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Molecular phylogenetic and taxonomic status of the large-eared desert shrew *Notiosorex evotis* (Eulipotyphla: Soricidae)

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Notiosorex is the only genus of shrews in North America with particular adaptations for arid habitats such as deserts. Five species currently are recognized in the genus, one of which, *Notiosorex evotis*, lives in deciduous rain forest from northern Sinaloa to the state of Jalisco in México. *Notiosorex evotis* originally was described as a subspecies of *N. crawfordi*; however, it was recently proposed as a valid species based on a discriminant function analysis of craniodental characters. Morphological differentiation between *N. evotis* populations and sympatric occurrences with *N. crawfordi* in northern Sinaloa have been recognized. Here, we used a phylogenetic analysis of a mitochondrial gene (*Cytb*; 1,140 bp) and the nuclear beta fibrinogen intron 7 (*β-fib I7*; 385 bp), as well as cranial geometric morphometrics, to assess the taxonomic status of *N. evotis*. We found sequences of *N. evotis* forming two main subclades: one that includes the populations of the state of Sinaloa, the other including populations of Nayarit and Jalisco. The boundaries between the two groups seem to be related to the ecotonal cline between the Pacific Lowlands province and the Trans-Mexican Volcanic Belt province, which acts as a geographical barrier. The discriminant function analysis revealed clear differences in skull shape between the three species of *Notiosorex* to the northwest of its distribution. Considering these multiple lines of evidence from our data set, we confirm that *N. evotis* is a monotypic species. Our results suggest that geometric morphometrics can be used successfully to identify sibling species by shape, especially in groups where determination by craniodental measurements is not possible.

Key words: ecotonal decline, endemic species, geometric morphometrics, Pacific Lowlands, shrews, sibling species, skull variation, Trans-Mexican Volcanic Belt

Notiosorex es el único género de musarañas en Norteamérica con adaptaciones especiales para zonas áridas como son los desiertos. Actualmente se reconocen cinco especies en el género, una de las cuales, *Notiosorex evotis*, vive en el bosque lluvioso caducifolio de México desde el norte de Sinaloa hasta el estado de Jalisco. *Notiosorex evotis* fue originalmente descrita como subespecie de *N. crawfordi*; sin embargo, se propuso recientemente como especie válida en base a un análisis discriminante utilizando caracteres craneodentales. Se ha reconocido la diferenciación morfológica entre poblaciones de *N. evotis* así como ocurrencias simpátricas en el norte de Sinaloa con *N. crawfordi*. Aquí, utilizamos un análisis filogenético de un gen mitocondrial (*Cytb*; 1,140 bp) y el gen nuclear intrón 7 de fibrinógeno beta (*β-fib I7*; 385 bp), junto con morfometría geométrica craneal, para evaluar el estado taxonómico de *N. evotis*. Encontramos que las secuencias de *N. evotis* forman dos subclados principales: uno que incluye las poblaciones del estado de Sinaloa, y el otro que comprende las poblaciones de Nayarit y Jalisco.

Los límites entre los dos grupos parecen estar relacionados con el cline ecotonal entre la provincia de la Costa del Pacífico y la provincia del Eje Volcánico Transversal, el cual actúa como una barrera geográfica. El análisis de función discriminante reveló claras diferencias en la forma del cráneo entre las tres especies de *Notiosorex* que constituyen el noroeste de la distribución del género. Teniendo en cuenta estas múltiples líneas de evidencia de nuestro conjunto de datos, confirmamos que *N. evotis* es una especie monotípica. Nuestros resultados sugieren que la morfometría geométrica es una herramienta útil para discriminar entre especies hermanas en función de su forma, y puede ser utilizada en otros grupos donde no es posible la determinación mediante mediciones craneodentales.

Palabras clave: Costa del Pacífico, cline ecotonal, Eje Volcánico Transversal, especies endémicas, especies hermanas, morfometría geométrica, musarañas, variación craneal

The taxonomy of species of the shrew genus *Notiosorex* is not yet clearly understood and has been subject to change in recent years (Baker et al. 2003; Carraway and Timm 2000; Carraway 2010; Camargo and Álvarez-Castañeda 2020). One of its putative species, *N. evotis* (Coues 1877), was described from a single specimen in poor condition from Mazatlán, Sinaloa. Coues assigned it a catalog number from the United States National Museum of Natural History (USNM 9066), but soon thereafter the specimen either was lost or misplaced (Merriam 1895:34). However, Dobson (1890: Pl XXIII) independently illustrated the teeth of a specimen of what he called *N. crawfordi* from Mazatlán, the type locality of *N. evotis*. At the time, Merriam (1895) had access to no other material of *N. evotis*. Based on the illustration by Dobson (1890), Merriam (1895:34) considered *N. evotis* to be a subspecies of *N. crawfordi* differentiated based on the fact that "the teeth are more crowded than in *N. crawfordi*, and the second or middle unicuspid smaller, being intermediate in height between the first and third. In *N. crawfordi* the first and second are essentially subequal." The presence of the skull of the type was recorded by Fisher and Ludwig (2015). However, in the visual inspection made 23 February 2021, only the skin is present (Woodmann N., in litt.).

