

First record of shrews (Eulipotyphla, Soricidae) in the Sierra de Otontepec, an isolated mountain in Veracruz, Mexico

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Knowledge of the diversity and distribution of mammals in tropical and subtropical regions remains incomplete. This is especially true for many small species inhabiting remote areas with limited access. Here we present the first record of small-eared shrews (Soricidae, *Cryptotis*) from the Sierra de Otontepec, Mexico, an isolated mountain in the Gulf Coastal Plain that has remained poorly explored (Figure 1). We conducted a short-term survey in the Sierra de Otontepec using pitfall and Sherman traps during August 2016. Taxonomic identification was based on morphological and molecular analyses (Appendix 1). We collected six shrew specimens from the cloud forests in the Sierra de Otontepec (Figure 2). Morphological and molecular characterization indicated that all specimens collected were small-eared shrews belonging to the *Cryptotis mexicanus* species group (Table 1, Figure 3), a clade which is closely associated with cloud forests. Based on the morphological and molecular evidence, we refer to this population from the Sierra de Otontepec as *C. mexicanus*. Our results corroborate the importance of continued fieldwork in remote tropical areas. Overall, this finding offers relevant information that could be used to better understand the evolution and biogeographic history of this species group and its habitat.

El conocimiento de la diversidad y distribución de los mamíferos en las regiones tropicales y subtropicales sigue siendo incompleto. Esto es particularmente notable para muchas especies pequeñas que habitan en áreas remotas de acceso limitado. Aquí presentamos el primer registro de musarañas (Eulipotyphla, Soricidae) de la Sierra de Otontepec, México, una montaña aislada en la llanura costera del Golfo que ha permanecido escasamente explorada (Figura 1). Nosotros realizamos un estudio a corto plazo en la Sierra de Otontepec utilizando trampas de caída y Sherman durante agosto de 2016. La identificación taxonómica se basó en análisis morfológicos y moleculares (Apéndice 1). Obtuvimos seis especímenes de musarañas en el bosque nuboso de la Sierra de Otontepec (Figura 2). La caracterización morfológica y molecular indicó que todos los especímenes recolectados eran musarañas de orejas pequeñas pertenecientes al grupo de especies de *Cryptotis mexicanus* (Tabla 1, Figura 3), un clado que está estrechamente asociado con los bosques nubosos. Con base en la evidencia morfológica y molecular, nos referimos a esta población de la Sierra de Otontepec como *C. mexicanus*. Nuestros resultados corroboran la importancia de continuar trabajo de campo en áreas tropicales remotas. En general, este hallazgo ofrece información relevante que podría usarse para comprender mejor la evolución y la historia biogeográfica de este grupo de especies y su hábitat.

Key words: cloud forests; *Cryptotis*; Mammalia; small-eared shrews.

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Introduction

Knowledge of the diversity and geographical distribution of mammals in tropical and subtropical regions remains incomplete (Ceballos and Ehrlich 2009). This is due, in large part, to the fact that many species inhabit remote areas with limited accessibility. This is particularly problematic when collecting small and secretive species that are difficult to find. Small-eared shrews (Eulipotyphla, Soricidae) of the genus *Cryptotis* Pomel, 1848 comprise a group of small- to medium sized species occurring from southeastern Canada to northern South America (Choate 1970). With about 44 species (Zeballos et al. 2013), *Cryptotis* is one of the most diverse genera of small mammals in the Americas. Discovery and description of new species, new distributional records, and range extensions for this genus are not unusual even today (Woodman 2010; Guevara et al. 2014; Guevara and Sánchez-Cordero 2018a).

Recently, while working on mammal inventories, our field team discovered a handful of specimens of small-

eared shrews in the cloud forest of the Sierra de Otontepec (Figure 1), an isolated mountain in Veracruz, Mexico, where biological inventories and research has been scarce (Hall and Dalquest 1963; Secretaría de Desarrollo Social y Medio Ambiente, SEDESMA, 2007). Here we provide morphological, molecular, and ecological information on these specimens that could be used to better understand the evolutionary and biogeographic history of the genus. Because the fauna in the Sierra de Otontepec is poorly known and most of the natural vegetation in the region has already been altered by human activity, we emphasize the biological relevance of this discovery.

