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New species of bonneted bat, genus *Eumops* (Chiroptera: Molossidae) from the lowlands of western Ecuador and Peru

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We describe and formally name a species of bonneted bat (genus *Eumops*), which is a member of the *E. glaucinus* complex. Closely related species are *E. glaucinus*, *E. ferox*, and *E. floridanus*. The conceptual basis for the description of this species is the Genetic Species Concept with speciation by the Bateson-Dobzhanzky-Muller model. The new species is distinguished from all other species of bats by its unique karyotype (2N = 38, FN = 54), sequence of the mitochondrial cytochrome-*b* gene, and genetic markers revealed through analysis of Amplified Fragment Length Polymorphisms. The series from the type locality (Ecuador, Guayas) is comprised of seven specimens. Morphologically, the new species is smaller than *E. floridanus* and *E. glaucinus*, but is indistinguishable from *E. ferox*. The new species is significantly smaller in size than *E. glaucinus* in six out of eight measurements and is distinguishable from *E. glaucinus* based on length of maxillary toothrow and zygomatic breadth. The geographic range of *E. wilsoni*, as currently documented, is the dry forests of southwestern Ecuador and adjacent northwestern Peru. We propose the common name for this species be Wilson's bonneted bat.

Key words: Genetic Species Concept, AFLPs, cytochrome-*b*, karyotypes, bonneted bats, *Eumops*, operational species criteria

INTRODUCTION

In addition to the 5,416 species of mammals recognized in Wilson and Reeder (2005) there remains in nature a substantial number of unrecognized mammalian species (Baker and Bradley, 2006). In a recent review of Wagner's bonneted bat using genetic [mitochondrial cytochrome-*b*, nuclear Amplified Fragment Length Polymorphisms (AFLPs), and karyotypic] and morphological data, McDonough *et al.* (2008) concluded that within *Eumops glaucinus*/*floridanus* (sensu Timm and Genoways, 2004) there were four species: *E. ferox*, *E. floridanus*, *E. glaucinus*, and an undescribed species. *Eumops ferox* is distributed in the Caribbean, Mexico, and Central America (Eger, 1977; McDonough *et al*., 2008), *E. floridanus* in southern Florida, *E. glaucinus* in South America east of the Andes, and the undescribed taxon is distributed in the western lowlands of the Andes in Ecuador and Peru. The purpose of this paper is to describe that taxon.

It is appropriate in describing a previously unrecognized species, in elevating a subspecies to the species level, or in synonymizing a currently recognized species, to define the conceptual and operational standard applied for specific recognition. There are over 20 species concepts (Mayden, 1997) and the criteria for recognition of species vary according to the conceptual application chosen. In the following description we are applying the Genetic Species Concept following Bradley and Baker (2001) and Baker and Bradley (2006). These authors define a genetic species as "a group of genetically compatible interbreeding natural populations that is genetically isolated from other such groups" (Baker and Bradley, 2006: 645). Further, they define speciation as the accumulation of genetic changes in two lineages (Bateson, 1909) that produce genetic isolation and protection of the integrity of the two respective gene pools resulting in each having independent evolutionary fates (Baker and Bradley, 2006).

Classically, morphological data have been used (Corbet, 1997; see Baker and Bradley, 2006) to recognize and describe species and even now the magnitude of morphological difference is the standard

against which most allopatric species-level decisions are made (Wilson and Reeder, 2005). Within the *E. glaucinus* complex the Morphological and Ecological Species Concepts were used as a basis for recognition of *E. floridanus* (Timm and Genoways, 2004; McDonough *et al.*, 2008). Although there is considerable variation within the *E. glaucinus* complex, morphologically *E. floridanus* is as unique from other members of the *E. glaucinus* complex as are most allopatrically distributed closely related species recognized in Wilson and Reeder (2005) where morphological characters are the only dataset available (see McDonough *et al.*, 2008 for justification).

With the advent of DNA sequencing and other genetic methods including karyotypes, other sources of data (mitochondrial and nuclear genomes) that are minimally linked are available for defining species presence/absence as well as establishing the genetic and geographic boundaries of species. da Silva and Patton (1998: 477) proposed that "for allopatric forms, as an operational procedure, we will recognize as species those reciprocally monophyletic clades of mtDNA haplotypes that have both regional coherence and diagnosability by characters other than the molecular ones that define them". Their definition of 'other' included morphology, chromosomes, and products of the nuclear genome that corroborate the inferences from the single gene phylogenies derived from mtDNA (da Silva and Patton, 1998). Today it is possible to generate sufficient mitochondrial and nuclear data to strengthen the operational definition for application of the Genetic Species Concept (Baker and Bradley, 2006). Therefore we employ an additional criterion that reciprocal monophyly of molecular phylogroups used to justify specific recognition be statistically supported (see also Mishler and Theriot, 2000 for comments on statistical support for implementation of the Phylogenetic Species Concept). This operational method (statistically-supported reciprocal monophyly in at least two relatively independent datasets) is more robust than previous methods that have been used (Corbet, 1997) for recognition of species that are allopatrically distributed phylogroups (Avise and Walker, 1999). We propose that at least two of the following operational critera be used for recognizing allopatrically distributed phylogroups as mammalian species: (i) statistically-supported reciprocal monophyly in mitochondrial molecular data, (ii) statistically-supported reciprocal monophyly in nuclear molecular data, (iii) statistically-supported morphological distinction, or (iv) karyotypes that distinguish the two. Ecological distinction will also strengthen justification of species recognition. The more of these criteria that distinguish two allopatrically distributed phylogroups, the stronger the justification for recognition of specific status. As with most conceptual and operational species criteria, monophyly must be preserved.

Materials and Methods

Methodology for taxon sampling, genetic and morphological analyses, and karyotypic preparation are presented in McDonough *et al.* (2008). Specimens examined are listed in the Appendix. Cytochrome-*b* sequences for all specimens examined herein were deposited in GenBank (EU349989–EU350041) by McDonough *et al*. (2008). All presented cranial measurements are in millimeters and follow Eger (1977). Eight cranial measurements were taken from 34 specimens (8 *Eumops* sp. nov., 6 *E. glaucinus*, and 20 *E. ferox* — sensu McDonough *et al.*, 2008). Statistical support for morphological measurements was performed using two-tailed *t*-tests in Microsoft Excel, ver. 11.6, with $\alpha = 0.05$. AFLP divergence among species was determined using principal coordinate analysis (PCOA) in GenAlEx, version 6.0, software (Peakall and Smouse, 2006) and a three-dimensional plot was generated using SigmaPlot, version 11.0 (SYSTAT Software, Inc., San Jose, California).