As long as no material other than Dobson's illustration was available, it was impossible to further refine the relationship between *N. evotis* and *N. crawfordi*. *Notiosorex c. evotis* remained a subspecies of *N. crawfordi* until a comparatively recent revision based on 36 specimens from the Pacific coastal region of México, in which *N. evotis* was elevated to species based on analysis of craniodental measurements (Carraway and Timm 2000). Since then, additional new species of living and fossil *Notiosorex* have been described: *N. villai* (Carraway and Timm 2000); *N. cockrumi* (Baker et al. 2003); *N. dalquesti* and *N. harrisi* (Carraway 2010); and *N. tataticuli*, which is divided into three subspecies (Camargo and Álvarez-Castañeda 2020). Thus, it has become apparent that *Notiosorex* has high levels of genetic subdivision and a great deal of previously hidden genetic diversity throughout its distribution in the arid regions of North America (McAliley et al. 2007).

Nevertheless, the taxonomic status of the desert shrews in the coastal region of México remains poorly resolved. Specimens from northern Nayarit and southern Sonora have intermediate morphological characteristics between *N. crawfordi* and *N. evotis* (Armstrong and Jones 1971). The southwestern limits of the distribution of *Notiosorex* have yet to be geographically defined and the phylogenetic relationships of *N. evotis* are undetermined. To resolve the taxonomic status of *Notiosorex evotis* and elucidate its phylogenetic relationships with respect to other species in the genus, we analyzed specimens from across its putative range, including newly collected material from its type locality and other key areas.

MATERIALS AND METHODS

Sample collection.—We conducted field trips in the Pacific coastal region of México ranging from northern Sinaloa to the southernmost part of the state of Jalisco. The Pacific coastal region is environmentally heterogeneous. The collecting area includes true coastal plains to the north, belonging to the Pacific Lowland biogeographic province and covered with thorn forest (bosque espinoso) and tropical deciduous forest (bosque tropical caducifolio). These coastal plains grade southwards into coastal highlands of the Sierra Madre Occidental that are part of the Trans-Mexican Volcanic Belt biogeographic province and are capped with mixed coniferous forests (bosque de coniferas y encinos; Fig. 1; Morrone et al. 2017). *Notiosorex* specimens were collected using pitfall traps following the technique of Camargo and Álvarez-Castañeda (2020). All animals were handled and euthanized following the recommendations of the American Society of Mammalogists (Sikes et al. 2016). Voucher specimens were deposited in the Centro de Investigaciones Biológicas del Noroeste (CIB).

Phylogenetic analyses.—The records came from four localities ranging from the northernmost end of the distribution of *N. evotis* in the state of Sinaloa to the southernmost part of the state of Jalisco (Loc 2, 7, 9, and 10; Fig. 1). In addition, we included a sample of *Megasorex gigas* as outgroup representative because it has been previously recognized as the sister genus of *Notiosorex*, and both taxa belong to the Notiosoricini tribe (Reumer 1984; Odhachi et al. 2006; Table 1).

From each specimen we sequenced the mitochondrial *Cytb* (1,140 bp) gene and the nuclear beta fibrinogen intron 7 (*β-fib I7*; 385 bp) locus. Detailed descriptions of our DNA extraction protocols, PCR amplification, and DNA sequencing are provided in Camargo and Álvarez-Castañeda (2020). All newly generated DNA sequences were deposited in GenBank (Table 1). We combined these new with previously published sequences from other taxa in our analysis (Baker et al. 2003; Camargo and Álvarez-Castañeda 2020).

