

Molecular Phylogeny of Short-Tailed Shrews, *Blarina* (Insectivora: Soricidae)

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Phylogenetic relationships among the three described species of short-tailed shrews (genus *Blarina*) were inferred based on mitochondrial DNA sequences of 16S rRNA (506 bp) and cytochrome *b* (1137 bp) from 38 specimens representing *B. brevicauda*, *B. hylophaga*, and *B. carolinensis*, from across their range in North America. Phylogenetic analyses of both data sets combined followed tests showing lack of incongruence between these fragments. Analysis of substitution patterns indicated saturation of transitions at third codon positions in cytochrome *b* when *Blarina* sequences were compared to those of *Sorex* and *Cryptotis*, used as outgroups. Maximum-likelihood and weighted parsimony supported the monophyly of the genus and placed *B. hylophaga* as its basal branch, sister to *B. brevicauda* + *B. carolinensis*. Phylogeographic analysis revealed a significant partition between eastern and western populations of *B. carolinensis* and *B. brevicauda*, on either side of the Mississippi basin. These results are discussed in relation to cytogenetic, morphological, and fossil data.

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Key Words: shrews; phylogeny; *Blarina*; mitochondrial DNA.

INTRODUCTION

Species of *Blarina* (Soricidae: Insectivora) are relatively common large semifossorial soricids which inhabit a broad range of habitats in the eastern half of North America (Fig. 1). Like all insectivores, they have a high metabolic rate and feed routinely on insects, snails, earthworms, and small vertebrates (George *et al.*, 1986; Genoways and Choate, 1998). Although species of *Blarina* are ubiquitous over much of their range, they are found only in areas where the moisture content is sufficient to keep the air in the soil and litter saturated (Getz, 1961). While species of *Blarina* have a rather uniform external morphology, they vary geographically in size and karyotype and several taxonomic revisions have been published since their original descriptions (Merriam, 1895; Genoways and

Choate, 1972; George *et al.*, 1981; Moncrief *et al.*, 1982; George, 1986). Morphological and chromosomal data support the designation of three species of short-tailed shrews. From largest to smallest body size, these are the northern short-tailed shrew, *Blarina brevicauda* (Say, 1823), Elliot's short-tailed shrew, *B. hylophaga* Elliot 1899, and the southern short-tailed shrew, *B. carolinensis* (Bachman, 1837). Presently, the three species have nearly parapatric distributions (Fig. 1) and in some cases they are known to hybridize along their contact boundaries (Genoways and Choate, 1972; Ellis *et al.*, 1978; Tate *et al.*, 1980; French, 1981; Braun and Kennedy, 1983; Qumsiyeh *et al.*, 1997; Benedict, 1999a,b).

Several studies have suggested that *Cryptotis parva* is the sister group to *Blarina* and that *Sorex* is the closest taxon to *Blarina* + *Cryptotis* (George, 1986; Tolliver and Robbins, 1987; Ruedi, 1998; Taylor, 1998). In agreement with the first appearance of fossil *Cryptotis*, close to nine million years ago (Mya) (Harris, 1998), *Adeloblarina*, the putative ancestral stock of short-tailed shrews, has been described from late Miocene deposits (5–10 Mya) of North America (Jones *et al.*, 1984; Harris, 1998). Specimens of *Blarina* are poorly known from the fossil record earlier than the Pleistocene. The earliest specimens that morphologically resemble present-day *B. brevicauda* were recovered from two-million-year-old Pleistocene faunas. These have been shown to have a narrowly sympatric distribution with fossil *B. carolinensis* found across the eastern half of North America during the early Pleistocene (Graham and Semken, 1976; Jones *et al.*, 1984). Throughout the Pleistocene, the distribution and differentiation of *Blarina* was affected by four major glacial and interglacial periods, but the most recent Wisconsin glacial event (20,000–40,000 years ago) may have had the most significant effect in shaping the current distribution pattern of *Blarina* (Graham and Semken, 1976; Jones *et al.*, 1984). Fossils resembling *B. hylophaga*, sympatric with some *B. carolinensis* in the Texas fauna, were assigned to early Wisconsin localities from Texas and Missouri as early as 35,000 years ago (Jones *et al.*, 1984). Fossil evidence also

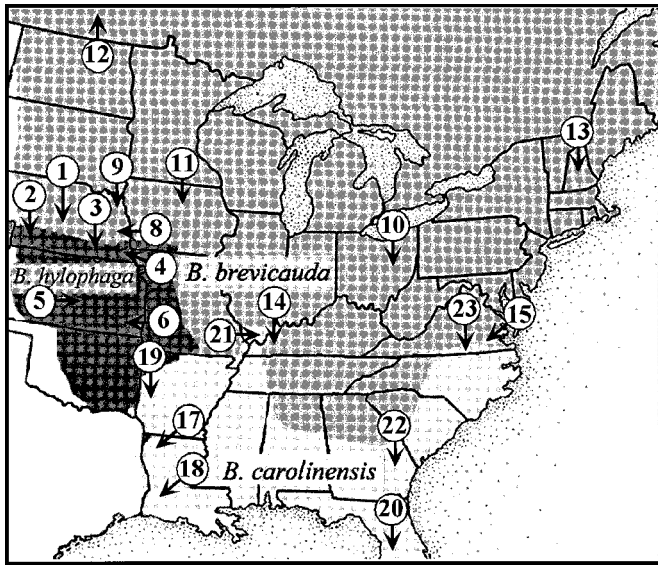


FIG. 1. Geographic distribution for three species of the genus *Blarina* showing sampling localities (numbers as in Table 1; George *et al.*, 1981; Genoways and Choate, 1998; Wilson and Ruff, 1999).

suggests that *B. carolinensis* was divided into eastern and western populations shortly after the Wisconsin glaciation and that subsequent subdivision may have given rise to *B. hylophaga* (Jones *et al.*, 1984). The fossil record of *Blarina* indicates a faunal succession that may not necessarily reflect the phylogenetic succession.

Classification and evolution of short-tailed shrews have been investigated extensively based on a variety of data and methods (e.g., Genoways and Choate, 1972, 1998; George *et al.*, 1981, 1982), yet no publication so far included an explicit phylogenetic analysis to evaluate relationships among the three species. Herein, we present a hypothesis of the phylogenetic relationships among the species in the genus *Blarina* using *Cryptotis* and *Sorex* outgroups, based on a cladistic analysis of two regions of the mitochondrial genome. Results are compared to morphological, paleontological, and cytogenetic studies.

MATERIALS AND METHODS

Specimens Examined

Specimens analyzed represent the three species of *Blarina* and the two outgroups. We collected data for 38 individuals representing five species and 19 populations (Table 1). Outgroup taxa included 2 individuals of the masked shrew (*Sorex cinereus*) and 2 individuals of the least shrew (*C. parva*). *Blarina* can be differentiated easily from *Sorex* by its characteristic short tail and from *Cryptotis* in that the latter lacks one unicuspid (George *et al.*, 1986). Short-tailed shrews were trapped in Sherman live traps, baited with peanut

butter and set along small mammal runs. *B. brevicauda* and *B. hylophaga* were trapped along roadside ditches with dense grass. *B. carolinensis* were trapped in mixed deciduous forest along fallen logs, mole holes, and small mammal runs in dense ground cover. Tissue was removed from trapped animals, placed immediately in 95% ethanol, and stored at -20°C for subsequent analysis.