Systematic Description

The species-level names available within the *Eumops glaucinus* complex (sensu Eger, 1977) are: (i) *Eumops glaucinus* (Wagner, 1843) — type purportedly from Cuyaba, Mato Grosso, Brazil (Carter and Dolan, 1978); (ii) *Molossus ferox* Gundlach, 1861 — type from Cuba; *Nyctinomus orthotis* H. Allen, 1889 — type from Spanishtown, Jamaica.

The names *Eumops glaucinus* and *Eumops ferox* are not available for this undescribed taxon because the type localities fall within the geographic range of other genetically defined species (McDonough *et al.*, 2008). *Nyctinomus orthotis* is a junior synonym of *E. ferox*. We conclude that there is no formal species level name available for the genetically defined populations from western Ecuador and Peru, therefore a formal description for a new name follows.

Eumops wilsoni sp. nov.

Holotype

Adult male; skin, skull (Fig. 1 and cover photo), postcranial skeleton, Museum of Texas Tech, TTU 103281 (specimen will be deposited in Pontificia Universidad Católica del Ecuador [PUCE], QCAZ 10600). Holotype collected from Ecuador,

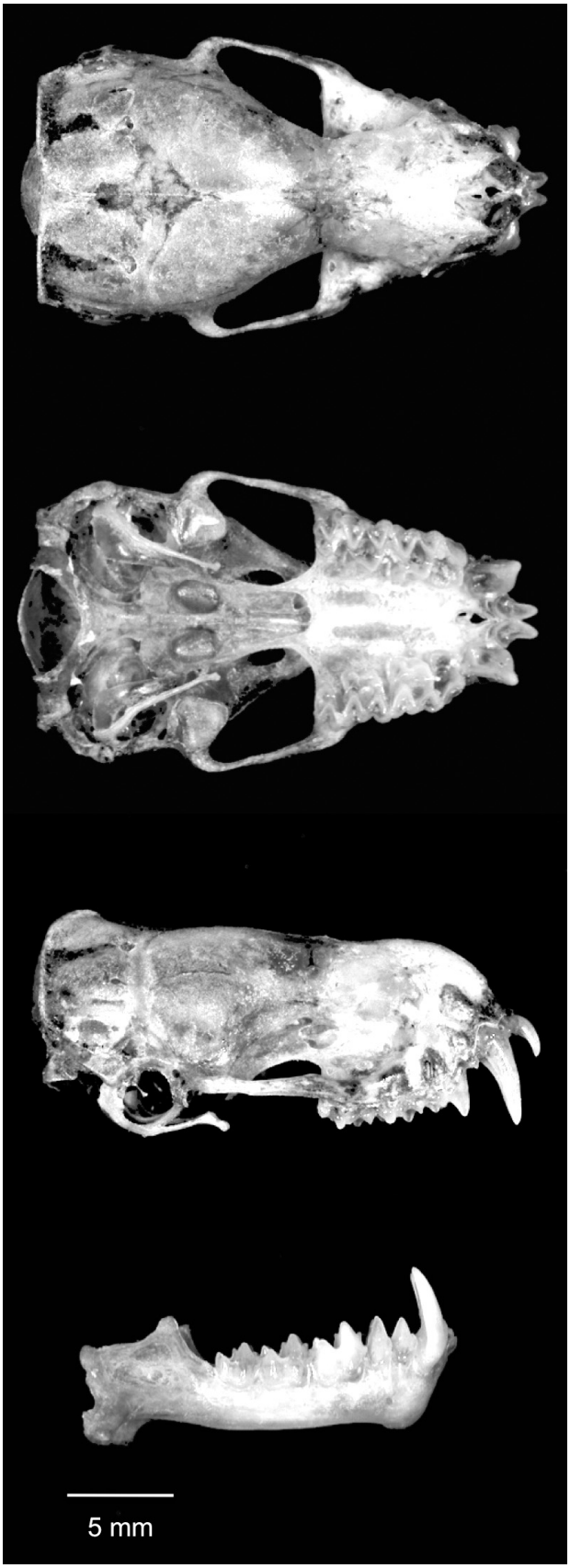

FIG. 1. Dorsal, ventral, and lateral view of the skull and lower jaw of the holotype of *Eumops wilsoni* (TTU 103281, QCAZ 10600)

Guayas, Bosque Protector Cerro Blanco, Centro de Visitantes (02°10'47.6"S, 80°01'17.7"W, 22 m elevation — Fig. 2). Collected on 4 July 2004, by a Texas Tech University (TTU)/PUCE field party on the Sowell Expedition. Original number, Juan Sebastián Tello Vasques (JSTV) 438. TK 134825 identifies tissue samples (preserved in lysis buffer and ethanol) deposited in the Natural Science Research Laboratory, TTU, and PUCE and karyotype preparations deposited at TTU.

Paratypes

An additional seven specimens (4 ♂♂ and 3 ♀♀) are included in the type series. Of these, six were collected at the type locality and one from Guayas, Isla Puna (02°45'34.3"S, 79°55'01.5"W, 10 m a.s.l. — Fig. 2). Three specimens consist of skins, skulls, and skeletons and four are alcohol preserved with the skull removed.

Distribution

From tropical dry forest of southwestern Ecuador and northwestern Peru (Fig. 2). Presently, the limits of the range of *E. wilsoni* cannot be determined beyond specimens identified by genetic analyses.

