The Condor 101:577–588

EVIDENCE SUPPORTING THE RECENT ORIGIN AND SPECIES STATUS OF THE TIMBERLINE SPARROW[1]

JOHN KLICKA AND ROBERT M. ZINK
J. F. Bell Museum of Natural History, University of Minnesota, 1987 Upper Buford Circle, St. Paul, MN 55108-6097, e-mail: klicka@biosci.umn.edu

JON C. BARLOW
Royal Ontario Museum, 100 Queen's Park, Toronto, ON M5S 2C6, Canada

W. BRUCE MCGILLIVRAY
Provincial Museum of Alberta, 12845-102 Avenue, Edmonton, AB T5N 0M6, Canada

TERRY J. DOYLE
Ten Thousand Islands National Wildlife Refuge, 3860 Tollgate Blvd., Suite 300, Naples, FL 34114

Abstract. The Timberline Sparrow (*Spizella taverneri*), although originally described as a species, is currently classified as a subspecies of the more widespread Brewer's Sparrow (*S. breweri*). We investigated the taxonomic status and recent evolutionary history of these species by comparison of both morphological and molecular characters. Morphometric comparisons using 6 external and 18 skeletal measurements show that *S. taverneri* specimens from two widely separated populations (Yukon and southwestern Alberta, Canada) are indistinguishable with respect to size yet are significantly larger (by 3%) than representatives of several *breweri* populations. Analysis of 1,413 base pairs of mitochondrial DNA (mtDNA) for 10 *breweri* and 5 *taverneri* samples representing widely scattered breeding populations revealed a maximum divergence among any *breweri-taverneri* pair of 0.21% and an overall average of 0.13%. In contrast, the average (± SE) pairwise distance among the other *Spizella* species is 5.7 ± 0.5%. We discovered that *breweri* and *taverneri* could be distinguished on the basis of a single, fixed nucleotide difference. Of an additional 11 *taverneri* and 8 *breweri* surveyed for this diagnostic site, a single bird (morphologically a *taverneri*) from northwest British Columbia did not sort to "type." Overall, 18 of 18 *breweri* and 15 of 16 *taverneri* were diagnosable. We interpret these results to suggest that gene flow does not currently occur between these two forms and that each is on an independent, albeit recently derived, evolutionary course. The molecular data are consistent with theoretical expectations of a Late Pleistocene speciation event. We believe that for passerine birds, this is the first empirical validation of this widely accepted evolutionary model. The data presented corroborate plumage, vocal, and ecological evidence suggesting that these taxa are distinct. As such, we suggest that *Spizella taverneri* be recognized as a species.

Key words: Late Pleistocene speciation, mtDNA, morphometrics, phylogeography, species limits, Spizella breweri, Spizella taverneri.

INTRODUCTION

The avian genus *Spizella* includes seven (AOU 1998) or eight (Sibley and Monroe 1990) small, drab, North American sparrow species that, with the possible exception of *S. arborea,* form a monophyletic group (Dodge et al. 1995). The taxonomic status of one member, the Timberline Sparrow (*Spizella taverneri*) is controversial. Swarth and Brooks (1925) thought it sufficiently distinct from its putative sister taxon, the Brewer's Sparrow (*S. breweri*), that they described it as a new species. Their conclusion was based on differences in "details of structure and coloration" but they also noted ecological (altitudinal distribution) and behavioral distinctions. Despite this evidence, the American Ornithologists' Union (AOU) Committee on Classification and Nomenclature has always classified this taxon as a subspecies. This is due, in part, to the influence of Grinnell (1932, also Grinnell et al. 1930) who observed that *breweri* taken in the northern United States displayed "minor tendencies toward *taverneri.*" Presumably, this remark was interpreted (AOU 1957) as evidence of hybridization at the zone of contact between these two forms. A recent taxonomic treatment (Sibley and Monroe 1990) once again affords the Timberline Sparrow species status. This conclusion was

[1] Received 11 August 1998. Accepted 12 February 1999.

based largely on new evidence regarding taxon-specific differences in vocalizations, morphology, and ecology (Barlow and McGillivray, unpubl. data). We summarize the distributional and ecological evidence that distinguishes these two forms and we present new morphological, molecular, and vocal evidence that support the classification of the Timberline Sparrow as a distinct, albeit recently evolved, species.