Laboratory Procedures

Total DNA was isolated from approximately 0.1 g of abdominal muscle tissue by standard proteinase K digestion and phenol/chloroform extraction (Sambrook *et al.*, 1989). Genomic DNA was purified using ethanol precipitation, by incubating at -20°C overnight, centrifuging for 20 min at maximum speed, rinsing the pellet with 70% ethanol, and finally resuspending in 50 μl of ddH₂O.

Two fragments of the mitochondrial genome were amplified using the polymerase chain reaction (PCR) (Saiki *et al.*, 1985). Conditions for amplification were as follows: 0.2 units *Taq* polymerase (Gibco), 1 \times reaction buffer (Gibco), 3 mM MgCl₂, 0.2 mM each dNTP, 0.4 μM each primer, and 100–500 ng of genomic DNA in a 25- μl final reaction volume. The complete cytochrome *b* gene and a fragment of the large subunit (16S) ribosomal DNA were sequenced for all samples listed in Table 1. The following primers were used for PCR and sequencing: 16S, 16S-L: 5'-CGCCTGTTTATCAAAA-CAT-3' and 16S-H: 5'-CCGGTCTGAACTCAGATCAC-GT-3' (Palumbi, 1996); cytochrome *b*, L14724: 5'-CG-AAGCTTGATAGAAAACCATCGTTG-3' and H15915: 5'-AACTGCAGTCATCTCCGGTTTACAAGAC-3' (Irwin *et al.*, 1991); plus an internal sequencing primer, cytbr1: 5'-GCTTCGTTGTTTGGAGGT-3'. PCR conditions for 16S amplifications were initial denaturing step at 94 $^{\circ}\text{C}$ for 1 min followed by 30 cycles of 94 $^{\circ}\text{C}$ (for 45 s), 55 $^{\circ}\text{C}$ (for 55 s), and 72 $^{\circ}\text{C}$ (for 55 s), and then a final extension step of 72 $^{\circ}\text{C}$ (for 7 min). PCR conditions for cytochrome *b* were similar but the annealing step was at 50 $^{\circ}\text{C}$ (for 45 s) and the extension step was at 72 $^{\circ}\text{C}$ (for 1 min 30 s). PCR products were visualized on a 0.8% agarose gel and then purified using ethanol precipitation. Approximately 50 ng of the purified PCR product was used for sequencing with each primer using the BigDye terminator kit and determined directly with a ABI 310 analyzer (Applied Biosystems). Forward and reverse strands were determined for consistency and the nucleotide sequence data obtained have been deposited in GenBank (Table 1).

Phylogenetic Analysis

The 16S rRNA sequences were aligned with ClustalW (Gibson *et al.*, 1996) using default gap penalties. Cytochrome *b* sequences were aligned by eye based on the amino acid sequences since no indels were observed. The final alignment of the mitochondrial re-

TABLE 1
Taxa Included in This Study

Specimens	ID No.	Collection locality (County, State)	Latitude–longitude (N; W)	Collection date	GenBank Accession No.
<i>Blarina brevicauda</i>					
BRE-8 ^a	1007	Lancaster, Nebraska	40.8585; 96.8573	Nov. 1997	AF395464
BRE-9	1027	Dixon, Nebraska	42.5909; 96.7537	Nov. 1997	AF395465
BRE-1	1042	Valley, Nebraska	41.6856; 98.9256	Nov. 1997	AF395461
BRE-10	1091	Wooster, Ohio	40.8045; 81.9834	May 1998	AF395469
BRE-10	1094	Wooster, Ohio	40.8045; 81.9834	May 1998	AF395472
BRE-12	1096	Manitoba, Canada	49.1000; 95.9833	Jul. 1998	AF395462
BRE-12	1097	Manitoba, Canada	49.1000; 95.9833	Jul. 1998	AF395463
BRE-11	1132	Allamakee, Iowa	43.1000; 91.1833	Aug. 1994	AF395466
BRE-14	1221	Trigg, Kentucky		Aug. 1998	AF395467
BRE-14	1223	Trigg, Kentucky		Aug. 1998	AF395468
BRE-13	1129	Grafton, New Hampshire	43.6761; 72.0610	Sept. 1998	AF395470
BRE-13	1152	Grafton, New Hampshire	43.6761; 72.0610	Sept. 1998	AF395471
BRE-15	1318	James City, Virginia	37.2301; 76.6230	Feb. 2000	AF395473
BRE-15	1320	James City, Virginia	37.2301; 76.6230	Feb. 2000	AF395474
<i>Blarina hylophaga</i>					
HYL-3	1046	Nuckolls, Nebraska	40.1750; 98.0858	Dec. 1997	AF395475
HYL-3	1054	Nuckolls, Nebraska	40.1750; 98.0858	Dec. 1997	AF395480
HYL-2	1064	Lincoln, Nebraska	41.0789; 100.5244	Nov. 1997	AF395481
HYL-2	1065	Lincoln, Nebraska	41.0789; 100.5244	Nov. 1997	AF395478
HYL-4	1119	Richardson, Nebraska	40.1134; 95.6989	Jan. 1999	AF395479
HYL-4	1124	Richardson, Nebraska	40.1134; 95.6989	Jan. 1999	AF395476
HYL-5	1180	McPherson, Kansas	38.5649; 97.7278	Sept. 1999	AF395477
HYL-6	1185	Montgomery, Kansas	38.5649; 97.7278	Sept. 1999	AF395482
<i>Blarina carolinensis</i>					
CAR-22	41	Georgia			AF395450
CAR-22	33	Georgia			AF395449
CAR-18	1155	Vernon, Louisiana	30.9964; 92.9508	Apr. 1999	AF395457
CAR-17	1154	Webster, Louisiana	32.5063; 93.3951	Apr. 1999	AF395455
CAR-19	1156	Polk, Arkansas	34.3698; 94.0310	Apr. 1999	AF395459
CAR-19	1157	Polk, Arkansas	34.3698; 94.0310	Apr. 1999	AF395456
CAR-20	1300	Highlands, Florida	27.1943; 81.3459	Apr. 2000	AF395453
CAR-20	1301	Highlands, Florida	27.1943; 81.3459	Apr. 2000	AF395454
CAR-23	1309	Lancaster, Virginia	37.6947; 76.3738	June 2000	AF395451
CAR-23	1311	Lancaster, Virginia	37.6795; 76.3422	June 2000	AF395452
CAR-21	1207	Jackson, Illinois		Apr. 1999	AF395460
CAR-21	1208	Jackson, Illinois		May 1998	AF395458

^a Numbers refer to collection localities in Fig. 1.

gions included 506 bp of 16S rRNA and 1137 bp of cytochrome *b*. Phylogenetic analyses were conducted for each data partition and the combined data set using PAUP* version 4.0b4 (Swofford, 2000). Maximum-parsimony (MP) analyses were performed using heuristic searches starting with stepwise addition trees and replicated 500 times, with each replicate starting with random input order of sequences. Branch swapping was performed by tree bisection–reconnection (TBR). Bootstrap analysis (Felsenstein, 1985) with 1000 pseudoreplicates was used to measure support of the resulting topologies; each pseudoreplicate used heuristic searches with a single replication (starting tree obtained by random stepwise addition of taxa and TBR branch swapping). An optimal model of nucleotide evolution for maximum-likelihood (ML) and minimum-evolution (ME) analyses was determined by using

Modeltest 3.0 (Posada and Crandall, 1998) for each of the two mitochondrial data sets and for the combined data set.

