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R. S. Pfau

*Tarleton State University, pfau@tarleton.edu*

D. Blake Sasse

*Arkansas Game and Fish Commission*

M. B. Connior

*South Arkansas Community College*

I. F. Guenther

*Arkansas Tech University*

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## Occurrence of *Blarina brevicauda* in Arkansas and Notes on the Distribution of *Blarina carolinensis* and *Cryptotis parva*

R.S. Pfau<sup>1</sup>, D.B. Sasse<sup>2</sup>, M.B. Connior<sup>3</sup>, and I.F. Guenther<sup>4</sup>

<sup>1</sup>Department of Biological Sciences, Tarleton State University, Stephenville, TX 76402

<sup>2</sup>Arkansas Game and Fish Commission, Mayflower, AR 72106

<sup>3</sup>Health and Natural Sciences, South Arkansas Community College, El Dorado, AR 71730

<sup>4</sup>Biological Sciences, Arkansas Tech University, Russellville, AR 72801

<sup>1</sup>Correspondence: pfau@tarleton.edu

### Abstract

We provide an update on the species and distribution of shrews occurring in Arkansas. Shrews were collected within Arkansas Game and Fish Commission Wildlife Management Areas and along the Buffalo National River. We also searched mammal collections at several institutional museums to provide additional locality records for *Cryptotis parva*. Specimens of *Blarina* were identified to species by DNA sequencing of the mitochondrial cytochrome *b* gene. Previously, *Blarina hylophaga* was believed to occur in the northwest corner of Arkansas and *B. carolinensis* throughout the rest of the state. However, our genetic analysis revealed that it is *B. brevicauda* that occupies the northwestern portion of the state. We also document several new county records for *B. carolinensis* and *C. parva* in Arkansas.

### Introduction

According to George et al. (1981, 1982), Garland and Heidt (1989), and Sealander and Heidt (1990), *Blarina hylophaga* (Elliot's short-tailed shrew) occurs in the northwestern corner of Arkansas and *B. carolinensis* (southern short-tailed shrew) occupies the remainder of the state. Initially, 2 of us (IFG and MBC) collected *Blarina* along the Buffalo National River, Newton County, as part of a larger ecological study. Because Newton County is along the probable contact zone between the 2 species, and because species of *Blarina* are difficult to distinguish based on morphology, we performed genetic analyses to determine whether the *Blarina* were *B. hylophaga* or *B. carolinensis*. Surprisingly, the initial specimens genetically matched *B. brevicauda* (northern short-tailed shrew). We then examined additional specimens of shrews from across the state of Arkansas to determine whether these initial specimens represented

an isolated population or were part of a broader distribution.

The purpose of this study was: 1) to determine the specific identity of *Blarina* spp. within Arkansas and 2) to provide additional records of distribution for shrews (*Blarina* spp. and *Cryptotis parva*) from previous small mammal surveys and unpublished museum records.

### Materials and Methods

Shrews (*Blarina* spp. and *Cryptotis parva*) were collected from the Buffalo National River and several Arkansas Game and Fish Commission (AGFC) Wildlife Management Areas (WMAs). To provide a more complete distributional record of shrews in Arkansas, we augmented our sample by investigation of unpublished shrew records from mammal collections at institutional museums.

Shrews were trapped along the Buffalo National River (Newton County) during 2 3-night sessions in April and October 2010 using a combination of a trapping web and pitfall traps in each field trapped. The trapping web consisted of a 128-m diameter web with 8 trap lines originating from the center. Sherman-live traps (7.6 x 8.9 x 22.9 cm) were placed 8 m apart with 2 additional traps located in the center for a total of 66 traps per web. Two drift fences (15 m length) were placed along the periphery of the field with a 19-liter bucket on each side of the fence at 7.5 meters and another at either end for a total of 4 buckets per fence.

Shrews were trapped, using Victor© mouse traps, from the WMAs during 3 4-night sessions from July-September in 2002 and 3 3-night sessions from July-September in 2003 and 2004. Five 150-m transects were placed in different habitat types on each WMA with 2 traps placed at each of 15 stations, spaced 10 m apart along each transect. Voucher specimens (including tissues) collected from WMAs have been

deposited in the Natural Science Research Laboratory, Texas Tech University.