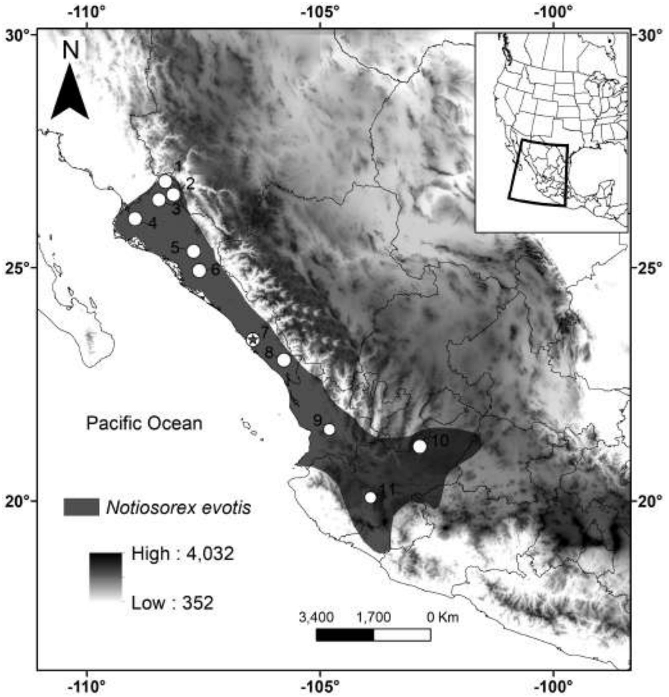

Fig. 1.—Geographic locations of the specimens of *Notiosorex* from the Pacific coastal region examined in the phylogenetic and morphological analyses. Numbers correspond to population samples listed in Table 1; the star indicates the locality where the topotype was collected.

To test the phylogenetic hypothesis for the monophyly of *N. evotis*, we constructed maximum likelihood (ML) phylogenetic trees using RAxML 8 (Stamatakis 2014). Bootstrap support values were based on 5,000 rounds of bootstrapping using the rapid bootstrap algorithm in RAxML, followed by a search of the best-scoring ML tree. To select the best-fit model of evolution, we used Akaike's information criterion (AIC) in jModelTest 2.1.1 (Darriba et al. 2012). The model selected for mtDNA was GTR + Γ + I, with the following nucleotide distributions: A = 0.2820, C = 0.2604, G = 0.1175, and T = 0.3399. HKY + Γ was the selected model for nDNA sequences, with the following nucleotide distributions: A = 0.2864, C = 0.2808, G = 0.2105, and T = 0.2182. We also estimated trees using Bayesian inference (BI), using MrBayes 3.2 (Hulsenbeck and Ronquist 2001). On the combined data set we implemented separate models for each gene. The Metropolis-coupled Markov chain Monte Carlo [$(MC)^3$] consisted of two independent runs of 10^7 generations in which trees were sampled every 100 generations. Convergence was assessed once the average standard deviation of split frequencies approached zero in the program TRACER 1.6 (Rambaut and Drummond 2013). The first 20% of the trees were discarded as burn-in, and a majority-rule consensus tree with values representing posterior probabilities was constructed with the remaining trees. Genetic distances were estimated using the Kimura 2-parameter (K2P) model of evolution (Kimura 1980) implemented in MEGA X (Kumar et al. 2018).

Geometric morphometrics.—We also assessed the taxonomy and morphological affinities of our new material using geometric morphometric analysis of skull shape. Our primary goal was to evaluate the taxonomy of 18 individuals from the Pacific coastal region, which putatively represent *N. evotis* (Fig. 1). To do this, we included these samples in our analysis as well as previously collected material of *N. cockrumi* (*n* = 13) and *N. crawfordi* (*n* = 49). We collected landmarks from a total of 79 skulls housed at CIB, University of Kansas Natural History Museum (KU), and Texas Tech University Natural Sciences Research Laboratory (TTU; Table 1). Only shrews classified as adults were included (Jackson 1928). Because part of our objective is taxonomic, we included type specimens of named taxa in our analysis whenever possible. Newly collected topotypes were used for *N. cockrumi* (*n* = 13); these first were identified using genetic markers because this species lives sympatrically with *N. crawfordi* and is difficult to distinguish using external characteristics (Baker et al. 2003). The holotype of *N. crawfordi* is preserved in alcohol (Merriam 1895), so instead we used an animal collected from as near the type locality as possible, 86 km NW (KU 764889). We also used 48 individuals from Texas to represent the morphological variation within that species (Table 1).

Nineteen two-dimensional landmarks were taken from the ventral side of the skull using a scheme modified from Polly (2007; Fig. 2; Table 2). Landmarks were digitized from images using TPSDig 2.16 (Rohlf 2010) and were superimposed using Procrustes generalized least square superimposition fitting to remove the effects of rotation, translation, and size, then projected into Euclidean tangent space (Rohlf and Slice 1990). Procrustes residuals were calculated by subtracting the mean or consensus shape, after which principal component analysis (PCA) was carried out on the covariance matrix of the residuals (Dryden and Mardia 1998). The resulting PC scores were used as shape variables for a multivariate analysis of variance (MANOVA) to test for shape differences among the groups and for discriminant function analysis (DFA). Cross-validation was used with DFA to determine the probability of identifying individuals using skull shape. Statistical analyses were undertaken using MorphoJ 1.6d (Klingenberg 2011) and PAST v. 2.17b (Hammer et al. 2001).