Following Wiens and Hollingsworth (2000), congruence between the two data partitions (16S + cytochrome *b*) was examined in three ways. (1) Data sets were analyzed separately and support for major clades was estimated using bootstrap analysis. Although not a statistical test of congruence, clades supported by both methods can be identified. (2) The partition homogeneity test (Farris *et al.*, 1994) using 1000 iterations was implemented to assess heterogeneity among data sets. This test evaluates the incongruence index (Mickevich and Farris, 1981) for the data sets when analyzed separately and then randomly partitions the combined data to generate a null distribution, assuming no significant conflict. Heuristic searches were per-

TABLE 2

Range and Mean Percentage Differences among DNA Sequences, within and among Taxa

Taxa	Cytochrome <i>b</i>		16S	
	Range	Mean	Range	Mean
BRE-BRE	0.18–2.9	1.7	0–2.0	0.71
CAR-CAR	0.08–3.9	2.3	0–3.6	1.45
HYL-HYL	0.0–4.3	1.6	0–1.0	0.35
BRE-CAR	6.5–8.5	7.2	1.0–4.3	2.1
HYL-CAR	6.2–9.2	7.1	1.2–4.2	1.9
BRE-HYL	8.6–11.4	9.4	1.2–3.0	1.7
BRE-CRY	15.0–16.0	15.5	6.1–7.3	6.7
BRE-SRX	15.7–17.0	16.3	8.7–10.5	9.4
BRE-OG	15.0–17.0	15.9	6.1–10.5	8.0
CAR-CRY	13.5–14.4	14.1	5.7–8.1	6.2
CAR-SRX	17.1–17.8	17.3	8.5–10.9	8.9
CAR-OG	13.5–17.8	15.7	5.7–10.9	7.6
HYL-CRY	14.5–18.2	15.3	5.5–6.3	5.8
HYL-SRX	16.4–18.4	17.4	8.3–9.1	8.6
HYL-OG	14.5–18.4	16.3	5.5–9.1	7.2

Note. BRE, *Blarina brevicauda*; CAR, *B. carolinensis*; HYL, *B. hylophaga*; CRY, *Cryptotis parva*; SRX, *Sorex cinereus*; OG, outgroups combined.

formed with stepwise addition trees with one random addition sequence replicate each and TBR branch swapping. A nonsignificant *P* value suggests that the data sets can be combined. (3) A constrained MP and ML search was completed to test whether a given data set significantly rejected the best tree from another data set using the Kishino and Hasegawa test for MP and ML (Kishino and Hasegawa, 1989) and Templeton's nonparametric test of parsimony (Templeton, 1983) using tree scores option in PAUP*.

To assess potential saturation of substitutions at third codon positions of the cytochrome *b* sequences, the numbers of transitions (TS) and transversions (TV) at third codon positions were plotted against the uncorrected *p*-distances for all pairwise comparisons. Tree-based distances were used to infer approximate dates of divergence. Node-to-tip distances for the main splitting events in the phylogeny, both between and within species, were calculated using ML.

RESULTS

General Pattern of Sequence Variation

A total of 1137 bp, comprising the complete coding sequence of cytochrome *b*, were analyzed for all 38 specimens. Base composition of these sequences was skewed toward a deficiency in guanine (12%), while the other three nucleotides were more balanced (thymine 29%, cytosine 29%, and adenine 30%). Analysis of the base content at each codon position revealed equal base frequencies (ca. 25%) at the first codon position, a high thymine content at the second codon position (42%),

and a deficiency in guanine content at the third codon position (ca. 5%). However, base composition across taxa and for each codon position was homogeneous ($\chi^2 = 27.72$, $df = 111$, $P = 1.0$). Animal mtDNA is typically biased in base composition and the pattern found in this study is consistent with the one seen in the cytochrome *b* of most vertebrates, including some insectivores (e.g., Kocher *et al.*, 1989; Irwin *et al.*, 1991; Taberlet *et al.*, 1994; Fumagalli *et al.*, 1999). Genetic differences among sequences (*p*-distances = proportion of different sites between two sequences) ranged from 0 to 4.3% within species, from 6.2 to 11.4% among *Blarina* species, and around 16% between *Blarina* and the outgroup (Table 2). Intraspecific divergences observed for *Blarina* fall into a typical range observed for intraspecific cytochrome *b* comparisons in mammals and other insectivores (e.g., Avise, 1994; Fumagalli *et al.*, 1999).

Evaluation of saturation of substitutions at third codon positions of the cytochrome *b* sequences showed that TS and TV substitutions within ingroup taxa accumulate almost linearly with increasing *p*-distance (positive slope) but that the TS substitutions between *Blarina* and the outgroup exhibit saturation, evidenced by the more or less constant number of TS despite increasing *p*-distance (Fig. 2). TS saturation becomes most apparent at *p*-distances above 15%, a value consistent with the pattern typically observed in many mammalian groups including, e.g., some insectivores (Irwin *et al.*, 1991; Fumagalli *et al.*, 1999). Therefore, a weighting scheme excluding or down-weighting TS at third codon positions seemed justified for MP analysis when using outgroup taxa to root the *Blarina*

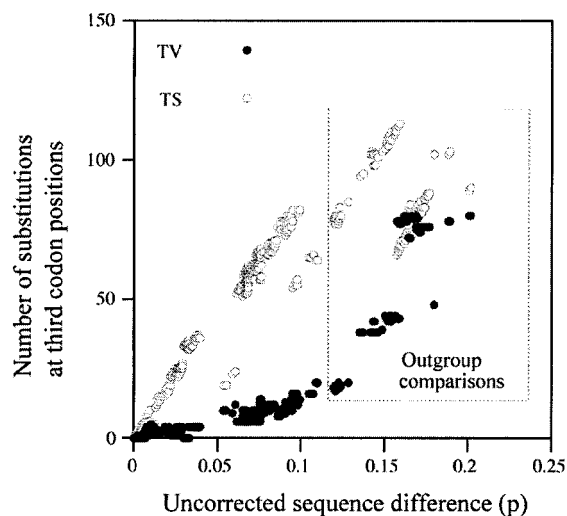


FIG. 2. Substitution pattern of cytochrome *b* gene at third codon positions. The number of transition (TS) and transversion (TV) substitutions at third codon positions is plotted against uncorrected sequence divergence (*p*) considering all sites. Each point represents a pairwise comparison among two taxa. Comparisons among outgroup and ingroup taxa show TS saturation.

