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Molecular phylogeny of long-tailed shrews (genus *Sorex*) from México and Guatemala

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Abstract

We present a molecular phylogeny of North American species of long-tailed shrews of the genus *Sorex.* Our focus is on Mexican and Guatemalan species to begin understanding their evolutionary relationships and to test the validity of nominal species. Seventy-seven sequences of the mitochondrial cytochrome *b* gene were analyzed, including 19 specimens representing nine Mexican and one Guatemalan species. Phylogenetic analyses using parsimony, maximum likelihood and Bayesian approaches revealed two major clades of North American species, all within the subgenus *Otisorex*. The first major clade includes *S. trowbridgii* and southern species (*S. macrodon* from Oaxaca*; S. veraecrucis* from Nuevo León, Michoacán, Chiapas, *S. saussurei* from Jalisco and Guatemala; *S. veraepacis* from Guerrero and Guatemala). Relatively deep branches among taxa characterize this clade and suggest that their early divergence from other North American shrews was soon after arrival of the ancestral stock from the Beringian region. The other major clade includes all other North American species of *Sorex* we examined, with two Mexican species, *S. milleri* and *S. emarginatus*, grouped in a subclade with the *S. cinereus* complex. *Sorex veraecrucis* is not, however, a monophyletic taxon because specimens of this nominal species were included in both the major clades. The Isthmus of Tehuantepec has likely played a role as a biogeographic barrier in the evolutionary history of Mexican shrews. This study of mitochondrial variation in southern North American shrews of the genus *Sorex* indicates there is substantial, previously undetected diversity that necessitates a revision of the taxonomy of *S. veraecrucis* and *S. veraepacis*.

Key words: biogeography, mammals, mitochondrial DNA, Soricidae, systematics

Introduction

Approximately 77 species of shrews in the genus *Sorex* (Soricomorpha, Soricidae) are currently recognized (Hutterer 2005). These diminutive species are often among the most abundant members of terrestrial mammalian communities in the boreal and mountainous regions of Europe, Asia, and North America. Phylogenetic relationships among some species have been studied using cranial, dental and other morphological characters (Junge & Hoffmann 1981; Carraway 1990), allozymes (George 1988), and molecular sequence data (Fumagalli *et al.*1999; Ohdachi *et al.* 2006; Dubey *et al.* 2007). A comprehensive assessment that includes all species will be difficult to complete due to limited availability of specimens for many species within this widely distributed group. Nevertheless, new species are described regularly (e.g., Dokuchaev 1997; Rausch *et al.* 2007; Nagorsen & Panter 2009) as more detailed assessments of geographic variation are completed.

The ancestral lineages of *Sorex* are hypothesized to have arisen in Eurasia (Repenning 1967; Storch *et al.* 1998) and then split between 7.0–18.4 million years ago (mya) into two principal lineages that are recognized as: a) subgenus *Sorex* (most Palearctic species plus Holarctic *S. tundrensis* Merriam 1900 and Nearctic *S. arcticus* Kerr 1792, *S. maritimensis* Smith 1939, and *S. yukonicus* Dokuchaev 1997) and b) subgenus *Otisorex* (chiefly Nearctic or Beringian taxa, Hutterer 2005; and possibly including *S. trowbridgii* Baird 1857,

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Fumagalli *et al.* 1999). Dubey *et al.* (2007) similarly estimated that these two subgenera diverged 10.2 – 17.5 mya (Table 1). Allozyme, immunological and chromosomal studies (e.g., George 1988; George & Sarich 1994; Ivanitskaya 1994) have generally recovered the two main clades of subgenera, but the placement of particular taxa (e.g., *S. trowbridgii*, *S. fumeus* G. M. Miller 1895, and most Mexican and Guatemalan species) has remained problematic (Hutterer 2005). Resolution of these relationships is critical to understanding the historical biogeography of the Holarctic, such as timing and impact of biotic exchange between Asia and North America.

TABLE 1. Species of subgenera *Sorex***,** *Otisorex* and species not previously classified (Hutterer 2005). Species included in this study (*).

Sorex (*Otisorex*)	*Sorex* (*Sorex*)	Unclassified
alaskanus	**alpinus*	*arizonae*
**bairdi*	*antinorii*	**emarginatus*
**bendirii*	*araneus*	*merriami*
**camtschatica*	**arcticus*	**mirabilis*
**cinereus*	*arunchi*	*planiceps*
dispar	*asper*	**saussurei*
**fumeus*	*averini*	*sclateri*
gaspensis	*bedfordiae*	*stizodon*
**haydeni*	*buchariensis*	*thibetanus*
**hoyi*	*caecutiens*	**trowbridgii*
**jacksoni*	*cansulus*	**ventralis*
leucogaster	*coronatus*	**veraecrucis*
**longirostris*	*cylindricauda*	
lyelli	*daphaenodon*	
**macrodon*	*excelsus*	
**milleri*	*gracillimus*	
**monticolus*	*granarius*	
nanus	*hosonoi*	
**neomexicanus*	*isodon*	
**oreopolus*	*kozlovi*	
orizabae	*maritimensis*	
**ornatus*	*minutissimus*	
**pacificus*	*minutus*	
**palustris*	*raddei*	
**portenkoi*	*roboratus*	
**preblei*	*samniticus*	
**pribilofensis*	*satunini*	
**sonomae*	*shinto*	
**tenellus*	*sinalis*	
**ugyunak*	**tundrensis*	
unguiculatus	*volnuchini*	
**vagrans*	*yukonicus*	
**veraepacis*		

For members of the predominantly Eurasian subgenus *Sorex,* two independent colonizations of the New World were hypothesized through Beringia; the first wave by *S. arcticus* occurred about 2.3 mya (0.9–4.5), whereas *S. tundrensis* represents an undated, but more recent, second wave (Fumagalli *et al.* 1999). For members of the subgenus *Otisorex*, phylogenetic studies based on mitochondrial sequences improved our understanding of the biogeographic history of this clade that includes mostly Nearctic species (Demboski & Cook 2001, 2003; Shafer & Stewart 2007). For example, the *cinereus* group (van Zyll de Jong & Kirkland 1989) is composed of two primary lineages (Demboski & Cook 2003). One is a predominantly Beringian (northern) clade comprised of Nearctic and a few Palearctic species with distributions close to Beringia, and *S. preblei* Jackson 1922 as a sister taxon. The other lineage is formed by species occurring from Alaska to the southeastern United States, with *S. longirostris* Bachman 1837 the sister taxon. Notably missing from previous analyses of this mostly Nearctic clade has been representatives of species of *Sorex* from Mexico and Central America.

To more completely understand the diversification of long-tailed shrews of the subgenus *Otisorex* in the New World, we need to test hypotheses related to several stages of their evolutionary and biogeographic history. For example, some nominal species of this subgenus harbor deep sequence divergence that likely reflects the presence of multiple cryptic or previously unrecognized species, while other nominal species show minimal molecular differentiation from allied species that questions the validity of currently accepted taxonomy (Demboski & Cook 2001). Along the west coast of North America, *S. bairdi* Merriam 1895, *S. pacificus* Coues 1877, *S. bendirii* Merriam 1884, *S. palustris* Richardson 1828, *S. sonomae* Jackson 1921, and one distinctive lineage of *S. monticolus* Merriam 1890 are closely related, while in New Mexico, *S. neomexicanus* Bailey 1913 and a distinct evolutionary trajectory of *S. monticolus* are closely allied, if not conspecific. In this case, further research is required to understand the phylogenetic position and species identity of the alleged wide-ranging *S. monticolus* and a number of closely related forms. Populations of *S. monticolus* from northwestern Mexico had not been examined previously. Indeed, Mexican and Guatemalan species of *Sorex* are generally poorly studied and have seldom been included in molecular phylogenetic analyses. Yet, these southern-most shrews are critical to tying previous phylogenetic studies within *Sorex* across their northern distribution (e.g., Shafer & Stewart 2007) to the overall history of *Sorex* diversification in North America.

We use mitochondrial sequence variation to investigate the evolutionary relationships among 11 (*S. emarginatus* Jackson 1925, *S. ixtlanensis* Carraway 2007, *S. macrodon* Merriam 1895, *S. monticolus*, *S. milleri* Jackson 1947, *S. oreopolus* Merriam 1892, *S. ornatus* Merriam 1895, *S. saussurei*, Merriam 1892, *S. veraecrucis* Jackson 1925, *S. ventralis* Merriam 1895, *S. veraepacis* Alston 1877) of the 14 recognized Mexican and Guatemalan long-tailed shrews (Hutterer 2005). Collectively, these 11 nominal species have a wide geographical distribution across Mexico and two species (*S. veraepacis, S. veraecrucis*) range southward to eastern Guatemala (Hall 1981; Hutterer 2005). Of these, *S. monticolus* is the only species that occurs north of Mexico. We constructed a molecular phylogeny for these southern North American species in an effort to complement and further our knowledge of their evolutionary relationships with other North American long-tailed shrews of the genus *Sorex*.

