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A NEW SPECIES OF DESERT SHREW, *NOTIOSOREX*, BASED ON NUCLEAR AND MITOCHONDRIAL SEQUENCE DATA

Editor's Comment: This paper describes a previously unrecognized species of shrew of the genus *Notiosorex*. This species is recognized based on DNA sequences from the cytochrome *b* gene of the mitochondria and an Intron from a nuclear gene (beta fibrinogen). Leslie Carraway and Robert Timm recently revised this genus and recognized three species (one new) based on classical morphological criteria. The new species described herein was placed within the species they recognized as *Notiosorex crawfordi* and if the new species is morphologically distinct from *N. crawfordi* it is not obvious from their data. It will be interesting to have specimens identified by genotype and see how discriminate scores from each genotype plot on a graph as in Table 3 of Carraway and Timm (2000). Two additional points merit some comment. First, from the molecular data, it is probable that a third species exists in Baja California. Also, because molecular data are available from so few individuals it will be difficult to determine the geographic range of these two? three? species. Because there is so much genetic difference in the cytochrome *b* gene it will be possible to identify voucher specimens from a small fragment of DNA that can be isolated from museum skins. However, to do this from hundreds of museum specimens will be labor intensive and it will be interesting to see how many museums will permit skin clips to identify specimens housed in their museums. The bottom line remains that if the kinds of data presented in Occasional Paper 222 are accurate in defining species boundaries, then a large number of unrecognized species of mammals remains to be discovered.

RJB

Front cover: Distribution of collection localities of samples for which cytochrome-*b* data are available. Red dots identify geographic localities where the phylogroups referable to *Notiosorex crawfordi* were collected. Asterisk identifies the type locality for *N. crawfordi*. Yellow circles identify the phylogroup herein described with the open square identifying the type locality. Triangles identify sites where a third phylogroup was collected.

A New Species of Desert Shrew, *Notiosorex*, Based on Nuclear and Mitochondrial Sequence Data

Robert J. Baker, Mark B. O'Neill, and L. Rex McAliley

The systematics of the genus *Notiosorex* recently was reviewed using classical skin and skull morphology by Carraway and Timm (2000), and they recognized three species; *N. crawfordi*, *N. evotis* and *N. villai* (Fig. 1). As defined by Carraway and Timm (2000), *N. villai* is restricted to isolated mountain valleys in Tamaulipas, Mexico and *N. evotis* occupies the southwestern part of the range of the genus in the Mexican states of Colima, Jalisco, Michoacan, Nayarit and Sinaloa (Fig 1). The geographic and ecological distribution of *N. crawfordi* is much more extensive than that described for the other two species (Fig. 1, Carraway and Timm 2000). For the remainder of this paper, we are concerned only with populations of *N. crawfordi* as defined by Carraway and Timm (2000).

We have sequenced the cytochrome *b* gene for 28 specimens of *N. crawfordi* and these results indicate that this species has at least three major phylogroups that are identifiable based on mitochondrial data with genetic distances between each group being greater than 10%. To better understand the significance of this level of differentiation in the mitochondrial genome, we sequenced more than 350 base pairs of Intron 7 of the nuclear beta fibrinogen gene (Wickliffe et al. 2003) from 10 individuals representing two of the phylogroups. These results indicate that the genetic differences distinguishing the two phylogroups are present in both the mitochondrial and nuclear genomes. At Leslie Canyon, National Wildlife Refuge in Cochise County, Arizona, the two

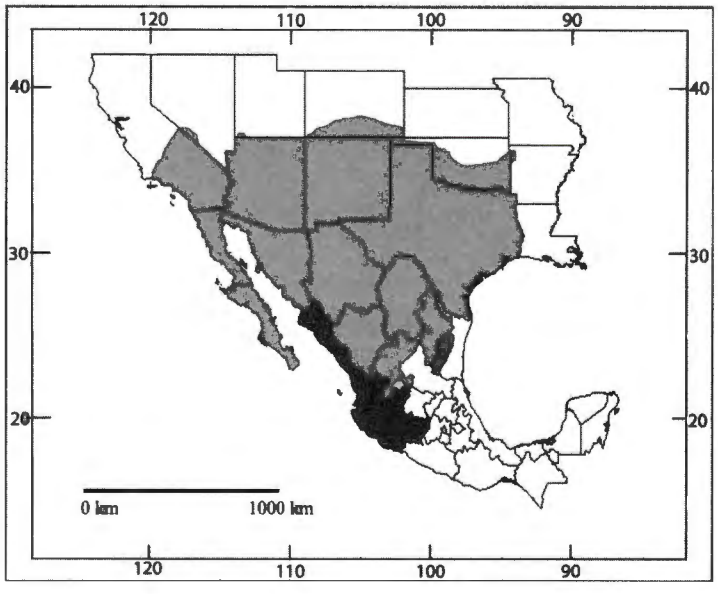

Figure 1. Geographic distribution of the genus *Notiosorex* (*sensu* Carraway and Timm, 2000). Light gray areas denote distribution of *Notiosorex crawfordi*. Medium gray shading denotes the geographic range of *N. villai* and dark gray shading is the geographic range of *N. evotis*. Ranges adapted from sampling localities of Carraway and Timm (2000).