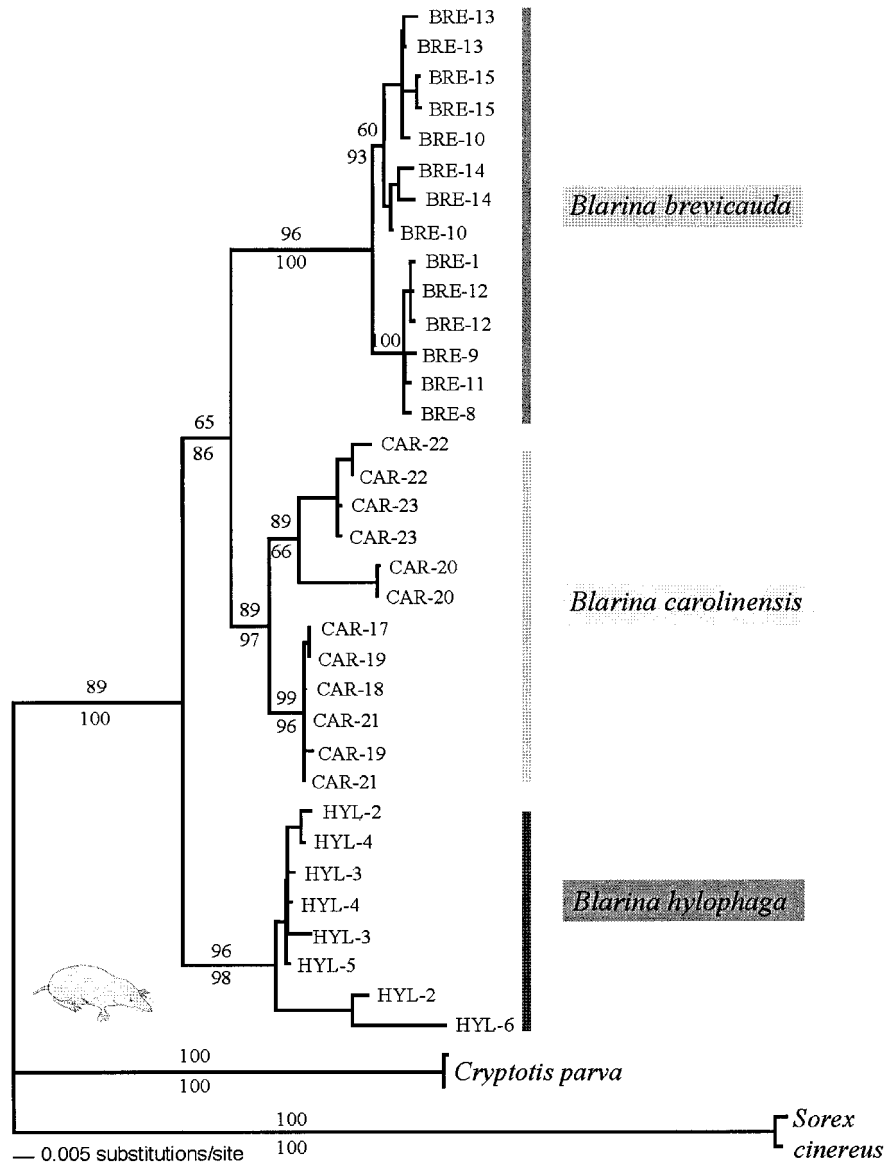


FIG. 3. Maximum-likelihood tree for *Blarina* based on cytochrome *b* + 16S rRNA sequences based on TrN+gamma model (see parameters in text). Bootstrap values (50% majority rule) for ML are shown above the branches; percentages below the branches are bootstrap values of the MP analysis of combined data excluding the third codon position TS. Letters and numbers follow the species and locality, respectively, following Table 1.

tree (i.e., to determine which of the three species is basal).

The cytochrome *b* data set contained 762 bp constant sites, 39 variable and parsimony-uninformative sites, and 336 parsimony-informative sites. This variation defined 34 different haplotypes among the 34 ingroup taxa. When TS at third codon positions were excluded, the number of informative characters for parsimony only decreased to 331. However, there were 346 TS of 491 changes at third codon positions reconstructed on the tree shown in Fig. 3 (using MacClade; Maddison and Maddison, 2000). The HKY+G model was selected by Modeltest as the best fit for the cytochrome *b* data

set (Hasegawa *et al.*, 1985; Posada and Crandall, 1998). Parameters estimated for this model were TS/TV = 3.4426, base frequencies: A = 0.3021, C = 0.2900, G = 0.1227, T = 0.2853; gamma distribution shape parameter = 0.2805.

Among the 34 ingroup taxa, 17 different haplotypes were identified for the 16S gene fragment. An alignment of length 506 bp that had no regions of ambiguous gap assignments was obtained with ClustalW. Percentage nucleotide composition of the 16S rRNA fragment among the shrews was relatively balanced among nucleotides, with 21% guanine, 26% thymine, 23% cytosine, and 31% adenine, and base composition

across taxa was homogeneous ($\chi^2 = 3.64$, $df = 111$, $P = 1.0$). Genetic differences ranged from 0 to 3.6% within species, from 1.0 to 4.3% among species, and around 8% between *Blarina* and the outgroup (Table 2). For this data set, 416 bp sites were constant, 22 sites were variable but parsimony uninformative, and only 68 sites were parsimony informative when alignment gaps were treated as "missing." Treating alignment gaps as a fifth base only increased the number of informative sites to 75 but resulted in the same tree topologies with similar bootstrap values (results not shown). The TrN+G model, with substitution types divided into transversions, transitions between purines and transitions between pyrimidines, plus gamma-distributed rates, was selected by Modeltest as the best fit for ML and ME searches (Tamura and Nei, 1993; Posada and Crandall, 1998). Rate matrix parameters estimated from Modeltest were $R(a) = 1.0$, $R(b) = 1.4423$, $R(c) = 1.0$, $R(d) = 1.0$, $R(e) = 5.9041$, $R(f) = 1.0$; base frequencies were $A = 0.3251$, $C = 0.2239$, $G = 0.1969$, $T = 0.2524$, and gamma distribution shape parameter = 0.2104.

Phylogenetic Inference

We used three measures of congruence to assess homogeneity between the two data partitions: first we analyzed each data set separately and compared the results, second we performed a partition homogeneity test, and third we compared tree topologies from the separate and combined analyses.

Maximum-parsimony analysis of cytochrome *b* sequences resulted in 24 equally parsimonious trees (615 steps) when all substitutions were equally weighted. When the third codon position TS were excluded, MP analysis resulted in 32 equally parsimonious trees (380 steps; similar to the tree shown in Fig. 3). In both cases; topological differences among the equally parsimonious trees involved closely related (intraspecific) terminals, but otherwise haplotypes from each species formed tight monophyletic groups. The major topological difference between these two weighting schemes (with or without third codon position TS) concerned the rooting of the ingroup: the placement of *B. brevicauda* (all substitutions considered) versus *B. hylophaga* (no TS at third codon positions) as the basal branch of the ingroup. Under weighted parsimony, the sister grouping of *B. brevicauda* + *B. carolinensis* received a bootstrap support of 63% and under equal weights, the alternative *B. hylophaga* + *B. carolinensis* received a bootstrap value of 61%. The HKY+G model was used for the ML analysis of cytochrome *b*, which supported the grouping of *B. brevicauda* + *B. carolinensis*, in agreement with weighted MP analysis excluding TS at third codon positions (but with bootstrap support <50%). The topology of the ME tree was identical to that of the ML tree. The monophyly of each of the three species was well supported by all analyses.

