 1.—Extended.

Species	TL	E	HW	SL	MW	ZW	IOW	PL	CM3U	M3M3WU	CM3L	ML	EUWC23	EUSC23
R. acuminatus (2)	22.8	19.5	7.5	20.8	10.0	10.9	2.7	2.3	8.2	8.1	8.8	14.3	0.205	0.107
pusillus (*Rhynophyllotis*)	22.5–23.0	19.0–20.0	7.4–7.6	20.3–21.3	9.8–10.1	10.7–11.0	2.7–2.7	2.2–2.4	8.0–8.4	8.0–8.1	8.7–9.0	14.0–14.5	0.082	0.169
R. affinis (3)	27.0 (2)	21.0 (2)	8.1	21.8	10.3	10.7	2.3	2.1	8.4	8.2	8.1	14.6		
megaphyllus (*Coelophyllus*)	26.0–28.0	20.0–22.0	8.1–8.3	21.6–22.0	10.2–10.4	10.5–10.9	2.2–2.4	2.0–2.1	8.1–8.7	8.2–8.3	7.6–8.4	13.6–15.2	0.174	0.090
R. alcyone (2)	24.0	23.5	10.5	22.6	10.7	12.0	2.7	2.8	8.8	8.6	9.4	15.7		
landeri (*Rhinolophus*)	21.0–27.0	23.0–24.0	9.7–11.3	22.0–23.2	10.5–10.9	11.6–12.5	2.7–2.8	2.6–2.9	8.4–9.2	8.3–8.9	9.0–9.9	15.2–16.3	0.078	0.038
R. blasii (1)	27.0	19.0	6.8	19.7	9.2	9.6	2.6	2.3	7.0	6.7	7.4	12.4		
landeri (*Rhinolophus*)	—	—	—	—	—	—	—	—	—	—	—	—		
R. xinanzhongguoensis, sp. nov. (5)	34.4	21.4	9.2 (4)	22.8	10.7	10.3	2.8	2.8	7.7	7.5	7.9 (4)	14.7 (4)	0.000	0.000
incertae sedis (*Rhinolophus*)	30.0–39.0	21.0–22.0	8.9–9.5	22.6–23.0	10.5–11.0	10.1–10.5	2.6–3.0	2.7–3.1	7.5–7.8	7.4–7.6	7.7–8.2	14.0–15.0	0.114	0.071
R. euryale (2)	24.5	20.5	6.4	18.6	9.4	9.6	2.2	2.1	6.3	6.7	6.7	12.0		
euryale (*Rhinolophus*)	24.0–25.0	20.0–21.0	6.2–6.5	18.5–18.8	9.4–9.5	9.5–9.7	2.2–2.3	2.1–2.1	6.2–6.4	6.7–6.7	6.6–6.7	11.7–12.2	0.027	0.082
R. ferrumequinum (5)	37.0	23.4	8.8 (4)	24.3	10.9	12.3	2.9	2.9	8.9	8.8	9.1	16.8		
ferrumequinum (*Rhinolophus*)	43.0–41.0	21.0–26.0	7.9–9.5	23.6–25.3	10.7–11.4	12.0–13.1	2.6–3.1	2.7–3.2	8.7–9.2	8.5–9.4	8.8–9.5	16.3–18.0	0.072	0.124
R. fumigatus (1)	28.0	23.0	9.3	22.8	10.3	11.5	2.3	2.9	8.7	8.6	9.6	15.6		
fumigatus (*Rhinolophus*)	—	—	—	—	—	—	—	—	—	—	—	—		
R. landeri (2)	23.5	18.0	6.8	18.1	9.0	9.4	2.6	1.9	6.4	6.5	7.1	12.0	0.152	0.077
landeri (*Rhinolophus*)	23.0–24.0	18.0–18.0	6.0–7.5	17.7–18.5	8.9–9.1	9.4–9.4	2.6–2.6	1.7–2.1	6.2–6.6	6.5–6.6	7.0–7.2	11.7–12.4	0.085	0.060
R. luctus (2)	54.3	35.8	13.7	30.5	13.4	15.0	3.5	4.4	11.5	10.4	12.1	21.7		
trifoliatus (*Aquias*)	52.0–56.5	35.0–36.5	12.9–14.5	30.0–31.0	13.0–13.8	14.3–15.8	3.3–3.6	4.4–4.5	11.1–11.9	10.1–10.8	11.1–13.1	21.2–22.3	0.055	0.027
R. mehelyi (2)	26.0	23.0	6.4	19.1	9.8	10.3	2.7	2.4	6.7	7.4	7.3	12.5		
euryale (*Rhinolophus*)	25.0–27.0	22.0–24.0	6.2–6.6	18.8–19.4	9.6–9.9	10.3–10.4	2.6–2.8	2.4–2.4	6.5–6.8	7.4–7.4	7.3–7.3	12.4–12.7	0.215	0.085
R. pearsonii (6)	22.7	21.0	7.6	23.5	10.9	12.1	2.5	3.1	9.1	8.9	9.3	16.5		
pearsonii (*Coelophyllus*)	22.0–23.0	19.0–22.0	6.8–8.7	23.1–23.9	10.6–11.2	11.8–12.5	2.4–2.6	2.9–3.4	8.9–9.2	8.5–9.2	8.9–9.5	16.1–16.9	0.064	0.050
R. simulator (1)	25.0	25.0	8.2	20.0	9.6	9.5	2.5	2.7	6.9	6.8	7.4	13.3		
capensis (*Rhinolophus*)	—	—	—	—	—	—	—	—	—	—	—	—		
R. sinicus (3)	25.7	16.7	7.7 (2)	20.1	9.5	10.1	2.8	2.3	7.5	7.6	7.5	13.7	0.166	0.063
rouxii (*Indorrhinolophus*)	25.0–26.0	16.0–17.0	7.4–8.0	18.7–20.9	9.0–9.7	9.5–10.4	2.7–2.8	2.1–2.6	7.1–7.8	7.3–7.7	7.2–7.7	12.5–14.3	0.163	0.063
R. thomasi (6)	23.0	16.8	7.0	20.0	9.8	10.6	2.8	2.4	7.4	8.1	8.0	14.0 (5)		
rouxii (*Indorrhinolophus*)	21.0–26.0	15.0–18.0	6.4–7.3	19.5–20.7	9.4–10.2	10.4–11.0	2.7–3.0	2.2–2.8	7.2–7.7	7.9–8.2	7.6–8.3	13.6–14.6	0.194	0.083
R. yunanensis (2)	27.5	24.5	10.1	25.3	11.5	12.4	2.5	3.9	10.2	9.3	10.4	18.1		
pearsonii (*Coelophyllus*)	27.0–28.0	22.0–27.0	9.3–10.9	25.2–25.3	11.5–11.6	12.3–12.5	2.4–2.6	3.8–3.9	10.0–10.3	9.2–9.5	10.3–10.5	18.0–18.1	0.131	0.083
R. trifoliatus (2)	32.5	26.9	10.8	22.2	10.3	11.2	2.4	3.1	8.4	8.2	9.0	15.3		
trifoliatus (*Aquias*)	29.7–35.5	25.9–27.8	10.5–11.0	21.6–22.8	10.2–10.4	10.7–11.7	2.4–2.4	2.9–3.4	8.2–8.7	8.0–8.4	8.7–9.4	14.8–15.8		

trees (sampled at stationarity) using the *sumt* command in MrBayes. If phylogenetic hypotheses of interest (those where the new species appeared as a sister group of any taxon in the Oriental lineages) were absent from the 99.9% credible set of trees, it could be rejected statistically (Brandley et al. 2005).

RESULTS

Fieldwork and museum collections.—One specimen of the new species was captured with a hand net during the survey of a cave at Wumulong. Another morphologically similar specimen was caught during the evening in a mist net set at a cave entrance across a stream during the survey in the Kuankuoshui Nature Reserve. Three more specimens were found in the mammal collection of the Kunming Institute of Zoology, which were collected on 27 October 1963 in Jinsha, Guizhou Province, China. Details of these specimens are provided in the formal description of the new species below.