The distribution of the *breweri/taverneri* complex is unique among North American birds. *Spizella breweri* is one of the characteristic birds of the arid sage (*Artemisia* spp.) country of the western United States and southwestern Canada, although it is not an obligate associate of this habitat type (Grinnell et al. 1930). *Spizella breweri*'s breeding distribution is relatively well defined and it is common to abundant throughout most of its range. The breeding range of *taverneri* is less perfectly known but seemingly consists of two disjunct populations (Godfrey 1986, McGillivray, unpubl. data), one encompassing southern Alaska, southwestern Yukon, and extreme northwestern British Columbia (Doyle 1997) and the other breeds on both slopes of the Rocky Mountains in Alberta and British Columbia (McTaggart-Cowan 1946, Nordin et al. 1988). McTaggart-Cowan (1946) considered *taverneri* an abundant breeding bird in this latter (more southerly) region, whereas Doyle's (1997) work suggests that it is far less common in the Alaska-Yukon area. We suspect that *taverneri* is more widely distributed than is currently known. The first Alaskan record for this taxon was established as recently as 1992 (Doyle 1997), and several additional Alaska and Yukon populations have been discovered since that time (Doyle, unpubl. data). The preferred alpine habitat (discussed below) is not uncommon in much of British Columbia, the Yukon, and the western edge of the Northwest Territories; however, most of it is not easily accessible.

At most localities, *taverneri* nests in dwarf birch (*Betula glandulosa*), the dominant woody shrub of many alpine and subalpine meadows, krummholz, and regenerating avalanche slopes. *Spizella taverneri* in Alaska appears to be restricted to a narrow vegetative band which occurs only on southeastern exposures at the transition between alpine and subalpine zones. Because Alaskan localities are at higher latitudes than *taverneri* sites elsewhere, the dominant shrub layer is a low to medium thicket of willow (*Salix glauca*), although dwarf birch is present in a lower vegetative layer (Doyle 1997). Nearly all *taverneri* breeding localities are associated with steep slopes; however, immediately east of Banff National Park in the Ya-Ha-Tinda grasslands (elev. 1,600 m) of southwestern Alberta, they also occur in flat to gently rolling terrain in patches of dwarf birch. These grasslands occupy a great "bowl" that is virtually surrounded by mountains. Here, *taverneri* is sympatric with the Clay-colored Sparrow (*S. pallida*), a congener that is likewise a common co-occupant with *breweri* in the sagebrush flats habitat of southeastern Alberta. In most respects, typical *taverneri* habitat contrasts with that of *breweri* in terms of plant dominants, but the woody shrubs (*Betula, Salix, Artemisia*) preferred for nesting by both taxa are structurally similar.

A key behavioral-ecological distinction between the two taxa is the timing of breeding. Even at the northern limits of the range of *breweri* in Alberta, territories are established in April (Pernanen 1994), clutches initiated in May, and broods fledged in June (Biermann et al. 1987). In contrast, the breeding habitat of *taverneri* in Alberta is often snow-covered and inaccessible to this species until mid-June (Nordin et al. 1988). Barlow and McGillivray (pers. observ.) encountered small flocks of *taverneri* in aspens (*Populus tremuloides*) in early June southwest of Calgary, Alberta, presumably waiting for the snow to melt in their normal breeding habitat more than 800 m higher in elevation. Doyle (1997) recorded a singing, migrant male *taverneri* at sea level at Hyder, Alaska on 5 June and determined the breeding season for this species in Alaska to be from mid-June to mid-July. Indeed, the few *taverneri* specimens taken during migration suggest a slow and deliberate northward spring migration, perhaps tracking some isotherm. Two specimens document migration in west Texas in early March (Oberholser 1974) and three such records exist from Washington in mid-April (Jewett et al. 1953).