phylogroups are sympatric. Within our limited sample of eight individuals from this area of sympatry, there is no evidence of hybridization. We interpret these data to indicate that as currently recognized, *N. crawfordi* contains at least two biological species. Below we provide a description for the species for which there is no available name.

Notiosorex cockrumi New Species

Holotype.—Adult female, skin, skull (Fig. 2), postcranial skeleton, and frozen tissues, Museum of Texas Tech University, TTU 100,000 from Arizona, Cochise County, Leslie Canyon National Wildlife Refuge, T21S, R28E Section NE ¼ 20, Elevation 4460. Original number M10 of L. Rex McAliley. Collected by Bill Radke in a pitfall trap. TK number 49918 identifies tissue samples deposited in the Natural Science Research Laboratory, Texas Tech University.

Selected Measurements of the Holotype.—External measurements (in mm) recorded by L. Rex McAliley at time of preparation were: total length 84, tail length 27, hind foot 10, ear 4, weight 3.5 g. Cranial measurements (in mm) of the holotype are as follows: greatest length of skull 16.16, rostral breadth 4.8, least interorbital breadth 3.61, cranial breadth 7.95, length of upper unicuspid toothrow 1.81, length of maxillary complex toothrow 3.81, width across M2-M2 4.84, length of mandibular toothrow 4.8, height of coronoid process 4.2, length of coronoid process-ventral point of upper condylar facet 3.39, and length of coronoid process-ventral point of lower condylar facet 3.54.

Distribution.—*Notiosorex cockrumi* is known from southeastern and south central Arizona to central Sonora, Mexico (Fig. 3). The actual range may be larger as genetic data are not available for specimens from Nevada, California, and Colorado as well as for specimens from areas of Central Mexico. What can be documented at this time is that *N. crawfordi* is distributed in Texas to southeastern and south central Arizona and that both *N. crawfordi* and *N. cockrumi* are sympatric over a substantial area of southeastern Arizona. Sequence data will be needed to determine how the ranges of *N. crawfordi* and *N. cockrumi* are defined. Fortunately, there is sufficient variation in the cytochrome *b* gene such that even a 200 base pair fragment would be adequate to properly identify the voucher specimen. Such small fragments often can be sequenced from museum skin biopsies.

Description of Type Locality.—As recorded by Radke, "riparian overstory of Arizona Walnut and Ash. Mesquite grassland dominated by Giant Sacaton" (*Sporobolus wrightii*).

Diagnosis.—Presently, the only basis for diagnosis is sequence data from the mitochondrial cytochrome *b* gene and Intron 7 of the beta fibrinogen nuclear gene. Table 1 presents sequence variation in the cytochrome *b* gene that distinguishes *N. crawfordi, N. cockrumi* and a phylogroup from Baja California from each other. Amino acid residues for the cytochrome *b* gene that distinguishes *N. crawfordi* from *N. cockrumi* are presented in Table 2. Variation in sequences distinguishing *N. crawfordi* from *N. cockrumi* for Intron 7 of the beta fibrinogen gene are presented in Table 3. These genetic characteristics define *N. cockrumi* as unique and serve as a diagnosis of *N. cockrumi* from all other species.

Justification for Specific Status.—Justification for recognizing *N. cockrumi* as a distinct species rather than a subspecies comes from two species concepts. Support is present in the application of the Genetic Species Concept. The Genetic Species Concept (Dobzhansky, 1950; Bradley and Baker, 2001; and Baker et al., 2002) employs measures of genetic distinctiveness to estimate specific status. For *N. crawfordi/cockrumi*, there are genetic data available from two sources; the mitochondrial cytochrome *b* gene and Intron 7 of the nuclear beta fibrinogen gene. Bradley and Baker (2001) reviewed the distance values for cytochrome *b* for populations, subspecies, sister species and other congeneric taxa. Based on 11 genera (involving 84 species) of rodents and bats, intraspecific populational variation ranged from 0.0 to 3.9% and subspecific variation ranged from 0.0 to 8.9%. Cytochrome *b* distance values for sister species ranged from 2.5 to 19% but of the 21 sister species comparisons, only two had distance values greater than 15%. Eighteen sister species comparisons had distance values less than 10%. When the distance values that distinguish *N. cockrumi* from *N. crawfordi* (12.96%, Table 4) are viewed against this summary, the values distinguishing *N. cockrumi* from *N.*