Morphological analysis.—A total of 47 specimens of 17 species of *Rhinolophus* were measured (Table 1; Appendix I). Loadings on the 1st principal component of the analyses for the wing and the skull variables were all positive and relatively uniform, indicating that this component represented variation in general body size. Although the percentage of variance explained by these 1st components was very high (77.0% and 88.1%, respectively), they represented only differences in size, not shape. Loadings on the next 2 components were variable in magnitude and sign, indicating representation of variation in shape.

In the analysis with the wing variables, components 2 and 3 explained 10.6% and 5.8% of the total variance, respectively. The variables that loaded most heavily on the 2nd wing component were P2-III, P2-IV, P2-V, and TL with positive correlations, and P1-V and P1-IV with negative correlations, indicating that this factor represented the breadth of the wing aspect ratio. P2-III, P2-IV, P1-V, and TL loaded most positively on the 3rd component. TB and P2-V loaded heavily on this same component but negatively, indicating that this factor represented primarily the shape differences between the wing and tail membranes (Table 2).

The species in the Afro-Palearctic clade had positive scores on the 2nd component, whereas species in the Asian lineages tended to have negative scores. The 3rd component did not distinctly separate the lineages, but species from highland and northern temperate areas had positive scores, whereas the tropical species tended to have negative scores. The Afro-Palearctic forms had a relatively higher ratio of length of 2nd to 1st phalanges of the wing. The Asian *R. luctus* and the new species were an exception because they plotted within the range covered by Afro-Palearctic species. Likewise, the species in the Afro-Palearctic lineage were connected to each other in the minimum-spanning tree with the only exceptions being the inclusion of the Asian *R. luctus* and the new species. The new species was connected to the Palearctic species *R. ferrumequinum* and *R. mehelyi*, which also had the shortest euclidean distances in the morphospace defined by components 2 and 3 (Table 1; Figs. 1A and 1B).

TABLE 2.—Factor loadings, percentage of variance explained (% VAR), and standard deviation (*SD*) for the first 4 principal components (PCs) of the analysis of wing variables. Variables were $\log_{10}$-transformed and a covariance matrix scaling was used for the analysis. Acronyms for the variables are listed in the "Materials and Methods."

| Variable | Component | | | |
	PC 1	PC 2	PC 3	PC 4
FA	0.232	0.031	0.038	−0.184
M-III	0.196	−0.021	0.130	−0.337
P1-III	0.346	−0.061	−0.065	0.097
P2-III	0.216	0.338	0.274	−0.089
M-IV	0.236	0.087	−0.018	−0.144
P1-IV	0.354	−0.715	0.140	0.050
P2-IV	0.237	0.464	0.225	−0.364
M-V	0.249	0.007	−0.004	−0.168
P1-V	0.250	−0.230	0.307	−0.191
P2-V	0.376	0.190	−0.711	0.141
TB	0.319	−0.095	−0.335	−0.150
TL	0.371	0.221	0.345	0.757
% VAR	77.0	10.6	5.9	3.9
SD	0.220	0.081	0.061	0.050

In the analysis with the skull variables, components 2 and 3 explained 5.2% and 4.6% of the total variance, respectively. The variables that loaded most heavily on the 2nd component were CM3U, M3M3WU, and CM3L, with positive correlations, and IOW and PL with negative correlations, indicating that this factor primarily represented the shape of the oral cavity. IOW loaded heavily and positively on the 3rd component and PL loaded heavily but negatively, indicating robustness of the rostral area (Table 3). The species were not clearly organized according to species groups in the space defined by components 2 and 3. The new species was most similar to Afro-Palearctic species characterized by a relatively long palate and wide interorbital region. *R. mehelyi* and *R. simulator* had the shortest euclidean distances to the new species in the morphospace defined by these 2 components. In the minimum-spanning tree, the new species is connected to taxa in the Afro-Palearctic lineage with its immediate neighbors being *R. simulator* from the *capensis* group and *R. blasii* from the *landeri* group (Table 1; Figs. 1C and 1D).

Molecular systematics.—Complete nucleotide sequences of *Cytb* (1,140 bp), except 3 samples that had a few nucleotides missing at the flanking ends, were generated for the holotype, 1 of the paratypes, and exemplar specimens representing 13 different species in the Afro-Palearctic lineage of the genus (Guillén-Servent et al. 2003). These sequences were submitted to GenBank (accession numbers EU391626, EU436667–EU436679, and EU750753; see Appendix II). No sequences had interior gaps or stop codons and their translations did not show noticeable changes among the amino acid charge categories, indicating that our samples did not include pseudogenes or nuclear copies of the gene.

The average base frequencies within *Rhinolophus* were A = 0.278, C = 0.325, G = 0.144, and T = 0.254, and were similar
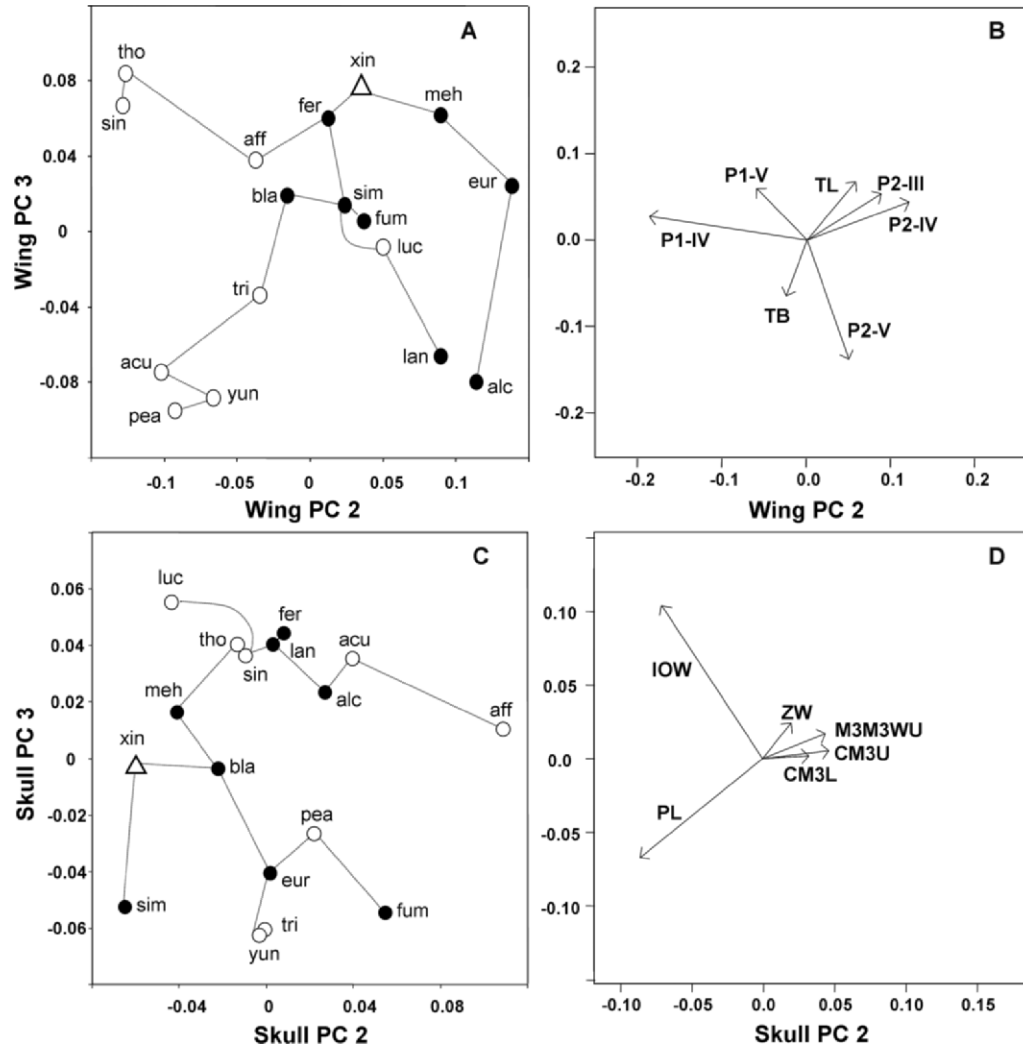
Fɪɢ. 1.—Bivariate plots of the A, B) wing and C, D) skull morphospaces as defined by principal components 2 and 3. A, C) The position of the species defined by their scores include the new species, *Rhinolophus xinanzhongguoensis* (△), species in the Afro-Palearctic lineage (●), and species in Asiatic lineages (○). Acronyms correspond to the first 3 letters of the species names. Lines connecting points represent the branches of the minimum spanning tree built by using the euclidean distances on the space defined by components 2–4. B, D) The length of the arrows indicates the proportion of the original variance explained by the variables in the 2 principal components defining the space portrayed. The direction of the arrows indicates the relative loadings of the variables on the components. Only variables with absolute loadings above 0.150 in at least 1 of the 2 components are portrayed.