Results

Genetic variation.—Phylogenetic analyses based on BI and ML confirmed the monophyly of *N. evotis*, mtDNA sequences grouping those specimens into a clade that had support of 1.0 by Bayesian posterior probability and 97% by bootstrap analysis (Fig. 3A). Our results also revealed four major clades within *Notiosorex*, with *N. tataticuli* as the sister species of *N. evotis* at a genetic distance (K2P) of 8.51% in mtDNA and 2.05% in nDNA. Phylogenetic analysis of only the mtDNA sequences yielded a topology congruent with that of the nDNA sequence analysis under both the BI and ML frameworks (Fig. 3).

Specimens from the Pacific coastal region group into two subclades in the nDNA phylogeny, one from the Pacific

Table 1.—List of sampling localities of all shrew specimens examined in this study. The first numbers correspond to the GenBank accession numbers of the mtDNA sequences, followed by the nDNA sequences included in the analyses; the museum specimen numbers in bold correspond to specimens used in the geometric morphometrics analyses. The numbers in parentheses correspond to the localities of *Notiosorex* of the Pacific coast referred to in Fig. 1.

Taxa	State	Locality	Specimens	GenBank accession numbers
Notiosorex cockrumi	Arizona	Leslie Canyon National Wildlife Refuge	TTU 83502	AY611587, AY611584
			TTU 82981–82984	AY611575, AY611599
			TTU 82986	
			TTU 82988	
			TTU 82991	
			TTU 82993–82994	
			TTU 83502	
			TTU 83504	
			TTU 83505	
Notiosorex crawfordi	Arizona	Leslie Canyon National Wildlife Refuge	TTU 82991	AY611582, AY611598
			TTU 82993	
	Chihuahua	3.5 mi SE of Los Lamentos	**KU 76488**	
	New Mexico	Vicinity of Antelope Pass	MVZ 191470	AY611573, MK895499
	Texas	Chaparral Wildlife Management Area	**TTU 80965**	AY611569, AY611601
			TTU 80807	
			TTU 88236-88241	
			TTU 88259–88264	
			TTU 97880–97894	
			TTU 98009	
			TTU 98152	
			TTU 98240	
			TTU 98282	
			TTU 98305–98322	
		Devils River State Natural Area	ASK 4277	MN061777, MK965951
		4.5 mi W, San Angelo	ASK 4530	ASK4350, MK965954
		5 mi N, 9.8 mi W, Mertzon	ASK 4571	MN061447, MK965952
Notiosorex from Pacific coastal region	Sinaloa	(1) 1 mi S of El Cajon	**KU 100319**	
		(2) 19 km W, Choix	**CIB 27876–27878**	MW182524–MW182526
			CIB 28196	MW182532–MW182534
				MW182527, MW182535
		(3) El Fuerte	**KU 75184**	
		(4) 5 mi NW of El Carrizo	**KU 105409**	
		(5) 10 km S, 38 km E, Sinaloa	**KU 125477**	
		(6) 20 km N, 5 km E, Badiraguato	**KU 96419–98889**	
		(7) 5.6 km N, 3 km W, Villa Union	**CIB 27875 Topotype**	MW182528, MW182536
		(8) Isla Palmito del Verde	**KU 98880**	
	Nayarit	(9) San Blas	**CIB 31460**	MW182529, MW182537
	Jalisco	(10) 6.41 km NW, Soyatlán del Oro	**CIB 29625**	MW182530, MW182538
		(11) 21 mi SW of Guadalajara	**KU 42583–42585**	
Notiosorex tataticuli	California	Camp Pendleton	MVZ 221962	MK956945, MN061488
	Baja California	Isla San Martín	CIB 24050	MK955168, MN061462
		2.6 km S, Punta Prieta	CIB 27884	MK895443, MN061462
	Baja California Sur	1.2 km S, 6.6 km E, El Sargento	CIB 27500	MK895492, MN895493

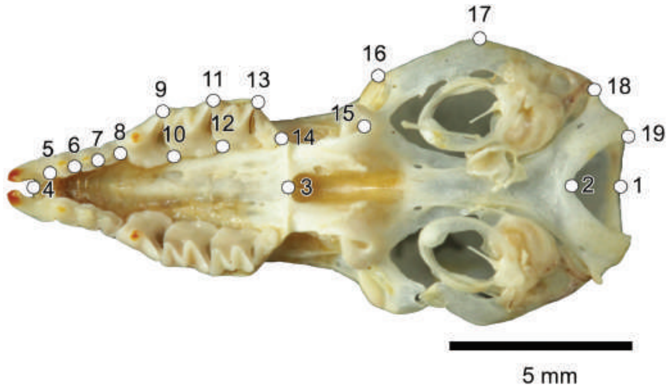

Fig. 2.—Position of landmarks for the ventral cranium of *Notiosorex*, modified from Polly (2007). Numbers refer to landmarks as defined in Table 2.