Materials and Methods

Study area. The Sierra de Otontepec (also known as Sierra de Tantima) lies on the eastern slope of the Sierra Madre Oriental, Veracruz, Mexico (Figure 1). It is a small, isolated volcanic mountain in the Gulf Coastal Plain that reaches an elevation of nearly 1,300 m, and with an extended range of nearly 20

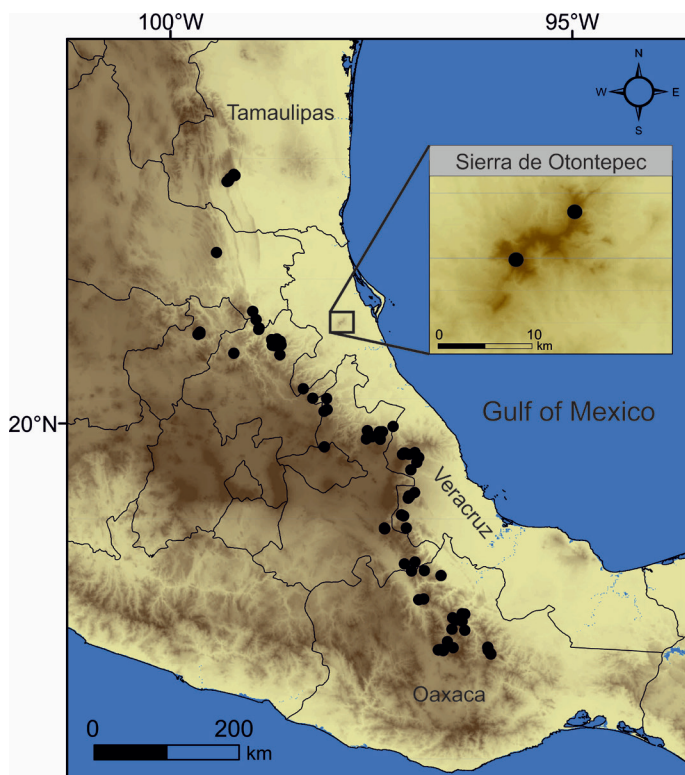


Figure 1. Map showing the known records (black dots) of the small-eared shrew, *Cryptotis mexicanus*, and the new records from the Sierra de Otontepec.

km (SEDESMA 2007). The summit vegetation begins at 900 masl and consists largely of cloud forest, making it one of the northernmost stands of cloud forest in the Neotropics.

Fieldwork. The Mexican Ministry of the Environment (SEMARNAT) authorized the capture of mammals under the scientific collector permit SGPA/DGVS/12142/16 issued to LLP. Fieldwork was carried out for 15 consecutive days in August 2016. We used 10 pitfall and 80 Sherman traps, which were placed next to rocks around the bases of the trees and next to fallen trunks. We conducted trapping at two locations covered by dense cloud forest (Site 1: 21.26855278 N, -97.84444 W, 1,080 m; Site 2: 21.24715 N, -97.895 W, 1,020 m), which have not been intensively surveyed for small mammals. We followed standard recommendations on specimen capture, sacrifice and preparation (Sikes et al. 2016). We measured (mm) and weighed (g) voucher specimens in the field and deposited skins, skeletons, and tissues samples at the Mammal Collection of the Museo de Zoología “Alfonso L. Herrera”, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC-M, Mexico City; Appendix 1).

Taxonomic identification. We focused morphological and molecular analyses on the comparison with members of *C. mexicanus* species group (*C. magnus*, *C. nelsoni*, *C. mexicanus*, and *C. phillipsii*) because of their greater similarity in pelage coloration, body size, size of forefeet and claws to the specimens from Sierra de Otontepec. We recorded the following cranial measurements for quantitative comparisons: condylobasal length (CBL), cranial breadth (CB), breadth of the palate across the second molars (M2B), and

breadth across the first unicuspid (U1B). All measurements were taken with a Mitutoyo electronic caliper at 0.01 mm precision under a stereomicroscope (Appendix 1). We performed a principal component analysis (PCA) on the matrix correlation of four cranial variables to describe and evaluate the degree of differentiation among the species of the *Cryptotis mexicanus* group.