Material and methods

Previous taxonomic reviews of the genus *Sorex* listed either 12 (Villa & Cervantes 2003) or 14 (Hutterer 2005) species in Mexico, two of which also occur in Guatemala. Our analysis includes ten of those species (excluding *S. arizonae* Diersing and Hoffmeister 1977, *S. orizabae* Merriam 1895, *S. sclateri* Merriam 1897, and *S. stizodon* Merriam 1895). Carraway (2007) recently proposed that a total of sixteen species of *Sorex* are distributed in Mexico (Hall 1981; Villa & Cervantes 2003; Hutterer 2005). Our study also included representatives of one of her newly recognized species, *S. ixtlanensis*.

For this study, 19 samples collected from Mexico were sequenced and 58 sequences were downloaded from GenBank for a total of 26 species of *Sorex* (Table 2). *Crocidura suaveolens* Pallas 1811 was designated

as outgroup for phylogenetic analyses (George 1988; Hutterer 2005), which allowed us to set the point of divergence between the subfamilies Crocidurinae and Soricinae (Reumer 1994).

TABLE 2. Collection localities, specimen numbers and GenBank accession numbers or (*) collection catalog number for specimens examined. Mammalian collection acronyms are as follows: CNMA or FAC = Colección Nacional de Mamíferos (Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City); KUNHM = University of Kansas (Lawrence, Kansas, USA); CDR = Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Durango (Instituto Politécnico Nacional, Mexico City); ECO-SC-M = El Colegio de la Frontera Sur (San Cristóbal de las Casas, Chiapas, México); MCNG = Museo de Ciencias Naturales, Universidad de San Carlos de Guatemala (Guatemala City); BYU = Brigham Young University (Provo, Utah, USA).

Species	Specimen identification code	Location	Specimen no. and GenBank number	Base pairs (bp)
Crocidura suaveolens (1)	0	AUSTRIA: Wien	AB077280	1140
Sorex alpinus (2)	0	SWITZERLAND: Pont de Nant, Vaud Canton	AB175120	1140
		SWITZERLAND: Col du Sanetsch, Valais Canton	AB175119	1140
Sorex arcticus (2)	2	CANADA: Quebec	AJ000428	1010
		CANADA: Windsor, Nova Scotia	AJ000427	1011
Sorex bairdi (2)	2	USA: Oregon, Yamhill County	AF238024	801
		USA: Oregon, Tillamook County	AF238023	801
Sorex bendirii (2)	2	CANADA: British Columbia, Fraser Valley	AY954947	1140
		CANADA: British Columbia	AY954946	1140
Sorex camtschatica (2)	0	RUSSIA: Magadan	AY014920	1140
		RUSSIA: Magadan	AY014919	1140
Sorex cinereus (2)	2	USA: Alaska: Kanuti National Wildlife Refuge	AY014951	1140
		USA: Minnesota, Goodhue County	AY014952	1140
Sorex emarginatus (1)	2	*MEXICO: State of Durango, Las Adjuntas	*KUNHM54346	647
Sorex fumeus (2)	2	USA: Pennsylvania, Westmoreland County	AB175116	1010
		CANADA: Edmundstone, New Brunswick	AJ000462	1010
Sorex haydeni (2)	2	USA: South Dakota, Davison County	AY014940	1010
		USA: South Dakota, Davison County	AY014939	1010
Sorex hoyi (2)	2	USA: Alaska, Hughes Quadrangle	AF238040	1140
		CANADA: AB, Seebe	AY310343	1010
Sorex hoyi thompsoni (2)	2	CANADA: Gogama	AY310344	1010
		CANADA: Toronto	AY310342	1010
Sorex pribilofensis (2)	2	USA: Alaska, St. Paul Island	AY014933	1140
		USA: Alaska, St. Paul Island	AY014932	1140
Sorex ixtlanensis (1)	3	*MEXICO: State of Oaxaca, San José de Cieneguilla	*FAC3181	1140
Sorex jacksoni (2)	2	USA: Alaska, St. Lawrence Island	AY014926	1140
		USA: Alaska, St. Lawrence Island	AY014925	1140
Sorex longirostris (2)	2	USA: Tennessee, Perry County	AY014954	1140
		USA: Virginia, Mecklenburg County	AY014953	1140
Sorex macrodon (1)	3	*MEXICO: State of Oaxaca, Vista Hermosa	*KUNHM121657	1081
Sorex milleri (3)	2	*MEXICO: State of Nuevo León, Cerro Potosí	*CNMA26549	1134
		*MEXICO: State of Coahuila, San Antonio de las Alazanas	*KUNHM67296	758

continued next page

TABLE 2. (continued)

Species	Specimen identification code	Location	Specimen no. and GenBank number	Base pairs (bp)
		*MEXICO: State of Coahuila, San Antonio de las Alazanas	*KUNHM67281	644
Sorex mirabilis (1)	0	South Korea	AB062737	1140
Sorex monticolus (7)	2	USA: Oregon, Tillamook County, Nestucca River	AJ000451	1010
		USA: Oregon, Tillamook County, Nestucca River	AJ000450	1010
		USA Colorado, Jackson County	AF238019	801
		USA: New Mexico, Cibola County	AF238018	801
		Canada: British Columbia, Barriere	AF238010	801
		Canada: British Columbia, Opax Mountain	AF238011	801
		*MEXICO: Durango	*CDR4360	1127
Sorex neomexicanus (2)	2	USA: New Mexico, Otero County	AF238030	801
		USA: New Mexico, Otero County	AF238029	801
Sorex oreopolus (1)	3	*MEXICO: Distrito Federal, Delegación Tlalpan, 1 km sur Parrés	*CNMA31955	1085
Sorex ornatus (2)	2	USA: California, San Diego County	AF238036	801
		USA: California, San Diego County	AF238035	801
Sorex pacificus (2)	2	USA: Oregon, Rock Creek, Lane County	AJ000453	1010
		USA: Oregon, Rock Creek, Lane County	AJ000452	1010
Sorex palustris (2)	2	Canada: Alberta, Alta, Calling Lake	AY954942	1140
		Canada: Alberta, Alta, Calling Lake	AY954941	1140
Sorex portenkoi (1)	0	RUSSIA: Provideniya	AY014921	1140
Sorex preblei (2)	2	USA: Oregon, Harney County	AY014937	1140
		USA: Oregon, Harney County	AY014936	1140
Sorex saussurei (2)	2	*MEXICO: State of Jalisco, Bolaños	*FAC3205	1140
	4	*GUATEMALA: Departamento Huehuetenango, Municipio San Mateo Ixtatán	*MCNG789	1140
Sorex sonomae (2)	2	USA: California, Humboldt County	AF238027	801
		USA: California, Humboldt County	AF238026	801
Sorex tenellus (2)	2	USA: California, Lassen Volcanic	DQ086472	983
		USA: California, Mono County	AY014955	1140
Sorex trowbridgii (3)	2	USA: Oregon	AJ000464	1010
		USA: Oregon	AJ000463	1010
		USA: Washington, Kittitas County	AY014956	1140
Sorex tundrensis (2)	1	Russia: Moneron Island	AB244646	1140
		Russia: Moneron Island	AB244645	1140
Sorex ugyunak (2)	2	USA: Alaska, Galbraith Lake	AY014930	1140
		USA: Alaska, Seward Peninsula	AY014928	1140
Sorex vagrans (2)	2	USA: California, Sagehen Creek, Nevada County	AJ000454	1010
		USA: Montana, Lake County	AF154551	1140
Sorex ventralis (1)	3	*MEXICO: State of Puebla, San Martín Texmelucan	*CNMA26543	939
Sorex veraecrucis (4)	3	*MEXICO: State of Michoacán, Pátzcuaro	*BYU15995	1127
	2	*MEXICO: State of Nuevo León, Galeana	*FAC2960	1140

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TABLE 2. (continued)

Species	Specimen identification code	Location	Specimen no. and GenBank number	Base pairs (bp)
	3	*MEXICO: State of Oaxaca, Santa María Pápalo	*CNMA39369	1127
	4	*MEXICO: State of Chiapas, San Cristóbal de las Casas	*CNMA42918	1127
Sorex veraepacis (4)	3	*MEXICO: State of Guerrero, Omiltemi	*CNMA41838	1140
		*MEXICO: State of Guerrero, Omiltemi	*CNMA41839	1126
		*GUATEMALA: Departamento Huehuetenango, Municipio Todos los Santos Cuchumatán	*MCNG806	1137
	4	*GUATEMALA: Departamento Huehuetenango, Municipio Todos los Santos Cuchumatán	*MCNG823	1134

DNA was extracted from frozen or alcohol-preserved tissues of field collected specimens, or skin samples from museum specimens, following a proteinase K-phenol- chloroform protocol (Darbre 1999; Surzycki 2000). Some extractions from skins were performed using the DNeasy® Tissue Kit. Extraction from museum specimens was preceded by cleaning with STE (sodium chloride-Tris-EDTA) to remove impurities (Hillis *et al.* 1996).