Maximum-parsimony analysis of the 16S rRNA sequences resulted in 30 equally parsimonious trees (123 steps). The strict consensus tree topology was unresolved for *Blarina* relationships, but the ingroup and each species were well supported by bootstrap analysis (data not shown). The TrN+G model selected by Modeltest was used for ML and ME searches (Tamura and Nei, 1993; Posada and Crandall, 1998). The most likely tree under this model supported a basal clade with *B. hylophaga*, sister to *B. brevicauda* + *B. carolinensis*, similar to that shown in Fig. 3. However, the *B. carolinensis* samples from Florida grouped within *B. brevicauda* in both ML and MP analyses. The topology of the ME tree was identical to that of the ML tree. The relatively conserved 16S rRNA sequences were not informative in revealing the basal species for the ingroup; therefore no substantial conflict in phylogenetic signal could be deduced from separate analyses of the data partitions.

In agreement with this observation, the partition homogeneity test (our second measure of congruence) failed to reject the null hypothesis that the cytochrome *b* and 16S data sets are significantly different from random partitions of the pooled data ($P = 0.4570$; Farris *et al.*, 1994). Since no substantial topological difference among data sets was found, the third criterion to assess character congruence among data partitions could not be applied and we therefore combined both data sets for all subsequent phylogenetic analysis. Because third codon position TS are saturated (Fig. 2) and were shown above to potentially misguide the analysis, they were excluded from the combined MP analysis. This analysis resulted in 32 equally parsimonious trees (380 steps) with relatively high bootstrap support for most branches (Fig. 3). Topological differences among the equally parsimonious trees and the tree in Fig. 3 involved closely related (intraspecific) terminals. The MP bootstrap analysis of the combined data set excluding third codon TS resulted in 86% bootstrap support for *B. brevicauda* + *B. carolinensis*, placing *B. hylophaga* at the base of the ingroup (Fig. 3). Using equal weights for all substitutions the same topology was obtained, albeit with a bootstrap value for the *B. brevicauda* + *B. carolinensis* clade <50%.

Given the different outcomes of Modeltest for each data partition, a mixed model for ML analysis would be preferable for the combined cytochrome *b* and 16S data sets, but this option is not currently available in PAUP*. Therefore, we used Modeltest again to find the best-justified model for the concatenated data set. The TrN+G model was selected with rate matrix parameters $R(a) = 1.0$, $R(b) = 4.5815$, $R(c) = 1.0$, $R(d) = 1.0$, $R(e) = 6.7196$, $R(f) = 1.0$, base frequencies $A = 0.3059$, $C = 0.2712$, $G = 0.1460$, $T = 0.2769$, and gamma distribution shape parameter = 0.2612. Using this model, the resulting ML tree topology showed strong support both for the monophyly of the ingroup and

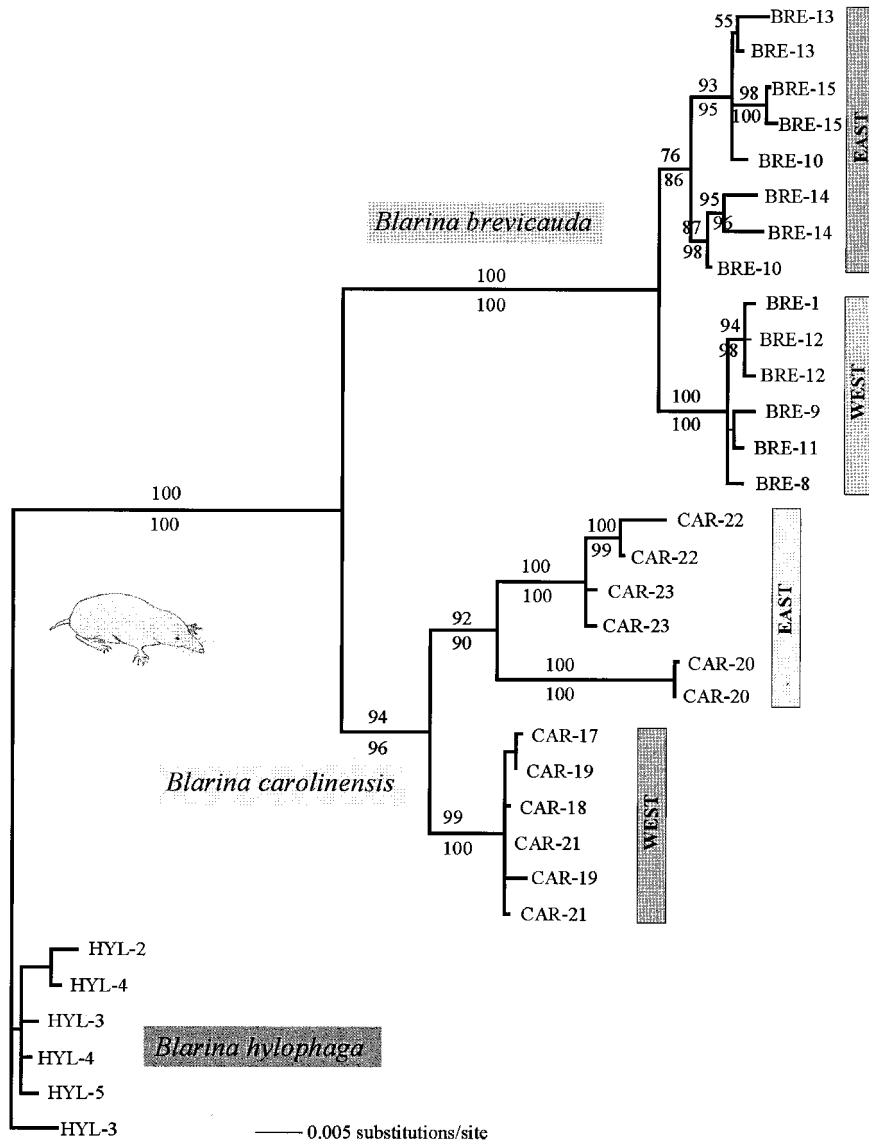


FIG. 4. Maximum-likelihood tree for the ingroup analysis of *Blarina* based on cytochrome *b* + 16S rRNA sequences. Bootstrap values (50% majority rule) for ML are above the branches and the bootstrap values below the branches are from MP including third codon position TS. Letters and numbers follow the species and locality, respectively, following Table 1.

each of the three species of shrews and for the placement of *B. hylophaga* in a basal position relative to *B. brevicauda* + *B. carolinensis* (Fig. 3). The topology of the ME tree was identical to that of the ML tree.

Exclusion of saturated TS changes at third codon positions to obtain an unbiased rooting for the ingroup was justified by analysis of substitution patterns (Fig. 2). However, with this weighting scheme a significant amount of variation that may otherwise provide phylogeographic information by resolving relationships among haplotypes within each species is not used. Unweighted parsimony and ML analysis of the ingroup were run using the combined data set with *B. hylophaga* as the outgroup (based on the previous anal-

yses; Fig. 3). Maximum-parsimony analysis resulted in 16 equally parsimonious trees (392 steps). The tree topology supports east and west clades for both *B. carolinensis* and *B. brevicauda*. Topological differences among the equally parsimonious trees involved closely related taxa within each of the well-supported east and west clades (i.e., nodes with no bootstrap support or polytomies). The TrN+G model was used for both the ML and the ME searches which each supported the east and west clades of both *B. carolinensis* and *B. brevicauda* (Fig. 4). The bootstrap values for MP are recorded on the ML tree (Fig. 4).