to those reported for *Cytb* in other species of bats (e.g. Guillén-Servent and Francis 2006; Stadelmann et al. 2004) with no significant differences among taxa ($\chi^2 = 14.608$, $d.f. = 72$, $P = 1.0$). Of the 1,140 bp, 676 (59.3%) sites were constant, 76 (6.7%) were variable but parsimony uninformative, and 388 (34.0%) were potentially parsimony informative characters. Plots of HKY85 distances against observed transition substitutions showed signs of saturation at high levels of divergence, which were not evident in tranversions or total number of substitutions. Genetic distances averaged 30.6% (22.9–39.6%) between the outgroup hipposiderids and the ingroup rhinolophids, and ranged from 5.7% to 27.3% among ingroup species-level taxa. The 2 sequences of the new species differed at only 5 nucleotide sites.

The transversion-weighted parsimony analysis rendered only 1 shortest tree with a length of 3,605 steps, a consistency index

of 0.46, and a retention index of 0.56. Bootstrap percentages were low at the deeper nodes; however, a monophyletic *Rhinolophus* clade was strongly supported. The 2 specimens of the new species formed a well-supported monophyletic clade, which was poorly supported (<50% bootstrap) as a sister to the clade consisting of species in the *ferrumequinum*, *fumigatus*, and *maclaudi* groups (Fig. 2).

In the Bayesian analysis, the chains converged quickly and the average standard deviation of split frequencies of the 2 runs was less than 0.01 after 343,000 generations, indicating that they were converging to a similar stationary distribution. We set this point as the burn-in sample size for discarding to calculate the posterior distribution of the topologies and parameters. The consensus tree was congruent with the highest likelihood score tree. The Bayesian tree was similar to the parsimony tree except that there were 2 poorly supported

TABLE 3.—Factor loadings, percentage of variance explained (% VAR), and standard deviation (*SD*) for the first 4 principal components (PCs) of the analysis of skull variables. Variables were log_{10}-transformed and a covariance matrix scaling was used for the analysis. Acronyms for the variables are as in the "Materials and Methods."

	Component			
Variable	PC 1	PC 2	PC 3	PC 4
SL	0.304	0.004	0.036	0.601
MW	0.223	−0.041	0.062	0.450
ZW	0.287	0.149	0.189	−0.129
IOW	0.124	−0.525	0.813	−0.130
PL	0.504	−0.637	−0.525	−0.197
CM3U	0.383	0.346	0.042	0.051
M3M3WU	0.299	0.328	0.132	−0.266
CM3L	0.362	0.242	0.014	−0.469
ML	0.373	0.091	0.063	0.266
% VAR	88.2	5.1	4.6	0.9
SD	0.175	0.042	0.040	0.018

incongruent relationships that were basal to the clade containing the new species (Fig. 2).

Species in the non–Afro-Palearctic groups and *R. hipposideros* appeared as functional outgroup clades to a lineage that contained all the Afro-Tropical and Mediterranean species plus *R. ferrumequinum* and the new species, but this clade was very weakly supported (<50% bootstrap, 0.60 posterior probability; Fig. 2). Within this lineage, *R. landeri* and *R. alcyone* formed a monophyletic lineage that was sister to a lineage that contained all other Afro-Palearctic species plus the new species, which had good support (77%, *P* = 0.97). The new species was sister to the common ancestor of a lineage with 2 clades that contained species in the groups *ferrumequinum*, *fumigates*, and *maclaudi* with strong posterior probability support (0.91) but very weak bootstrap support (<50%).

The *Cytb* nucleotide sequences of the new species were quite distinctive. The average HKY85 distance of the new species to other *Rhinolophus* not in the Afro-Palearctic lineage was 18.8%. The distance to the Asian *R. sinicus* was only 13.0%, which suggests differences in the rates of molecular evolution among lineages. Average distance to the clade formed by *R. alcyone* and *R. landeri* was 18.5%, whereas distances to the other member of the *landeri* group (*R. blasii*, 14.1%), to *R. simulator* (16.6%) from the *capensis* group, and to *R. euryale* (12.7%) from the *euryale* group, were smaller. *R. mehelyi*, from the *euryale* group, showed the shortest interspecific distance (11.3%) to the new species. Average distances of the new species to the lineages within its sister group were 13.0% to the clade composed of *R. ferrumequinum* and *R. clivosus* and 15.6% to the clade consisting of the members of the *fumigatus* group plus *R. ruwenzorii* and *R. darlingi* (Fig. 2).

In the corresponding credible set of trees, the new species was never a sister taxon to species or a group of species not in the Afro-Palearctic clade of Guillén-Servent et al. (2003). Therefore, the hypothesis that it does not belong in the Afro-Palearctic lineage could be rejected with a confidence of 99.9%.

The morphological and molecular analyses indicate that the 5 specimens collected in the Chinese provinces of Yunnan and Guizhou represent a distinctive undescribed species of *Rhinolophus*.

Family Rhinolophidae Gray, 1825

Genus *Rhinolophus* Lacépède, 1799

Rhinolophus xinanzhongguoensis, sp. nov.

Etymology.—The species name is constructed from the romanized pinyin Chinese words for west (*xi*), south (*nan*), and China (*Zhongguo*; literally translated as "Middle Kingdom"), to mean horseshoe bat from southwestern China. Order of the geographical prefixes follows the regular Chinese grammatical use. Pronunciation in English is "shee-nan-joong-guo-en-sis."

Holotype.—Adult male, KIZ 0505003 in Kunming Institute of Zoology; preserved in alcohol, skull removed, baculum stored in glycerol. Collected by D. C. Ouyang on 13 May 2005 (measurements in Table 1). The nucleotide sequence of the mitochondrial gene *Cytb* has been deposited in GenBank with accession number EU391626. Right dentary of the holotype is missing p2.

Type locality.—Wumulong, Yongde County, Yunnan Province, China 24°22′N, 99°39′E 1980 m above sea level (Fig. 3).

Paratypes.—ROM 117760 (Fig. 2), an adult female, is a skin, skull, and partial postcranial skeleton preparation in good condition, and with frozen tissue samples stored at −80°C. It was collected on 22 April 2006 by Burton K. Lim and Judith L. Eger (field number F47617) at 0.6 km W of Kuankuoshui Nature Reserve Headquarters (28°13′38″N, 107°9′13″E, 1,500 m above sea level), Suiyang County, Guizhou Province, China. There is an associated parasite number 1275 of Sarah E. Bush (University of Utah). The nucleotide sequence of the mitochondrial gene *Cytb* has been deposited in GenBank with accession number EU750753. Other paratypes include KIZ 631388 (adult female), and KIZ 631386 and KIZ 631387 (adult males), which were preserved as museum skins and skulls. They were collected on 27 October 1963 in Jinsha, Guizhou Province, China (27°27′N, 106°12′E) but the elevation was not recorded (see measurements in Table 4).

Diagnosis.—A large horseshoe bat (FA 58–61 mm [Tables 1 and 4]) with a pointed hornlike connecting process. As in all horseshoe bats, this laterally flattened structure of the nose leaf connects the sella (dorsoventrally flattened forward projection of skin located above the nostrils) with the base of the lancet (triangular posterodorsal projection of skin, dorsoventrally flattened, with its base between the eyes [Figs. 4 and 5]). The new species is larger than all of the sympatric species of the *pusillus* group, which also have pointed connecting processes. The pointed connecting process distinguishes *R. xinanzhongguoensis* from the large and sympatric *R. pearsonii*, *R. trifoliatus*, and *R. yunanensis*, and other similar-sized allopatrically distributed *Rhinolophus*, which have rounded connecting processes.