Amplification of the mitochondrial cytochrome *b* gene (cyt *b*) was performed via polymerase chain reaction (PCR). Several primer pairs were used for PCR amplification and sequencing of cyt *b* gene (Table 3). Two protocols were used for the PCR: 1. Primers L-14115(A-Soricidae)/H-Sorex770 and L-14764Sorex/H-15895(E) were used, with an initial denaturation at 94°C for 1 min, annealing at 45 or 50°C for 1 min, and extension at 72°C for 1.5 min. All amplifications were performed for 30 cycles (Lessa & Cook 1998). 2. Primers L-14115(A-Soricidae), MVZs, UNMF14 and UNMR17, with an initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min (Francisco X. González pers. comm.). In both cases the concentrations were: MgCl 3mM, Primers 0.4uM, Buffer 1X, dNTP's 2mM, Taq 1ul. PCR products were purified and sequenced in both directions with 25 cycles of denaturation at 95°C for 10 sec, annealing at 50°C for 5 sec, and extension at 60°C for 4 min. We used 2µl Big Dye, 2µl 5 x Buffer, 1µl 1pM each primer and 2 ul of PCR product. Sequences were run on an Applied Biosystems 3100 DNA sequencer and assembled and aligned with Sequencher Version 4.7 and Bio Edit version 7.0.5 (Hall 1999).

An uncorrected ("*p*") genetic distance matrix was generated using PAUP* version 4.0b (Swofford 1999) to compare with other reports (e.g., Demboski & Cook 2001). The best fit model of DNA substitution was determined by hierarchical likelihood-ratio tests using MODELTEST Version 3.06 (Posada & Crandall 1998). Phylogenies and nodal support (posterior probabilities) were estimated using MrBayes, version 3.1 (Huelsenbeck & Ronquist 2001). Bayesian analysis was initiated with random starting trees, and run for 5 x 10^6 generations with four chains sampling every 1000 generations. Two independent replicates were conducted (Huelsenbeck & Imennov 2002). The stationary stage of the Markov chain was determined by plotting log-likelihood values against number of generations. The first 1000 trees sampled from generations preceding stationary were discarded as burn-in (Huelsenbeck & Ronquist 2001). Data collected following burn-in were used to estimate nodal support as posterior probabilities.

Maximum likelihood (ML) and bootstrap analysis (Felsenstein 1985) were used to infer the phylogeny and nodal support using PhyML 3.0 (Guindon & Gascuel 2003). Because the sequences have different lengths and missing data in different positions, we tested if sequence size variation might produce different tree topologies. Therefore, we ran two analyses, one with sequences from 647 to 1140 bp and the other with sequences from 647 to 827 bp.

Maximum likelihood was used to infer divergence times of different lineages. Molecular clock was estimated using r8s version 1.70 (Sanderson 2003); only one or two individuals for each species were used (43

sequences total). The divergence date of 20 million years between the subfamilies Crocidurinae and Soricinae was used as reference for the calibration of the molecular clock, this date has been used in previous studies of molecular clock for Soricidae shrews as an estimate of the time of the split between Crocidurinae and Soricinae subfamilies as suggested by the fossil record (Reumer 1994; Fumagalli *et al*. 1999).

TABLE 3. Primers used for PCR amplification. Due to the antiquity of some museum samples, we used primers in the following combinations: L-14115-MVZ06, MVZ03-MVZ04, MVZ05-MVZ04, MVZ05-MVZ10, MVZ03-MVZ10, MVZ45-MVZ16, MVZ45-MVZ26, MVZ23-MVZ16, MVZ03-H-Sorex770, MVZ17-MVZ14, MVZ23-MVZ16, UNMF14-MVZ14, MVZ17-UNMR17.

Primer name	Primer Sequence 5' ---> 3'	Source
L-14115	GACATGAAAAATCATCGTTG	Endo *et al.* (2004)
H-*Sorex*770	TTGAGGGGATTAGCGGGTGT	J. Demboski (pers. comm.)
L-14764*Sorex*	GGMGTVCACCTMCTATTCCT	J. Demboski (pers. comm.)
H-15895(E)	TAGAATGTCAGCTTTGGGTGCT	Ohdachi *et al.* (2001)
UNMF14	GGMGGHGTMCTAGCYYTA GTC	Designed in this study
UNMR17	TATYASGCTDCGTTGTTTRGATGT	Designed in this study
MVZ03	GCTTCCATCCAACATCTCAGCATGATG	Smith & Patton (1993)
MVZ04	GCAGCCCCTCAGAATGATATTTGTCCTC	Smith & Patton (1993)
MVZ05	CGAAGCTTGATATGAAAAACCATCGTTG	Smith & Patton (1993)
MVZ06	GCTGTGTCTGATGTGTAGTGTAT	Smith & Patton (1993)
MVZ07	AACCCCATCTAACATTTCLTCYTGATG	Smith & Patton (1993)
MVZ10	TATGAGCCGTAGTARAKKCCTC	Smith & Patton (1993)
MVZ14	GGTCTTCATCTYHGGYTTACAAGAC	Smith & Patton (1993)
MVZ16	AAATAGGAARTATCAYTCTGGTTTRAT	Smith & Patton (1993)
MVZ17	ACCTCCTAGGAGAYCCAGAHAAYT	Smith & Patton (1993)
MVZ23	TACTCTTCCTCCACGAAACJGGNTC	Smith & Patton (1993)
MVZ26	AGATCTTTGATTGTGTAGTAGGGGT	Smith & Patton (1993)
MVZ45	ACJACHATAGCJACAGCATTCGTAGG	Smith & Patton (1993)

We assessed probabilities of the biogeographic origin of the clades through reconstruction of ancestral geographical origins with a maximum likelihood approach using Mesquite 2.0 software (Maddison & Maddison 2007). We followed the method used by Dubey *et al*. (2007) and coded the current geographical distribution of the species considering significant biogeographic barriers: (0) only Eurasia, (1) Eurasia and America, (2) North America north of the Trans Neovolcanic Belt of Mexico, (3) between Trans Neovolcanic Belt and the Tehuantepec Isthmus of Mexico and (4) south of the Tehuantepec Isthmus (Table 2). We used the Mk1 model of evolution (Lewis 2001) which assumes an equal rate of change between any two character states, and used the phylogenetic tree obtained from r8s.

Results

Mitochondrial DNA (mtDNA) sequences

Cytochrome *b* gene was sequenced from 19 Mexican samples belonging to 11 species of *Sorex*. Due to marginal preservation conditions for some samples, different size fragments were obtained. Skins from museum specimens were the most difficult samples to assay, however, 15 sequences are > 1000 base pairs (bp) long, and four are 644–939 bp. Available GenBank sequences also differed in length (800–1140 bp; Table 2).

Phylogenetic analysis

Fifty six models were tested by MODELTEST and GTR + I + G (-lnL = 11678.6426, A:0.3245, C: 0.3211, G:0.0970, T:0.2574, gamma = 1.4578) was the model chosen for Bayesian and maximum likelihood (ML) analysis (Figs. 1, 2). Bayesian and ML analyses showed very similar topologies, however, support values differed (Figs. 1, 2).

Bayesian analysis using short sequences of similar size (644 – 827 bp) produced a phylogenetic tree (not shown) similar to that generated by longer sequences size (644 – 1140 bp; Fig. 1). The former tree showed less structure, with polytomies and lower posterior probabilities possibly due to a reduction of phylogenetic signal, so we used the phylogenetic tree based on sequences ranging from 644 to 1140bp in length.

New World shrews of the genus *Sorex* are composed of two major clades, A and B (Fig. 1). Clade A consists of species of the *vagrans* group (Fig. 1-A.1; Hennings & Hoffmann 1977) and *S. hoyi*, the *cinereus* group (Fig. 1-A.2; van Zyll de Jong & Kirkland 1989), a few Mexican shrews and shrews from California and the Appalachian region of North America (Fig. 1-A.3).