Both of these sparrows are thought to be completely migratory although their winter destinations are unclear. *Spizella breweri* winters commonly in small flocks in southernmost California, Arizona, and New Mexico, and in extreme west Texas. It ranges south in the Mexican highlands as far as Jalisco and Guanajuato and also occurs in the Pacific lowlands of western and northern Mexico and throughout Baja California

TABLE 1. Comparisons of external morphological character measurements of male *Spizella breweri* and *taverneri* specimens. Values given are means (± SE); sample sizes are shown in parentheses. All lengths are in millimeters, mass in grams.

Character	*breweri*	*taverneri*	*t*	*P*
Tarsus length	17.19 ± 0.08 (36)	17.84 ± 0.14 (45)	4.08	<0.001
Total length	134.54 ± 0.41 (110)	137.37 ± 0.88 (43)	2.92	<0.01
Wing length	63.12 ± 0.17 (136)	64.67 ± 0.21 (57)	5.13	<0.001
Tail length	62.33 ± 0.20 (112)	63.64 ± 0.43 (45)	3.14	<0.01
Bill length	9.86 ± 0.05 (108)	9.95 ± 0.12 (46)	0.70	ns
Mass	11.06 ± 0.06 (130)	11.66 ± 0.10 (57)	5.63	<0.001

(Rising 1996). The winter range of *taverneri* is thought to overlap to some degree. With the exception of a single aberrant record (Rea, 1967; a male *taverneri* in song on 14 February, San Diego County, California), we know of no November–February specimen records for *taverneri*. The paucity of winter *taverneri* specimens suggests that either they exist unrecognized in collections or that the main body of *taverneri* individuals winters south of most *breweri*, in Mexico, where relatively few specimens have been taken in winter. Winter distributions for both of these taxa are poorly understood and their relationship (if one exists) on the wintering ground is unknown.

Evidence from vertebrate paleontology (Anderson 1973), botany (Vitt and Horton 1979, Murray 1992), invertebrate zoology (Delorme et al. 1977), and palynology (Ritchie 1976, Luckman and Kearney 1986, Vance et al. 1995) points to a warmer, drier climate in western North America approximately 8,000 to 6,000 years before the present (BP). The distribution of grasslands and associated bushes, like sage, was extended north and up-slope relative to current ranges. Other evidence (Vereschagin and Baryshnikov 1982) points to a warm interglacial period 42,000 to 37,000 years BP with concomitant vegetation changes. Burns (1996) argues for widespread grassland in the middle Wisconsin 22,000–35,000 years BP in Alberta, paralleling the Eurasian evidence (Vereschagin and Baryshnikov 1982). On the assumption that in these two warm periods the distribution of sage included the slopes of the northern Rockies and parts of Yukon and Alaska, it is reasonable to think that the range of "*breweri*" expanded with it. Subsequent cooling and increased precipitation eliminated most of the sage (although relict populations can be found) forcing *breweri/taverneri* to shift from sage to dwarf birch and willows for nesting cover, thereby creating the present-day habitat disjunction.

METHODS

MORPHOMETRICS

For morphometric comparisons, 6 external (Table 1) and 18 skeletal (Table 2) measurements were taken on *breweri* and *taverneri* collected in Idaho, Washington, British Columbia, Alberta, and Yukon (the combined distribution is depicted in Fig. 1). The objective of sampling was to compare birds from near a potential *breweri-taverneri* contact area (Alberta) and to then compare these with birds taken nearer the centers of *breweri's* (Idaho) and *taverneri*'s (Yukon) respective ranges. The birds from Washington and southern British Columbia are of interest because they represent the only *breweri* in this sample from west of the Rocky Mountains and hence directly west of *taverneri* populations in southeastern Alberta. Morphometric analyses were run using SAS (SAS Institute 1989). All specimens measured were prepared similarly by one person (Gary Erickson) and all measurements were taken by McGillivray using the protocol outlined by Johnston and Selander (1971) and Johnston (1973).

GENETICS

To represent genetic variation occurring among and within these two sparrow taxa, we sampled birds from widely separated breeding localities (Fig. 1). Potential areas of introgression were sampled as were regions that may be isolated by geographic barriers. An initial sample of 15 birds was chosen for a general assay of genetic variation. For these specimens, tissues were excised in the field and placed as soon as possible on dry ice or in liquid nitrogen and subsequently housed in the laboratory at −70°C or in ethanol. DNA was isolated from minute portions of heart

TABLE 2. Comparisons of skeletal character measurements of male *Spizella breweri* and *taverneri* specimens. Values shown are means (± SE) with sample sizes in parentheses. All lengths are in millimeters.