Description (Figs. 4–7; Table 4).—The ears are brown, semitranslucent, and small (do not reach the tip of the nose
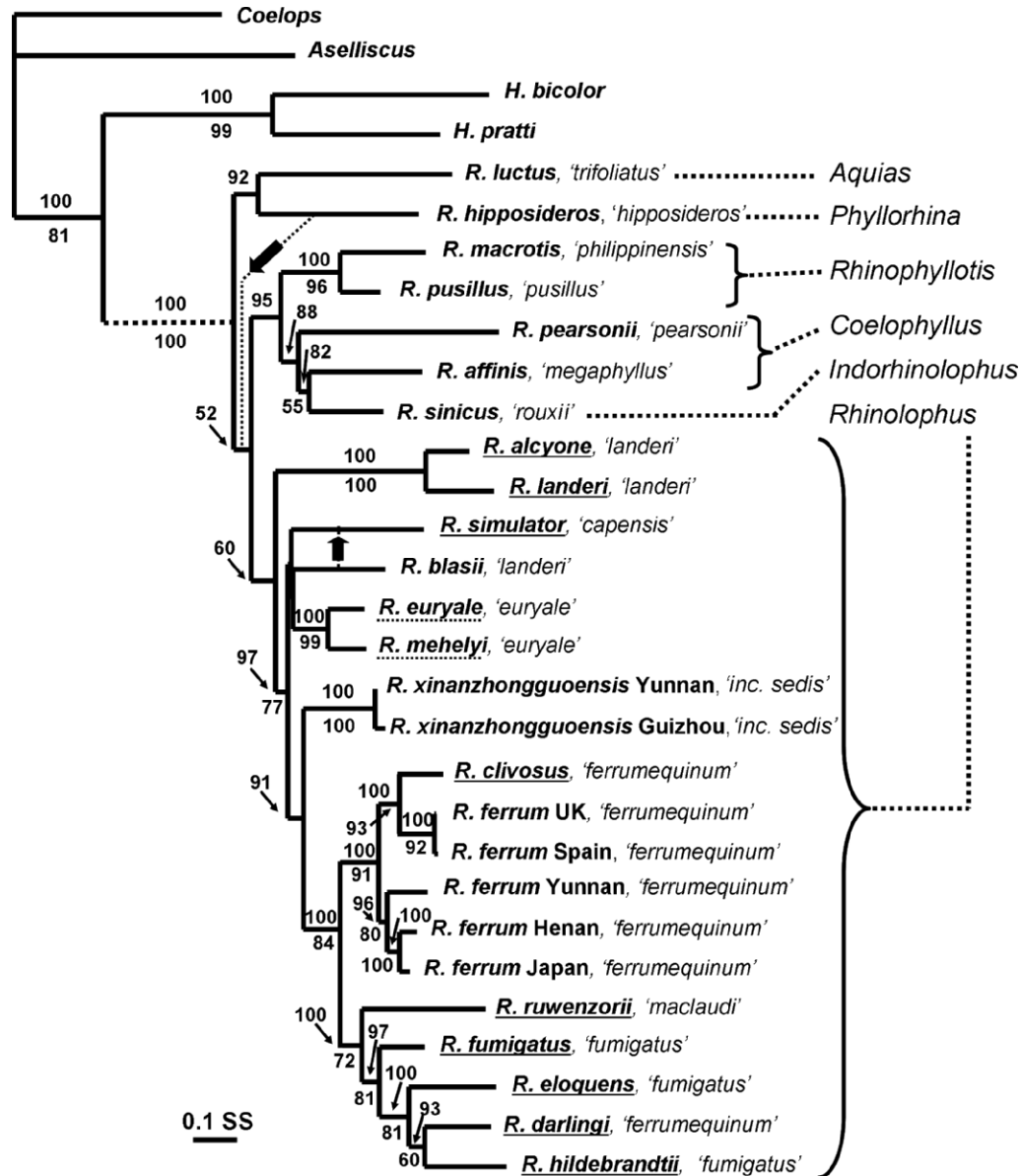
Fɪɢ. 2.—Phylogenetic relationships of *Rhinolophus* obtained from the analyses of complete cytochrome-*b* sequences. The terminal taxa corresponding to the new species are labeled as *R. xinanzhongguensis*. The tree portrayed is the one with highest likelihood obtained with the Bayesian inference. Posterior probabilities (×100) are provided next to and above the corresponding nodes when >0.50. Bootstrap values for the heuristic search under weighted-parsimony criterion (transversions weighted 6 times over transitions) also are provided below the nodes when >50%. Branch lengths indicate the relative number of substitutions per site, except the dotted line, which is 0.866 substitutions per site long. The 2 thick arrows indicate the only incongruences between the best hypothesis under the weighted-parsimony criterion and the Bayesian tree. In the most-parsimonious tree, the clade at the arrow's origin appears as sister to the clade at the arrow's end. Species names are followed by the names of species groups of Csorba et al. (2003) between quotation marks. Broken lines after the species group names connect to the names of the lineages identified by Guillén-Servent et al. (2003—*Rhinolophus* refers to the Afro-Palearctic lineage). Species whose distributions are mostly sub-Saharan are underlined with solid lines. Species whose distributions are mostly Mediterranean are underlined with broken lines.

when laid forward). The lower lip has 3 mental grooves. The horseshoe is relatively wide (8.9–9.5 mm) but does not cover the whole muzzle (Fig. 4). The sella is parallel sided near the base, slightly constricted medially, and gradually narrows distally to a wedge-shaped rounded tip. The connecting process is fairly high and pointed with the anterior surface concave in profile and the posterior surface slightly convex.

The lancet is hastate and tapers with concave sides to a pointed tip (Figs. 4 and 5). The tail ranges from 30 to 39 mm in length. In the wing, the 3rd metacarpal is the shortest (41.87–42.21 mm), and the 4th and 5th metacarpals are longer and subequal in length (42.50–45.78 mm). The dorsal fur is dull medium brown with the bases of the hairs light brown for three-fourths of the length and the tips medium brown for the
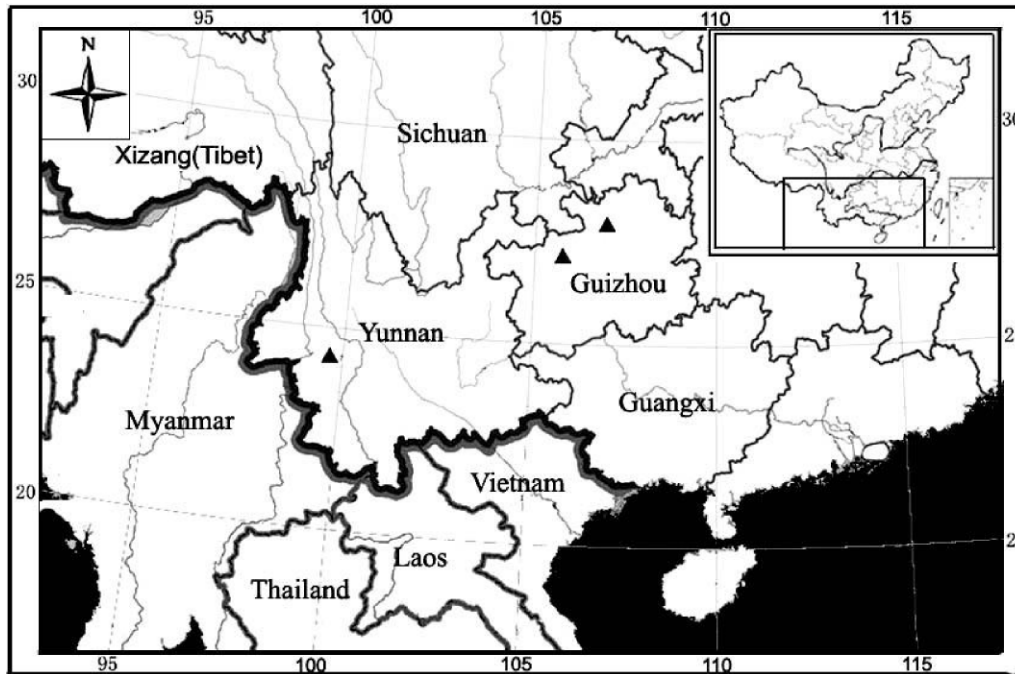
Fig. 3.—Map of southwestern China showing the localities where *Rhinolophus xinanzhongguoensis*, sp. nov., has been collected.

remaining one-fourth of the length. The underparts are paler brown with slightly darker bases. The flight membranes are a uniform dark brown.