Lowlands province, the other from the higher elevation Trans-Mexican Volcanic Belt province (Fig. 3B); this split is strongly supported, with a Bayesian posterior probability of 1.0 and bootstrap confidence of 91% (Fig. 3A). The divergence in the DNA sequences between intraspecific populations is 2.21% for *Cytb* and 1.84% for *β-fib I7*. Pairwise comparisons of *N. evotis* indicated a high mean sequence divergence from *N. crawfordi* (17.71% *Cytb*; 4.05% *β-fib I7*), *N. cockrumi* (16.69%; 3.44%), and *N. tataticuli* (8.51%; 2.05%).

Cranial morphometrics.—Principal component analysis of ventral skull shape separates *N. cockrumi*, *N. crawfordi*, and *N. evotis* (Fig. 4A). The first five PCs, which collectively explain 65.10% of the total variance, are significant based on the results of the broken stick method (Frontier 1976; Jackson 1993). The first and second PC axes of the cranial morphometric analysis

Table 2.—Landmark numbers correspond with the configurations illustrated in Fig. 2.

Landmark	Description
1	Dorsal midline of the foramen magnum
2	Ventral midline of the foramen magnum
3	Midline of posterior margin of palate
4	Midline of anterior margin of premaxilla
5	Posterior margin of upper incisor
6	Posterior margin of upper incisor
7	Posterior margin of first antemolar
8	Posterior margin of second antemolar
9	Posteriormost point of metastylar blade of upper fourth premolar
10	Posterior margin of fourth antemolar
11	Posteriormost point of metastylar blade of upper first molar
12	Posteriormost point of hypoconid of upper first molar
13	Posteriormost point of metastylar blade of upper second molar
14	Posterior margin of palate at lateral junction of pterygoid process
15	Center of depression in glenoid fossa
16	Anteriormost point of the lateral braincase
17	Lateral point of widest point of braincase
18	Paroccipital process
19	Lateral edge of foramen magnum

explained 23.73% and 15.14% of the variance, respectively (Fig. 4).

Despite considerable variation within each taxon, there is little overlap between the three *Notiosorex* species. PC1 describes a posterior displacement of the glenoid fossa relative to the rest of the skull in *N. crawfordi* compared to the other taxa, and PC2 describes an anteroposteriorly compressed palate and posteriorly shifted braincase wall in *N. evotis* and *N. crawfordi* compared to *N. cockrumi* (Fig. 4B; Table 3). *Notiosorex crawfordi* was completely nonoverlapping with the other two species, and there was only slight morphometric overlap between *N. evotis* and *N. cockrumi* (Fig. 4A). Mean skull shape was significantly different among the three species (MANOVA, $F = 10.85$, $P < 0.01$). Discriminant function analysis (also known as canonical variates analysis, or CVA) also found significant differences ($P < 0.0001$) in the ventral view of the skull. Discriminant function analysis maximizes the differences among groups and finds linear functions that discriminate between the groups so as to classify specimens. These groups were so distinctive that DF was able to correctly classify every individual in the analysis. Discriminant function analysis further showed that *N. crawfordi* differs from *N. evotis* and *N. cockrumi* in having a more generally elongated skull with a compressed pharyngeal region (Fig. 5). In addition, *N. evotis* differs from both *N. crawfordi* and *N. cockrumi* in having a relatively short palate relative to a long pharyngeal region and a compressed braincase, all in the anteroposterior direction (Fig. 5).