Node-to-tip distances for the main splitting events in the phylogeny, both between and within species, were

TABLE 3

Comparisons of Cytochrome *b* Sequence Differences and Dates of Divergences among Ingroup Clades

Taxonomic divergences	Kimura two-parameter sequence divergences ^a	Mean absolute (%) genetic differences across all sites	Node-to-tip distance based on ML for all substitutions ^b	Date of divergence (Myr)
<i>Blarina</i> / <i>Cryptotis</i>	0.0815	15	0.08039	9 ^c
<i>B. hylophaga</i> / <i>B. carolinensis</i> + <i>B. brevicauda</i>			0.04134	4.6
<i>B. brevicauda</i> / <i>B. carolinensis</i>	0.0773	7.2	0.0333	3.7
<i>B. brevicauda</i> / <i>B. hylophaga</i>	0.107	9.4		
<i>B. carolinensis</i> / <i>B. hylophaga</i>	0.0809	7.1		
<i>B. carolinensis</i>				
E/W	0.0351	3.4	0.01902	2.13
Within East	0.0201	2.0		
Within West	0.0253	0.2		
<i>B. brevicauda</i>				
E/W	0.0256	2.5	0.00192	1.02
Within East	0.0104	1.02		
Within West	0.0048	0.5		

Note. E, eastern clades; W, western clades (Fig. 4).

^a Kimura (1980).

^b Substitution/site.

^c Based on earliest fossils of *Cryptotis* at 9 Mya (Harris, 1998); therefore substitution rate = 0.08039/9 My = 0.009 substitutions/site/My.

calculated using ML. These tree-based distances were used to infer approximate dates of divergence, given an independent dating based on fossil evidence. Using the first appearance of fossil *Cryptotis* dated at 9 Mya (Harris, 1998) as an external calibration point, we obtained the following dates of divergence: *B. hylophaga* versus *B. brevicauda* + *B. carolinensis* diverged 4.6 Mya and *B. brevicauda* and *B. carolinensis* diverged 3.7 Mya. These and other measures of divergence are given in Table 3.

DISCUSSION

The present analysis supports the conclusion of a monophyletic origin for the genus *Blarina*. Also, the mtDNA genealogy clearly clusters haplotypes from each species into well-supported monophyletic groups (Fig. 3). Furthermore, within the *B. brevicauda* and *B. carolinensis* clades, a manifest subdivision of haplotypes reveals eastern and western geographic structuring (Fig. 4). The distinctive monophyly of each species stands out despite reported instances of hybridization among *Blarina* species along several contact zones (Genoways and Choate, 1972; Ellis *et al.*, 1978; Tate *et al.*, 1980; French, 1981; Braun and Kennedy, 1983; Qumsiyeh *et al.*, 1997; Benedict, 1999a,b). Even though a relatively dense sampling was done close to the contact zone between *B. hylophaga* and *B. brevicauda* in Nebraska, no instance of introgression of mtDNA among species was detected (i.e., heterospecific mtDNA haplotypes were not observed in morphologically defined species). It should be noted that species identifications

for this study were based on morphological criteria and performed prior to the molecular analysis. Interspecific hybrids with intermediate morphology are not uncommon along a narrow contact zone between *B. hylophaga* and *B. brevicauda* but they may have low fertility (Benedict, 1999a,b). Previous cytogenetic and morphometric analyses also support the monophyly of the three species (Genoways and Choate, 1970, 1972, 1998; Tate *et al.*, 1980; French, 1981; George *et al.*, 1981, 1982, 1986; Moncrief *et al.*, 1982; Jones *et al.*, 1984; George, 1986; Tolliver and Robbins, 1987).

Morphometric analyses generally have suggested that *B. carolinensis* and *B. hylophaga* are more similar to each other than either is to *B. brevicauda* (George *et al.*, 1981; Jones *et al.*, 1984). However, none of these studies used explicit cladistic methodology. Our MP analyses (combined cytochrome *b* + 16S data) favored a *B. brevicauda* + *B. carolinensis* clade with strong bootstrap support (86%; Fig. 3), when TS at third codon positions were excluded. This result only was contradicted by unweighted MP analysis of cytochrome *b* alone, which favored a grouping of *B. hylophaga* + *B. carolinensis* (61% bootstrap). This alternative rooting may be misguided by homoplasy due to saturation of third position transitions (Fig. 2). All other analyses of the combined data set, including ML and ME using justified models of DNA substitution (Posada and Crandall, 1998), support the tree shown in Fig. 3. Our results do not reflect the patterns of tooth morphology favoring *B. carolinensis* + *B. hylophaga* (Jones *et al.*, 1984).

Phylogenetic analysis of mtDNA haplotypes shows

that *B. brevicauda* and *B. carolinensis* each are divided into well-defined groups to the east and west of the Mississippi basin (Fig. 4). This pattern is reminiscent of the commonly found phylogeographic category I, "with prominent genetic gaps distinguishing deep allopatric lineages in a gene tree" (Avise *et al.*, 1987). Numerous examples of small mammals with this kind of phylogeographic pattern have been reported from all continents (reviewed by Avise, 2000). In Europe, a study of two species of hedgehogs (genus *Erinaceus*) revealed a major division between western and eastern populations separated by karyotypic differences and cytochrome *b* divergences of up to 11.7% between species and 5–6% within species (Santucci *et al.*, 1998). In the eastern United States, geographic populations of the woodrat, *Neotoma floridana*, have been shown to fall into sharply distinct phylogroups based on analyses of mtDNA restriction sites (Hayes and Harrison, 1992).

Shrews in general, and soricine shrews in particular, are known for their remarkable inter- and intraspecific karyotypic diversity. It has been suggested that chromosomal rearrangements may promote hybrid infertility and result in diversification or speciation in some groups of shrews (e.g., Zima *et al.*, 1998). Cytogenetic studies reporting the diploid number ($2N$) of chromosomes for species and populations of *Blarina* and the outgroups were published by Meylan (1967), Genoways *et al.* (1977), George *et al.* (1981, 1982), and Moncrief *et al.* (1982). The amount of karyotypic variation for *Blarina* is extensive, with $2N$ of 37–39, 46, or 50–52 for *B. carolinensis*, 48–50 for *B. brevicauda*, and 52 for *B. hylophaga*. The diploid numbers observed for *C. parva* (52) and *S. cinereus* (66) suggest higher ancestral chromosome numbers (Genoways *et al.*, 1977; Zima *et al.*, 1998). It may be noted that the chromosome number in *B. hylophaga* ($2N = 52$) is the same as the ancestral state in *C. parva* ($2N = 52$), consistent with our placement of *B. hylophaga* as a basal clade of *Blarina*. However, it should be mentioned that the acrocentric complement of chromosomes in *C. parva* resembles that in *B. brevicauda* (Genoways *et al.*, 1977). One of the many studies on the *Sorex araneus* group suggested a tendency of karyotypic evolution to progress from high to low chromosome numbers by means of Robertsonian and tandem translocations and that a similar process may be important in the evolution of the *S. cinereus* group (Volobouev, 1989; Volobouev and Jong, 1994). Interspecific hybrid sterility or low viability due to chromosomal rearrangements may explain the strongly supported mtDNA clades that we found for each species and the lack of mtDNA introgression among species despite reported cases of hybridization. The number of morphologically similar, yet karyotypically different, species of shrews suggests that chromosomal speciation may have been a significant process in the diversification of shrews (Zima *et al.*, 1998).

