Occurrence of the northern short-tailed shrew (Mammalia: Soricomorpha: Soricidae: *Blarina brevicauda*) in Oklahoma

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Three species of shrews of the genus *Blarina* inhabit the central and eastern U.S. —*B. brevicauda*, *B. hylophaga*, and *B. carolinensis.* These shrews are quite similar morphologically, and prior to 1972 they were considered a single species (*B. brevicauda*). Genoways and Choate (1972) and Genoways et al. (1972) separated *B. brevicauda* into two species (*B. brevicauda* and *B. carolinensis*) by elevating *B. brevicauda carolinensis*. Later, George et al. (1981, 1982) split *B. carolinensis* into two species (*B. carolinensis* and *B. hylophaga*) based on karyotypic and morpholologic data. They described the distribution of *B. brevicauda* as extending south to Platte, Randolph, and Ralls counties of Missouri (George et al. 1982), with a broad area of sympatry with *B. hylophaga* occurring across southern Iowa and northern Missouri. Thompson et al. (2011) examined this purported area of sympatry (using karyotypic and nuclear DNA markers) and found that the two species occurred parapatrically rather than sympatrically. Within their study area, *B. hylophaga* was found to occur in two counties of southern Iowa (Fremont and Page) and three counties of northern Missouri (Atchison, Holt, and Nodoway). Since shrews from the southernmost extent of their study area (Platte, Clay, and Ray counties) were identified as *B. brevicauda*, the southern limit of the range of *B. brevicauda* must be south of those counties. Additionally, Webster et al. (2011) extended the distribution of *B. brevicauda* into northeastern Kansas (based on morphologic data) where it is considered disjunct, occurring in the valleys of the Kaw River and its tributaries in Douglas, Jefferson, and Leavenworth counties.

Pfau et al. (2011) used mitochondrial DNA sequences to discover that *B. brevicauda* actually occurs as far south as northern Arkansas (Madison, Newton, Pope, Sharp, and Van Buren counties). Based on the genetic identification of the specimens examined, Pfau et al. (2011) hypothesized that *B. brevicauda* is restricted to the Ozark Plateau and Boston Mountains of Arkansas with *B. carolinensis* occurring in the remainder of the state. They further hypothesized that *B. brevicauda* may occur in eastern Oklahoma. Since no specimens that they examined from Arkansas were identified as *B. hylophaga*, Pfau et al. (2011) concluded that *B. brevicauda* had been misidentified as *B. hylophaga* in Arkansas since the recognition of *B. hylophaga* as a species by George et al. (1981, 1982), most likely due to size of southern specimens of *B. brevicauda* being more similar to that of *B. hylophaga*.

In Oklahoma, it was thought that *B. hylophaga* occured throughout the eastern two-thirds of the state (Caire et al. 1989; Stangl et al. 1992; Stangl and Carr 1997; Braun and Revelez 2005; McDonald et al. 2006), with *B. carolinensis* restricted to McCurtain and Le Flore counties of southeastern Oklahoma (Caire et al. 1989; Braun et al. 2011). However, as in Arkansas, the identification of specimens was based only on size, which is probably unreliable—at least in the southern parts of their distribution (Pfau et al. 2011). In order to determine the species of *Blarina*

inhabiting Oklahoma and to clarify further the distribution of *B. brevicauda*, *B. hylophaga*, and *B. carolinensis* in the central U.S., we sequenced a portion of the cytochrome *b* gene from museum specimens. Frozen tissue, snips from prepared skins or ribs, or molars from skulls in owl pellets were obtained from specimens deposited in the Oklahoma State University Collection of Vertebrates, the Sam Noble Museum Collection of Mammals, Sam Noble Museum Oklahoma Collection of Genomic Resources, and the University of Central Oklahoma Natural History Museum. See Appendix for details regarding specimens examined.