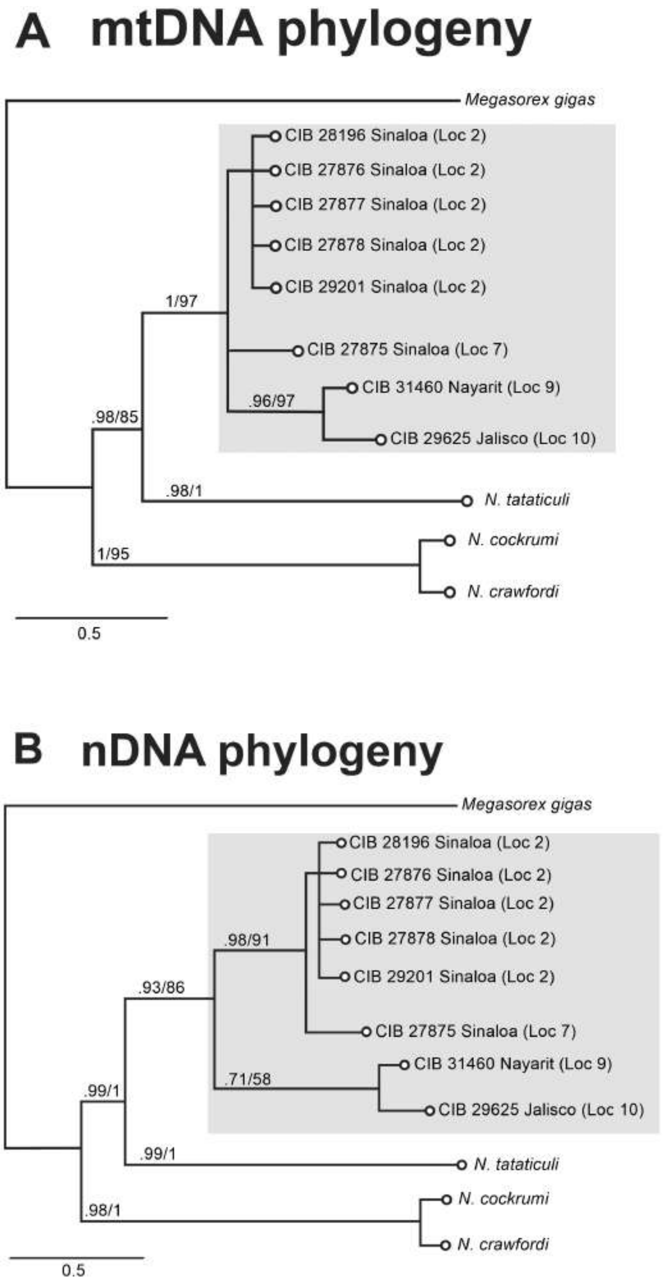

Fig. 3.—(A) Phylogenetic hypothesis of *Notiosorex* based on cytochrome *b* (1,140 bp); and (B) the phylogeny derived from the nuclear intron 7 of the beta fibrinogen gene (385 bp). In both trees Bayesian inference with posterior probabilities are shown above diagonal and maximum likelihood analyses with bootstrap support values below diagonal. The area marked in gray corresponds to *N. evotis*.

DISCUSSION

Notiosorex evotis forms a clade in the Pacific coastal regions subdivided into two subclades, the first containing populations from the Pacific Lowlands province, the second containing populations from the Trans-Mexican Volcanic Belt province. These two groups have genetic distances of 2.21% for *Cytb* and 1.84% for *β-fib I7*. The divergence between these subclades may be due to dispersal barriers associated with topographical and environmental differences between two biogeographical provinces: the lowland clade is restricted to the Pacific Lowlands province in Sinaloa, and the highlands populations to Nayarit and Jalisco in the Trans-Mexican Volcanic Belt province (Morrone et al. 2017). *Notiosorex evotis* is restricted on the eastern margin of its distribution by the Sierra Madre Occidental. This geographic barrier is associated with speciation processes in other mammal species such as the pocket mouse *Chaetodipus artus*

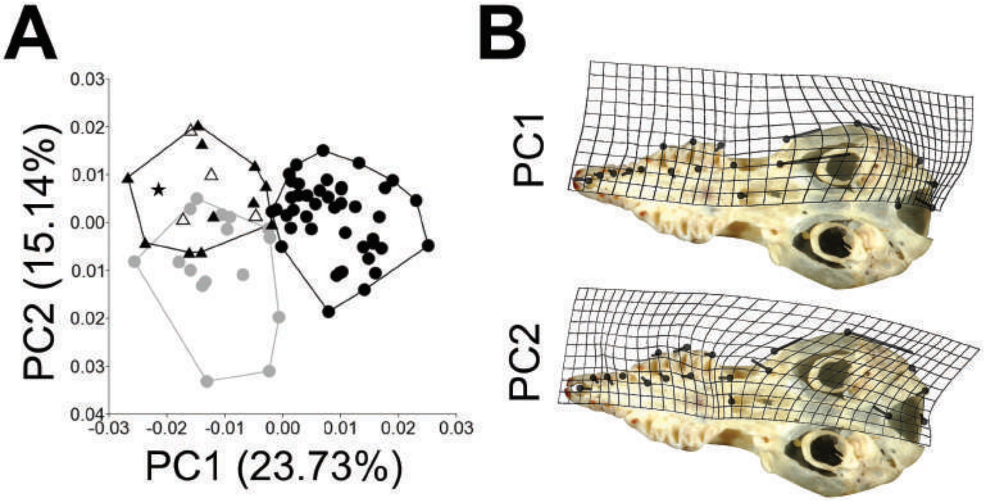

Fig. 4.—Scatterplots for principal components (PCs) of shape variation. Displacement of landmarks corresponding to first and second PCs of variation in the ventral view. Gray dots indicate *N. cockrumi*, black dots represent *N. crawfordi*, black triangles are *Notiosorex* from Pacific Lowlands province populations, and white triangles *Notiosorex* from Trans-Mexican Volcanic Belt province populations; the star denotes the topotype.