Character	*breweri*	*taverneri*	*t*	*P*
Sternum length	15.64 ± 0.05 (99)	16.21 ± 0.07 (41)	3.84	<0.001
Sternum depth	7.57 ± 0.03 (98)	7.87 ± 0.06 (39)	4.41	<0.001
Sternum width	6.71 ± 0.04 (103)	7.00 ± 0.09 (39)	3.31	<0.002
Coracoid length	13.87 ± 0.04 (107)	14.15 ± 0.05 (42)	3.76	<0.001
Keel length	14.42 ± 0.07 (96)	14.85 ± 0.09 (39)	3.55	<0.001
Mandible length	16.67 ± 0.09 (100)	16.97 ± 0.15 (41)	1.75	ns
Premaxilla length	5.80 ± 0.08 (99)	6.21 ± 0.08 (41)	3.00	<0.004
Skull width	13.28 ± 0.03 (101)	13.12 ± 0.06 (40)	-2.83	<0.006
Skull length	25.08 ± 0.08 (98)	25.63 ± 0.11 (39)	3.78	<0.001
Dentary length	4.60 ± 0.08 (100)	4.93 ± 0.11 (39)	2.20	<0.023
Tarsus length	24.02 ± 0.06 (97)	24.90 ± 0.09 (40)	7.79	<0.001
Metatarsus length	17.34 ± 0.05 (103)	17.99 ± 0.08 (38)	6.97	<0.001
Synsacrum width	9.16 ± 0.03 (97)	9.52 ± 0.05 (30)	6.38	<0.001
Synsacrum length	11.47 ± 0.04 (101)	11.50 ± 0.08 (37)	0.40	ns
Scapula length	15.72 ± 0.04 (104)	16.12 ± 0.09 (42)	4.38	<0.001
Humerus length	14.80 ± 0.04 (105)	14.90 ± 0.06 (41)	1.31	ns
Ulna length	17.50 ± 0.05 (103)	17.74 ± 0.07 (42)	2.61	<0.01
Femur length	14.16 ± 0.04 (107)	14.43 ± 0.06 (40)	3.75	<0.001

or pectoral muscle via incubation in Chelex/Proteinase K, a modification of Ellegren's (1992) method. We used the polymerase chain reaction (PCR) to amplify a 1,050 base pair (bp) segment of the mitochondrial DNA (mtDNA) cytochrome *b* gene (cyt *b*) using primers L14841 (Kocher et al. 1989) and H4A (Harshman 1996) and a 363 bp segment of the mtDNA control region I (CR1, the putative hypervariable region; Baker and Marshall 1997) using primers LGL2 and H417 (Tarr 1995). Each of these segments was amplified for each of the 10 *breweri* and 5 *taverneri* specimens (see Fig. 2 and the Appendix). Double-stranded manual sequencing was done using the above-listed primers in conjunction with a set of nested primers that were, with one exception (H15424, Hackett 1996), developed by Klicka specifically for use on New World nine-primaried oscines. These include: (1) HCBC (AGGGGGCGGAAGGTTATTGATCG), (2) LCBA (TTACAAACCTATTCTCAGC), (3) LCBB (CTACTCGTCTCACTAGCCCT), and (4) LCBC (CACACATCAAAACTACGATC). A map of primer locations is available from the authors upon request. To minimize sequencing error, both light and heavy strands of DNA were sequenced for all individuals. Gels were read manually and sequence was visually aligned. Alignment was straightforward because the cyt *b* sequences all coded and there were no indels in the CR1 sequence. Using the computer program MEGA (Kumar et al. 1993), we computed pairwise percent sequence distances among haplotypes, neighbor-joining trees, and basic sequence statistics. Parsimony analysis was conducted using the program PAUP (3.1.3; Swofford 1993). Sequence data are deposited in Genbank (accession numbers AF118231–AF118239, AF122949–AF122953 inclusive).