For specimens represented by tissues, the entire mitochondrial cytochrome *b* (cyt *b*) gene was amplified by polymerase chain reaction (PCR) using the primers LGL765 (5′-gaa aaa cca ycg ttg twa ttc aac t-3′) and LGL766 (5′-gtt taa tta gaa tyt yag ctt tgg g-3′; Bickham et al. 1995, 2004). For specimens represented by skin snips, ribs, or molars, PCR amplifications of the entire cyt *b* gene was assumed to not be possible because of DNA degradation (based on preliminary attempts of a few specimens). Thus, PCR primers were designed to anneal to conserved regions of the cyt *b* gene to amplify short segments. Two internal primers were designed: *Blarina*_cytb_Int-1 (5′-atg ggt gac aga tga aaa ggc ag-3′) and *Blarina*_cytb_Int-2 (5′-ttt gcg tgt aga tag cgg-3′), which, in combination with primer LGL765, were predicted to amplify 204 bp and 254 bp fragments, respectively. Preliminary tests showed that both primers successfully amplified fragments of the correct size. We chose, primarily, to use the Int-1 primer because it amplified the shortest fragment and thus would have the highest likelihood of success with highly degraded samples. All PCR products were sequenced with primer LGL765 using a Beckman-Coulter CEQ8000 Genetic Analysis System (Beckman-Coulter, Inc., Fullerton, California).

Care was taken to minimize the chance of PCR contamination due to the small amounts of degraded DNA extracted from museum specimens. DNA extractions, PCRs, and gel electrophoresis/sequencing reactions were conducted in separate rooms using separate instruments. PCRs were conducted in a fume hood where instruments were exposed to UV radiation. At least four negative control PCRs were used with each set of reactions. Sets of reactions in which negative controls were positive for a PCR product were repeated following bleach or UV treatment of instruments and with new reagents.

Once DNA sequences were obtained, 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 using trimmed sequences of up to 160 bp using PAUP*4.0b10 (Swofford 2000) with the PaupUp interface (Calendini and Martin 2005). Reference sequences were included in the phylogenetic analysis representing *B. hylophaga*, *B. brevicauda,* and *B. carolinensis* from Kansas, Iowa, and Arkansas, respectively (GenBank accession numbers JF912177, JF912169, and JF912173). Selected cyt *b* sequences obtained in this study were deposited in GenBank (accession numbers: KF735659 – KF735665; see Appendix).

All but three specimens of *Blarina* from Oklahoma were identified as either *B. hylophaga* or *B. carolinensis* (Fig. 1). Counties with specimens identified as *B. hylophaga* included Atoka, Cleveland, Comanche, Cotton, Johnston, Kingfisher, Logan, Nowata, Osage, Ottawa, Pawnee, Payne, Pittsburg, Rogers, Tulsa, and Washington (see Appendix). Counties with specimens identified as *B. carolinensis* included Le Flore and McCurtain (see Appendix).

Three specimens were identified genetically as *B. brevicauda* (Figure 1). All three specimens were from the northeastern portion of Oklahoma: two from Muskogee County (OMNH 31929 and OMNH 31930) and the other from Ottawa County (OK 8305; see Appendix). The Muskogee