Table 3.—Correlation values obtained for the most important morphological characters (bold) of the first five PCs for *Notiosorex* species and coefficients of canonical variables. PC = principal component; DF = discriminant function.

Landmark	PC1	PC2	PC3	PC4	PC5	DF1	DF2
1	−0.3883	−0.0172	−0.1775	−0.3034	−0.4605	0.3804	**−0.0745**
2	−0.0540	−0.2298	−0.2041	**0.7011**	0.0608	0.3170	−0.0712
3	0.1087	−0.0494	0.1544	−0.0600	−0.0432	−0.0059	−0.0561
4	−0.2286	0.1825	**−0.5970**	−0.0432	0.1758	−0.3011	−0.0419
5	−0.1201	0.1609	−0.1759	0.0464	0.1649	−0.2744	−0.0205
6	−0.0530	0.1349	−0.0591	0.0487	0.0427	−0.2507	−0.0168
7	−0.1100	0.0251	0.0519	0.0227	−0.0447	−0.2261	−0.0132
8	−0.0731	−0.0832	0.0906	−0.0821	−0.1008	−0.2003	−0.0070
9	−0.0106	−0.1162	0.1394	−0.0160	0.0042	−0.1563	0.0362
10	0.0099	−0.0576	0.1809	−0.0744	−0.1158	−0.1447	−0.0108
11	−0.0247	−0.1700	0.1717	0.0260	0.0194	−0.0961	0.0468
12	−0.0169	−0.1369	0.2249	−0.0260	−0.0595	−0.0895	−0.0050
13	−0.0632	−0.1901	0.2040	0.0241	0.0743	−0.0425	0.0425
14	0.0418	−0.1502	0.2194	−0.0447	0.0358	−0.0206	−0.0002
15	**0.6946**	−0.3085	−0.3445	−0.0528	0.0710	0.0649	0.0084
16	0.4027	0.3297	−0.1374	−0.2485	**−0.2980**	0.0916	0.0657
17	0.1215	**0.6838**	0.2257	0.2458	0.0309	0.2185	0.1015
18	−0.0449	0.0747	0.1377	−0.0640	**0.3024**	0.3489	0.0338
19	−0.1918	−0.0826	−0.1050	−0.0997	0.1403	**0.3869**	−0.0176
Percent variance explained	23.7313	15.1492	11.3694	8.0793	6.7778	79.3	20.3

(Best and Lackey 1992), the deer mice *Peromyscus schmidlyi* (López- González et al. 2014) and *P. simulus* (Schmidly and Bradley 1995), and the wood rat *Hodomys alleni* (Edwards and Bradley 2002). The northern limit of the distribution of *N. evotis* is bounded by two geographic barriers: the Sierra de Barobampo and the Rio Fuerte (Hafner and Riddle 2011); both of these barriers could be impermeable to connectivity by desert shrew species.

The sister species of *N. evotis* is *N. tataticuli* (Fig. 3; BI: 0.91; Bootstrap support [BS]: 85%), the range of which is farther north: in the Pacific coastal region in the California desert. The *N. evotis* clade is deeply separated from both *N. crawfordi* and *N. cockrumi*.

We detected interspecific differences between the shape of the skull among the three *Notiosorex* species analyzed. The shape of the skull of *N. evotis* differs from that of *N. cockrumi* and *N. crawfordi* in having a wider, shorter palate and broader tympanic area than *N. crawfordi*. Carraway and Timm (2000) mentioned that *Notiosorex* populations of the northern Pacific coast of México show intermediate characteristics between *N. evotis* and *N. crawfordi*. Our results of variation in the shape of the skull are consistent in recognizing the three distinct *Notiosorex* species because in all of our analyses the differences between these two species are consistent. Analysis of variation in the shape of the skull of the populations of *Notiosorex* in the Pacific coast region indicates that *N. evotis* has an allopatric distribution relative to remaining *Notiosorex* species. Jones et al. (1962) recorded *N. crawfordi* in Sinaloa (KU 75184); however, our analysis does not support this specimen's taxonomic identification, which we reclassified as *N. evotis*. We therefore consider all *Notiosorex* populations on the Pacific coast of Sinaloa and Nayarit to be *N. evotis* (Fig. 5).