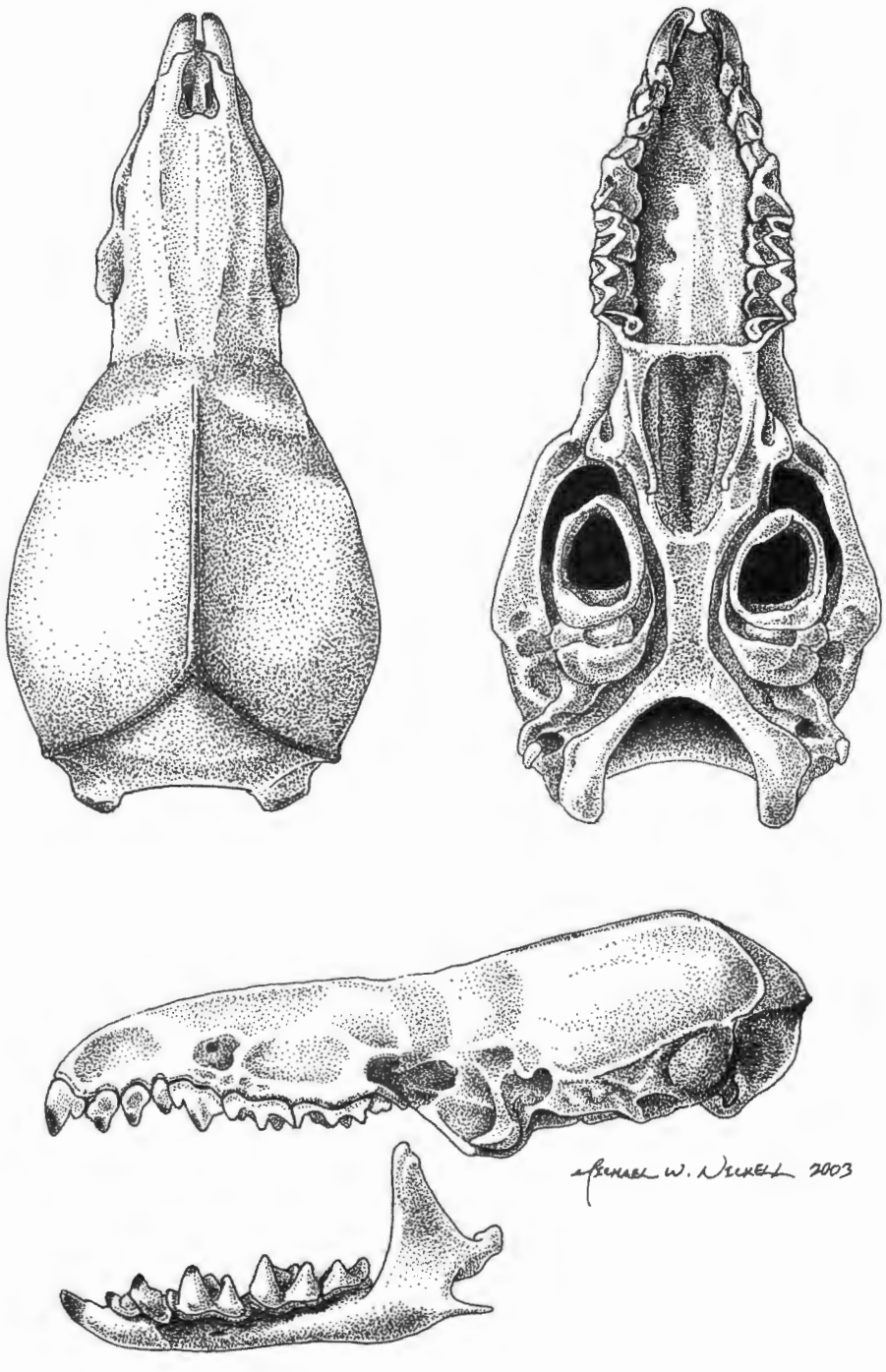

Figure 2. Drawing of the skull of the holotype of *Notiosorex cockrumi*, TTU100,000.