Diagnosis

Diagnosis is based on karyotypic differences (Fig. 3) and statistically-supported reciprocally monophyletic clades in both mitochondrial [cytochrome-*b* — see Fig. 1 in McDonough *et al.* (2008: 1309) and Fig. 4 of this paper] and nuclear [AFLP — see Fig. 2 in McDonough *et al.* (2008: 1310) and Fig. 5 of this paper] phylogenies. The karyotype of the holotype (TK 134825) has a diploid number of 38 and the fundamental number is 54 (Fig. 3). The autosomes are comprised of nine pairs of biarmed chromosomes (six pairs of large metacentrics and three pairs of medium-sized metacentrics) and nine pairs of acrocentrics. In some metaphase spreads, intra-individual variation is present within several of the acrocentrics, where a small second arm may be visible. The X is a medium metacentric and the Y is the smallest acrocentric.

Selected Measurements

External measurements (in mm) recorded in the field by JSTV are: total length — 126.9; tail length — 46.0; hindfoot — 13.1; ear — 21.8; tragus — 2.5. Body mass was 26.3 g. The following measurements taken by RJB from the holotype are: length of forearm on the dried specimen — 58.2 and cranial measurements of the holotype (Fig. 1) are: greatest

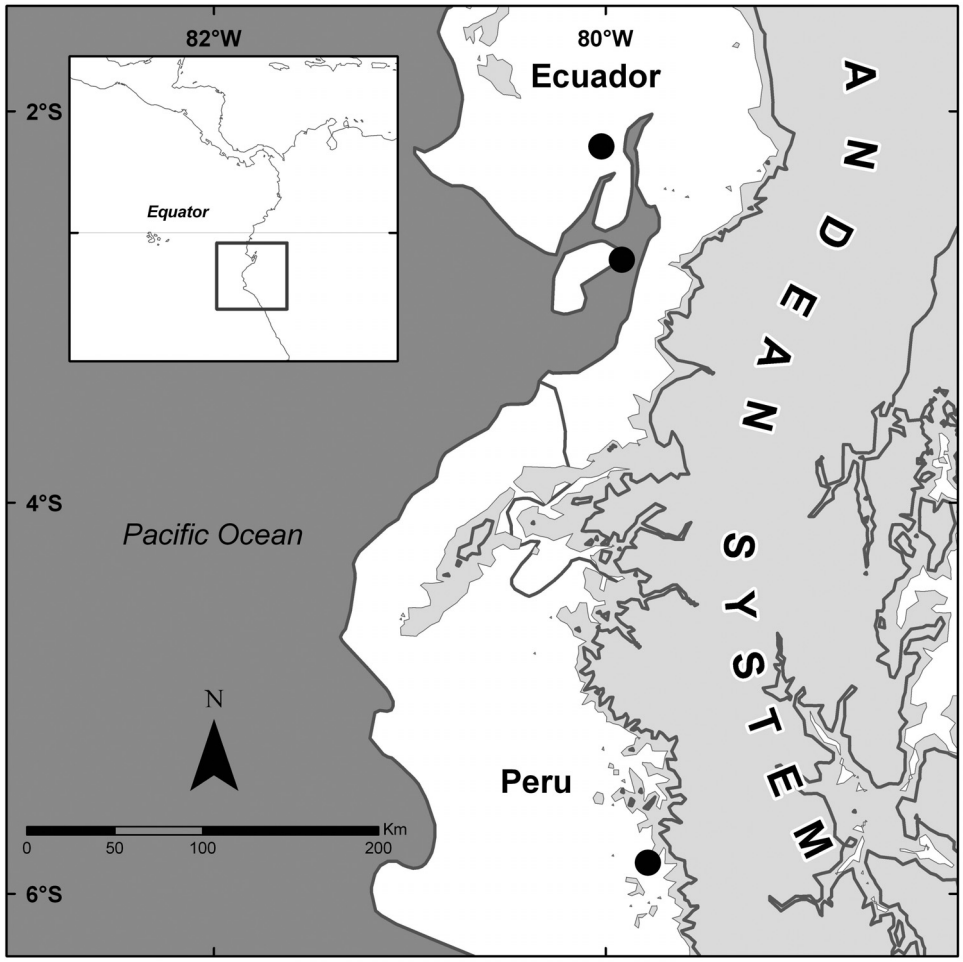

FIG. 2. Collecting localities (indicated by closed circles) of *E. wilsoni* confirmed with genetic data. Localities from north to south are: Ecuador: Guayas, Bosque Protector Cerro Blanco; Ecuador: Guayas, Isla Puna; and Peru: Piura, Piura

length of skull — 23.5; condylobasal length — 22.8; zygomatic breadth — 13.2; mastoid breadth — 12.7; breadth of braincase — 11.1; depth of braincase — 8.3; palatal length — 9.2; breadth across upper molars — 9.9; length of maxillary toothrow — 9.4; width across upper canines — 5.7; postorbital constriction — 5.2; length of mandible — 16.7; length of mandibular toothrow — 10.4.

Average measurements from four males and three females from Ecuador are (holotype not included – see also Table 1): total length — 117.3; tail length — 45.3; hindfoot — 11.8; ear — 23.7. Average body mass is 29.5 g. Average length of forearm is 59.3. Average cranial measurements are as follows: greatest length of skull — 23.1; condylobasal length — 22.2; zygomatic breadth — 14.1; mastoid breadth — 12.6; breadth of braincase — 11.0; depth of braincase — 8.3; palatal length — 10.2; breadth across upper molars — 10.1; length of maxillary toothrow — 9.3; width across upper canines — 5.6; postorbital constriction — 4.9; length of mandible — 16.9; length of mandibular toothrow — 11.1. Of the eight specimens examined, including the holotype, no significant difference exists between cranial measurements of males and females.

Karyotypic Data

Six specimens of *E. wilsoni* were karyotyped (TK 134793, 134816, 134825, 134826, 134832, and 134989) and their diploid number was 38 and fundamental number (herein defined as the number of arms in the autosomal complement) was 54 (Fig. 3). Other than sex chromosomal differences that distinguish karyotypes between males and females and the previously noted intra-individual variation in short arms of the acrocentric pairs, no intra-populational variation was detected.