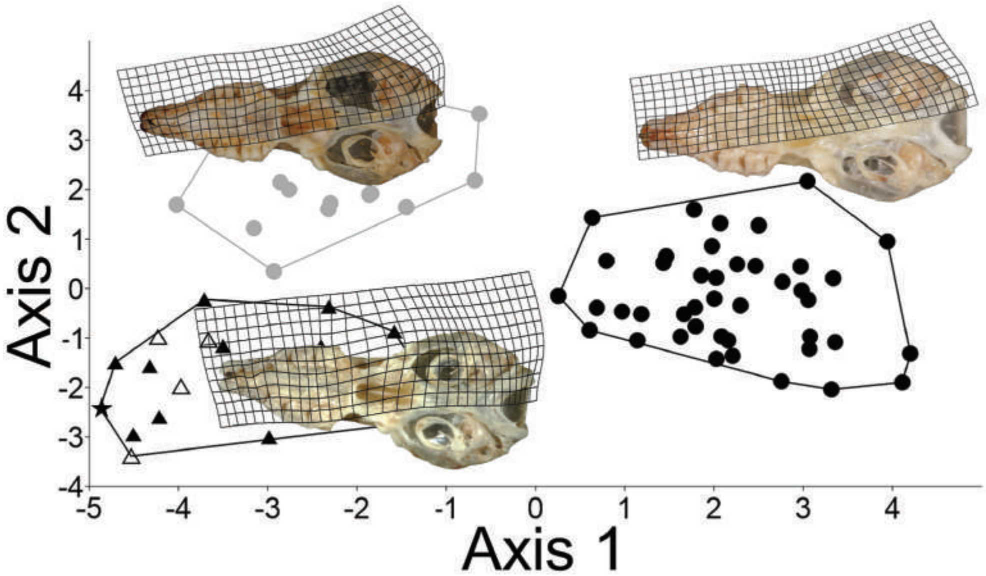

Fig. 5.—Intraspecific canonical analyses of sets of variables in a plot of skull shape variation of *Notiosorex*. Gray dots indicate *N. cockrumi*, black dots represent *N. crawfordi*, and black triangles are *Notiosorex* from Pacific Lowlands province populations. *Notiosorex evotis* and white triangles are *Notiosorex* from Trans-Mexican Volcanic Belt province populations.

We found that the species of desert shrew are not only phylogenetically distinct, but that they are also morphologically distinct. Species of *Notiosorex* have been considered to be "cryptic" because their differences were difficult to detect using traditional morphometric techniques, all of which are substantially influenced by size (Carraway 2007, 2010). Geometric morphometrics, which focuses solely on shape, has been shown to be powerful at differentiating even the most closely related shrew populations and has been used recently to study speciation and evolution in shrews (Polly and Wójcik 2019).

The potential for selection or drift to cause phenotypic changes in shrews is considerable due to their low vagility and their ability to persist in localized microhabitats (Polly 2007; Polly et al. 2013). We did not detect strong differences between the populations of *N. evotis* in the lowlands and highlands, which suggests either very recent divergence or high levels of gene flow. Currently, the populations may not be interconnected because of fragmentation of their habitats due to intensive agriculture in Sinaloa (38% of the surface territory; INEGI 2017).

The taxonomic status of *Notiosorex* has been poorly understood and hampered by the lack of voucher specimens in scientific collections and several misplaced type specimens. *Notiosorex evotis* is a rare and difficult-to-trap species with a spotty distribution, associated with deciduous forest along the Pacific coastal plain (Baker 1962). This study has added new material and analyzed it in the context of other museum specimens, supporting the elevation *N. evotis* to species status. However, the problem of its partially missing holotype (USNM 9066) still plagues its taxonomy (Merriam 1895; Carraway and Timm 2000). Because of the current absence of the skull of the type (Woodmann N., in litt.), we give information of the species and photos of a topotype (Fig. 6). *Notiosorex evotis* is a monotypic endemic species of Mexico protected by the Mexican government (NOM-059) in the threatened category. Our results in the field reflect that it is a species that is not very abundant

Fig. 6.—From top to bottom: right lateral, ventral, and dorsal views of the skull and labial view of the mandible of the topotype of *Notiosorex evotis* (CIB 27875; 5.6 km N, 3 km W Villa Unión, area of Mazatlán, Sinaloa, México; 23.2148°N, 106.1069°W). Scale = 5.0 mm.

and could be subject to pressure from habitat loss due to the intense agriculture in its geographical distribution.

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