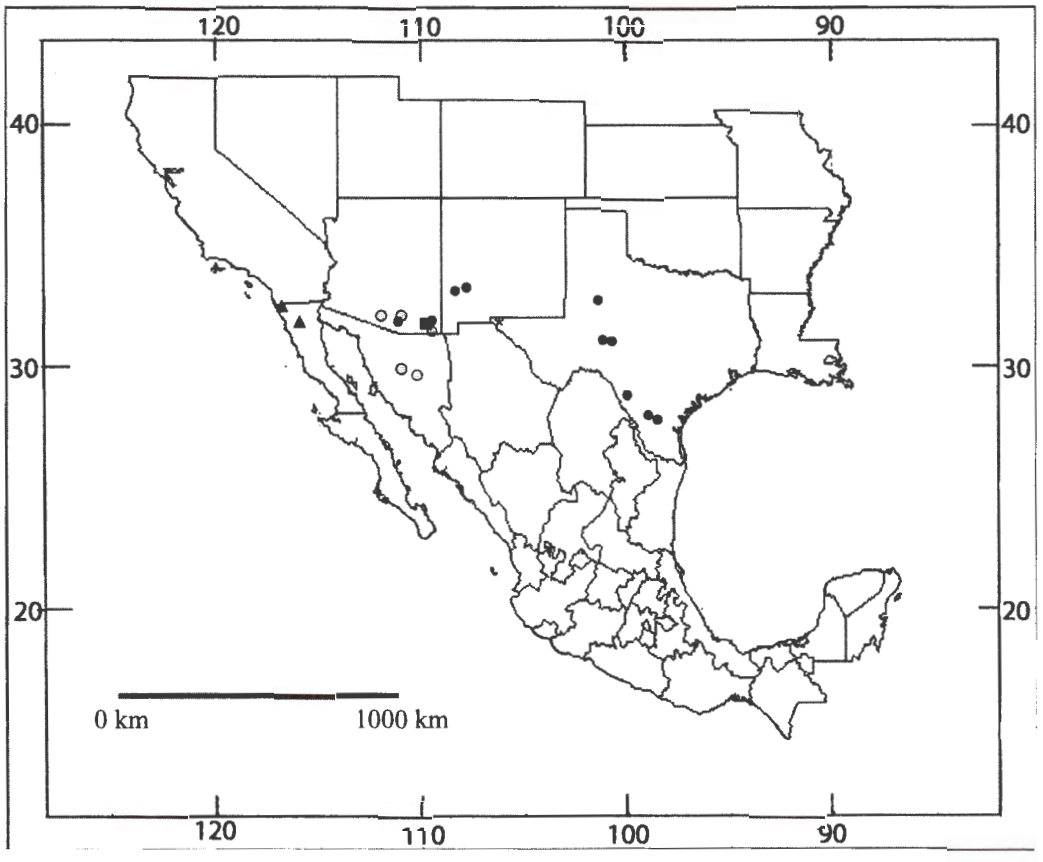

Figure 3. Distribution of collection localities of samples for which cytochrome-*b* data are available. Solid dots identify geographic localities where the phylogroups referable to *Notiosorex crawfordi* were collected. Asterisk identifies the type locality for *N. crawfordi*. Shaded circles identify the phylogroup herein described as *N. crockrumi* with the open square identifying the type locality. Triangles identify sites where a third phylogroup was collected. As presently defined *N. cockrumi* is isolated to regions in which shaded circles are plotted.

crawfordi are in the range of values more typical of sister species comparisons rather than levels present in intraspecific comparisons. Bradley and Baker (2001) concluded that in cases where distance values were 7-11% the default mode should be to recognize the phylogroups as distinct biological species unless there is compelling evidence to the contrary. Are there other data that suggest that these two phylogroups should be recognized as subspecies? The data most compatible with the subspecies status for *cockrumi* that we are aware of is the pattern of morphological variation reported by Carraway and Timm (2000). They detected no geographically discernable patterns in skin and skull characteristics and measurements across the range of *N. crawfordi* (Fig. 1). However, there are other examples of mammals that cannot be distinguished by skin and skull characteristics but are recognized as being distinct species based on genetic data such as karyotypes. Such examples include *Rhogeessa genowaysi* (Baker, 1984) and *Microtus rossiaemeridionalis* (Fredga et al. 1990; Zagorodnyuk 1991).