In the cyt *b* sequence for the initial 15 birds sampled, a nucleotide difference that allowed *breweri* and *taverneri* forms to be distinguished was identified (Klicka and Zink 1997). To investigate further the utility of this difference as a diagnostic marker, 19 additional specimens (8 *breweri*, 11 *taverneri*) were obtained, 10 of which consisted of feathers and fragments of skin taken from study skins. For this subset of specimens (identified in the Appendix), total genomic DNA was extracted from feather quills using a Qiaquick tissue extraction kit (Qiagen, Chatsworth, California) and the manufacturer's protocol. These samples were screened using the sequencing procedures previously described, but only for the cyt *b* fragment containing the diagnostic marker.

In addition, we characterized these two taxa by summarizing available information on distribution, habitat selection, and song. Many of these observations are based on recent field studies by McGillivray and Barlow (unpubl. data), and Doyle (1997).

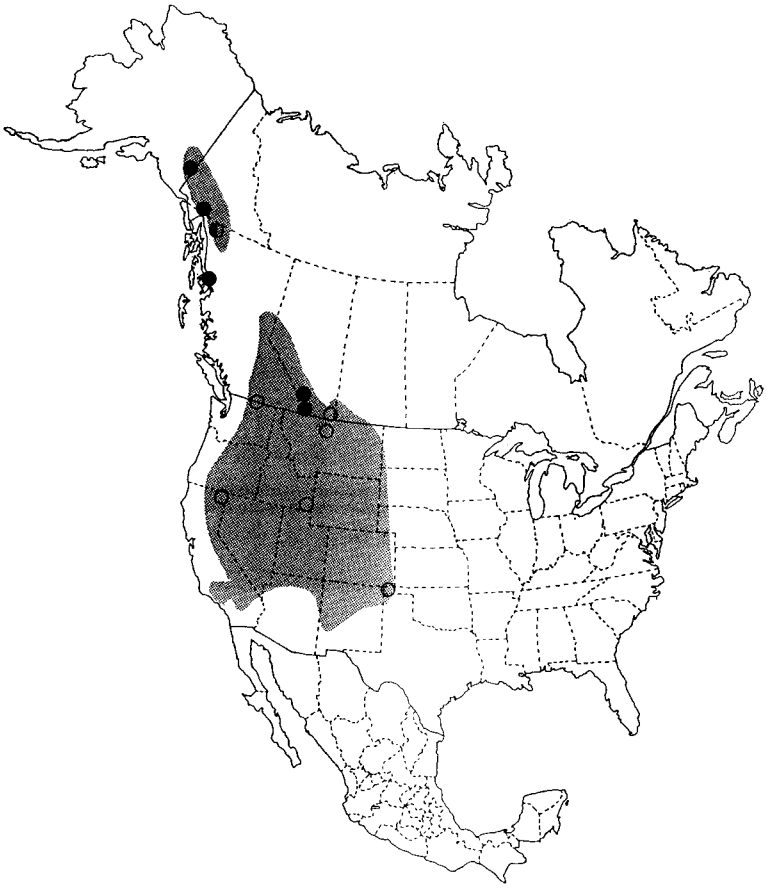

FIGURE 1. Gray shading represents the approximate known breeding distribution for members of the *Spizella breweri-S. taverneri* complex. Sampling distribution for specimens used in the genetic portion of this study is depicted. *Spizella breweri* and *taverneri* localities are depicted by open and solid circles, respectively. The origin of the single *taverneri* (on morphological and distributional grounds) specimen with a *breweri* mtDNA marker is denoted by a half-solid circle. For complete locality data see the Appendix.

RESULTS

MORPHOMETRICS

Univariate comparisons between pooled samples of *breweri* and *taverneri* show that for five of six standard external morphological character measurements (Table 1), *taverneri* is significantly larger than *breweri.* Fourteen of the 18 skeletal variables (Table 2) are significantly larger in *taverneri* than in *breweri.* When these are combined to produce an index of size comparable to a Principal Component 1 score (McGillivray and Johnston 1987, Rising and Somers 1989), *taverneri* is 3% larger than *breweri* (93.13 vs. 90.47 $t_{102} = 5.71$, $P < 0.001$). The area of least difference is in the skull where only three of five measures are significantly larger in *taverneri.* Mandible length is not significantly different between types and surprisingly, skull width is significantly larger in *breweri* (Table 2).