We also conducted a Bayesian Inference to determine the genetic affinity of the specimens from Sierra de Otontepec within the *Cryptotis mexicanus* group. We amplified the mitochondrial gene *cytochrome b* (*cytb* = 1,140 bp) by a polymerase chain reaction (PCR) using the pair of primers MVZ05 and H15915. The PCR profile included 3 min of initial denaturation at 95° C, followed by 35 cycles of 30 s of denaturation at 95° C, 1 min of annealing at 49° C, and 2 min for extension at 72° C. Finally, we included a step of 5 min of final extension at 72° C. We used the Montage PCR purification kit from Millipore, and the ABI PRISM BigDye Terminator cycle sequencing kit with AmpliTaq DNA polymerase for sequencing the PCR products in an ABI 3730XL sequencer (Applied Biosystems, Seoul, Korea). Both, cleaning of PCR products and sequencing reaction was performed in Macrogen Inc. (Seoul, Korea) following the standard protocols. The sequences generated were deposited in the GenBank database (Accession #'s MK286564-MK286565). We included 21 *cytb* sequences from the *C. mexicanus* species group and one sequence of *C. parvus* (used as outgroup) housed in the GenBank database (see accession numbers



Figure 2. A) Specimen of a small-eared shrew collected in Sierra de Otontepec, Mexico. B) Typical cloud forest habitat of the Sierra de Otontepec, where small-eared shrews were collected.

on Figure 4, downloaded on 07 November, 2018). Using PartitionFinder 2 (Lanfear et al. 2017), we selected the best scheme of partition and evolution model ($cytb_{[1]} = \text{SYM}+\Gamma$; $cytb_{[2]} = \text{F81}+\Gamma$; $cytb_{[3]} = \text{GTR}+\Gamma$). We then incorporated this partition and evolution model in a phylogenetic analysis using Bayesian inference (MrBayes 3.2.3; Ronquist et al. 2012). We used three hot and one cold chains in two independent runs of 10 million generations, sampling data every 1,000 iterations. The final topology was obtained using a majority tree consensus and considering a burn-in of 25 %. We checked the convergence of our results and a good sampling (ESS > 200) in Tracer 1.6.

Results

We obtained a total of six shrew specimens in the cloud forests from the Sierra de Otontepec; four were collected with pitfall traps and two with Sherman traps. The sample included three adult males (MZFC-M 16221, 16223-16224) and three adult females (MZFC-M 16219-16220, 16222), one of which was pregnant with five embryos (MZFC-M 16222). Other genera of mammals collected in this short-term survey were *Handleyomys* (rice rat), *Sigmodon* (cotton rat), and *Marmosa* (mouse opossum). The shrew specimens displayed previously reported external diagnostic characters of the *Cryptotis mexicanus* species group (Choate 1970; Guevara and Sánchez-Cordero 2018b). Principal components 1 and 2 explained 95% of the variation in the data (PC 1 = 90 % and PC 2 = 5 %). The breadth of the palate across the second molars (M2B) and condylobasal length (CBL) contributed strongly to the first factor axis, providing a measure of overall cranial size. A plot of the two PCs showed that the specimens from Sierra de Otontepec tended to plot among the smaller species within the *C. mexicanus* species group (*C. phillipsii* and *C. mexicanus*) and overlapped partly with previously known specimens of *C. mexicanus* (Table 1; Figure 3). Bayesian Inference indicated that specimens from the Sierra de Otontepec are nested within *C. mexicanus*, which is supported by a high posterior probability (Figure 4). The rest of the relationships within the *C. mexicanus* species group resemble previous phylogenetic hypothesis (Guevara and Cervantes 2014; He et al. 2015).

Discussion and conclusions

Morphological characterization indicates that the specimens collected in Sierra de Otontepec belong to the *Cryptotis mexicanus* species group, a clade comprising four species highly associated with cloud forests (*C. magnus*, *C.*

Table 1. Loadings of four cranial measurements on the first two axes (PCs 1 and 2) of a principal component analysis of small-eared shrews (*Cryptotis*) from Mexican cloud forests.