Molecular Data

Phylogenetic analyses of both mitochondrial and nuclear markers of species of *Eumops* (McDonough *et al.*, 2008) demonstrated that *E. wilsoni* and other

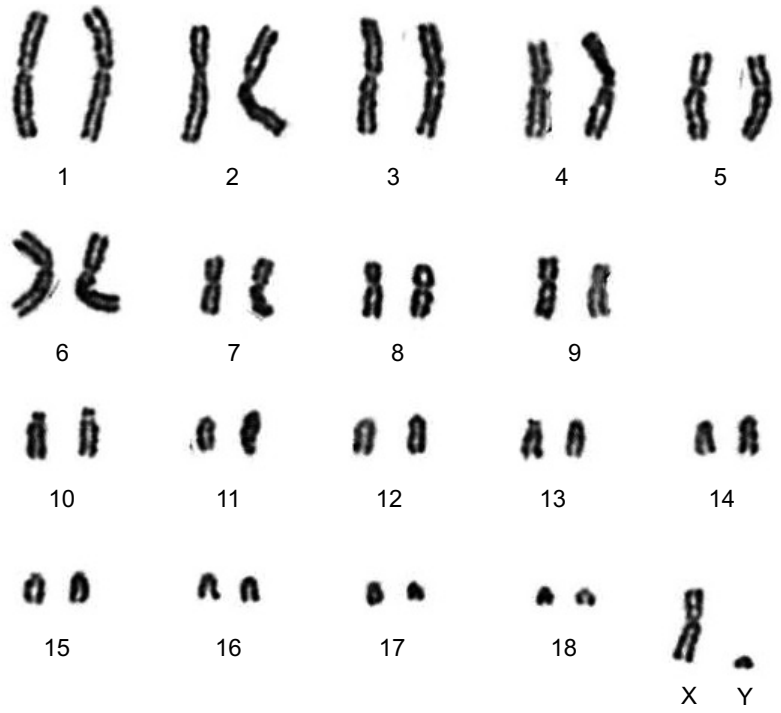

FIG. 3. Karyotype of the holotype (adult ♂, no. TK 134825) of *E. wilsoni*

members of the *E. glaucinus* complex (sensu Eger, 1977) formed a monophyletic group. Therefore, in this molecular description, our comparison of relationships of *E. wilsoni* to other species includes members of the *E. glaucinus* complex (*E. ferox*, *E. floridanus*, and *E. glaucinus*) as well as two outgroup taxa (*E. perotis* and *E. underwoodi*).

Of the 351 scored AFLP bands in McDonough *et al.* (2008), 20 bands were unique to *E. wilsoni* when compared to the other three species of the *E. glaucinus* complex and outgroups. The numbers of unique

TABLE 1. Descriptive statistics for eight cranial measurements (in mm) of *E. wilsoni* (n = 8), *E. glaucinus* (n = 6), and *E. ferox* (n = 20). Numbers presented include mean ± SD, and range. *P*-values indicate a significant difference in size based on a two-tailed *t*-test between species. Asterisks identify measurements of *E. glaucinus* that do not overlap with *E. wilsoni.* ns = not significant

Cranial measurements	*E. wilsoni*	*E. glaucinus*	*E. ferox*	*P*-level		
				E. wilsoni vs. *E. glaucinus*	*E. wilsoni* vs. *E. ferox*	*E. ferox* vs. *E. glaucinus*
Greatest length of skull	23.11 ± 1.02 21.81–24.50	24.73 ± 0.56 23.69–25.30	22.85 ± 0.94 21.64–24.82	≤ 0.05	ns	≤ 0.05
Condylobasal length	22.37 ± 1.24 20.98–23.79	24.03 ± 0.46 23.37–24.56	21.73 ± 1.15 20.21–23.64	≤ 0.05	ns	≤ 0.05
Zygomatic breadth	14.02 ± 0.42 13.44–14.80	15.00 ± 0.12 14.86–15.21*	14.39 ± 0.57 13.52–15.31	≤ 0.05	ns	≤ 0.05
Postorbital constriction	4.91 ± 0.08 4.82–5.05	4.95 ± 0.15 4.71–5.10	4.81 ± 0.13 4.60–5.08	ns	≤ 0.05	≤ 0.05
Mastoid breadth	12.61 ± 0.26 12.16–12.90	13.10 ± 0.41 12.52–13.60	12.83 ± 0.35 12.22–13.52	≤ 0.05	ns	ns
Palatal length	10.13 ± 0.54 9.55–10.99	11.10 ± 0.28 10.79–11.44	10.07 ± 0.51 9.13–10.86	≤ 0.05	ns	≤ 0.05
Length of maxillary toothrow	9.34 ± 0.27 9.07–9.72	9.93 ± 0.14 9.78–10.12*	9.29 ± 0.45 8.52–9.98	≤ 0.05	ns	≤ 0.05
Breadth across M^1–M^1	10.02 ± 0.19 9.73–10.23	10.24 ± 0.21 9.99–10.58	9.83 ± 0.34 9.18–10.38	ns	ns	≤ 0.05

bands displayed by *E. wilsoni* is comparatively higher than *E. ferox/floridanus* (16 unique bands) and lower than *E. glaucinus* (25 unique bands). The first three axes of the AFLP PCOA (Fig. 5) contained 80% of the total variation and *E. wilsoni* formed a distinct cluster separate from members of the *E. glaucinus* complex and the outgroup taxa. Nei-Li genetic distances (Nei and Li, 1979) of *E. wilsoni* are greater than 5% when compared to the *E. glaucinus* complex (see Table 2 of McDonough *et al*., 2008: 1310). This distance is greater than the genetic distances seen between *E. glaucinus* and *E. ferox/floridanus* that ranges from 3.6 to 4.0%. The cytochrome-*b* gene sequence of *E. wilsoni* is unique from all other species of bats. The sequence of the holotype is deposited in GenBank (accession no. EU349994). Within species variation, calculated using the Kimura 2-parameter model (Kimura, 1980), ranges from 0.13–0.83% (average is 0.3%) for individuals from Ecuador. The cytochrome-*b* gene sequence divergence of *E. wilsoni* is greater than 6% from other species of *Eumops* within the *E. glaucinus* complex (Table 2). Of the 705 bases of the cytochrome-*b* gene sequenced by McDonough *et al*. (2008), *E. wilsoni* exhibits 34 fixed nucleotide differences when compared to members of the *E. glaucinus* complex (Table 3).