The utility of Intron 7 of the nuclear beta fibrinogen gene in phylogenetic analyses has been demonstrated in several taxa (Johnson and Clayton 2000, Prychitko and Moore 2000, Seddon et al. 2001, Weibel and Moore 2002, Wickliffe et al. 2003). In the genus *Picoides* (woodpeckers), Weibel and Moore (2002) reported intraspecific variation to range from 0.00 to 0.30%; whereas Prychitko and Moore (2000) found intraspecific variation to range from 0.00 to 0.12%, values well below those reported in Table 5 for *N. crawfordi/N. cockrumi* (1.29 and 1.39% respectively).

Table 1. List of fixed and polymorphic positions in the mitochondrial cytochrome b *gene among the species* Notiosorex crawfordi, N. cockrumi, *and samples from the phylogroup from Baja California, Mexico. Complete sequence is in GenBank, accession number AY305379.*

Position	*N. crawfordi*	*N. cockrumi*	*Notiosorex* Baja	Position	*N. crawfordi*	*N. cockrumi*	*Notiosorex* Baja
5	T	C	T	291	C	T	T
12	C	T	C	294	C	A	C
21	A	A	G	303	C	A	T
27	T	C	C	309	C	T	C
40	G	A	A	315	A	A	G
42	T	C	T	318	T	A	C
47	A	A	G	321	C/T	T	T
48	C	C	T	333	G	A/G	G
57	T	C/T	T	342	T	T	C
60	T	C	T	345	T	C	T
61	C	T	C	348	C/T	T	T
66	C	A	T	351	T	A	C
69	C	T	C	352	C	T	T
78	A	A	C	357	A	A	T
81	C	C/T	T	364	A	G	G
87	T	C	T	369	C	C	T
99	T	T	C	381	C/T	C	C
108	C	C/T	C	390	A	A	G
109	T	T	C	396	T	C/T	T
117	C	C	T	399	T	C	T
121	C	T	C	402	A	T	A
129	C	T	T	411	A	A	G
132	A	A	G	417	A	C	A
135	C	T	T	432	A	C	A
138	C	T	T	438	T	T	C
144	C	T	C	444	C	C	T
145	C	T	T	447	C	T	A
150	T	T	C	456	T	C	T
151	T	T	C	459	C	T	C
156	A/G	A	A	462	C	T	C
162	T	C	T	465	T	C	C
174	T	C	T	468	C/T	C	C
177	T	T	C	471	T	C	C
178	C	T	T	474	T	A	C
186	T	C	T	477	C	T	C
198	C	A	C	478	T	C	C
201	C	T	T	480	A	A/G	A
204	T	C	C	483	T	C	C
210	T	C	C	492	T	C	C
216	C	C	T	498	G	G	A
219	A	A	T	501	C	T	T
222	C	T	T	504	T	T	C
225	C	C	T	519	A	C	T
228	C	C	G	522	A	C	A
232	C	C/T	T	525	C	C/T	T
234	C	A	A	528	C	T	C
240	C	T	T	537	C	C	T
243	T	T	C	540	T	C	T
246	C/T	C	T	543	T	T	C
255	T	C	C	553	T	T	C
264	A	T	A	558	T	T	A
270	T	T	C	561	C	T	C
276	C	T	T	564	T	C	T
279	C	T	T	567	T	C/T	C
282	T	A	C	570	T	A/G	A
285	C	T	C	579	A	T	T
286	C	C	T	582	A	A/G	A

Table 1 (cont.)

Position	*N. crawfordi*	*N. cockrumi*	*Notiosorex* Baja
585	T	C	C
588	T	C	C
592	T	C	C
600	T	C/T	C
603	C	T	T
609	A	C	A
615	T	A	C
621	T	T	C
627	C	T	C
630	A	A	T
636	C	T	C
642	C	C	T
645	T	T	C
648	T	C	T
652	A	A	A/T
654	T	C	C
660	T	C	C
666	T	A	A
672	C	C/T	T
684	C	T	C
687	T	C	T
693	A	T	T
697	C/T	C	C
699	A	T	T
705	T	C	T
708	C	C	T
709	C	T	C
712	A	A	T
713	C	C	T
717	C	T	C
718	T	G	G
724	C	T	C
729	C	C	A
730	T	C	T
735	C	T	T
744	C	T	C
745	A	T	T
747	G	A	A
750	C	T	T
756	C/T	T	C
759	T	A	A
762	C	T	C
771	A	A	C
774	A	T	A
777	C	C/T	C
780	T	T	C
786	T	A/G	A
789	C	C	T
795	C	T	T
798	A	C/T	A
801	C	T	T
804	T	C	C
813	A	A/G	A
828	C/T	C	C
834	T	T	C
846	A	T	C
849	T	T	C
852	T	C	C
855	T	C	T
858	C	T	C
867	A	A	G
870	T	A	T
873	C	C	T
874	C	C/T	C
879	T	C	C
885	C/T	C	C
886	C/T	C	C
891	T	A/C	T
894	C	T	T
895	T	T	C
897	G	A	A
901	C	C	T
903	A	T	A
906	C	C	T
912	T	C	C
918	C	C	T
921	C	C	T
924	C	T	T
930	T	C/T	C
936	G	A	A
942	C	C	T
960	C	T	T
962	C	C	T
969	T	C	C
981	C	T	T
987	A	G	A
993	C	T	C
994	C	T	C
999	A	C	T
1003	T	T	C
1005	A	A	T
1014	T	T	C
1020	C	C	T
1023	A	G	G
1029	T	C	T
1032	A	G	A
1035	C	T	C
1038	C	C	T
1041	C	C	T
1047	T	C	C
1053	T	A	T
1057	C/G	G	C
1068	C	T	C
1069	T	T	C
1074	T	C	T
1080	C	A	C
1081	T	T	C
1083	A	A	G
1086	T	C	C
1087	T	T	C
1090	A/G	A	G
1095	T	C	T
1101	C	C	T
1104	T	T	C
1110	C/T	C	T
1116	C	C	T
1122	C	T	C
1126	C	A	T