	PC 1	PC 2
CBL	-0.955133	0.180397
CB	-0.941390	0.231200
M2B	-0.964130	-0.088268
U1B	-0.933362	-0.326616

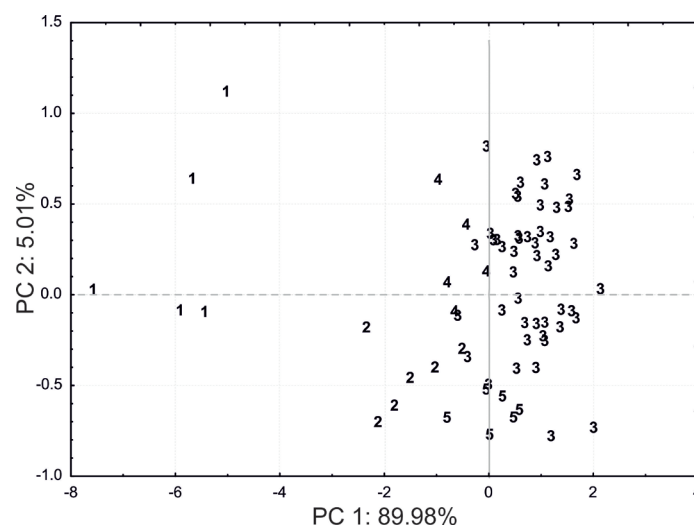


Figure 3. Plot of the first 2 principal components from a principal component analysis (PCA) of four cranial measurements from *C. magnus* (1), *C. nelsoni* (2), *C. mexicanus* (3), *C. phillipsii* (4), and the specimens from Sierra de Otontepec (5).

mexicanus, *C. nelsoni*, and *C. phillipsii*; González-Ruiz et al. 2014; Guevara and Sánchez-Cordero 2018b). The fact that specimens from Sierra de Otontepec are morphological similar and are phylogenetically nested within *C. mexicanus* implies that there is no justification for the recognition of a new species for this new population. Therefore, we refer to the population from the Sierra de Otontepec as *C. mexicanus*. Interestingly, this first record of small-eared shrews in the Sierra de Otontepec is consistent with the potential distribution of the *C. mexicanus* group based on models of its climatic requirements using correlative techniques (Guevara and Sánchez-Cordero 2018b).

Our discovery corroborates the importance of continued surveys in remote tropical areas (Guevara et al. 2014). Very little is known about the biota and biogeographic history of the Sierra de Otontepec (SEDESMA 2007), which has gone largely unnoticed in the study of cloud forest vegetation. Hence, this discovery of small-eared shrews could also be used to better understand the origin and history

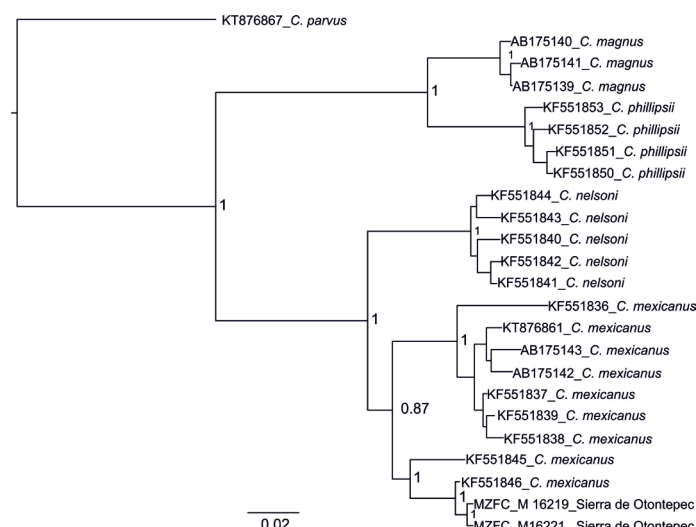


Figure 4. Majority-rule consensus tree from a Bayesian Inference showing the position of the specimens from Otontepec within the *Cryptotis mexicanus* species group. The numbers on clades indicate the posterior probability for each clade. The accession numbers of GenBank precede the scientific names.

of one of the northernmost islands of cloud forest in the Neotropical region. Finally, we highlight the relevance of montane cloud forest fragments like the Sierra de Otontepec because they are important reservoirs of endemic and endangered species that have a critical role in maintaining landscape-level biodiversity in an ecosystem with rapid rates of clearing and global conservation priority (Wilson and Rhemtulla 2018).