Shrews were examined and identified as either *Blarina* or *Cryptotis* based on dentition (Sealander and Heidt, 1990). For the 52 specimens identified as *Blarina*, the entire mitochondrial cytochrome *b* (*cytb*) gene was amplified by polymerase chain reaction (PCR) using the primers LGL765 (GAA AAA CCA YCG TTG TWA TTC AAC T) and LGL766 (GTT TAA TTA GAA TYT YAG CTT TGG G; Bickham et al. 1995, 2004) and sequenced (with primer LGL 765 only) using a Beckman-Coulter CEQ8000 Genetic Analysis System (Beckman-Coulter, Inc., Fullerton, California). Specimens were initially identified to species using BLAST searches at the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). To verify BLAST identifications, a neighbor-joining tree was constructed from Jukes-Cantor distances of selected specimens using PAUP\*4.0b10 (Swofford 2000) with the PaupUp interface (Calendini and Martin 2005). Reference sequences were included in the phylogenetic analysis, including *B. hylophaga* and *B. brevicauda* (from Kansas and Iowa, respectively; Thompson et al. 2011) and *B. hylophaga*, *B. brevicauda*, and *B. carolinensis* from GenBank (AF395480, AB175134, and AF395457). Selected *cytb* sequences obtained in this study were deposited in GenBank.

In order to rule out the possibility of mitochondrial introgression between species of *Blarina* (and thus confounding identification based on *cytb*), we performed amplified fragment length polymorphism (AFLP) analysis on 2 Newton County specimens identified as *B. brevicauda* (based on *cytb*) and compared them with reference specimens of *B. hylophaga* and *B. brevicauda* (from Kansas and Iowa respectively; Thompson et al. 2011) using principal coordinate analysis (PCoA) implemented in the software GenAlEx 6.4.1 (Peakall and Smouse 2006). AFLP analysis was conducted following Thompson et al. (2011).

Specimens from WMAs are referred to by the specimen (TTU) and tissue (TK) numbers (Natural Science Research Laboratory, Texas Tech University). A specimen housed at the University of Central Oklahoma is referred to by the individual collector number (GMW).

## Results

### Identification of *Blarina* specimens

All specimens of *Blarina* were identified as either *B. carolinensis* or *B. brevicauda* based on 452 base pairs of the *cytb* gene (GenBank accession numbers JF912160-JF912178). Based on phylogenetic analysis of selected specimens (Fig. 1), BLAST searches identified specimens correctly. The 2 specimens of *B. brevicauda* included in the AFLP analysis grouped with reference specimens of *B. brevicauda* in the PCoA (Fig. 2). No specimen was identified as *B. hylophaga* based on *cytb*.

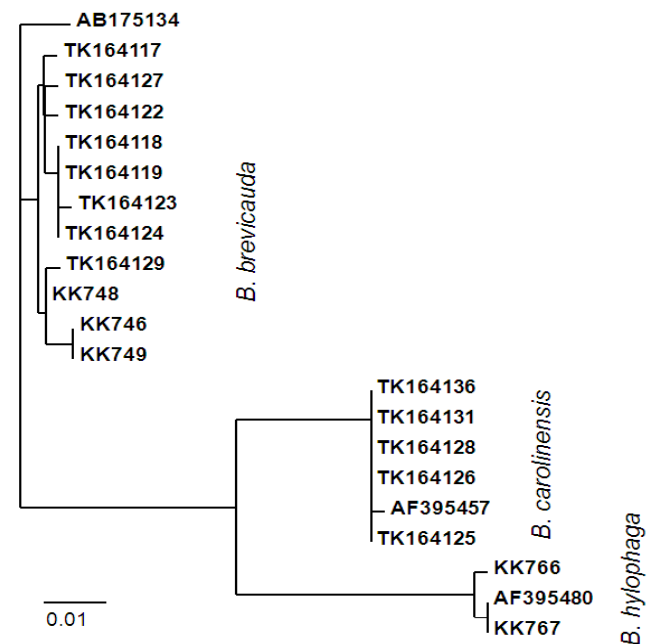


Figure 1. Unrooted neighbor-joining tree based on Jukes-Cantor distances for the cytochrome *b* gene from selected *Blarina* specimens obtained for this study (TK numbers), reference specimens from Thompson et al. 2011 (KK numbers), and GenBank (AB and AF numbers).