Table 2. Amino acid variation in the mitochondrial cytochrome b *gene for* Notiosorex crawfordi, N. cockrumi *and a phylogroup from Baja California, Mexico.*

Position	*N. crawfordi*	*N. cockrumi*	*Notiosorex* Baja
2	Isoleucine	Threonine	Isoleucine
14	Valine	Isoleucine	Isoleucine
16	Asparagine	Asparagine	Serine
122	Threonine	Alanine	Alanine
238	Threonine	Threonine	Leucine
240	Serine	Alanine	Alanine
249	Methionine	Leucine	Leucine
303	Leucine/Phenylanine	Leucine	Phenylanine
353	Leucine/Valine	Valine	Leucine
364	Isoleucine/Valine	Isoleucine	Isoleucine
376	Leucine	Methionine	Leucine

Table 3. List of fixed and polymorphic sites in ~386 bp of the nuclear gene beta fibrinogen Intron 7. Between positions 211 to 228 there is an indel present in Notiosorex crawfordi *that is absent in* N. cockrumi. *Complete sequence is in GenBank accession number AY305380.*

Position	*N. crawfordi*	*N. cockrumi*
22	A	G
32	A	A/C
39	A/C	A/T
42	A	A/G
44	A/T	A
51	C/T	T
64	A	G
68	A/C	A
73	A/G	A
75	C/G	C
98	C	T
112	T	C
133	T	A
154	C	C/T
164	A	T
211-228	Present	Absent
240	A	A/T
259	G	A
263	G	C/G
264	T	C/T
281	C	T
292	G	A/G
298	C	C/G/T
310	A	A/C
312	T	A/T
345	C	T
359	A/G	G
377	G	C
382	C/T	T
384	T	A/T

Table 4. Average genetic distances (uncorrected) between samples of Notiosorex crawfordi, N. cockrumi, *and a phylogroup found in Baja California, Mexico for the mitochondrial cytochrome* b *gene.*

	S. cinereus	*N. crawfordi*	*N. cockrumi*	*Notiosorex* Baja
S. cinereus	—			
N. crawfordi	16.74	0.96		
N. cockrumi	18.51	12.96	1.30	
Notiosorex Baja	16.15	13.55	13.78	0.0

Johnson and Clayton (2000) found genetic distances values between species of columbiforms (doves and pigeons) to range between 0.27 and 7.30%. In an analysis of the glacial effects on distributions of the European hedgehogs, *Erinaceus europaeus* and *E. concolor* Seddon et al. (2001)., reported distance values ranging from 0.91 to 1.24% between species. Genetic distance values seen herein between *N. crawfordi* and *N. cockrumi* (4.40%, Table 5) exceed values reported for other genera of mammals and birds. However there certainly is not sufficient sequence data from Intron 7 of the beta fibrinogen gene to make a strong statement concerning specific status relative to the Genetic Species Concept.

The second basis for recognition of *N. cockrumi* at the species level comes from application of the Biological Species Concept (Mayr 1963). The observation that genetic distinction of the two phylogroups is present and correlated in the nuclear and mitochondrial genomes greatly reduces the probability that the observed variation in the maternally inherited cytochrome *b* gene is a polymorphism within a single species (Fig. 4). However, the most significant observation that supports specific status of the two phylogroups is that the two phylogroups occur in sympatry and that for the eight individuals for which mitochondrial and nuclear data are available, there is no evidence of hybridization. Integrity of the gene pools of sympatric taxa is widely recognized as justification for the conclusion that two sympatric taxa have achieved specific status. In summary, two lines of evidence (from application of the Genetic Species Concept and the Biological Species Concept) in concert provide justification for recognition of *N. cockrumi* as a distinct biological species.