The skull is moderately built and slender in shape with the mastoid width slightly greater than the zygomatic width.

The anterior median nasal swellings are high and prominent, protruding slightly forward. The lateral and posterior nasal compartments are well defined. The sagittal crest is low but distinct. The frontal depression is only moderately deep but well defined. The supraorbital crests are poorly developed. The

TABLE 4.—Measurements of the holotype and the 4 paratypes of *Rhinolophus xinanzhongguoensis*, sp. nov. All measurements are in millimeters except for body mass in grams. Acronyms for the variables are described in the ''Materials and Methods.''

Variable	KIZ 0505003 holotype	KIZ 631386 paratype	KIZ 631387 paratype	KIZ 631388 paratype	ROM 117760 paratype
Sex	♂	♂	♂	♀	♀
M	—	22	26	20	23
HB	59	62	62	60	70
FA	60.19	58.74	59.79	60.39	60
M-III	42.21	41.94	41.87	41.90	40.7
P1-III	19.66	19.79	18.92	19.17	19.2
P2-III	33.62	34.85	31.89	34.58	34.4
M-IV	44.99	45.78	42.50	44.74	42.8
P1-IV	10.77	11.51	11.27	11.21	11.1
P2-IV	21.71	21.27	21.53	20.97	20.6
M-V	44.88	44.98	43.91	45.47	43.5
P1-V	14.69	15.04	14.91	14.54	14.4
P2-V	16.05	16.36	16.71	17.33	16.0
TB	24.03	25.51	25.86	25.76	23.2
TL	33	30	35	35	39
E	21	22	21	21	22
HW	9.54	9.09	—	8.87	9.4
SL	22.96	22.81	22.87	22.58	22.9
MW	10.95	10.78	10.77	10.58	10.5
ZW	10.47	10.34	10.08	10.23	10.2
IOW	2.67	2.96	2.75	2.86	2.6
PL	2.67	2.92	2.78	3.07	2.8
CM3U	7.53	7.72	7.70	7.76	7.8
M3M3WU	7.36	7.42	7.53	7.40	7.6
CM3L	7.87	7.86	—	7.68	8.2
ML	14.87	14.92	—	15.02	14.0

Fig. 4.—Photograph of the new species, *Rhinolophus xinanzhong-guoensis* (paratype ROM 117760). Photo by Judith L. Eger © Royal Ontario Museum.

palatal bridge is moderate in length, about 35–40% of the maxillary toothrow, and essentially spans the length of the first 2 upper molars. In profile, there is a marked depression on the posterodorsal surface of the braincase (Fig. 6).

The dentition has the typical formula for the genus: i 1/2, c 1/1, p 2/3, m 3/3, total 32. The upper canines are moderately developed. The anterior upper premolar is small, laterally displaced in the toothrow, and separates the canine from the 2nd premolar (P4). The middle lower premolar is very small and slightly external to the toothrow. The 1st (p2) and 3rd (p4) lower premolars are separated by a small gap (Fig. 6).

The baculum has a large basal cone, subequal in width and height, and slightly compressed dorsoventrally. The basal cone is deeply emarginated both in the ventral and dorsal margins but slightly less in the dorsal margin. The shaft is straight and cylindrical toward the tip, which has a rounded point (Fig. 7).

Comparisons with other species.—According to the identification key to the species groups of *Rhinolophus* by Csorba et al. (2003), the new species would belong in the *landeri* group. This group consists of the species *R. alcyone*, *R. landeri*, *R. guineensis*, and *R. blasii*, all of which are smaller and have a predominantly Afro-tropical distribution, with only 1 species, *R. blasii*, extending into the Western Palearctic. *R. xinan-zhongguoensis* is similar to species of the *landeri* group in terms of having relatively small ears, hairy triangular connecting process, short 3rd metacarpal of the wing, 4th metacarpal subequal to or slightly shorter than the 5th, anterior median nasal swellings medium sized or pronounced, and frontal depression shallow or moderately deep (Figs. 4–6; Table 1). However, these species in the *landeri* group have a moderately large 1st upper premolar only slightly external to the toothrow, instead of reduced and laterally displaced as in *R. xinanzhongguoensis*. More specifically, *R. blasii* is most
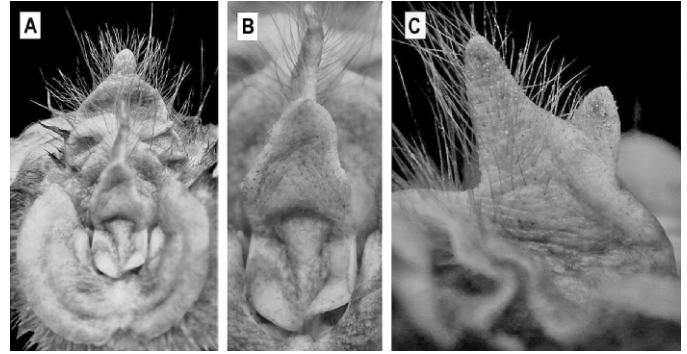

Fig. 5.—A) Frontal view of the nose leaf of *Rhinolophus xinanzhongguoensis*, sp. nov. (holotype, KIZ 0505003); B) frontal view of the cuneated sella; and C) lateral view of the pointed connecting process.

similar morphologically to the new species, sharing with it the wedge-shaped sella, relatively slender skull, anteriorly projecting nasal swellings, depression in the posterodorsal braincase, and the shaft of the baculum is cylindrical in cross section (Fig. 7). However, the new species is larger in all measurements than *R. blasii*.

Rhinolophus xinanzhongguoensis differs from the sympatric species of *Rhinolophus* in the *pusillus* group, which have
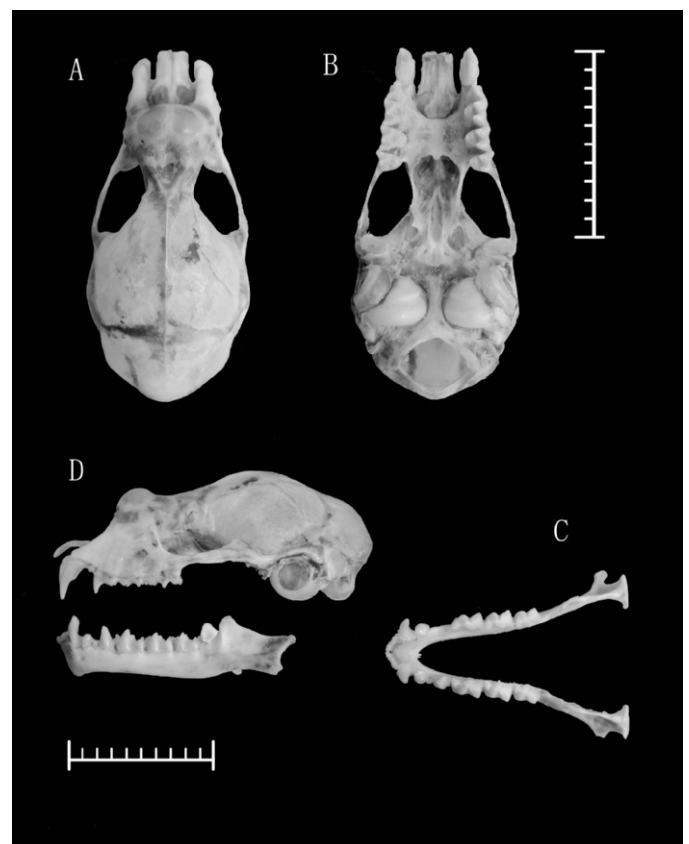