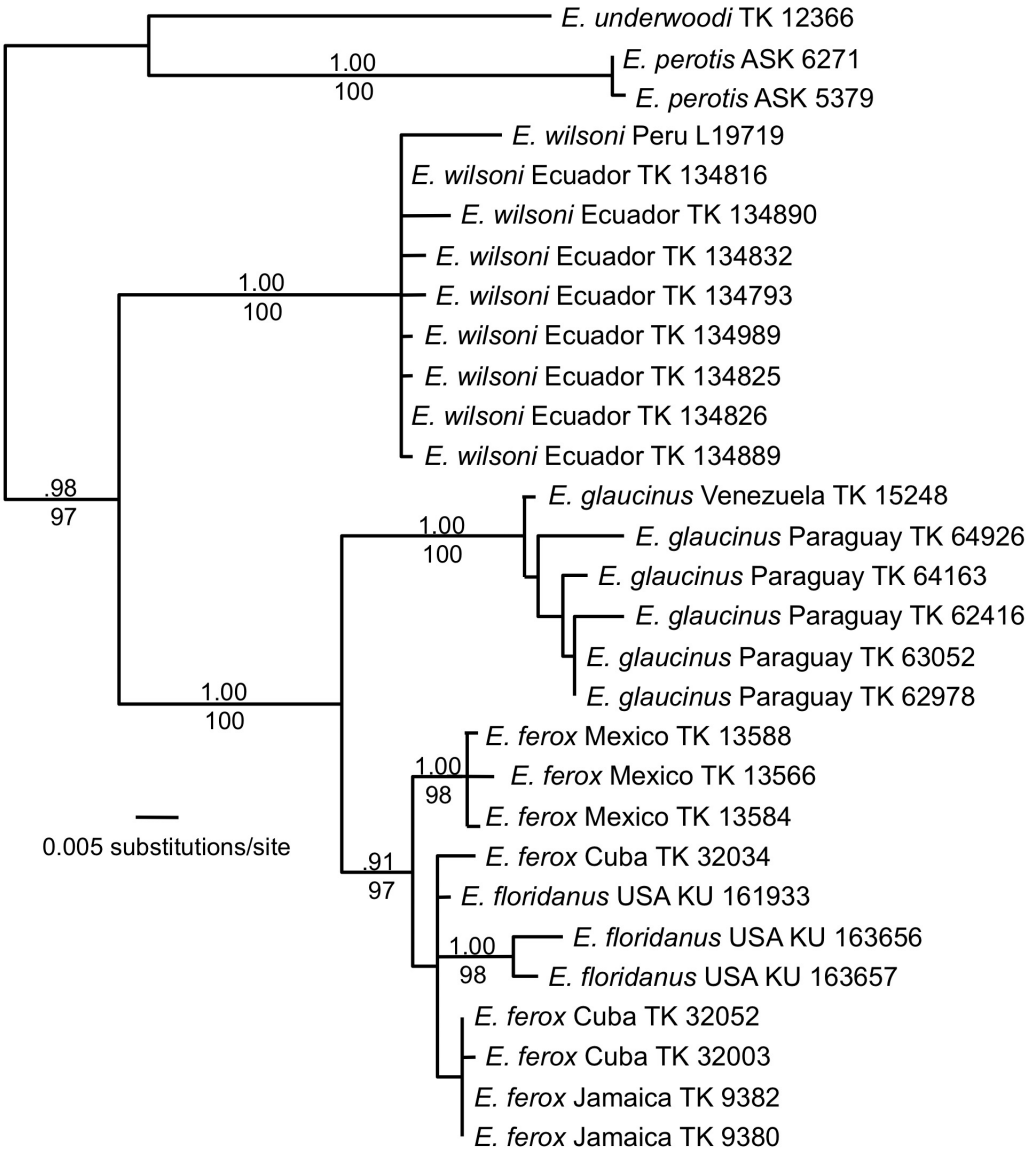

FIG. 4. Bayesian phylogram of 29 sequences of the cytochrome-*b* gene (705 base pairs). *Eumops underwoodi* and *E. perotis* were used as outgroups. Support based on Bayesian posterior probabilities (top score) and parsimony bootstrapping (bottom score) are shown along branches

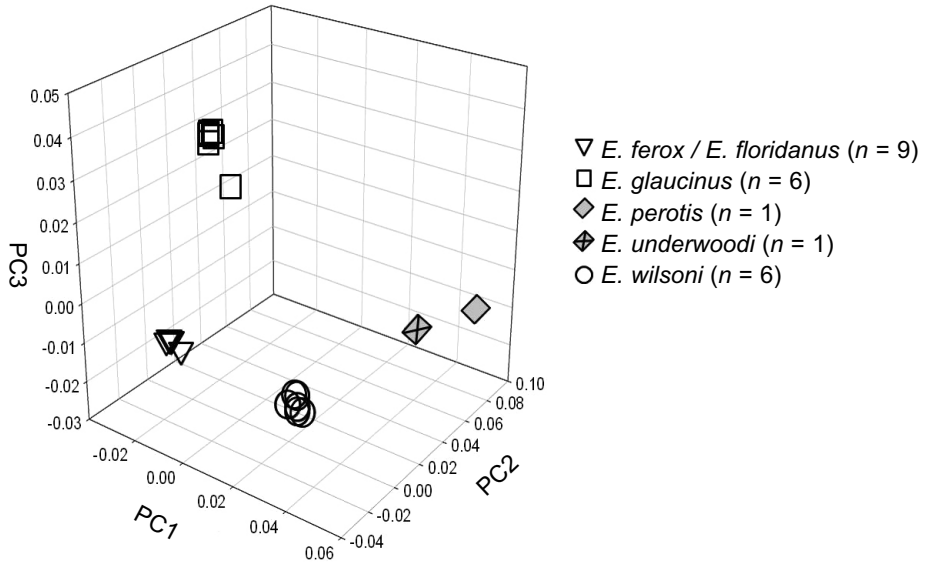

FIG. 5. Plot of the first three coordinates of a principal coordinate analysis based on 351 AFLP bands of 23 specimens (see Appendix)

Etymology

Don E. Wilson has made outstanding contributions to many different areas of the science of mammalogy, including conservation and systematics, as well as education of professional mammalogists, natural historians, and the general public. His work on the production of a thorough and consistently updated list of mammals of the world continues to have a worldwide impact. It is our pleasure to name this species for him in recognition of his significance to mammalogy. We propose the common name for this species be Wilson's bonneted bat.