Clade A.1 indicates that *S. monticolus* from Durango (northern Mexico; Figs. 1, 3), is sister taxon to *S. monticolus* (New Mexico)—*S. neomexicanus*. This population of *S. monticolus* from Durango had not been included in previous phylogenetic analyses. Moreover, *S. monticolus* from British Columbia groups with *S. bairdi*. In addition, the species of the *vagrans* group share a common ancestor with the clade *S. hoyi* Baird 1857, although this split is supported by a relatively low posterior probability value.

Clade A.2 shows that the Mexican shrews *S. milleri* and *S. emarginatus* (Fig. 3) are monophyletic, but nested within *S. cinereus* Kerr 1792 from Alaska and Minnesota. *Sorex longirostris* (from Tennessee and Virginia) is sister to this group. Other members of clade A.2 represent northern members of the Beringian clade of the *cinereus* group. These did not group with Mexican samples of this clade.

An unresolved group of basal species, A.3, shows *S. fumeus* and *S. tenellus* Merriam 1895 closely related to *S. oreopolus* and *S. ventralis*, which are sister species. These last two shrews are morphologically similar and are sympatric in the forests of the Trans Neovolcanic Belt of Central Mexico (Fig. 3). The samples of *S. veraecrucis* and *S. ixtlanensis* are from two mountain ranges of Oaxaca in southeastern Mexico (Sierra de Juárez in the north and Sierra Madre del Sur in the south), but group together (S1).

In contrast, the second major clade (B) includes only Mexican species of shrews and *S. trowbridgii* from California, which are sister to *S. saussurei* and *S. veraecrucis*. Samples of *S. saussurei* (S2) from mountain ranges in western (Sierra Madre Occidental) and samples of *S. veraecrucis* from eastern (Sierra Madre Oriental) and central (Trans Neovolcanic Belt) Mexico group together. Other samples of *S. veraecrucis* and *S. saussurei*, occurring in southeastern Mexico (Chiapas) and Guatemala beyond the Tehuantepec Isthmus, share a common ancestor (Fig. 3).

Another unresolved clade (Fig. 1-B.2) shows that *S. macrodon*, a highly endemic shrew of eastern (Veracruz) and southeastern (Oaxaca) Mexico is closely related to *S. veraepacis*. One of the samples of the *S. veraepacis* comes from a mountain range in Sierra Madre del Sur (Guerrero), southern Mexico, whereas the other sample (Guatemala) represents the southernmost distribution of the genus *Sorex* in the New World; these localities are east and west, respectively, of the Tehuantepec Isthmus (Fig. 3).

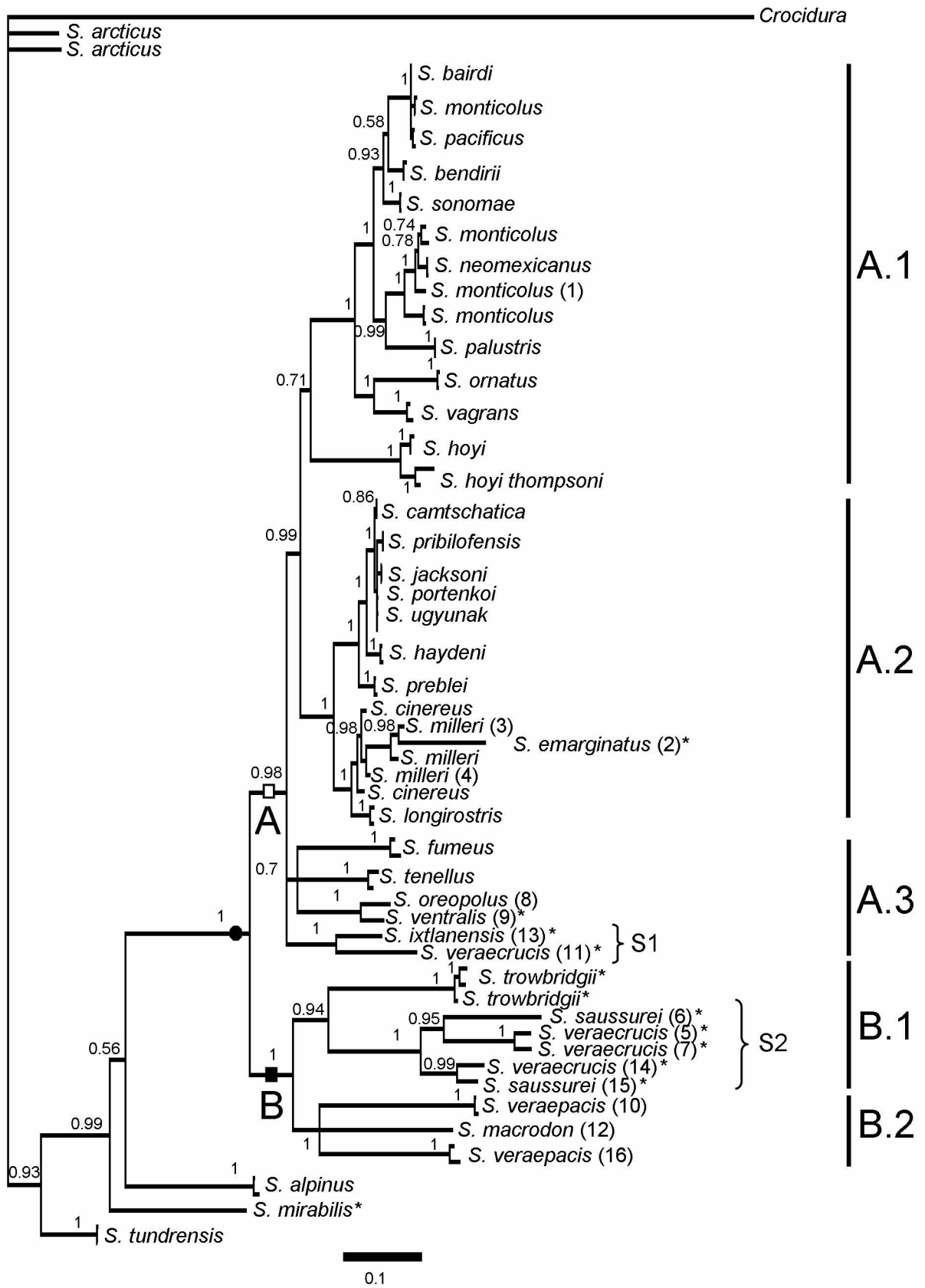

FIGURE 1. Phylogenetic relationships among long-tailed shrews of the genus *Sorex* from Mexico based on 77 mtDNA sequences of the cytochrome *b* gene. The tree represents the Bayesian analysis estimated through 6,000,000 generations. Numbers on branches indicate posterior probability values of nodal support. The numbers in parenthesis refer to the localities of samples from México (see Fig.3). Symbols point to the ancestral nodes of primary clades: A–B (●), A (□), B (■). ***** Not yet classified to either subgenera *Sorex* or *Otisorex* (see Table 1).