Fig. 6.—Photographs of the skull of the holotype of *Rhinolophus xinanzhongguoensis* (KIZ 0505003). A) Dorsal view; B) ventral view; C) dorsal view of mandible; and D) lateral view of skull with mandible. White scale bar represents 10 mm.
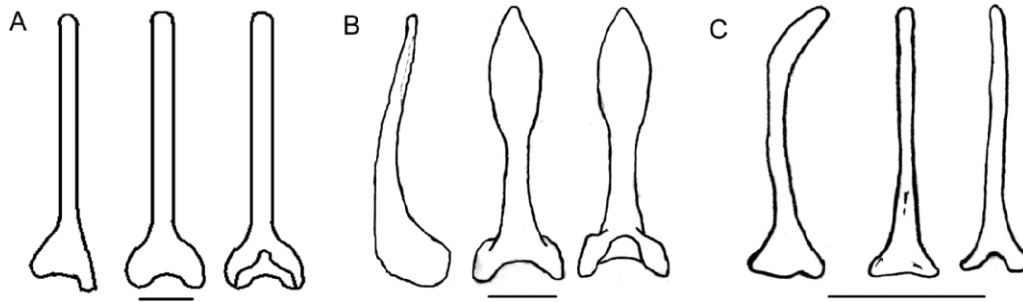
FIG. 7.—Bacula of selected *Rhinolophus*. Lateral, dorsal, and ventral views from left to right within. A) *R. xinanzhongguoensis* holotype KIZ 0505003; B) *R. ferrumequinum* (after Csorba et al. 2003); and C) *R. blasii* (after Csorba et al. 2003). Scale = 1 mm.

pointed connecting processes, by its much larger size as summarized by forearm length (≥58 mm) and skull length (≥22 mm). In addition, the smaller species have a medium to well-developed 1st upper premolar (P2), and medium-sized 2nd lower premolar (p3). *R. ferrumequinum*, *R. pearsonii*, *R. trifoliatus*, and *R. yunanensis* are similar-sized species sympatric with *R. xinanzhongguoensis* in the provinces of Yunnan and Guizhou (Table 1). *R. xinanzhongguoensis* differs from *R. pearsonii*, *R. trifoliatus*, and *R. yunanensis* by a higher and pointed connecting process with narrow triangular profile. In *R. ferrumequinum*, the connecting process is also high, but bluntly rounded, and this species is usually larger in all the external and cranial measurements. Cranially, *R. ferrumequinum* and other members of its group are further distinguishable from *R. xinanzhongguoensis* by the presence of a medium to prominent sagittal crest, low and less inflated anterior median nasal swellings, small anterior upper premolar (P2) that is external to the toothrow or missing, and small or missing middle lower premolar (p3). The end of the shaft of the baculum of *R. ferrumequinum* is flattened and laterally expanded and distinct from the narrow and cylindrical shaft of *R. xinanzhongguoensis* (Fig. 7). The species in the *euryale* group (*R. euryale* and *R. mehelyi*) have a connecting process shaped as an elevated triangle with a pointed tip, similar to that in *R. xinanzhongguoensis*. However, these species are characterized by parallel-sided sellae and are smaller in body size.

Distribution.—Known from Jinsha and Suiyang counties, Guizhou Province, China, and the type locality in Yongde County, Yunnan Province, China (Fig. 3).

Habitat and ecology.—The holotype was the only specimen of the new species that was caught roosting together with a group of about 100 bats in a flooded cave, approximately 50 m deep, during the daytime. During random sampling with a hand net, all other bats caught were *R. affinis*. The cave was in a gorge near farmland with tea plantations surrounded by subtropical montane mixed forest, which were not surveyed for bats. One of the paratypes (ROM 117760) was caught during the evening in a mist net set at a cave entrance across a stream. The weather during the time of capture in April was cool and wet, with temperatures ranging from an estimated 5°C to 10°C, and there was light rainfall during most days. The general habitat was subtropical lower montane mixed forest on karst formations; however, there were extensive agricultural fields primarily of tea plantations within the area. The cave entrance narrowed into a tunnel, which opened into a large cavern. There was no evidence of bats using the cave as a day roost; however, 9 bats representing 5 other species were caught at the mouth of the cave during several nights of sampling, including 3 *Myotis laniger*, 3 *M. ricketti*, 1 *M. siligorensis*, 1 *R. pearsonii*, and 1 *Miniopterus fuliginosus*. The habitat where the 3 other paratypes were collected near Jinsha, Guizhou, was not recorded.

The type locality in Yongde County is located in the alpine gorge of Nujiang-Langcang, at the southern end of the Nushan Mountains in Yunan Province. The paratype locality in Suiyang County is situated in the central area of the Loushan Mountains, and Jinsha County is at the meeting point of the Wumeng and the Loushan mountains in Guizhou Province. These 2 paratype areas are characterized by an eroded karstic landscape with many caves. All 3 areas are in the seasonal and mesic subtropical monsoon climate belt. Average annual precipitation is 1,300, 1,160, and 1,050 mm, respectively. There is a large elevational gradient from around 500 m above sea level to 3,500 m at Yongde, to 1,800 m at Suiyang, and to 1,900 m at Jinsha.

Reproductive data.—Adult female ROM 117760, collected in the spring, was pregnant with an embryo with a crown–rump length of 15 mm.

Conservation status.—The new species is a large, distinctive species of horseshoe bat that cannot be easily confused with any other species, and which is known from only 5 specimens collected over a 45-year span in a forested landscape highly modified by humans and fragmented by farmland. The species is probably rare because there are about 1,200 specimens of other rhinolophids collected from the mountainous areas of the provinces of Yunnan, Guizhou, Sichuan, and Guangxi during the last 50 years and deposited in Chinese museums. These provinces are relatively well represented by records of *Rhinolophus* specimens compared with other regions in China (Smith and Xie 2008; Wang 2003; Zhang et al. 1997). The species of *Rhinolophus* most commonly collected in these areas were *R. affinis*, *R. ferrumequinum*, *R. pearsonii*, *R. pusillus*, and *R. sinicus*. These species also have been commonly reported from the Himalayan montane areas in the northern fringe of the Indian subcontinent (Bates and Harrison 1997). Based on this information, *R. xinanzhong-*

guoensis may be a rare species restricted to the mountainous regions of Yunnan and Guizhou. However, some regions around the Tibetan Plateau, such as northern Myanmar, Assam State in India, and western Sichuan, have not been very well surveyed for bats (Bates and Harrison 1997; Struebig et al. 2005; Zhang et al. 1997), and the new species may prove to also occur in these areas. Immediate study is needed to ascertain its geographic range and population size to assess potential conservation concerns and criteria for inclusion on the International Union for the Conservation of Nature and Natural Resources Red List of Threatened Species (Hutson et al. 2001).

DISCUSSION

The influential classification of *Rhinolophus* by Andersen (1905a, 1905b, 1918) identified several species groups, based primarily on morphology, but putatively reflecting evolutionary relationships (Andersen 1905b). Many of these groups consisted of species living in disparate areas of the Old World tropics, implying a pervasive historical dispersal between the African and the Indo-Australian tropics.

The recent molecular systematic study by Guillén-Servent et al. (2003) showed that many of the species groups recognized by previous studies based on morphological analyses did not adequately reflect the phylogenetic history of the genus. Guillén-Servent et al. (2003) proposed a modified arrangement of the species groups, organizing them into 6 subgenera based on monophyletic lineages. Their molecular systematic approach identified lineages geographically more restricted than Andersen's (1918) groups, including a monophyletic Afro-Palearctic lineage. The species in this lineage included all the sub-Saharan African forms, plus the circum-Mediterranean populations of *R. blasii*, *R. euryale*, and *R. mehelyi*, populations of *R. blasii*, *R. bocharicus*, and *R. clivosus* from the Middle East to the Indus Division, and *R. ferrumequinum*, a species with a wide distribution in the Palearctic from Britain to Japan. *R. ferrumequinum* was the only representative of the Afro-Palearctic clade known to occur east of the Himalayas and the Ural Mountains (Csorba et al. 2003). The unique combination of morphological characters aligns *R. xinanzhongguoensis* with the species in the Afro-Palearctic lineage and this relationship is corroborated by the molecular data. *R. xinanzhongguoensis* represents the 2nd species in the Afro-Palearctic lineage that is also present in southern China, and adds further to the higher level taxonomic diversity of the horseshoe bats in this region.