Karyology.—Two karyotypes (Baker and Hsu 1970) have been reported for *N. crawfordi* as defined by Carraway and Timm (2000). These two karyotypes differ not only in diploid and fundamental number, but also in chromosomal morphology. One of these karyotypes has a diploid number of 68 and a fundamental number of 102. This karyotype was reported from specimens collected from Post, Garza County, Texas, which is well within the range of the phylogroup that is herein assigned to *N. crawfordi* (Fig. 3). A second karyotype has a diploid number of 62 and a fundamental number of 94. The second karyotype was present in a male specimen from Santa Rita Experimental Range, Pima County, Arizona. At the Pima County locality, both *N. crawfordi* and *N. cockrumi* most likely are present, but this second karyotype that is unique from *N. crawfordi* from Texas possibly will be characteristic of *N. cockrumi.*

Table 5. Average genetic distances (uncorrected) between and within samples of Notiosorex crawfordi *and* N. cockrumi *based on the nuclear beta fibrinogen Intron-7 gene.*

	S. cinereus	*N. crawfordi*	*N. cockrumi*
S. cinereus	—	—	
N. crawfordi	21.05	1.29	—
N. cockrumi	21.38	4.40	1.39

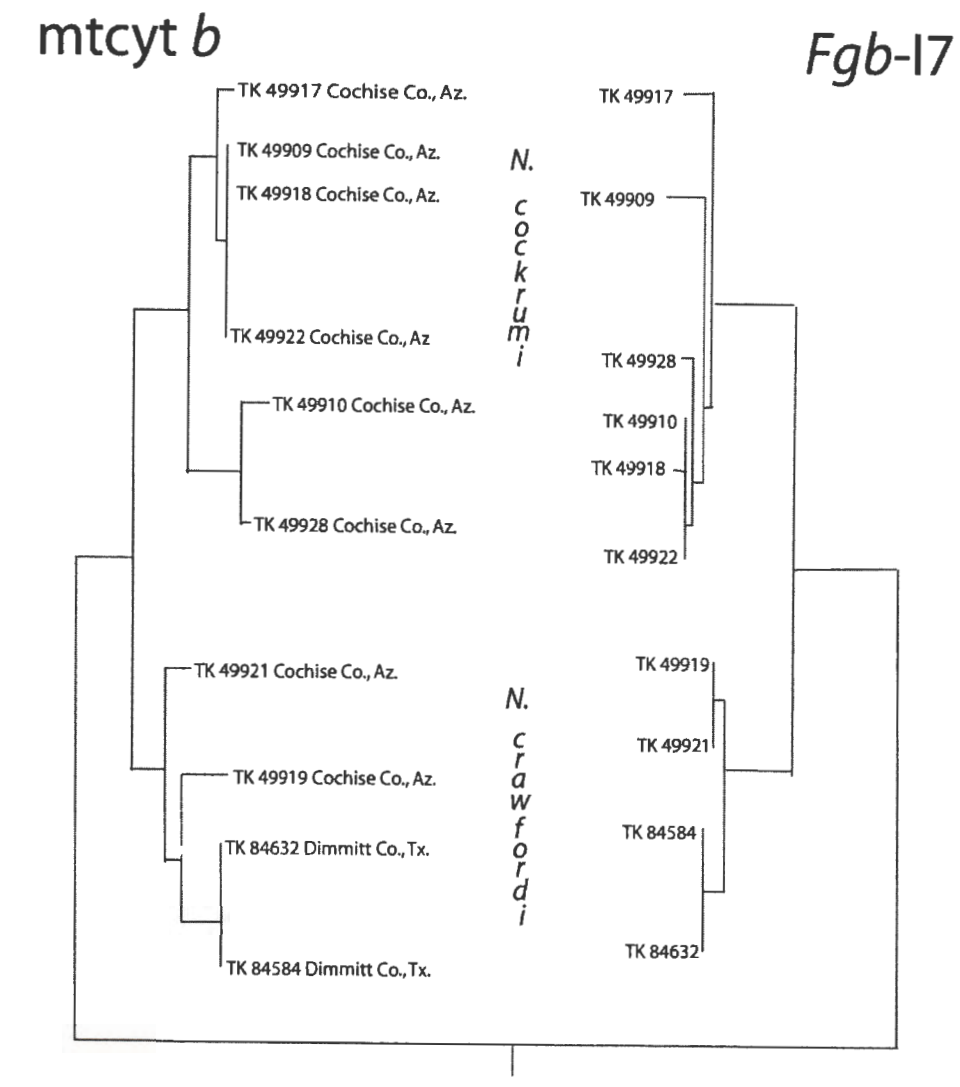

Figure 4. Comparison of neighbor joining trees generated from Tamura-Nei distance values for *Notiosorex crawfordi* and *N. cockrumi* samples using the molecular markers, cytochrome *b* and beta fibrinogen Intron-7. Although based on distance values, trees are not to scale in order to facilitate design of the figure. Distance values for major clades are reported in Tables 4 and 5.

Etymology.— The specific epithet *cockrumi* was chosen to recognize and to honor Dr. E. Lendell Cockrum for his lifetime of research on mammals and for his commitment to the education of students in Mammalogy and General Biology. Examples of Dr. Cockrum's research on the distribution and biodiversity of mammals include *Mammals of Kansas* (1952), and *The Recent Mammals of Arizona* (1960). Examples of university textbooks include *Introduction to Mammalogy* (1962), and *Zoology* (Cockrum and McCauley, 1965). Additionally, he has written a number of books for the general public on a variety of subjects including mammals as well as health issues (Cockrum, 1998).

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LIST OF SPECIMENS EXAMINED