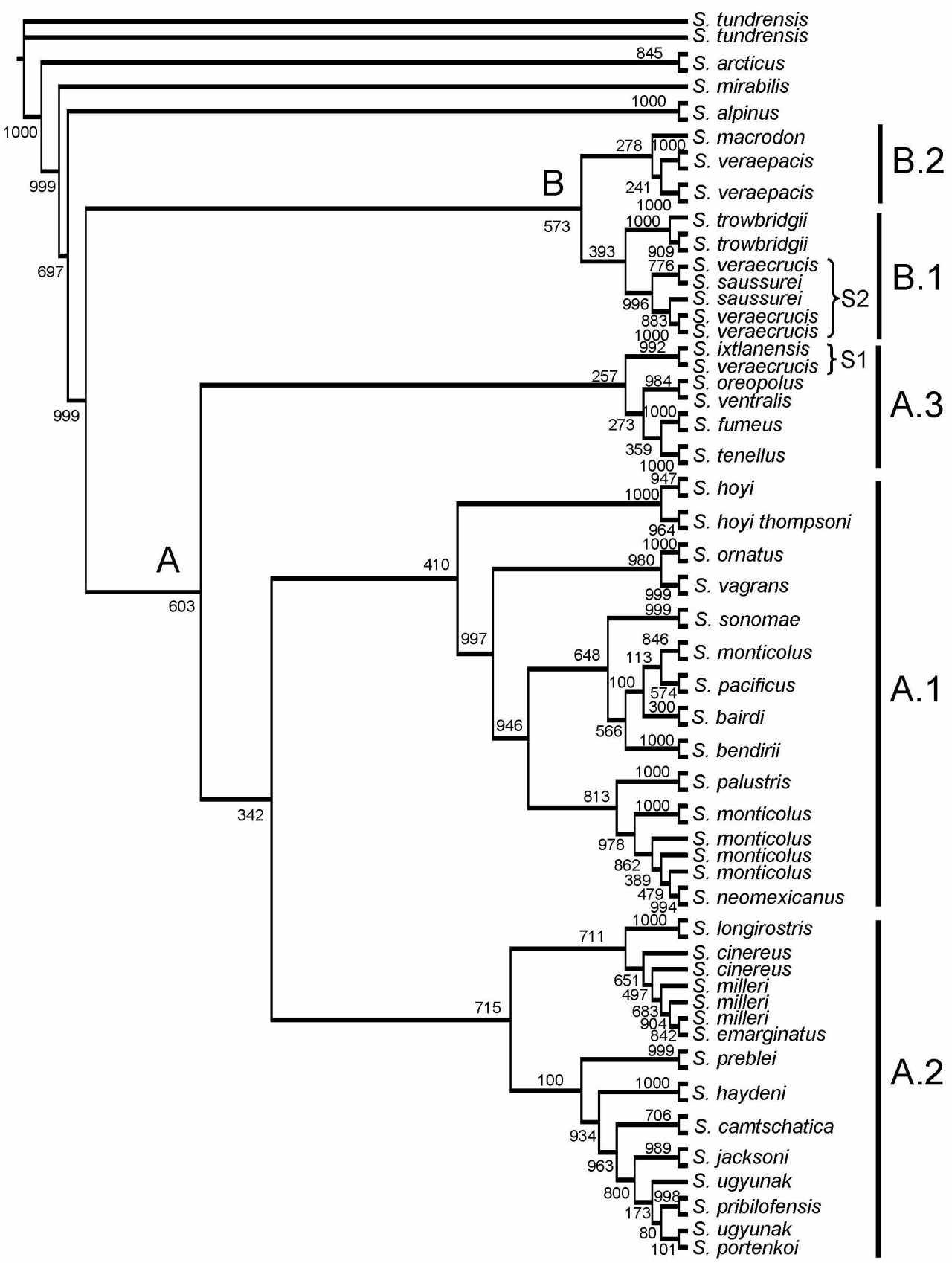

FIGURE 2. Phylogenetic relationships among long-tailed shrews of the genus *Sorex* from Mexico based on 77 mtDNA sequences of the cytochrome *b* gene. The tree represents the maximum likelihood analysis. Numbers indicate bootstrap probability values of nodal support. Bayesian method identified the same supported clades.

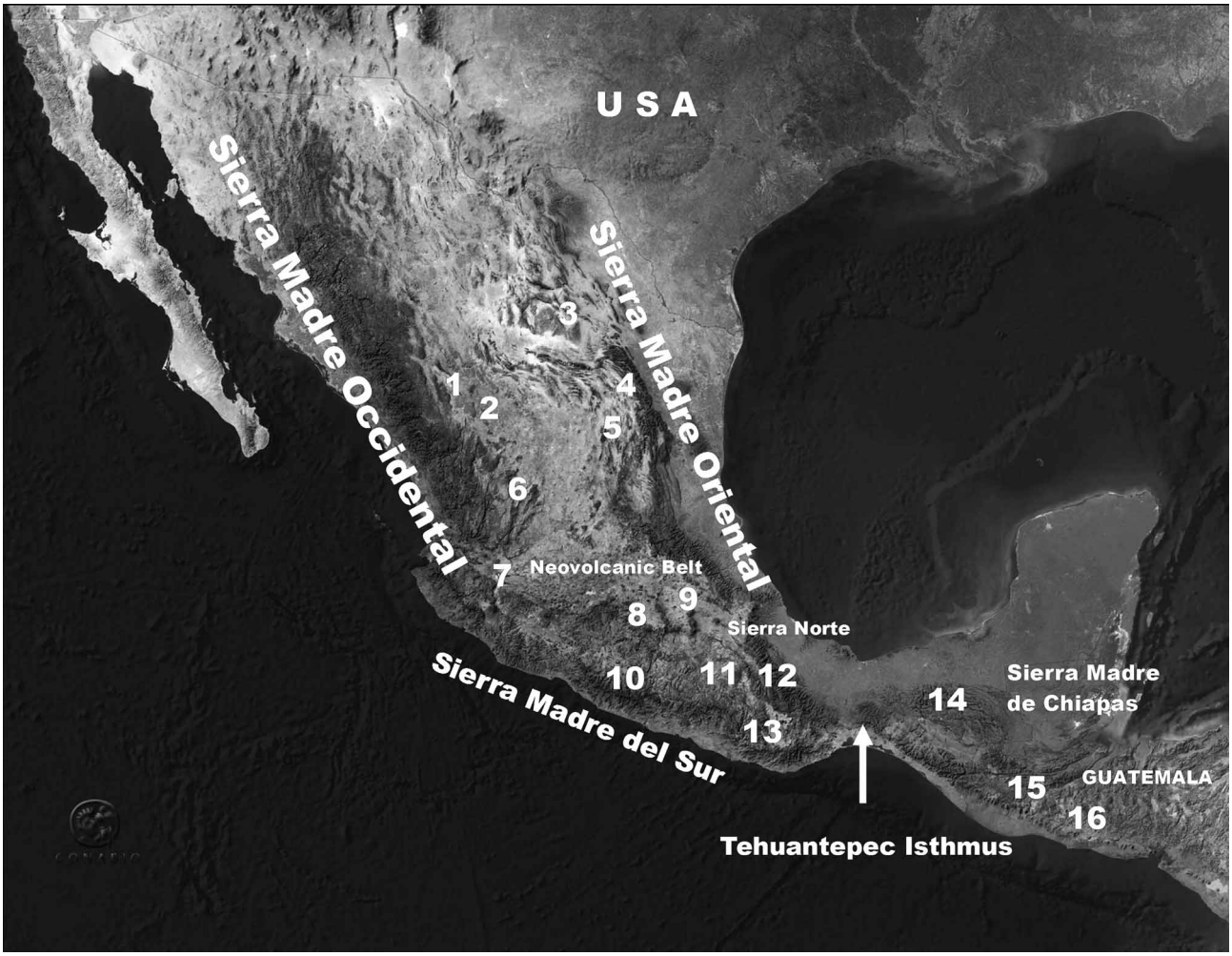

FIGURE 3. Major mountain ranges of México (Conabio 2003) and geographical location of the collecting localities for Mexican samples of long-tailed shrew species of the genus *Sorex*. Durango: 1= *S. monticolus*, 2= *S. emarginatus;* Coahuila: 3= *S. milleri*; Nuevo León: 4= *S. milleri*, 5= *S. veraecrucis*; Jalisco: 6= *S. saussurei*; Michoacán: 7= *S. veraecrucis*; Distrito Federal: 8= *Sorex oreopolus*; Puebla: 9 = *S. ventralis*; Guerrero: 10= *S. veraepacis*; Oaxaca: 11= *S. veraecrucis*, 12 = *S. macrodon*, 13= *S. ixtlanensis*; Chiapas: 14= *S. veraecrucis*; Guatemala: 15= *S. saussurei*; 16 = *S. veraepacis.*

Molecular clock and biogeographic analysis

Diversification of the *Otisorex* shrew taxa examined herein (i.e., divergence between major clades A and B; Fig. 4) was estimated to have begun 8.91 mya (Miocene) and ended 0.68 mya (Pleistocene). Separation of the *monticolus-vagrans* and *cinereus* clades (A), bifurcation between the populations from Sierra Norte and Sierra Madre del Sur of Oaxaca (S1), and divergence between *S. oreopolus* and *S. ventralis* (A.3) were dated in the Pliocene (1.6 mya). In contrast, the split between *S. cinereus* and *S. milleri* occurred more recently during the Pleistocene (680,000 years ago; - A.2).

Diversification of Clade B (Fig. 4) was estimated during the Miocene (5–10 mya). The split between *S. trowbridgii* and *S. saussurei-veraecrucis*, as well as the divergence between *S. macrodon* and *S. veraepacis* was during the late Miocene. Similarly, populations of *S. veraepacis* from Guerrero and Guatemala diverged about 5.81 mya. Finally, divergence between populations of this species from the Trans Neovolcanic Belt – Sierra Madre Oriental group and those from the Chiapas and Guatemala group was in the Pliocene (Fig. 4-B).

The ML assessment of the ancestral biogeographic origins indicates that *Otisorex* shrews have an American origin (Fig. 5). The probability that the ancestral node of the A-B clade has an American origin is

0.84, whereas the ancestral node of the A clade showed a probability of 0.93 of an American origin; a comparatively smaller value (0.82) suggests that the ancestral node of the B clade has the same origin.