For *B. brevicauda*, the western group identified by mtDNA analysis (Fig. 4) agrees with the currently recognized subspecies *B. b. manitobensis* and *B. b. brevicauda*, and the eastern clade includes all other subspecies (George *et al.*, 1986). However, the eastern and western clades within *B. brevicauda* and *B. carolinensis* (Fig. 4) are not distinguished by karyotypic differences (George *et al.*, 1982). The two phylogroups of *B. brevicauda* also have been designated two semi-species, *B. brevicauda brevicauda* in the west (dated by fossil evidence to the late Pleistocene, about 200,000 years ago) and the much older *B. b. talpoides* in the east (Jones *et al.*, 1984). Interestingly, mean cytochrome *b* sequence variation within the western clade of *B. brevicauda* (0.5%; see Table 3) is lower than variation within the eastern clade (1.02%), consistent with a recent range expansion in the western clade (Jones *et al.*, 1984). Similarly, the *B. carolinensis* mtDNA clades represent two recognized subspecies, *B. c. carolinensis* in the east and *B. c. minima* in the west of the distribution area (Genoways and Choate, 1998). However, there are some specimens assigned to *B. c. minima* that occur east of the Mississippi river valley (Genoways and Choate, 1998). Collecting specimens from Mississippi would help clarify the boundary between the two subspecies of *B. carolinensis* and help to reevaluate the current subspecific designations.

Also like the pattern in *B. brevicauda*, the western clade of *B. carolinensis* has lower mean within-group sequence variation than the eastern clade (0.20% vs 2.0%; Table 3). Comparing sequence divergence values within species, the mean divergence between eastern and western clades of *B. carolinensis* is 3.4%, larger than the 2.5% mean divergence observed between the eastern and the western clades of *B. brevicauda*. These divergence values suggest an older separation between eastern and western populations in southern North America (*B. carolinensis*) than between populations further north (*B. brevicauda*). Although there is strong support for east–west groups, sequence divergence values (Table 3) do not suggest that these groups should be considered separate species since they fall within typical values of intraspecific variation recorded for mammals (Johns and Avise, 1998; Avise and Walker, 1999). These results are in agreement with the current revisions of the taxonomic status of both *B. brevicauda* and *B. carolinensis* (Jones *et al.*, 1984; George *et al.*, 1986; Genoways and Choate, 1998).

In contrast to these, *B. hylophaga* has no geographic structuring and a most restricted distributional range. Geological evidence for the formation of the Mississippi basin may be relevant to an understanding of the effect of landscape formation on the distribution of genetic variation among North American shrew populations. Cytochrome *b* genetic differences among several species of *Sorex* in Europe were 3.7 to 9% and in the lesser white-toothed shrew, *Crocidura suaveolens*, within-

species differences were about 6.5% (Taberlet *et al.*, 1998).

Under the assumption of a molecular clock, mtDNA data can provide information about branching time in addition to branching order. The substitution rate for cytochrome *b* inferred using the first fossil appearance of *Cryptotis* (9 My) is 0.009 substitutions per site per Myr (Table 3). Node-to-tip distances were used to estimate time of divergence for the major clades discussed above (Table 3). Our results suggest that all speciation events within *Blarina* occurred around 3.7 to 4.6 Mya, well before the onset of Pleistocene glaciations, and that the east/west subdivision within species dates to 1–2 Mya. The latter could have been affected by Pleistocene events. Fossil data suggest later origins since the earliest fossils of *B. brevicauda* date to the Pliocene (~2 Mya), the earliest fossils of *B. carolinensis* are from the late Pliocene/early Pleistocene (~1.7 Mya), and the earliest fossils of *B. hylophaga* date to late Pleistocene (Harris, 1998).

CONCLUSIONS

Cytochrome *b* and 16S rRNA sequence data presented here are used to produce the first explicit phylogenetic analysis for this group of short-tailed shrews. Major findings of this study include (i) support for the monophyly of the genus and each species of *Blarina*, (ii) a basal position for *B. hylophaga*, (iii) a sister group relationship between *B. brevicauda* and *B. carolinensis*, and (iv) an earlier origin (pre-Pleistocene) for species of *Blarina* than previously estimated from fossil data. Furthermore, our study suggests that at the intraspecific level the formation of the Mississippi drainage had a marked effect on the mtDNA phylogeographic pattern for *B. carolinensis* and *B. brevicauda*. Future work may benefit from additional sampling in the area between the eastern/western clades of these two species. It would be also informative to sample areas of sympatry or contact and, perhaps more importantly, to study the area where all three species converge to gain a better reconciliation between the molecular data and the fossil history of short-tailed shrews.

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REFERENCES

- Avise, J. C. (1994). "Molecular Markers, Natural History, and Evolution." Chapman & Hall, New York.
- Avise, J. C. (2000). "Phylogeography: The History and Formation of Species." Harvard Univ. Press, Cambridge, MA.
- Avise, J. C., and Walker, D. (1999). Species realities and numbers in sexual vertebrates: Perspectives from an asexually transmitted genome. *Proc. Natl. Acad. Sci. USA* **96**: 992–995.
- Avise, J. C., Arnold, J., Ball, R. M., Jr., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., and Saunders, N. C. (1987). Intraspecific phylogeography: The mitochondrial DNA bridge between populations genetics and systematics. *Annu. Rev. Ecol. Syst.* **18**: 489–522.
- Benedict, R. A. (1999a). Morphological and mitochondrial DNA variation in a hybrid zone between short-tailed shrews (*Blarina*) in Nebraska. *J. Mammal.* **80**: 112–133.
- Benedict, R. A. (1999b). Characteristics of a hybrid zone between two species of short-tailed shrews (*Blarina*). *J. Mammal.* **80**: 135–141.
- Braun, J. K., and Kennedy, M. L. (1983). Systematics of the genus *Blarina* in Tennessee and adjacent areas. *J. Mammal.* **64**: 414–425.
- Ellis, L. S., Diersing, V. E., and Hoffmeister, D. F. (1978). Taxonomic status of short-tailed shrews (*Blarina*) in Illinois. *J. Mammal* **59**: 305–311.
- Farris, J. S., Källersjö, M., Kluge, A. G., and Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10**: 315–319.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783–791.
- French, T. W. (1981). Notes on the distribution and taxonomy of short-tailed shrews (Genus *Blarina*) in the southeast. *Brimleyana* **6**: 101–110.
- Fumagalli, L., Taberlet, P., Stewart, D. T., Gielly, L., Hausser, J., and Vogel, P. (1999). Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* **11**: 222–235.
- Genoways, H. H., and Choate, J. R. (1970). Additional notes on some mammals from eastern Nebraska. *Trans. Kan. Acad. Sci.* **73**: 120–211.
- Genoways, H. H., and Choate, J. R. (1972). A multivariate analysis of systematic relationships among populations of the short-tailed shrew (genus *Blarina*) in Nebraska. *Syst. Zool.* **21**: 106–116.
- Genoways, H. H., and Choate, J. R. (1998). Natural history of the southern short-tailed shrew, *Blarina carolinensis*. *Occas. Pap. Mus. Southwest. Biol.* **8**: 1–43.
- Genoways, H. H., Patton, J. C., III, and Choate, J. R. (1977). Karyotypes of shrews of the genera *Cryptotis* and *Blarina* (Mammalia: Soricidae). *Experientia* **33**: 1294–1295.
- George, S. B. (1986). Evolution and historical biogeography of soricine shrews. *Syst. Zool.* **35**: 153–162.
- George, S. B., Choate, J. R., and Genoways, H. H. (1981). Distribution and taxonomic status of *Blarina hylophaga* Elliot (Insectivora: Soricidae). *Ann. Carnegie Mus.* **50**: 493–513.
- George, S. B., Choate, J. R., and Genoways, H. H. (1986). *Blarina brevicauda*. *Mammal. Species* **261**: 1–9.
- George, S. B., Genoways, H. H., Choate, J. R., and Baker, R. J.