Geographic variation was explored by separating specimens into five groups: Yukon and northern British Columbia *taverneri,* Alberta *taverneri,* Alberta *breweri,* Idaho *breweri,* and Washington and southern British Columbia *breweri.* A body-core measure (coracoid), wing bone (ulna), leg bone (tibiotarsus), and an index of size were compared among groups using ANOVA with an *a posteriori* comparison of means (Duncan's multiple range test). *Spizella breweri* from the south and west are consistently smaller than birds from either population of *taverneri.* Alberta *breweri* appear somewhat intermediate

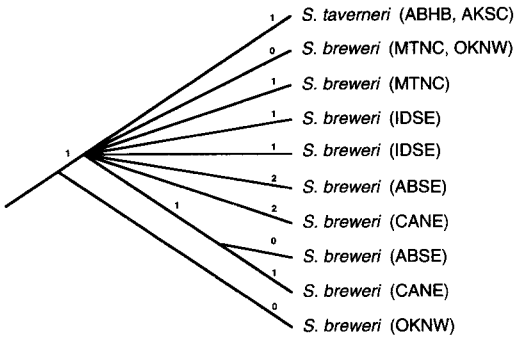

FIGURE 2. Single most parsimonious tree of *breweri* and *taverneri* haplotypes from an initial sample of 15 specimens for which the entire 1,413 bp complement of mtDNA sequence is available. This tree was rooted with Clay-colored Sparrow (*Spizella pallida)* although qualitatively identical results are obtained using any combination of congeners as outgroups. Numbers on branches indicate level of character support. No nodes shown had greater than 55% support in 500 bootstrap replicates. Localities are in parentheses (see Appendix for specifics).

although in two of the four comparisons, they are not significantly different from the other populations of *breweri* (Table 3).

VOCALIZATIONS

Song differences between *breweri* and *taverneri* parallel morphological divergence. Both have short (ca. 2.4 sec) to long songs that average 8–10 sec in length, although a *breweri* song can exceed 16 sec. Short songs of *breweri* have a buzzy prefix, always with a greater frequency and modulation than the trill-like prefix of *taverneri.* The prefix of *breweri* has a low of 2,500 Hz and a high of 7,000 Hz, whereas that of *taverneri* has a low of 3,000 Hz and a high of about 6,000 Hz. The overall minimum range for any song of *breweri* or *taverneri* was 2,000 Hz, but the maximum frequency for *taverneri* was 7,000 Hz compared to 8,000 Hz for *breweri* (Fig. 3).

Comparison of songs of 18 *breweri* and 9 *taverneri,* both aurally and spectrographically, showed that *breweri* song was always more frequency modulated. These songs are basically linear although slightly ascending or descending overall and less melodic than those of *taverneri.* Doyle (1997) found *breweri* songs to be comprised of trills that more closely resembled the buzzy song of *S. pallida,* a finding also reported by Pernanen (1994). In contrast, *taverneri* song was less strongly frequency modulated and instead, trilling and melodic. Overall, songs of 18 *breweri* included 19 song types, only one of which was shared with *taverneri.* From nine *taverneri,* 12 additional song types were recorded, all of which were lacking among the *breweri* sample.

GENETICS

Of the 1,413 bps sequenced for the initial *breweri/taverneri* sample (n = 15), 11 variable positions were detected including 7 third base transitions in cyt *b* and 4 transitions in CR1. Overall nucleotide composition was biased towards cytosine (46.1%), followed by adenine (33.5%), thymine (13.8%), and guanine (6.6%). In a maximum parsimony analysis in which all species of *Spizella* were represented (not shown), the 15 *S. breweri* and *S. taverneri* haplotypes occurred together in 100% of 500 bootstrap replicates. An extremely shallow phylogeographic history was observed. All *taverneri* specimens shared an identical haplotype, whereas 9 of 10 *breweri* haplotypes were unique. Average uncorrected sequence divergence among *breweri* haplotypes was 0.1%, with a maximum of 0.28% (four bp changes). *Spizella breweri* and *S. taverneri* are separable by a single, fixed nucleotide difference, a third position transition. Maximum divergence among any *breweri-taverneri* haplotype pair was 0.21% (three bp changes), and the