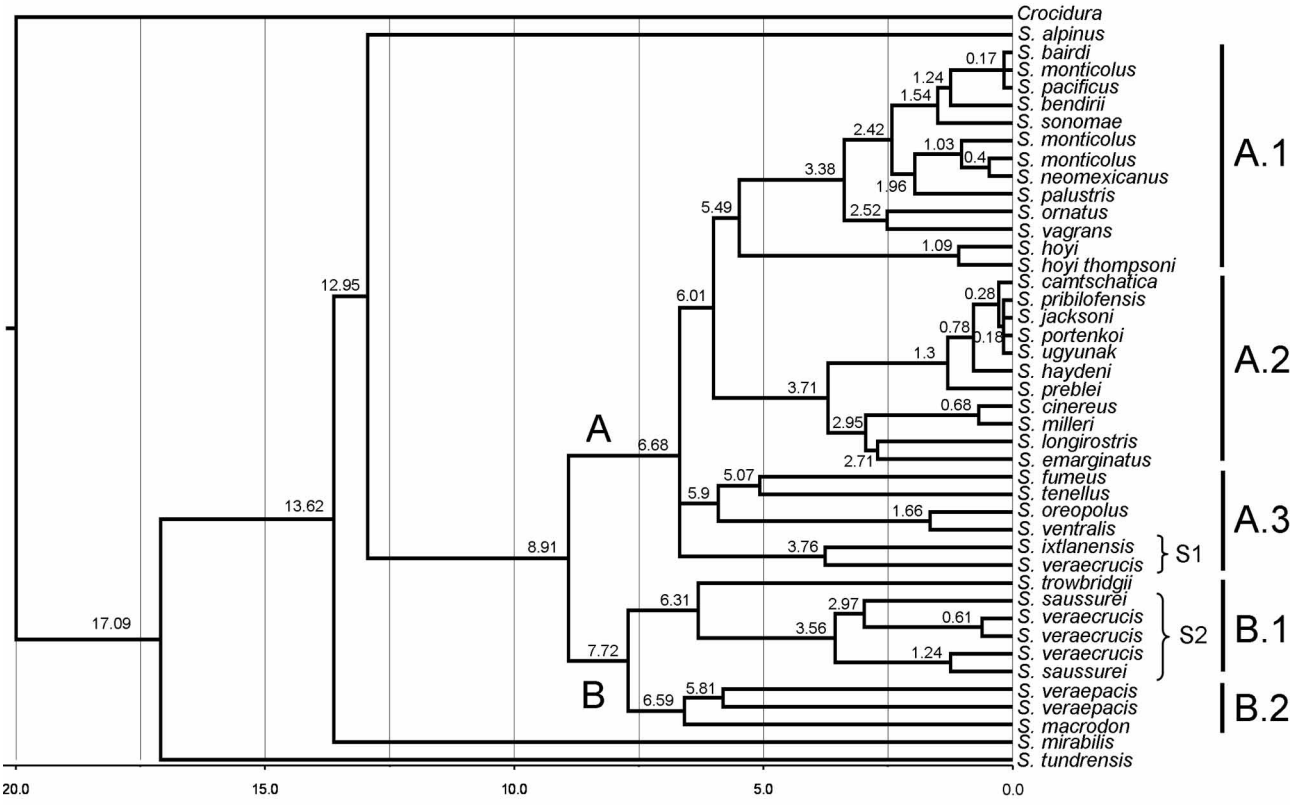

FIGURE 4. Chronogram tree with the divergence time estimated of speciation events derived from the r8s test (version 1.7; Sanderson 2003). Numbers indicate millions of years.

Discussion

Molecular systematics often provides key insight into species-level diversity, especially when applied to complex taxonomic groups that have otherwise proven problematic. Such is the case of southern North American shrews of the genus *Sorex;* a group that, based on this initial view of mitochondrial variation, exhibits substantial previously undetected diversity.

Origin and diversification of North American long-tailed shrews, *Sorex (Otisorex)*. The cytochrome *b* phylogenetic reconstruction presented herein is the most comprehensive molecular assessment of New World species of *Sorex* to date, even though a few North American species were not included. We identify a primary evolutionary split between most Palaearctic and Nearctic species of *Sorex* (Fig. 1). With the exception of *S. tundrensis* (found in northern Alaska and eastern Russia), all New World species we examined grouped in a clade that includes species in the subgenus *Otisorex.* This result is consistent with earlier molecular studies (Fumagalli *et al*. 1999; Ohdachi *et al.* 2006; Dubey *et al*. 2007; Shaffer & Stewart 2007). Also included in this clade are two Palaearctic endemics (*S. camtschatica* Yudin 1972, and *S. portenkoi* Stroganov 1956) of eastern Siberia. However, these two species are part of a previously identified Beringian clade within the *cinereus* complex that includes both Palaearctic and Nearctic species (Demboski & Cook, 2003). Relative to subgenus assignment, the previously enigmatic *S. trowbridgii* and *S. fumeus* (Findley 1955; George 1988; Ivanitskaya 1994) are part of the *Otisorex* clade. A rigorous test of whether the two nominal subgenera, *Otisorex* and *Sorex*, are valid will eventually require more complete worldwide sampling of all species in the genus *Sorex*. We identify two major clades within the North American *Otisorex* species (Fig. 1) that appear to be the result of an early split in North American soricine shrews. This division identifies clade A, which includes most North American (and Beringian) species of *Otisorex*, and clade B, which consists of *S. trowbridgii* (a Pacific

coastal species ranging from southern British Columbia to northern California) and three species found at the southern limits in North America of species of *Otisorex* (southern Mexico and Guatemala). Within *Otisorex*, the deep split into two clades has resulted in members of both clades reaching the southern latitudes of central Mexico likely via mountain ranges that included the Trans Neovolcanic Belt in Mexico. Only members of Clade B extend beyond the Isthmus of Tehuantepec, perhaps reflecting the influence of this important biogeographic barrier (Sullivan *et al*. 1997; Conroy *et al*. 2001).