A high Bayesian posterior probability and reduced interspecific genetic distances supported the inclusion of *R. xinanzhongguoensis* in the crown group of species of the Afro-Palearctic lineage (all species in this lineage excepting *R. alcyone* and *R. landeri* [Fig. 2]). Most of these species are linked to seasonally dry environments in Africa or the Mediterranean region (Csorba et al. 2003; Guillén-Servent et al. 2003). Within this crown group, the nodes connecting the clades leading to *R. blasii*, *R. euryale* + *R. mehelyi*, *R. simulator*, and the common ancestor of *R. xinanzhongguoensis* + *R.*

ferrumequinum + *R. fumigatus* are separated by very short branches. This makes difficult to resolve the historical relationships among these relatively deep nodes with only the relatively short mitochondrial sequence used in this study, as it is indicated by the poor support values in this area of the phylogeny. Regardless of the node order, the very short internodes suggest a relatively rapid diversification at that time. Guillén-Servent et al. (2003) dated the diversifications of these lineages at around 10 million years ago, within the late Miocene, when the warming climate could have made the Sahara, the Middle East, and the northern areas of the Indian subcontinent more suitable for tropical rhinolophids than they are today (François et al. 2006; Zubakov and Borzenkova 1990). This paleoenvironment may have facilitated dispersal of these bats between the Ethiopian and the Western and Eastern Palearctic regions, as has been documented for other mammalian taxa (Koufos et al. 2005; Prothero 2006). The phylogeny suggests that the common ancestor of *R. xinanzhongguoensis* and the *R. ferrumequinum* plus *R. fumigatus* clades could have had a Palearctic or Indomalayan origin. This scenario would make the biogeographical history of the Afro-Palearctic lineage of the horseshoe bats more complex than the 2 independent colonization events from Africa into the Palearctic region (1 for the circum-Mediterranean species and 1 for *R. ferrumequinum*) hypothesized by Guillén-Servent et al. (2003) before the existence, distribution, and phylogenetic position of *R. xinanzhongguoensis* were known. However, longer mitochondrial sequences and more slowly evolving nuclear gene sequences are needed to clarify if the unresolved basal relationships in the Afro-Palearctic clade represent a hard polytomy indicating a rapid radiation, or a soft polytomy caused by homoplasy or phylogenetically noisy data, as well as to resolve the age order of the nodes and test the historical biogeography hypotheses.

The identification of *R. xinanzhongguoensis* as a new species was initially facilitated by its morphological distinctiveness from sympatric taxa and the distant allopatry of morphologically similar species. However, discrimination among rhinolophid bats has been problematic because of subtle morphological differences among taxa (Bogdanowicz 1992; Csorba et al. 2003). For example, the specific status of *R. clivosus* and *R. ferrumequinum* has been a vexing taxonomic problem (see Csorba et al. [2003] for details). Examination of our genetic data indicates that the Western Palearctic European populations of *R. ferrumequinum* are more closely related to the allopatrically and primarily African-distributed *R. clivosus* than to the Eastern Palearctic populations of *R. ferrumequinum*. This casts new doubts on the specific status of these 2 taxa (they may represent only 1 or up to 3 different species) and also the African forms (*R. hillorum* and *R. sakejiensis*) that are putatively closely related to *R. clivosus* (Cotterill 2002), but have not been sequenced for *Cytb*. When the morphological similarity of taxa is high, distributions are allopatric or parapatric, or specimens are scarce, or both, as often happens with tropical Rhinolophidae, genetic data may become fundamental for facilitating species identification and classification.

In groups of mammals that show high morphological similarity between species, such as the horseshoe bats (Bogdanowicz 1992), genetic information also may be essential for delimiting higher level taxa that reflect evolutionary history. The initial examination of external morphology placed *R. xinanzhongguoensis* in the *landeri* species group of Csorba et al. (2003). However, more-detailed morphological analyses were less conclusive when trying to ascribe the species to the groups in Csorba et al. (2003). *R. xinanzhongguoensis* showed characters intermediate among the *capensis*, *euryale*, *ferrumequinum*, *fumigatus*, and *landeri* species groups. An examination of the ladderlike structure of the phylogenetic tree obtained with the molecular systematic analysis exposes the difficulties in splitting the species in this lineage into natural sets, using only morphological characters that may have evolved in parallel in the different sublineages (i.e., reduction and lateral displacement of premolar teeth, blunting of the projection of the connecting process of the nose leaf, etc.). We concur with the genetic concept of species by Baker and Bradley (2006) as an operational criterion to identify phylogroups putatively representing independent evolutionary units and to reveal their evolutionary relationships. This idea can be extended to assist in the classification of higher-level taxa. In this respect, the reorganization of the genus *Rhinolophus* into 6 subgenera corresponding to phylogenetic lineages identified with molecular characters, as proposed by Guillén-Servent et al. (2003), seems appropriate. The molecular data provided in this work may serve as comparative information for future studies with Afro-Palearctic horseshoe bats. Molecular data are accumulating at a rapid pace for many groups of bats and are providing a test not only of species-level diversity but also of higher levels of classification derived from previous morphological-based systematic hypotheses.

Resumen

Se describe una nueva especie de murciélago de herradura (Chiroptera: Rhinolophidae) del suroeste de China. La sella en forma de cuña y el proceso conectivo puntiagudo en la hoja nasal alinean la nueva especie con otras del grupo *landeri*, en el linaje Afro-Paleártico del género *Rhinolophus*. Sin embargo, se diferencia claramente de estas especies alopátricas por su tamaño corporal notablemente mayor. Las demás especies simpátricas de tamaño grande tienen procesos conectivos redondeados. Los análisis de sistemática molecular con secuencias del gen mitocondrial citocromo *b* confirmaron la afinidad de la nueva especie con el linaje Afro-Paleártico, pero en un clado más relacionado con las especies de los grupos *ferrumequinum*, *fumigatus* y *maclaudi*. De estas especies, tan sólo *R. ferrumequinum* está presente en Asia, donde su extensa área de distribución incluye a la de la nueva especie. *R. ferrumequinum* es similar en tamaño y apariencia externa, pero la nueva especie se diferencia claramente en las características de las hojas nasales, el cráneo y el báculo. La presencia de una nueva especie del linaje Afro-Paleártico en el suroeste de China indica que la biogeografía histórica del género *Rhinolophus* es más compleja de lo que se suponía. Las dificultades en adscribir la nueva especie a uno de los grupos fenéticos de especies tradicionales apuntan la conveniencia de utilizar una organización sistemática con orientación filogenética para el género *Rhinolophus*.

Acknowledgments

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APPENDIX I

Specimens examined.—Institution acronyms correspond to: EBD: Estación Biológica de Doñana, Sevilla, Spain; IEX: Instituto de Ecología, Xalapa, Veracruz, México; and KIZ: Kunming Institute of Zoology, Kunming, Yunnan, China. *Rhinolophus acuminatus* Peters, 1871: IEX M0130 ♀, M0131 ♂ (Champasak, Lao People's Democratic Republic); *Rhinolophus affinis* Horsfield, 1823: KIZ 61013 ♀, 640160 ♂, 640178 ♀ (Yunnan, China); *Rhinolophus alcyone* Temminck, 1852: EBD 13931 ♀, 16801 ♂ (Equatorial Guinea); *Rhinolophus blasii* Peters, 1866: EBD 23260 ♂ (Agadir, Morocco); *Rhinolophus euryale* Blasius, 1853: EBD 19563 ♂, 19572 ♀ (Cádiz, Spain); *Rhinolophus ferrumequinum* Schreber, 1774: KIZ 610087 ♂, 630448 ♀, 630449 ♂, 640570 ♂, 73411 ♂ (Yunnan, China); *Rhinolophus fumigatus* Rüppell, 1842: EBD 14921 ♀ (Equatorial Guinea); *Rhinolophus landeri* Martin, 1838: EBD 13907 ♀, 14918 ♂ (Equatorial Guinea); *Rhinolophus luctus* Temminck, 1834: KIZ 57165 ♀ (Yunnan, China), IEX M0148 ♀ (Louang Nam Tha, Lao People's Democratic Republic); *Rhinolophus mehelyi* Matschie, 1901: IEX M0156 ♂ (Huelva, Spain), M0157 ♀ (Valencia, Spain); *Rhinolophus pearsonii* Horsfield, 1851: KIZ 73239 ♂, 73240 ♀, 73242 ♀, 73243 ♀, 73244 ♀, 73254 ♀ (Yunnan, China); *Rhinolophus simulator* Andersen, 1904: EBD 14949 ♀ (Equatorial Guinea); *Rhinolophus sinicus* Andersen 1905: KIZ 7319 ♂, 78320 ♂, 73608 ♀ (Yunnan, China); *Rhinolophus thomasi* Andersen, 1905: KIZ 640172 ♂, 640173 ♂, 640174 ♂, 640177 ♂, 640568 ♀, 76718 ♂ (Yunnan, China); *Rhinolophus trifoliatus* Temminck, 1834: EBD 23913 ♂ (Sabah, Malaysia), IEX M0194 ♂ (Krabi, Thailand); *Rhinolophus yunanensis* Dobson, 1872: KIZ 73279 ♂, 73280 ♂ (Yunnan, China).