DISCUSSION

Comparisons: Genetic Systems

Karyotypes

Based on the combination of fundamental number and diploid number, *E. wilsoni* has a karyotype (Fig. 3) unlike any other molossid bat. The diploid number of 38 matches the lowest reported thus far of any species of *Eumops* (2N = 38 in *E. ferox*). The fundamental number is 54 or 56 depending on assessment of the second arm of the acrocentric designated as number 10 (Fig. 3). Nine pairs of near acrocentrics distinguish the karyotype of *E. wilsoni* from all other members of the *E. glaucinus* complex [for comparisons see Fig. 3a and Fig. 3b of McDonough *et al.* (2008: 1311)]. The karyotype of *E. glaucinus* has been described as 2N = 40, FN = 64, although if the short second arm on a near acrocentric is not counted, then the FN would be 62 (Warner *et al.*, 1974; Morielle-Versute *et al.*, 1996). The X chromosome in all individuals of *E. glaucinus* (sensu McDonough *et al.*, 2008) thus far karyotyped has been biarmed and the Y chromosome has been an acrocentric (Warner *et al.*, 1974; Morielle-Versute *et al.*, 1996; Genoways *et al.*, 2005; McDonough *et al.*, 2008). The diploid number for *E. ferox* has been described as 2N = 38 and FN = 64 or 62 (Warner *et al.*, 1974; Genoways *et al.*, 2005; McDonough *et al.*, 2008). The X chromosome on specimens from

TABLE 2. Kimura 2-parameter genetic distances (in percentages) based on 702 base pairs of the cytochrome-*b* gene. Intraspecific values are in bold along the diagonal

No	Species	*n*	1	2	3	4	5	6
1.	*Eumops wilsoni*	8	**0.3**					
2.	*E. ferox*	8	6.8	**0.7**				
3.	*E. floridanus*	3	7.4	1.4	**1.2**			
4.	*E. glaucinus*	6	8.1	3.9	4.6	**0.9**		
5.	*E. perotis*	2	10.0	11.4	11.9	12.2	**0.1**	
6.	*E. underwoodi*	1	10.5	9.5	10.4	10.4	9.9	–

Jamaica, Cuba, and Chiapas Mexico is a biarmed element and in those from Costa Rica and Honduras has been described as possibly polymorphic (enough specimens of both sexes were not examined to eliminate the possibility that this was an autosomal polymorphism). Assuming the polymorphism involves the X, one X chromosome morph is like that found in Jamaica, Cuba, and Chiapas specimens and the other X chromosome morph is an acrocentric with a short second arm. The Y chromosome from specimens from southern Mexico and Costa Rica is a small metacentric or submetacentric, whereas specimens from Jamaica are described as either a minute acrocentric or an equally minute biarmed chromosome.

In an overview relative to the recognition of *E. glaucinus*, *E. ferox*, and *E. wilsoni* as species, chromosomal rearrangements potentially are an isolation mechanism. The role of different types of chromosomal rearrangements (heterochromatic shortarm additions, pericentric inversions, and whole arm translocations, etc.) has not been documented for the evolution of the karyotype present in *E. ferox*, *E. glaucinus*, and *E. wilsoni*. Clearly multiple types of chromosomal rearrangements are required to explain the different karyotypes in this complex. This is further documented by Finato *et al.* (2000) who studied the chromosomal location of the telomere repeat $(TTAGGG)_n$ in two species of *Eumops* (*E. glaucinus* and *E. perotis*). In *E. perotis* and *E. glaucinus*, telomeric sequences were found within the telomeres and interstitially (centromeric and pericentromeric) on multiple chromosomes. In both *E. glaucinus* and *E. perotis* the interstitial telomeric sequences were observed as part of the heterochromatin, although in *E. glaucinus* (sensu McDonough *et al.*, 2008) the signals of the telomeric repeats in the centromeres of the six largest pairs of autosomes and the X and Y were indicative of Robertsonian fusions. The significance of this observation is explained by Finato *et al.* (2000) that these telomeres were from ancestral chromosomes that participated in fusion events.

Sequence and AFLP Data

Specimens of *E. wilsoni* show 34 fixed nucleotide differences out of 705 bases of the cytochrome-*b* gene when compared to its closest relatives, *Eumops glaucinus*, *E. ferox*, and *E. floridanus* (Table 3). Twenty-eight of the changes are transitions and six are transversions. These base differences result in fixed amino acid differences in the cytochrome-*b* protein at positions 237, 238, and 241 (valine, alanine, and methionine respectively in *E. wilsoni*) when compared to *E. glaucinus*, *E. ferox*, and *E. floridanus* (alanine, threonine, and threonine).

The significance of genetic distance values for cytochrome-*b* gene sequences, relative to species justification, needs to be placed in context. Most species listed in Wilson and Reeder (2005) were described based on the level of morphological distinctiveness from all other closely related species. Bradley and Baker (2001) and Baker and Bradley (2006) reviewed cytochrome-*b* gene trees for sister taxa of species of mammals recognized by morphological distinction and determined that few sister species recognized based on classical morphology

TABLE 3. Fixed nucleotide changes in cytochrome-*b* among *E. wilsoni* (n = 8), *E. glaucinus* (n = 6), *E. ferox* (n = 8), and *E. floridanus* (n = 3). A = adenine, C = cytosine, G = guanine, and T = thymine. Position refers to the nucleotide position in the cytochrome-*b* gene from the complete mitochondrial genome of *Artibeus jamaicensis* (Pumo *et al*., 1998)