Specimens examined and their geographic localities are given below: TK numbers correspond to samples from the frozen tissue collection at the Natural Science Research Laboratory, Texas Tech University Lubbock; MVZFC numbers correspond to samples from the Museum of Vertebrate Zoology, Berkley, California; ASK numbers correspond to samples from Angelo State Natural History Collection San Angelo, Texas. Numbers given in bold type refer to samples for which we have mitochondrial and nuclear data. TTU numbers (where available) refer to specimen catalog numbers in the Museum, Texas Tech University.

Notiosorex crawfordi.—MEXICO: 10 Miles Southeast El Rosario Baja California, Mexico (MVZFC 4830); 9 miles South Rosarito Baja California Sur, Mexico (MVZFC 4829); UNITED STATES: 4.5 miles West of San Angelo, Tom Green County, Texas (ASK 4530); 5 miles North, 9.8 miles West Mertzon, Irion County, Texas (ASK 4571); Devils River State Natural Area, 29 degrees, 58.4' N, 100 degrees, 58.2' W Val Verde County, Texas (ASK 4277); Leslie Canyon National Wildlife Reserve T21S, R28E Section 20 Northeast 1/4 Cochise County, Arizona (**TK 49919; TTU 82991, TK 49921; TTU 82993**); Chaparral Wildlife Management Area Dimmit County, Texas (**TK 84584; TTU 80965, TK 84585; TTU 80966, TK 84632; TTU 80807**); 8 miles S Post, Garza County, Texas (TK 22986; TTU 40000).

Notiosorex cockrumi.—MEXICO: 14.6 miles East of Mazocahui Sonora, Mexico (MVCFC 2652); 4.1 miles Northwest Nacari Chico Sonora, Mexico (MVZFC 2653); UNITED STATES: Leslie Canyon National Wildlife Reserve T21S, R28E Section 20 NE 1/4 Cochise County, Arizona (**TK 49909; TTU 82981, TK 49910; TTU 82982**, TK 49912; TTU 82984, TK 49914; TTU 82986, TK 49915; TTU 82987, **TK 49917; TTU 82989, TK 49918; TTU 100000, TK 49922; TTU 82994**, TK 49924; TTU 82996, TK 49926; TTU 82998, **TK 49928; TTU 83502**, TK 49929; TTU 83503, TK 49930; TTU 83504, TK 49931; TTU 83505); 1 mile past 1012 Southern Pacific Railroad near Cienega Creek, T16S, R17E, NE1/4 Pima County, Arizona (TK 90849); SE Tucson at exit 275 on I10 (TK 90852).

Sorex cinereus.—COLORADO: Montrose County 2 miles E of Cimarron, Highway 50 (**TK 28042; TTU 41918**).

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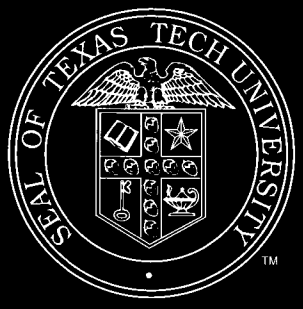
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