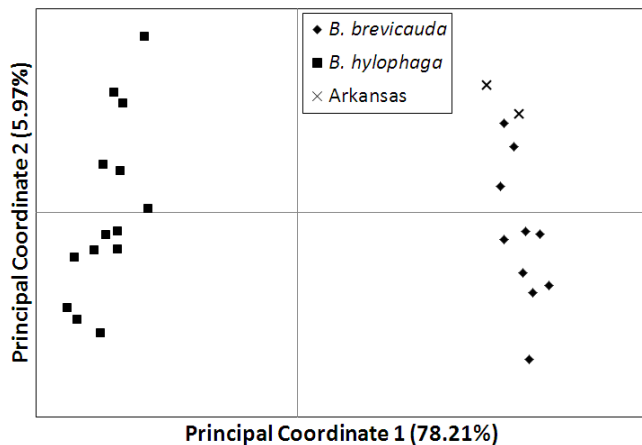
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Figure 2. Plots of the first 2 coordinates of principal coordinate analysis based on AFLPs of 2 Arkansas specimens and reference specimens of *B. hylophaga* and *B. brevicauda*.

### County records of *Blarina brevicauda* (Say) – Northern short-tailed shrew

Twelve of the specimens were identified as *B. brevicauda* based on *cytb* sequences. County records are listed below and shown in Fig. 3.

**Madison County:** Madison County WMA (UTM 15S 435254E 4009705N), 21 July – 19 August 2004, 3 specimens (TTU115289, TK 164118; TTU115290, TK 164119; TTU115288, TK 164123). **Newton County:** Gene Rush WMA (UTM 15S 498268E 3979340N), 13 August 2003, 2 specimens (TTU115291, TK 164116; TTU115292, TK 164117); (15S 501985E 3980908N), 14 August 2003, (TTU115293, TK 164122); Buffalo National River (UTM 15S 485148.30E 3990400.19N), 11 April 2010, 2 specimens (no vouchers). **Pope County:** Piney Creeks WMA, 6 January 2006, (GMW 2666). **Sharp County:** Harold Alexander WMA (UTM 15S 642854E 4010935N), 23 July 2002, (TTU115294, TK 164127); (UTM 15S 639840E 4011859N), 12 August 2002, (TTU115295, TK 164129). **Van Buren County:** Gulf Mountain WMA (UTM 15S 530679E 3936347N), 31 August 2004, (TTU115296, TK 164124).

### New county record of *Blarina carolinensis* (Bachman) – Southern short-tailed shrew

Forty-one of the specimens were identified as *B. carolinensis* based on *cytb* sequences. A single new county record is listed below and shown in Fig. 3 (additional specimens examined that are not new county records are listed in the Appendix).

**Yell County:** Petit Jean River WMA (UTM 15S 477499E 3882712N), 27 July 2003 (TTU115336); (UTM 15S 476497E 3882996N), 11 September 2002 (TTU115337, TK 164125).

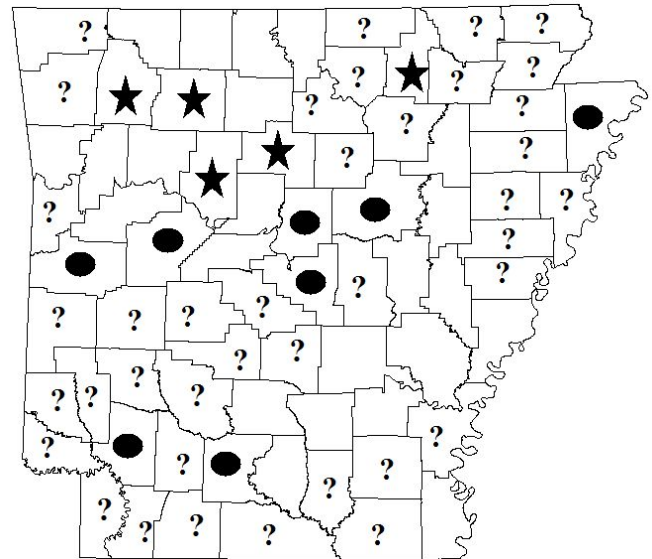


Figure 3. Distribution of *Blarina* in Arkansas. Stars represent *Blarina brevicauda* (confirmed by *cytb*), dots represent *Blarina carolinensis* (confirmed by *cytb*), and question marks represent historical records of genetically unidentified *Blarina* spp.). Historical records are from Connior et al. (2008), Garland and Heidt (1989), Sealander and Heidt (1990), Tumilson et al. (1992), and Tumilson and Robison (2010).