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Appendix 1.

Specimens examined and measurements (CBL-U1B) used for the morphological comparison (see methods for details). Specimens examined are housed in the following collections (followed by their abbreviations): National Collection of Mammals, Mexico City (CNMA); The University of Kansas Natural History Museum, Lawrence, Kansas (KU); Museum of Zoology 'Alfonso L. Herrera', Mexico City (MZFC); Collection of Mammals, Universidad Autónoma Metropolitana, Iztapalapa, Mexico City (UAMI); and National Museum of Natural History, Washington, District of Columbia (USNM).

SPECIES	MUSEUM	CATALOGUE NUMBER	CBL	CB	M2B	U1B							
<i>Cryptotis mexicanus</i>	MZFC	637	18.5	9.7	5.2	2.5	<i>Cryptotis mexicanus</i>	USNM	68525	18.4	10.0	5.2	2.4
<i>Cryptotis mexicanus</i>	MZFC	638	18.9	9.6	5.2	2.5	<i>Cryptotis mexicanus</i>	USNM	68523	18.4	9.8	5.3	2.3
<i>Cryptotis mexicanus</i>	MZFC	639	19.1	10.2	5.3	2.5	<i>Cryptotis mexicanus</i>	USNM	68526	18.4	10.2	5.1	2.4
<i>Cryptotis mexicanus</i>	MZFC	640	18.5	9.4	5.3	2.5	<i>Cryptotis mexicanus</i>	USNM	68532	18.5	10.0	5.2	2.4
<i>Cryptotis mexicanus</i>	KU	29548	18.4	10.0	5.6	2.6	<i>Cryptotis mexicanus</i>	USNM	68528	18.7	9.9	5.3	2.3
<i>Cryptotis mexicanus</i>	KU	29561	18.4	10.0	5.3	2.5	<i>Cryptotis mexicanus</i>	UAMI	11169	19.2	10.1	5.4	2.7
<i>Cryptotis mexicanus</i>	KU	29552	18.5	9.8	5.3	2.5	<i>Cryptotis mexicanus</i>	UAMI	11170	19.2	10.2	5.2	2.5
<i>Cryptotis mexicanus</i>	KU	29528	18.6	10.3	5.2	2.5	<i>Cryptotis mexicanus</i>	UAMI	11172	18.8	9.8	5.1	2.4
<i>Cryptotis mexicanus</i>	KU	29555	18.7	9.9	5.2	2.5	<i>Cryptotis mexicanus</i>	UAMI	11176	19.0	10.1	5.2	2.4
<i>Cryptotis mexicanus</i>	KU	29554	18.7	10.0	5.3	2.5	<i>Cryptotis mexicanus</i>	UAMI	11177	19.6	10.1	5.4	2.5
<i>Cryptotis mexicanus</i>	CNMA	42741	17.6	9.4	5.1	2.4	Otontepec	MZFC	16224	18.6	9.7	5.3	2.6
<i>Cryptotis mexicanus</i>	CNMA	42733	18.1	9.8	5.1	2.4	Otontepec	MZFC	16223	18.9	10.0	5.4	2.7
<i>Cryptotis mexicanus</i>	CNMA	42748	18.3	9.5	5.2	2.4	Otontepec	MZFC	16222	19.5	10.1	5.4	2.8
<i>Cryptotis mexicanus</i>	CNMA	42756	18.8	9.8	5.1	2.4	Otontepec	MZFC	16221	18.6	9.9	5.4	2.7
<i>Cryptotis mexicanus</i>	CNMA	42744	18.9	10.0	5.3	2.4	Otontepec	MZFC	16220	18.8	9.8	5.3	2.6