- (1982). Karyotypic relationships within the short-tailed shrews, genus *Blarina*. *J. Mammal.* **63**: 639–645.
- Getz, L. L. (1961). Factors influencing the local distribution of shrews. *Am. Midl. Nat.* **65**: 67–88.
- Gibson, T., Higgins, D., and Thompson, J. (1996). ClustalW. EMBL, Heidelberg, Germany and EMBL/EBI, Hinxton, UK.
- Graham, R. W., and Semken, H. A. (1976). Paleocological significance of the short-tailed shrew (*Blarina*), with a systematic discussion of *Blarina ozarkensis*. *J. Mammal.* **57**: 433–449.
- Harris, A. H. (1998). Fossil history of shrews in North America. In "Evolution of Shrews" (J. M. Wójcik and M. Wolsan, Eds.), pp. 133–156. Mammal Research Institute, Polish Academy of Sciences, Białowieża.
- Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **21**: 160–174.
- Hayes, J. P., and Harrison, R. C. (1992). Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. *Syst. Biol.* **41**: 331–344.
- Irwin, D. M., Kocher, T. D., and Wilson, A. C. (1991). Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* **32**: 128–144.
- Johns, G. C., and Avise, J. C. (1998). A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. *Mol. Biol. Evol.* **15**: 1481–1490.
- Jones, C. A., Choate, J. R., and Genoways, H. H. (1984). Phylogeny and paleobiogeography of short-tailed shrews (genus *Blarina*). *Spec. Publ. Carnegie Mus. Nat. Hist.* **8**: 56–148.
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111–120.
- Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of the Hominoidea. *J. Mol. Evol.* **29**: 170–179.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 6196–6200.
- Maddison, D. R., and Maddison, W. P. (2000). *MacClade 4*: analysis of phylogeny and character evolution. Version 4. Sinauer, Sunderland, MA.
- Merriam, C. H. (1895). Revision of the shrews of the American genera *Blarina* and *Notiosorex*. *North Am. Fauna* **10**: 102–107.
- Meylan, A. (1967). Formules chromosomiques et polymorphisme Robertsonien chez *Blarina brevicauda* (Say) (Mammalia: Insectivora). *Can. J. Zool.* **45**: 1119–1127.
- Mickevich, M. F., and Farris, J. S. (1981). The implications of congruence in *Menidia*. *Syst. Zool.* **30**: 351–370.
- Moncrief, N. D., Choate, J. R., and Genoways, H. H. (1982). Morphometric and geographic relationships of short-tailed shrews (genus *Blarina*) in Kansas, Iowa, and Missouri. *Ann. Carnegie Mus.* **51**: 157–180.
- Palumbi, S. R. (1996). Nucleic Acids II: the polymerase chain reaction. In "Molecular Systematics" (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), pp. 205–247. Sinauer, Sunderland, MA.
- Posada, D., and Crandall, K. A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Qumsiyeh, M. B., Choate, J. L., Peppers, J. A., Kennedy, P. K., and Kennedy, M. L. (1997). Robertsonian chromosomal rearrangements in the short-tailed shrew, *Blarina carolinensis*, in western Tennessee. *Cytogenet. Cell Genet.* **76**: 153–158.
- Ruedi, M. (1998). Protein evolution in shrews. In "Evolution of Shrews" (J. M. Wójcik and M. Wolsan, Eds.), pp. 269–294. Mammal Research Institute, Polish Academy of Sciences, Białowieża.
- Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N. (1985). Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**: 1350–1354.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual" Cold Springs Harbor Laboratory Press, Cold Springs Harbor, NY.
- Santucci, F., Emerson, B. C., and Hewitt, G. M. (1998). Mitochondrial DNA phylogeography of European hedgehogs. *Mol. Ecol.* **7**: 1163–1172.
- Searle, J. B., and Wójcik, J. M. (1998). Chromosomal evolution: The case of *Sorex araneus*. In "Evolution of Shrews" (J. M. Wójcik and M. Wolsan, Eds.), pp. 219–268. Mammal Research Institute, Polish Academy of Sciences, Białowieża.
- Smith, M. F., and Patton, J. L. (1993). The diversification of South American murid rodents: Evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biol. J. Linn. Soc.* **50**: 149–177.
- Swofford, D. L. (2000). PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4, Sinauer, Sunderland, MA.
- Taberlet, P., Fumagalli, L., and Hausser, J. (1994). Chromosomal versus mitochondrial DNA evolution: Tracking the evolutionary history of the southwestern European populations of the *Sorex araneus* group. *Evolution* **48**: 623–636.
- Taberlet, P., Fumagalli, L., Wust-Saucy, A., and Cosson, J. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol.* **7**: 453–464.
- Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**: 512–526.
- Tate, C. M., Pagels, J. F., and Handley, C. O. (1980). Distribution and systematic relationship of two kinds of short-tailed shrews (Soricidae: *Blarina*) in south-central Virginia. *Proc. Biol. Soc. Wash.* **93**: 50–60.
- Taylor, J. R. E. (1998). Evolution of energetic strategies in shrews. In "Evolution of Shrews" (J. M. Wójcik and M. Wolsan, Eds.), 309–346. Mammal Research Institute, Polish Academy of Sciences, Białowieża.
- Templeton, A. R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and apes. *Evolution* **37**: 221–244.
- Tolliver, D. K., and Robbins, L. W. (1987). Genetic variability within *Blarina carolinensis*, and among three sympatric species of shrews (Insectivora: Soricidae). *J. Mammal.* **68**: 387–390.
- Volobouev, V. T. (1989). Phylogenetic relationships of the *Sorex araneus-arcticus* species complex (Insectivora, Soricidae) based on high-resolution chromosome analysis. *J. Hered.* **80**: 284–290.
- Volobouev, V. T., and van Zyll de Jong, C. G. (1994). Chromosome banding analysis of two shrews of the *cinereus* group: *Sorex haydeni* and *Sorex cinereus* (Insectivora, Soricidae). *Can. J. Zool.* **72**: 958–964.
- Wiens, J. J., and Hollingsworth, B. D. (2000). War of the Iguanas: Conflicting molecular and morphological phylogenies and long branch attraction in iguanid lizards. *Syst. Biol.* **49**: 143–159.
- Wilson, D. E., and Ruff, S. 1999. "The Smithsonian Book of North American Mammals." Random House (Smithsonian Inst. Press) New York.
- Zima, J., Lukáčová, L., and Macholán, M. (1998). Chromosomal evolution in shrews. In "Evolution of Shrews" (J. M. Wójcik and M. Wolsan, Eds.), pp. 175–218. Mammal Research Institute, Polish Academy of Sciences, Białowieża.