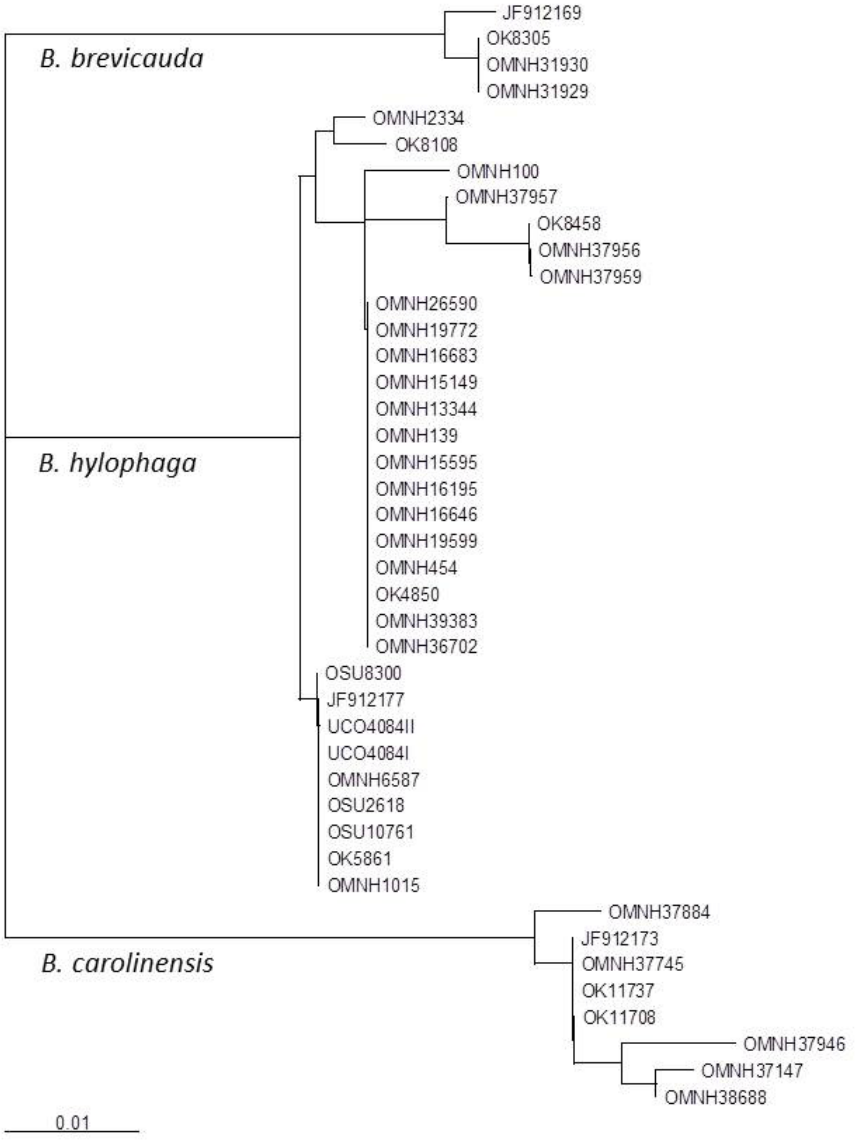

Figure 1. Midpoint-rooted neighbor-joining tree based on Jukes-Cantor distances for the cytochrome *b* gene from *Blarina* specimens obtained for this study and from Genbank (JF912177, JF912169, and JF912173).

County specimens were collected near the western-most extent of the Boston Mountain ecoregion as it transitions into the Central Irregular Plains ecoregion (Woods et al. 2005). The specimen from Ottawa County (OK 8305) was collected near the western-most extent of the Ozark Highland ecoregion (Woods et al. 2005). A second specimen from Ottawa County (OSU 10761), collected just northeast of OK 8305 in the Central Irregular Plains ecoregion, was identified as *B. hylophaga*; thus a contact zone between these two specimens likely occurs in Ottawa County.

These findings represent a significant update to the known distribution of *Blarina brevicauda* in Oklahoma and the central U.S. Prior to this study, only two species of *Blarina* thought to occur in Oklahoma: *B. hylophaga* and *B. carolinensis*. It is now known that a third species, *B. brevicauda*, occurs in the extreme northeast counties of the state. Although sample sizes for this study are limited, the occurrence of *B. brevicauda* relative to geographical and ecological features in Oklahoma corresponds to that in Arkansas, where *B. brevicauda* appears to be restricted to the Ozark Highlands and Boston Mountains ecoregions (Pfau et al. 2011). These results highlight the importance of utilizing molecular data in addition to morphologic, karyotypic, and ecologic data, together with museum voucher specimens, in furthering our knowledge of mammalian species diversity in Oklahoma and the region.

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APPENDIX

List of specimens examined, including locality, voucher/tissue number, and type of tissue from which DNA was extracted. Voucher/tissue numbers refer to the following institutions: University of Oklahoma, Sam Noble Museum, Oklahoma Collection of Genomic Resources (OCGR), University of Oklahoma, Sam Noble Museum, Collection of Mammals (OMNH), Oklahoma State University, Collection of Vertebrates (OK and OSU), and University of Central Oklahoma, Collection of Vertebrates (UCOCV). Genbank numbers (beginning with KF) are listed following specimen numbers.