### New county records of *Cryptotis parva* (Say) – Least shrew

New county records for *C. parva* (including unpublished county records from institutional museums) are listed below and shown in Fig. 4.

**Benton County:** 14.5 km (9 mi) N of Benton, 28 October 1962, University of Kansas Museum of Natural History (KUM 92635). **Calhoun County:** 0.6 km (0.4 mi) E Locust Bayou, 03 January 1992, Arkansas State University Museum of Zoology Mammal Collection (ASUMZ 26159). **Clay County:** Piggot, 03 July 1982, (ASUMZ 27222). **Cleveland County:** 14.5 km (9 mi) N of Warren on AR St. Hwy 8, 26 March 1973, Museum of Texas Tech University (TTU 22461). **Logan County:** 17.1 km (11 mi) W New Blaine, jct. SR 197 & SR 22, 20 March 1990, Cornell University Museum of Vertebrates (CU 14774). **Madison County:** Madison County WMA (UTM 15S 435254E; 4009705N), 18 August 2004

(TTU115369). **Mississippi County:** Big Lake WMA (UTM 15S 763134E; 3975196N), 26 September 2002, (TTU115374). **Nevada County:** 6.4 km (4 mi) W Laneburg near Hwy 299, 11 February 1985, (ASUMZ 12948). **Ouachita County:** 3.2 km (2 mi) N Harmony Grove, 24 April 1992, (ASUMZ 26273). **Sharp County:** Harold Alexander WMA (UTM 15S 643140E; 4012969N); 23 July 2002, (TTU115395). **White County:** Russell, 15 November 1994, (ASUMZ 27524).

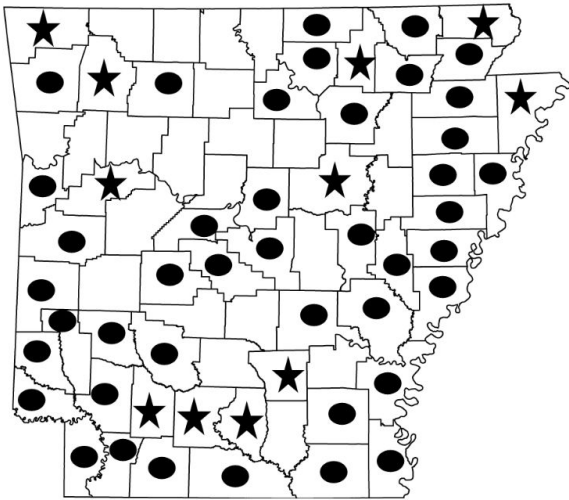


Figure 4. Distribution of *Cryptotis parva* in Arkansas. Stars represent new county records and dots represent historical records. Historical records are from Connor (2010), Garland and Heidt (1989), Sealander and Heidt (1990), Steward et al. (1988), Tumilson and Robison (2010), and Whitsett and Tappe (2009).

## Discussion

### *Identification and distribution of Blarina ssp.*

Based on DNA sequences, we report herein the occurrence of *B. breviceauda* in Arkansas in areas formerly thought to be occupied by *B. hylophaga*. Because the potential exists for historical introgression of the mitochondrial genome between species (and other limitations of using mtDNA to identify species; Moritz and Cicero, 2004), AFLP analysis was conducted on 2 specimens from Newton County to verify the *cytb*-based identifications. AFLPs reveal variation in the nuclear genome, allowing us to independently confirm the identification of specimens identified as *B. breviceauda* based on *cytb*. When compared with AFLP data for *B. hylophaga* and *B. breviceauda* reference specimens (Thompson et al.

2011), the 2 specimens clearly grouped with the *B. breviceauda* reference specimens in a PCoA (Fig. 2), confirming the status of these specimens as *B. breviceauda* and ruling out the possibility of mitochondrial introgression (or other factors) confounding the identifications based on *cytb*.