Position	*E. wilsoni*	*E. glaucinus*	*E. ferox* and *E. floridanus*
106	A	T	T
129	A	G	G
141	C	T	T
165	T	C	C
198	C	T	T
216	C	T	T
222	C	T	T
228	C	T	T
232	T	C	C
255	T	C	C
285	C	T	T
291	T	C	C
315	G	A	A
318	A	C	C
354	C	T	T
357	T	A	A
358	C	T	T
438	T	C	C
501	C	G/T	T
525	A	C	C
534	T	C	C
540	C	T	T
555	C	T	T
558	C	A	A
579	T	C	C
592	C	T	T
678	T	C	C
700	T	C	C
706	T	C	C
710	T	C	C
712	G	A	A
722	T	C	C
786	A	C	C
789	T	C	C

had a genetic distance value less than 5%. Baker and Bradley (2006) advanced the idea that speciation in mammals was generally a time dependent process resulting from the genetic divergence of the two respective genomes of two allopatric populations that speciated by the Bateson-Dobzhanzky-Muller (BDM) model. Baker and Bradley (2006) concluded that the time required to evolve greater than 5% distance value in the cytochrome-*b* gene was adequate to evolve sufficient genetic divergence in the respective genomes to result in genetic isolation by the BDM model. Relative to other members of the *E. glaucinus* complex, the fact that *E. wilsoni* has a greater than 6% genetic divergence within the cytochrome-*b* gene, a karyotype that is unique, and 6% unique bands (of 351 bands analyzed) with a greater than 5% genetic distance in AFLP analysis is presented here as evidence that the allopatric *E. wilsoni* has achieved genetic isolation and merits recognition as a species.

A sequence fragment (528 nucleotides) of the cytochrome-*b* gene from *E. glaucinus* (LSUMZ 27212) on Genbank (L19719) from Peru [Fig. 2 herein; Sudman *et al.* (1994)] has the same fixed differences that were present in the specimens of *E. wilsoni* from Ecuador and is therefore genetically assigned as a specimen of *E. wilsoni*. There are more than 30 specimens that are potentially *E. wilsoni* from this region in Peru housed in museums at Louisiana State University Museum of Natural Science, the Smithsonian Institution, and the American Museum of Natural History, but the specific identity of these specimens needs to be confirmed with genetic data (see Eger, 1977 and McDonough *et al*., 2008).

Comparisons: Morphology

McDonough *et al*. (2008) indicated that morphologically *E. wilsoni* was not distinguishable from *E. glaucinus*. This position was based on the variation in *E. glaucinus* (sensu Eger, 1977). When the morphological measurements are broken into monophyletic groups identified by molecular data, and the measurements are restricted to individuals that have been identified by molecular data, *E. glaucinus* sensu stricto is larger in two cranial measurements (length of maxillary toothrow and zygomatic breadth — Table 1) than any member of *E. wilsoni*. Further, Eger (1977) measured two specimens from Guayaquil, Ecuador and six specimens from Piura, Peru that are distinguished from *E. glaucinus* (from eastern Colombia and western Venezuela) and these need to be analyzed genetically to define the geographic distributions of *E. wilsoni*, *E. glaucinus*, and *E. ferox*. In light of the molecular data, and the need for monophyly in the context of species, a morphological review of inter- and intra-species variation of the *E. glaucinus* complex is merited. Presently there are no morphological diagnostic characters that distinguish *E. wilsoni* from *E. ferox* (Eger, 1977; Timm and Genoways, 2004; McDonough *et al*., 2008).

Justification for Recognition of Four Species within the Eumops glaucinus *Complex*

In the most recent edition of Mammals of the World, Simmons (2005) recognized the variation within the *E. glaucinus* complex as a single species. Timm and Genoways (2004) elevated *E. floridanus* as a distinct species based on morphology and fossil record. *Eumops floridanus* was justified as a distinct species primarily because it was morphologically larger than the remainder of the *E. glaucinus* complex and this morphological distinctiveness was as great as that used to justify many species recognized on a morphological basis in Wilson and Reeder (2005). In their molecular review of the *E. glaucinus* complex, McDonough *et al.* (2008) followed and supported the position of Timm and Genoways (2004) pending additional study.

McDonough *et al*. (2008), using molecular data from the mitochondrial and nuclear genomes, morphology, and karyotypes, divided *E. glaucinus* into four species. Three of these four species were genetically defined phylogroups (Avise and Walker, 1999) with geographic cohesiveness. Only *E. floridanus* failed to exhibit reciprocal monophyly based on molecular data. If *E. floridanus* and *E. ferox* speciated by the Bateson-Dobzhansky-Muller model as proposed by Baker and Bradley (2006), then a separate portion of the genome (than is identified by the AFLP sample of McDonough *et al.*, 2008) must be involved as an isolating mechanism. Therefore, using the operational criteria outlined in the introduction of this description, *E. floridanus* meets the fewest criteria (ecological and morphological distinction) for species recognition (Table 4). However, the karyotype of *E. floridanus* has not been described. *Eumops glaucinus*, *E. ferox*, and *E. wilsoni* are distinguished from each other by unique karyotypes. *Eumops glaucinus* and *E. wilsoni* are also distinguished from each other by statistically supported morphological differences in two cranial

TABLE 4. Presence of operational criteria to justify species status. Asterisk indicates data presented in McDonough *et al*. (2008)

Species	Statistically supported reciprocal monophyly: *Cyt-b*	Statistically supported reciprocal monophyly: AFLP*	Morphologically distinct	Karyotypically distinct	Species monophyly preserved	Ecologically distinct
E. wilsoni vs. *E. ferox*	Yes	Yes	No	Yes	Yes	Yes
E. wilsoni vs. *E. floridanus*	Yes	Yes	Yes	Unknown	Yes	Yes
E. wilsoni vs. *E. glaucinus*	Yes	Yes	Yes	Yes	Yes	Yes
E. ferox vs. *E. floridanus*	No	No	Yes	Unknown	No	Yes
E. ferox vs. *E. glaucinus*	Yes	Yes	Possibly	Yes	Yes	Yes
E. floridanus vs. *E. glaucinus*	Yes	Yes	Yes	Unknown	Yes	Yes

measurements (length of maxillary toothrow and zygomatic breadth; see Table 1), by statistically supported reciprocal monophyly defined by mitochondrial cytochrome-*b* gene sequence, and by statistically supported monophyly in the AFLP data (McDonough *et al*., 2008). *Eumops wilsoni* and *E. ferox* meet the criteria of being distinguished by unique karyotypes and by statistically supported reciprocal monophyly of a mitochondrial gene and the nuclear AFLP data, but are not morphologically distinguished from each other using the morphological features examined herein and in Eger (1977). In summary, there are four potential species in this complex of bats, and using the above operational criteria, a combination of justification for species recognition of each is evident (Table 4).