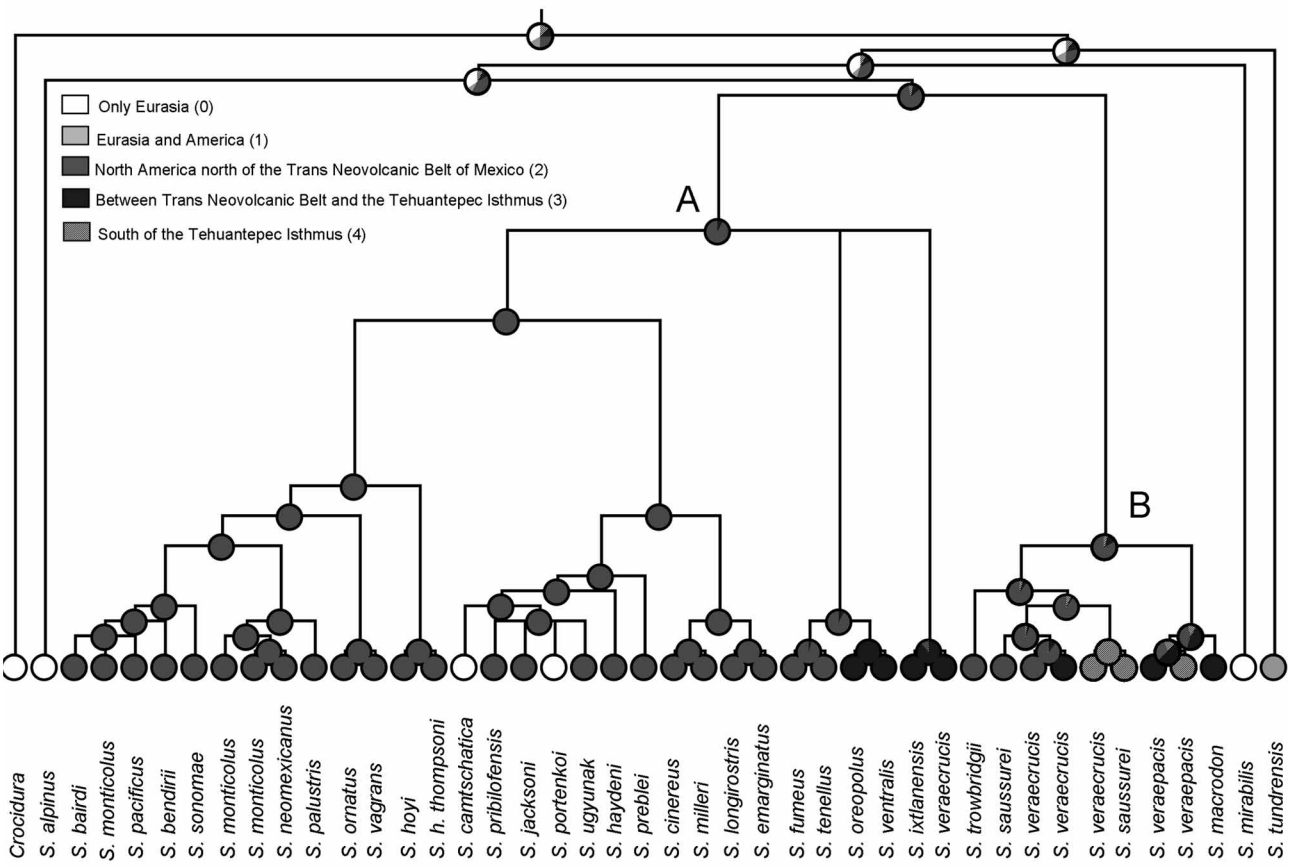

FIGURE 5. ML reconstruction of ancestral geographical origins using Mesquite 2.0 software (Maddison & Maddison 2007). The current geographical distribution of the species was coded: (0) only Eurasia, (1) Eurasia and America, (2) North America north of the Trans Neovolcanic Belt of Mexico, (3) between Trans Neovolcanic Belt and the Tehuantepec Isthmus of Mexico and (4) south of the Tehuantepec Isthmus. The pie charts represent the proportional likelihoods of each character state.

Phylogenetic relationships of long-tailed shrews (subgenus *Otisorex*) from México. The *vagrans* group (A.1). In this group, specimens of *S. monticolus* from Mexico (Fig. 1) are allied with the continental *monticolus* clade proposed by Demboski & Cook (2001). The results are consistent with previous work that showed *S. monticolus* encompasses two distinct clades, coastal and continental, that are paraphyletic with respect to several other species of *Sorex* (Demboski & Cook 2001). In this case, *S. bairdi*, *S. pacificus*, *S. bendirii*, and *S. sonomae* Jackson 1921 are more closely allied with the coastal clade (Demboski & Cook 2001) of *S. monticolus*, while *S. palustris* Richardson 1828 and *S. neomexicanus* are more closely allied to the continental clade of *S. monticolus*. Hence, *S. monticolus* likely constitutes at least two independent species.

The position of *S. hoyi* has been debated and some (e.g., Findley 1955) indicated that this species formed a third distinct radiation, independent of the *Otisorex* and *Sorex* clades. George (1988) suggested this species belonged in *Otisorex* and previous mtDNA studies have supported this classification (Fumagalli *et al*. 1999; Ohdachi *et al*. 2006). The close association of *S. hoyi* and *S. thompsoni* Baird 1858 (Fig. 1-A.1) has been noted previously (Long 1974; Diersing 1980; Stewart *et al.* 2003; Shafer & Stewart 2007). Hutterer (2005) considered these species to be synonymous, consistent with the suggestion that *S. thompsoni* is a subspecies of *S. hoyi* (Long 1974; Diersing 1980). Genetic distances among *S. hoyi* samples, including *S. hoyi thompsoni*

(between 2.5 and 3.8%) found herein are not high (Table 4), but these relatively low values are similar to genetic distances among a few other nominal species of *Sorex*, which may raise questions related to rates of morphological and molecular divergence (e.g., *S. sonomae* and northern continental *S. monticolus*; Demboski & Cook 2001). Our molecular data suggest that *S. hoyi* and *S. thompsoni* may be conspecific. With regard to *S. hoyi*, the allozyme study of George (1988) identified a clade of *S. hoyi* and *S. vagrans* Baird 1857. In contrast, our mtDNA study (Fig. 1) places *S. hoyi* as sister taxon to the other species in the A.1 group, although this position is weakly supported.

Clade A.2 supported the relationship between the Russian species, *S. camtschatica* and *S. portenkoi*, and other North American members of the *cinereus* group (*S. ugyunak* Anderson and Rand 1945, *S. pribilofensis* Merriam 1895, *S. jacksoni* Hall and Gilmore 1932, *S. haydeni* Baird 1857 and *S. preblei*; Demboski & Cook 2003; Hutterer 2005). The majority of these species are found at high latitudes with the exception of *S. haydeni* and *S. preblei*. This topology is congruent with the molecular study of Demboski & Cook (2003).

Our study showed that *S. cinereus* is most closely related to *S. milleri* and *S. emarginatus.* In the *cinereus* complex (clade A.2), *S. milleri* has been considered a relict species derived from *S. cinereus* (van Zyll de Jong & Kirkland 1989). *Sorex cinereus* has boreal affinities whereas the southerly distribution of *S. milleri* may indicate a tolerance of warmer climatic conditions (Hall 1981; Villa & Cervantes 2003; Hutterer 2005). Although these shrew populations are now separated by considerable geographical distance (ca. 2200 km), the genetic distance computed herein between these taxa is low (2%). The low genetic distance may reflect a much wider range for *S. cinereus* in southern North America when Pleistocene climatic conditions were cooler and wetter. Subsequent climatic shifts may have isolated *S. milleri* in northern Mexico (Kurtén & Anderson 1980; Toledo 1982; Luna & Alcántara 2001).

Sorex emarginatus (clade A.2) is an endemic species from northern Mexico with a close relationship to *S. milleri* and *S. cinereus* (Hall 1981; Villa & Cervantes 2003; Hutterer 2005). This first view of the phylogenetic position of *S. emarginatus* shows that this shrew is highly differentiated relative to other members of this clade and, more generally, to other species of *Sorex*. The genetic distance between *S. milleri* and *S. emarginatus* averages 6.7% (Table 4). The northern representative of this clade, *S. cinereus*, occurs widely across Canada and the United States, while the southern representative, *S. milleri*, is found in northern Mexico. *Sorex emarginatus* (southeastern United States) appears to have diverged from the *S. milleri-S. cinereus* group as early as the Pliocene (Fig. 4), but additional samples of *S. emarginatus* and multiple loci should be examined to more rigorously assess this point.

Though still a part of clade A, there are six species of *Sorex* that together are sister taxa to the A.1-A.2 groups of shrews (Fig.1-A.3). The phylogenetic positions of these six species are not well defined, although four are in a weakly supported clade that includes *S. fumeus* and *S. tenellus* as sister to a well supported subclade of *S. oreopolus* and *S. ventralis*. *Sorex oreopolus* and *S. ventralis* are morphologically distinct species (Villa & Cervantes 2003; Carraway 2007), that are sympatric in the Trans Neovolcanic Belt. The mtDNA sequences reveal that they are about 4% different (Table 4). Also in this group (S1) are *S .ixtlanensis* and a few samples of *S. veraecrucis. Sorex veraecrucis* is polyphyletic in this mtDNA analysis and clearly in need of further taxonomic study. *Sorex ixtlanensis* is endemic to Guerrero and the mountain ranges in northern and southern Oaxaca. The sample examined here came from the south of Oaxaca where *S. veraepacis* and *S. veraecrucis* also occur. These three taxa are morphologically similar to each other (Carraway 2007), however, *S. ixtlanensis* is genetically distinct (7.8% ; Table 4) from its sister species, *S. veraecrucis* from northern Oaxaca.

The second major group (Clade B) of the subgenus *Otisorex*, includes species that had not previously been assigned to a subgenus. Clade B includes *S. trowbridgii*, samples of *S. saussurei* from Jalisco and Guatemala, sequences of *S. veraecrucis* from Nuevo Leon, Michoacan, Chiapas, *S. macrodon* from Oaxaca, and *S. veraepacis* from Guerrero and Guatemala. Significantly, this clade unites the enigmatic *S. trowbridgii* with a suite of southern shrews, thus revealing that the historical biogeography of *Otisorex* shrews in North America is complex, with multiple episodes of southern colonization of Mexico.

This mtDNA analysis places *S. trowbridgii* and *S. veraecrucis* and the southern branch of the paraphyletic *S. saussurei* (Clade S2) as sister taxa. This is the oldest clade and displays a wide geographical distribution.

Sorex trowbridgii occurs farther north along the coast, valleys and slopes of coastal mountains from California to southern British Columbia, *S. veraecrucis* is distributed from northern Mexico down to Guatemala, whereas *S. saussurei* is endemic to central Mexico (Hall 1981; Wilson & Ruff 1999). Previous studies placed *S. trowbridgii* in various nodes throughout the phylogenetic tree of the North American species ranging from sister taxon to an all-inclusive clade of both the subgenera *Otisorex* and *Sorex* (George 1988), to sister taxon to all species of only *Otisorex* (Fumagalli *et al*. 1999), to sister taxon to *S. saussurei* (Ohdachi *et al*. 2006). Inclusion of Mexican specimens has helped to resolve this long standing taxonomic question.