Because all specimens of *Blarina* were identified as either *B. carolinensis* or *B. breviceauda* in our study (Fig. 1), and no specimen was identified as *B. hylophaga*, it is possible that *B. hylophaga* may not occur in Arkansas. Rather, all specimens historically recognized by previous researchers as *B. hylophaga* within Arkansas are likely *B. breviceauda* (or *B. carolinensis*), but were assumed to be *B. hylophaga* based on George et al. (1981, 1982).

The question remains why George et al. (1981, 1982) concluded that *Blarina* in northwestern Arkansas were *B. hylophaga*. Their conclusions regarding the distributional limits of *Blarina* spp. were based only on morphometric analysis of the skull and mandible, thus it is likely that clinal size variation, in combination with an absence of *B. breviceauda* reference specimens in their study, resulted in erroneous identifications.

Given the distribution of *B. breviceauda* and *B. carolinensis* based on our results (Fig. 3), we hypothesize that, in Arkansas, *B. breviceauda* is restricted to the Ozark Plateau and Boston Mountains, with *B. carolinensis* occupying the remainder of the state. However, the possibility still exists of *B. hylophaga* occurring in portions of western Arkansas as the precise distributional limits of this species remain unknown.

Our results put into question the published distribution of *Blarina* in adjacent states. Thompson et al. (2011) documented *B. breviceauda* as far south as the Missouri river in northeastern Missouri (the southern-most extent of their study). Given that specimens have not been examined genetically from the southern three-fourths of Missouri, it is possible that most of Missouri is occupied by *B. breviceauda* and not *B. hylophaga*, as is currently thought (George et al., 1981; 1982). In Oklahoma, it is thought that *B. hylophaga* occupies the eastern half of the state, with *B. breviceauda* being absent (Claire et al. 1989). However, our results suggest that *B. breviceauda* could occur in eastern Oklahoma.

### *Distribution of Cryptotis parva*

*Cryptotis parva* occurs throughout the central and eastern United States and southward into Central America (Whitaker 1974). This shrew commonly

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inhabits grassy and brushy areas (Whitaker 1974; Sealander and Heidt 1990) but may go undetected, unless specifically targeted, because it is often difficult to trap. We provide several new distributional records confirming that this shrew is widely distributed in Arkansas and may be very abundant locally. For instance, Connior et al. (2008) captured 89 least shrews during 1 trapping season in a drift fence array consisting of 2 33-m drift fences with 8 19 liter pitfall buckets each.

**Conclusions**

The distribution of *B. brevicauda*, formerly treated as *B. hylophaga* in Arkansas (Garland and Heidt 1989), is larger than previously thought. Our results suggest that *B. brevicauda* occurs throughout the Ozarks and is replaced by *B. carolinensis* to the south and east of the Ozark Plateau and Boston Mountains. However, further sampling throughout the Ozark Uplift, Crowley's Ridge (where isolated populations of *B. brevicauda* may occur), the Arkansas River Valley, and the Mississippi Alluvial Plain is needed to determine the present contact zone between *B. carolinensis* and *B. brevicauda*. Our results also show that *Cryptotis parva* is common throughout Arkansas, agreeing with Sealander and Heidt (1990).

**Acknowledgments**

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## Appendix

Specimens of *B. carolinensis* for which *cytb* sequences were obtained, but that were not new county records. Specimen (TTU) and tissue (TK) numbers (Natural Science Research Laboratory, Texas Tech University) are in parentheses.

**Faulkner County:** Bell Slough WMA, 2 specimens (TTU115297, TK164126; TTU115298, TK164128). **Hempstead County:** Hope Upland WMA, 8 specimens (TTU115299-115306). **Ouachita County:** Two Bayou WMA, 9 specimens (TTU115307-115314, TTU115315). **Pulaski County:** Holland Bottoms WMA, 9 specimens (TTU115316, TK164131; TTU115317, TK164136; TTU115318, TK164134; TTU115319, TK164133, TTU115320; TTU115321-115323; TTU115324, TK164138). **Scott County:** Cedar Creek WMA, 3 specimens (TTU115325, 115326, 115327). **White County:** Henry Gray WMA, 8 specimens (TTU115328-115334, TTU115335).