APPENDIX II

Taxa, taxonomic affiliation, geographic localities, GenBank accession numbers, and tissue and voucher numbers of cytochrome-b sequences used in the molecular systematics analysis.—Initials for the genera correspond to: A: *Aselliscus*; C: *Coelops*; H: *Hipposideros*; and R: *Rhinolophus*. Species groups are those used by Csorba et al. (2003), and lineages are those described by Guillén-Servent et al. (2003). Under the "Region" column we have listed the general biogeographic regions where the species occurs (i.e., after Wallace 1876). We used more-detailed geographical names when the distribution is relatively restricted within a region. Acronyms correspond to: EPA: Eastern Palearctic; ET: Ethiopian; IN (Indus division, transitional region between the Oriental and the Western Palearctic regions); ME: Mediterranean (subregion within the Western Palearctic); NOR: North Oriental; TC: Trans-Caucasian (within the Western Palearctic); and WPA: Western Palearctic. Accession numbers marked with an asterisk in the "GenBank" column correspond to the new sequences contributed in this study. The legend "Not in GenBank" in the "Tissue Specimen" and "Voucher" column entries means that location of the samples was not indicated in the GenBank record and the corresponding publication. Institution acronyms in the last 2 columns correspond to: CN: Carnegie Museum of Natural History, Pittsburgh, Pennsylvania; DM: Durban Museum, Durban, South Africa; EBD: Estación Biológica de Doñana, Sevilla, Spain; ECNU: Zhang S.Y., School of Life Science, East China Normal University; FMNH: Field Museum of Natural History, Chicago, Illinois; IEX: Instituto de Ecología, Xalapa, Veracruz, México; IZCASB: Zhang S.Y. (Institute of Zoology, Chinese Academy of Sciences, Beijing); KIZ: Kunming Institute of Zoology, Kunming, Yunnan, China; ROM: Royal Ontario Museum, Toronto, Ontario, Canada; SBSUB: Jones G. (School of Biological Sciences, University of Bristol, United Kingdom); and TTU: Museum of Texas Tech University, Lubbock, Texas.

Species	Species group (lineage)	(Area), Province, Country	Region	GenBank	Tissue Specimen	Voucher
A. stoliczkanus	Hipposiderid Outgroup	Guizhou, China	Oriental	DQ888677	Isolate JJ006	Not in GenBank
C. frithii	Hipposiderid Outgroup	(Dayuanshan), Kenting, Taiwan, China	Oriental	DQ888674	Isolate CF	Not in GenBank
H. bicolor	Hipposiderid Outgroup	(Khao Nor Chuchi), Krabi, Thailand	Oriental	DQ054808	IEX AGS970408n03	IEX M0071
H. pratti	Hipposiderid Outgroup	Guangxi, China	Oriental	DQ297584	Not in GenBank	IZCASB DBP
R. affinis	*megaphyllus (Coelophyllus)*	Guizhou, China	Oriental	DQ297582	Not in GenBank	IZCASB B004
R. alcyone	*landeri (Rhinolophus)*	(Institute d'Ecologie Tropicale), Ivory Coast	Ethiopian	*EU436667	ROM 100491	ROM 100491
R. blasii	*landeri (Rhinolophus)*	Agadir, Morocco	ET, ME, TC, IN	*EU436669	EBD COI316	EBD 23260
R. clivosus	*ferrumequinum (Rhinolophus)*	(Chome Forest), Kilimanjaro, Tanzania	Ethiopian, ME	*EU436674	FMNH 151424	FMNH 151424
R. xinanzhongguoensis	incertae sedis *(Rhinolophus)*	(Yongde), Yunnan, China	North Oriental	*EU391626	Alcoholic voucher	KIZ 0505003
R. xinanzhongguoensis	incertae sedis *(Rhinolophus)*	(Suiyang County), Guizhou Province, China	North Oriental	*EU750753	ROM 117760	ROM 117760
R. darlingi	*ferrumequinum (Rhinolophus)*	(Mlawula Reserve), Swaziland	Ethiopian	*EU436675	DM 5821	DM 5821
R. eloquens	*fumigatus (Rhinolophus)*	(Nakuru), Rift Valley, Kenya	Ethiopian	*EU436677	TTU TK33126	CM 97948
R. euryale	*euryale (Rhinolophus)*	Málaga, Spain	Mediterranean	*EU436671	EBD 24814	EBD 24814
R. ferrumequinum	*ferrumequinum (Rhinolophus)*	United Kingdom	Western Palearctic	U95513+14	Not in GenBank	Not in GenBank
R. ferrumequinum	*ferrumequinum (Rhinolophus)*	Cádiz, Spain	Western Palearctic	*EU436673	EBD 24818	EBD 24818
R. ferrumequinum	*ferrumequinum (Rhinolophus)*	Henan, China	EPA, NOR	EF544404	Not in GenBank	Not in GenBank
R. ferrumequinum	*ferrumequinum (Rhinolophus)*	(Oshima), Tokyo, Japan	EPA, NOR	AB085723	Not in GenBank	Not in GenBank
R. ferrumequinum	*ferrumequinum (Rhinolophus)*	Yunnan, China	EPA, NOR	DQ297575	Not in GenBank	IZCASB 68
R. fumigatus	*fumigatus (Rhinolophus)*	(Kwale), Coastal Province, Kenya	Ethiopian	*EU436678	TTU TK33203	CM 97951
R. hildebrandtii	*fumigatus (Rhinolophus)*	(East Usambara Mts.), Tanga Region, Tanzania	Ethiopian	*EU436676	FMNH 151422	FMNH 151422
R. hipposideros	*hipposideros (Phyllorhina)*	Upper Langford, United Kingdom	WPA, TC, IN	DQ297586	Not in GenBank	SBSUB DRH
R. landeri	*landeri (Rhinolophus)*	(Nakuru), Rift Valley, Kenya	Ethiopian	*EU436668	TTU TK33121	CM 97952
R. luctus	*trifoliatus (Aquias)*	Huibei, China	Oriental	DQ297596	Not in GenBank	IZCASB T234
R. macrotis	*philippinensis (Rhinophyllotis)*	Yunnan, China	Oriental	EF517312	Not in GenBank	ECNU CB00011
R. mehelyi	*euryale (Rhinolophus)*	(Cazalla de la Sierra), Sevilla, Spain	Mediterranean	*EU436672	IEX AGS970109n04	EBD 24813
R. pearsonii	*pearsonii (Coelophyllus)*	Yunnan, China	Oriental	EF517310	Not in GenBank	ECNU B00014
R. pusillus	*pusillus (Rhinophyllotis)*	Guangdong, China	Oriental	DQ297597	Not in GenBank	IZCASB T245
R. ruwenzorii	*maclaudi (Rhinolophus)*	(Ruwenzori Mts.), Western Province, Uganda	Ethiopian	*EU436679	FMNH 144309	FMNH 144309
R. simulator	*capensis (Rhinolophus)*	(Chome Forest), Kilimanjaro, Tanzania	Ethiopian	*EU436670	FMNH 153928	FMNH 153928
R. sinicus	*rouxii (Indorhinolophus)*	Guizhou, China	Oriental	EF517304	Isolate CA066	ECNU A066