B. brevicauda

Muskogee Co., Camp Gruber, north of Two Ponds on north side of Hilltop Road and east of Two Ponds road, 35° 45'29.7"N, 95° 09'42.7"W (OMNH 31930 [skin], KF735659); Camp Gruber, LCTA 57, UTM 15S 302460E 3960129N (OMNH 31929 [skin], KF735660). **Ottawa Co.**, 4 mi N, 8 mi E Miami (OK 8305 [frozen tissue], KF735665).

B. carolinensis

Le Flore Co., 3 mi E Honobia (OCGR 5465/OMNH 37147, 5558/37745 [KF735664], 7644/37884, 7696/37946, 8866/38688 [frozen tissue]). **McCurtain Co.**, 1.2 mi N, 5 mi W of Tom in Red Slough WMA (OK 11708 [frozen tissue]); 1.9 mi N, 4.6 mi W of Tom in Red Slough WMA (OK 11737 [frozen tissue], KF735663).

B. hylophaga

Atoka Co., Atoka WMA, Array A off of Chilly Bend Rd. (OCGR 7706/OMNH 37956 [frozen tissue], OCGR 7707/OMNH 37957 [frozen tissue]); Atoka WMA, Array B in public hunting area along D3917 Rd. (OCGR 7709/OMNH 37959 [frozen tissue]). **Cleveland Co.**, 12 mi E Norman (OMNH 15595 [skin]); Norman, Hwy 9, north of Oliver's Woods (OMNH 19772 [skin]); Norman (OCGR 5298/OMNH 36702 [frozen tissue]); 3 mi S, 7.5 mi E Norman (OMNH16646 [skin]). **Comanche Co.**, Wichita Mountains Wildlife Refuge, Cache Gate (OMNH 19599 [skin]); 2 mi S Meers, Wichita Mountains Wildlife Refuge (OMNH 16195 [skin]); 7 mi S, 0.25 mi E Elgin, T2N, R10W, Sec 6, NE 1/4 (OMNH 26590 [ribs]). **Cotton Co.**, Waurika Wildlife Management Area, T3S, R9W, SW1/4, Sec.4 (OK 4850 [frozen tissue], KF735661). **Johnston Co.**, 9 mi E Tishomingo, Blue River (OMNH 15149 [skin], OMNH 16883 [skin]). **Kingfisher Co.**, T16N, R5W, Sec. 27, NW ¼ (OCGR 9465/OMNH 39383 [frozen tissue]). **Logan Co.**, T16N, R4W, Sec 11 (OMNH 100 [skin], OMNH 139 [skin], OMNH 454 [skin]). **Nowata Co.**, 2 mi S, 2 mi E Nowata, Double Creek Cove (OK 8458 [frozen tissue]); 1.5 mi N, 3 mi E Watova (UCOCV 4084-I and UCOCV 4084-II [molars from two skulls in same owl pellet]). **Osage Co.**, Tall Grass Prairie Preserve, 17 mi N Pawhuska (OK 5861 [frozen tissue]). **Ottawa Co.**, 1.5 mi S, 1.5 mi W of Picher (OSU 10761 [skin]). **Pawnee Co.**, 2.3 mi N, 2.5 mi E Jct Hwy 177 and Hwy 15E (OMNH 13344 [skin]). **Payne Co.**, 2 mi N, 9 mi W of Stillwater, Lake Carl Blackwell (OK 8108 [frozen tissue], KF735662). **Pittsburg Co.**, 9 mi SE Eufala (OMNH 2334 [skin]). **Rogers Co.**, 7 mi N of Claremore (OSU 8300 [skin]). **Tulsa Co.**, 7 mi S, 4 mi E Tulsa (OMNH 1015 [skin]). Sequoyah Lake, Tulsa (west end of lake) (OSU 2618 [skin]). **Washington Co.**, Bartlesville (OSU 6587 [skin]).

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