REMARKS

Based on our results and the morphological analysis of Eger (1977), the exact limits of the distribution of *E. wilsoni* will require further collecting and genetic analyses but it is possible that *E. wilsoni* may be in contact with *E. ferox* on the western side of the Andes, somewhere between Panama and Ecuador. Further, the range of *E. wilsoni* may extend across the Andes in northern Peru or southern Ecuador where contact with *E. glaucinus* is possible. The cytochrome-*b* gene genetic distance values that distinguish *E. wilsoni* from these two species, as well as the chromosomal uniqueness of *E. wilsoni*, suggest that if hybridization occurs, genetic isolation will be achieved by hybrids that are sufficiently less fit than are pure parental types.

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Appendix

Specimen, locality, tissue number, catalog number, GenBank (EU and L) accession number, and analysis method for specimens used in morphometric (M), cytochrome-*b* (C), Amplified Fragment Length Polymorphism (A), and karyotype (K) datasets. ASK (tissue number) and ASNHC (catalog number) = Angelo State Natural History Collection; CM = Carnegie Museum; LSUMZ = Louisiana State University Museum of Natural Science; RMT (tissue number) and KU (catalog number) = University of Kansas; TK (tissue number) and TTU (catalog number) Natural Science Research Laboratory, Texas Tech University; QCAZ (catalog number) = Pontificia Universidad Católica del Ecuador

Species	Geographic origin	Catalog no.	Tissue no.	GenBank no.	Analysis
Eumops ferox	Cuba: Guantánamo Province; Guantánamo Bay Naval Station	TTU 52635	TK 32001	EU350007	C, A
– " –	– " –	TTU 52642	TK 32002	EU350008	M
– " –	– " –	TTU 52643	TK 32003	EU350009	M, C
– " –	– " –	TTU 52636	N/A	N/A	M
– " –	– " –	TTU 52612	TK 32018	EU350010	M
– " –	– " –	TTU 52637	TK 32019	EU350011	M, C, A
– " –	– " –	TTU 52639	TK 32032	EU350012	M
– " –	– " –	TTU 52613	TK 32033	EU350013	M
– " –	– " –	TTU 52640	TK 32034	EU350014	M, C, A
– " –	– " –	TTU 52617	TK 32052	EU350015	M, C, A
– " –	– " –	TTU 52618	TK 32053	EU350016	M
– " –	– " –	TTU 52619	TK 32054	EU350017	M
– " –	– " –	TTU 52627	TK 32112	EU350019	M
– " –	– " –	TTU 52628	TK 32113	EU350020	M
– " –	Jamaica: Queenhythe; St, Ann Parish	CM 44612	TK 9378	EU350022	C, A
– " –	– " –	CM 44614	TK 9380	EU350021	C
– " –	– " –	CM 44616	TK 9382	EU350023	C
– " –	– " –	TTU 22081	N/A	N/A	M
– " –	Mexico: Mérida; Campestre Country Club	N/A	TK 13566	EU350030	C
– " –	– " –	N/A	TK 13584	EU350035	C
– " –	– " –	TTU 47519	TK 13585	EU350036	M, C, A
– " –	– " –	TTU 47520	TK 13588	EU450039	M, C
– " –	– " –	TTU 47521	TK 13589	EU350040	M
– " –	– " –	TTU 47522	TK 13590	EU350041	M
– " –	– " –	TTU 29075	N/A	N/A	M
– " –	– " –	TTU 29076	N/A	N/A	M
E. floridanus	United States: Florida; Dade County, Coral Gables	KU 161933	N/A	EU350026	C, A
– " –	United States: Florida; Lee County, North Fort Meyers	KU 163656	RMT 4610	EU350024	C, A
– " –	– " –	KU 163657	RMT 4611	EU350025	C, A
E. glaucinus	Paraguay: Alto Paraguay; Estacia Tres Marias	TTU 79823	TK 62416	EU350000	M, C, A
– " –	Paraguay: Boquerón; Base Naval Pedro P. Peña	TTU 79955	TK 62978	EU350001	M, C, A
– " –	– " –	TTU 79957	TK 63051	EU350002	M
– " –	– " –	TTU 79957	TK 63052	EU350003	M, C, A
– " –	Paraguay: Concepción; Parque Nacional Serrania de San Luis	TTU 80255	TK 64163	EU350005	M, C, A
– " –	Paraguay: Presidente Hayes; Estancia Samaklay	TTU 80542	TK 64926	EU350004	C, A
– " –	Venezuela: Guárico; 48 km S Calabzo	TTU 33408	TK 15248	EU350006	M, C, A
E. perotis	United States: Texas; Brewster County, Big Bend National Park	ASNHC 12238	ASK 5379	EU349990	C, A
– " –	– " –	ASNHC 13295	ASK 6271	EU349991	C
E. underwoodi	Nicaragua: Boaca; Los Cocos, 14 km S Boaco, Rio Los Cocos	TTU 29311	TK 123366	EU349989	C, A
E. wilsoni	Ecuador: Guayas Province; Bosque Protector Cerro Blanco	TTU 103255	TK 134793	EU349992	M, C
– " –	– " –	TTU 103278	TK 134816	EU349993	M, C, A, K
– " –	– " –	TTU 103281/			
– " –	– " –	QCAZ 10600	TK 134825	EU349994	M, C, A, K

APPENDIX. Continued

Species	Geographic origin	Catalog no.	Tissue no.	GenBank no.	Analysis
E. wilsoni	Ecuador: Guayas Province; Bosque Protector Cerro Blanco	TTU 103282	TK 134826	EU349995	M, C, A, K
– " –	– " –	TTU 103286	TK 134832	EU349996	M, C, A, K
– " –	– " –	TTU 103302	TK 134889	EU349997	M, C, A, K
– " –	– " –	TTU 103303	TK 134890	EU349998	M, C
– " –	Ecuador: Guayas Province; Isla Puna	TTU 103466	TK 134989	EU349999	M, C, A, K
– " –	Peru: Departmento Lambayeque; 12 km N Olmos	LSUMZ 2721	N/A	L19719	C