<i>Cryptotis mexicanus</i>	MZFC	8354	18.3	10.0	5.2	2.6	Otontepec	MZFC	16219	18.8	9.7	5.3	2.6
<i>Cryptotis mexicanus</i>	MZFC	8332	18.3	9.9	5.0	2.3	<i>Cryptotis nelsoni</i>	CNMA	41961	19.0	10.1	5.6	2.6
<i>Cryptotis mexicanus</i>	MZFC	8386	18.4	10.2	5.3	2.5	<i>Cryptotis nelsoni</i>	CNMA	41993	19.1	10.4	5.5	2.8
<i>Cryptotis mexicanus</i>	MZFC	8400	18.5	9.8	5.0	2.3	<i>Cryptotis nelsoni</i>	CNMA	41959	19.4	10.6	5.8	2.9
<i>Cryptotis mexicanus</i>	USNM	69733	18.4	9.7	5.4	2.4	<i>Cryptotis nelsoni</i>	CNMA	41956	19.5	10.3	5.8	2.8
<i>Cryptotis mexicanus</i>	USNM	69615	18.4	9.7	5.3	2.4	<i>Cryptotis nelsoni</i>	CNMA	41992	19.9	10.3	5.7	2.8
<i>Cryptotis mexicanus</i>	USNM	69609	18.4	9.8	5.3	2.5	<i>Cryptotis nelsoni</i>	CNMA	41960	19.9	10.6	5.9	2.8
<i>Cryptotis mexicanus</i>	USNM	69605	18.6	9.9	5.4	2.4	<i>Cryptotis magnus</i>	CNMA	29468	23.0	11.3	6.3	3.1
<i>Cryptotis mexicanus</i>	CNMA	29429	18.9	9.8	5.3	2.4	<i>Cryptotis magnus</i>	CNMA	29469	22.7	11.3	6.3	2.9
<i>Cryptotis mexicanus</i>	CNMA	29431	19.0	10.0	5.3	2.4	<i>Cryptotis magnus</i>	CNMA	33609	23.3	11.7	6.9	3.3
<i>Cryptotis mexicanus</i>	CNMA	29427	19.3	10.4	5.2	2.5	<i>Cryptotis magnus</i>	CNMA	29823	22.4	11.3	6.5	3.2
<i>Cryptotis mexicanus</i>	USNM	68299	18.5	9.5	5.2	2.3	<i>Cryptotis magnus</i>	CNMA	43057	22.2	11.3	6.3	3.2
<i>Cryptotis mexicanus</i>	USNM	68311	18.7	9.9	5.2	2.4	<i>Cryptotis phillipsii</i>	CNMA	44725	19.4	10.0	5.4	2.5
<i>Cryptotis mexicanus</i>	USNM	68303	18.7	9.6	5.3	2.3	<i>Cryptotis phillipsii</i>	CNMA	44727	20.0	9.9	5.8	2.5
<i>Cryptotis mexicanus</i>	USNM	68305	18.8	9.7	5.0	2.3	<i>Cryptotis phillipsii</i>	CNMA	44728	20.2	10.3	5.5	2.5
<i>Cryptotis mexicanus</i>	CNMA	29989	17.5	9.7	5.0	2.3	<i>Cryptotis phillipsii</i>	CNMA	44729	19.9	10.0	5.5	2.5
<i>Cryptotis mexicanus</i>	CNMA	35261	18.6	10.0	5.1	2.3	<i>Cryptotis phillipsii</i>	CNMA	44730	19.7	10.0	5.6	2.6
<i>Cryptotis mexicanus</i>	CNMA	35260	18.7	10.2	5.1	2.5							
<i>Cryptotis mexicanus</i>	CNMA	34859	18.8	10.0	5.2	2.4							
<i>Cryptotis mexicanus</i>	CNMA	29991	19.0	10.1	5.2	2.5							
<i>Cryptotis mexicanus</i>	CNMA	29986	19.0	10.3	5.6	2.6							
<i>Cryptotis mexicanus</i>	CNMA	43060	18.4	9.7	5.1	2.3							
<i>Cryptotis mexicanus</i>	CNMA	43059	18.4	9.6	5.2	2.4							
<i>Cryptotis mexicanus</i>	CNMA	43053	19.1	9.9	5.3	2.5							