The polyphyletic nature of *S. veraecrucis* suggests further sampling and analyses are needed. In addition to the placement of an Oaxacan sample of *S. veraecrucis* in clade A, three populations of *S. veraecrucis* (S2) in clade B are deeply divergent from each other. Specimens of *S. veraecrucis* from Nuevo Leon and Michoacan form a clade and are distinct from the Jalisco population of *S. saussurei*. In turn, this clade is distinctive from populations of *S. veraecrucis* from Chiapas and *S. saussurei* from Guatemala. This southern clade may reflect isolation produced by the formation of the Isthmus of Tehuantepec as a geographical barrier. Hence, there is considerable geographic structure in this single nominal species with representatives from Mexico through the Sierra Madre Oriental, Sierra Madre Occidental, in central Mexico in the Transversal Neovolcanic Belt, and then as far south as Guatemala.

Sorex veraepacis, as currently delimited, also shows considerable variability and is included in the second subclade within Clade B (Fig. 1). This species is morphologically similar to *S. veraecrucis* and *S. ixtlanensis* (from north and south of Oaxaca), however, molecular data suggest comparatively deep divergence among them (up to 15%; Table 4). Similarly *S. veraepacis* has a disjunct distribution with populations found to the west and east of the Isthmus of Tehuantepec that showed a considerable genetic distance of 15% (Table 4). *Sorex macrodon* is a poorly known shrew that occurs in Veracruz and Puebla, Mexico (Hall 1981; Villa & Cervantes 2003). This first perspective on its evolutionary affinities suggests that it is distantly related to *S. veraepacis* (Table 4).

Further research is needed to elucidate the molecular relationships and species limits of *S. saussurei* and *S. veraecrucis*. Morphological evidence indicates that *S. saussurei* likely represents multiple species (Carraway 2007). In addition, our molecular study showed deep intraspecific genetic differences among the samples of *S. veraecrucis* from Sierra Madre Oriental, Transversal Neovolcanic Belt and Oaxaca.

Biogeography and molecular clock. Diversification among North American long-tailed shrews has been attributed to vicariance events associated with environmental changes (George 1988; Demboski & Cook 2003; O'Neill *et al.* 2005). Our analysis is tentative as it is based on a single locus, but these preliminary data indicate that initial events related to the origin of *Sorex* shrews occurred during the Miocene, with the emergence of a large number of species in the Pliocene and then further diversification in the climatically variable Pleistocene (Clades A & B). This conclusion is largely in line with what other researchers have hypothesized (Findley 1955; George 1988; Churchfield 1990; Harris 1998). The split between the two major clades of *Otisorex* took place in North America during the Miocene, when most of the mountain ranges of the United States, Mexico and Guatemala were formed (Maldonado-Koerdell 1964; Halffter 1987; Ferrusquía-Villafranca 1993; González-Medrano 1998; Campbell 1999; Centeno-García 2004); our data support this scenario (Fig. 4-A.1 & A.2). The structured topology within and among species of *Otisorex* (Fig. 4) likely reflects the influence of biogeographic barriers that appear to be most critical in species with potentially low dispersal capability (Avise 2000; Hewitt 2004), but critical testing of individual species responses will require much more detailed geographic sampling and examination of independent genes.

The distribution of *S. monticolus* is wide and two primary clades have been identified. Demboski and Cook (2003) hypothesized that the northern and southern continental populations form a clade to the exclusion of Pacific coastal populations. Our results showed that Mexican populations of *S. monticolus* are more closely related to the southern continental group, than they are to the coastal forms. Southern continental populations of *S. monticolus* are located in the Rocky Mountains, thus our work extends the distribution of members of this clade southward to the Sierra Madre Occidental. Also in this major clade are the central Mexican species, *S. oreopolus* and *S. ventralis*, which are estimated to have diverged in the Miocene. These divergent taxa provide the first evidence of an early colonization of this phylogenetic lineage south to at least Oaxaca.

TABLE 4. Percent divergence in cytochrome *b* gene sequences among long-tailed shrews (genus *Sorex*) from Mexico and Guatemala.

	Sorex monticolus (Durango)	*Sorex milleri*	*Sorex emarginatus*	*Sorex oreopolus*	*Sorex ventralis*	*Sorex ixtlanensis* (Oaxaca south)	*Sorex veraecrucis* (Oaxaca north)	*Sorex saussurei* (Jalisco)	*Sorex veraecrucis* (Nuevo León)	*Sorex veraecrucis* (Michoacán)	*Sorex veraecrucis* (Chiapas)	*Sorex saussurei* (Guatemala)	*Sorex macrodon*	*Sorex veraepacis* (Guerrero)	*Sorex veraepacis* (Guatemala)
Sorex monticolus (Durango)	-------														
Sorex milleri	11.0–12.2	-------													
Sorex emarginatus	13.9	4.8–8.4	--------												
Sorex oreopolus	11.5	9.4–9.8	10.7	--------											
Sorex ventralis	12.0	6.9–9.7	11.3	4.1	------										
Sorex ixtlanensis (Oaxaca south)	11.3	10.6– 11.7	12.2	9.9	11.7	--------									
Sorex veraecrucis (Oaxaca north)	13.2	11.2–12.0	13.4	11.7	12.8	7.8	--------								
Sorex saussurei (Jalisco)	15.2	14.6–15.5	11.0	13.3	15.1	13.6	14.3	--------							
Sorex veraecrucis (Nuevo León)	14.9	15.1– 17.2	15.6	14.0	15.5	14.2	15.6	10.7	--------						
Sorex veraecrucis (Michoacán)	15.9	15.5–17.2	16.8	14.2	15.9	14.4	16.2	10.9	2.9	--------					
Sorex veraecrucis (Chiapas)	14.1	15.4–17.3	14.9	13.5	14.8	15.3	16.0	10.7	9.6	9.7	--------				
Sorex saussurei (Guatemala)	14.0	15.6–17.5	14.8	13.2	15.0	13.6	14.6	10.1	9.5	9.7	4.0	--------			
Sorex macrodon	15.0	9.0–11.3	10.3	10.9	12.7	12.7	14.6	14.7	15.1	15.4	14.8	14.0	--------		
Sorex veraepacis (Guerrero)	15.0	13.0–13.8	14.5–14.9	15.1	15.5–15.6	15.3–15.4	15.1 – 15.3	15.9	14.9–15.0	15.0–15.2	14.3–14.5	14.6	14.0	--------	
Sorex veraepacis (Guatemala)	15.7	14.1–15.7	18.1–18.6	14.0–14.6	15.3	14.0–14.2	15.1–15.4	15.5–15.9	13.1–13.2	13.0–13.2	14.1–14.2	12.6–12.7	13.0–13.3	14.5–14.7	

The second major clade (B) of Mexican and Guatemalan shrews in the subgenus *Otisorex* includes *S. veraepacis* and *S. saussurei,* which had not been previously classified (Fig. 1). In both of these species, the Isthmus of Tehuantepec, may have played a role as a biogeographic barrier in the evolutionary divergence of each of these neotropical shrew taxa. The southernmost distribution of the long-tailed shrews of the genus *Sorex* in the New World is *S. veraepacis* of the highlands of Guatemala (Matson 2008). These species, *S. macrodon,* and *S. trowbridgii* form clade B (Fig. 4) and this arrangement reinforces the idea that the Mexican/ Guatemalan shrews belong to one of the oldest evolutionary lineages of Soricidae in North America (7.72 mya). Our data suggest two distinct arrivals of independent lineages of *Sorex* to southern Mexico that differ by at least one million years. The first wave (7.72 mya) reached the mountain region of the highlands of Guatemala and gave rise to species identified as *S. veraepacis* (Guerrero and Guatemala), *S. macrodon* (Oaxaca), *S. veraecrucis* (Nuevo Leon, Michoacan, Chiapas), *S. saussurei* (Jalisco and Guatemala), and *S. trowbridgii* (United States). The second migration event (6.68 mya) colonized areas only as far south as southwestern Mexico and this lineage eventually differentiated into the *vagrans* group, *cinereus* group, *S. fumeus, S. tenellus, S. oreopolus, S. ventralis* and *S. veraecrucis* and *S. ixtlanensis.*

In summary, this paper establishes an initial view of diversification in Mexican and Guatemalan shrews and provides an opportunity to further test and refine our understanding of their evolution. Expanded research initiatives that are focused on these southern species of long-tailed shrews are necessary to understand the pattern and tempo of diversification at the southern limit of their distribution in the New World.

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