TABLE 3. ANOVA comparing mean values for three skeletal variables and a composite variable (size) for five groups of *breweri* and *taverneri.* Sample sizes are Yukon (23), Alberta *taverneri* (23), Alberta *breweri* (37), Idaho (38), and Washington and south central British Columbia (32).

	taverneri		*breweri*			Overall	
Measurement	Yukon	Alberta	Alberta	Idaho	Washington	*F*	*P*
Coracoid	14.15 A[a]	14.14 A	14.06 A	13.77 B	13.74 B	7.1	<0.001
Ulna	17.94 A	17.88 A	17.55 B	17.31 B	17.31 B	13.6	<0.001
Tibiotarsus	24.97 A	24.82 A	24.18 B	23.95 B	23.93 B	16.3	<0.001
Size	92.96 A	93.39 A	91.48 B	90.04 C	90.01 C	10.6	<0.001

[a] Values with the same letter do not differ significantly according to Duncan's multiple range test.

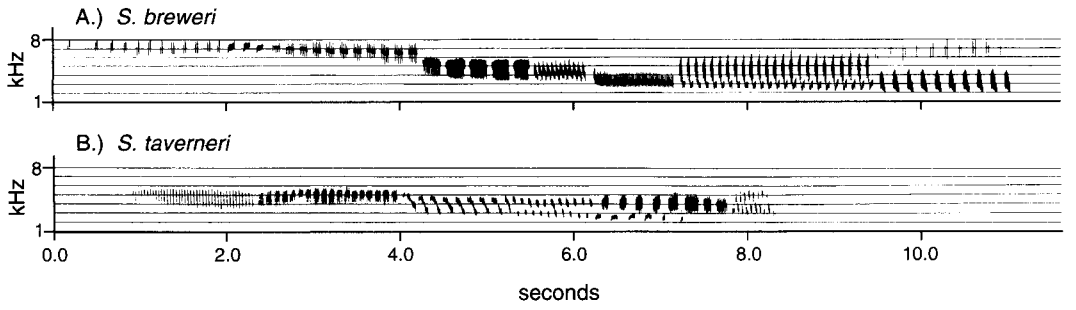

FIGURE 3. Sonagrams of long songs for direct comparison. The *breweri* song (A) was recorded at Manyberries, Alberta (ABSE); the *taverneri* song (B) at Hailstone Butte, Alberta (ABHB).

average was 0.13%. The average (± SE) corrected pairwise distance among the other *Spizella* species, 5.7 ± 0.5% (Klicka and Zink 1997), provides a perspective on the close similarity of *breweri* and *taverneri.*

In neighbor-joining (not shown) and parsimony trees (Fig. 2), the *taverneri* haplotype was more closely related to a *breweri* haplotype than many *breweri* haplotypes were to each other. That is, *breweri* haplotypes appear paraphyletic with respect to *taverneri,* although we cannot reject a tree with all haplotypes united at a single node. No genetic geographic structure was apparent within *breweri.* Nearly all of the variation uncovered within *breweri* represented singleton changes. Only one California and one Alberta bird shared an uniquely variable site. The only two *breweri* with identical haplotypes represent Montana and Oklahoma populations.

Because both the CR1 and cyt *b* data sets are relatively depauperate in terms of genetic variability, we are disinclined to think of either as "hypervariable." Nevertheless, the CR1 sequence is ca. 1.7 times as variable as the cyt *b* sequence, a finding consistent with a growing body of evidence (Baker and Marshall 1997) regarding evolution of the avian mtDNA control region.

From an overall sample (the initial 15 birds sequenced and 19 subsequently sequenced only for the diagnostic marker) of 16 *taverneri* and 18 *breweri,* we detected a single individual (a *taverneri*) that did not sort to type using this marker. This bird was not taken at the southerly edge of the *taverneri* range where *breweri-taverneri* interbreeding, if it occurs, would likely take place. Rather, it was one of two birds collected near Atlin, British Columbia, where Harry Swarth and Allan Brooks first collected *taverneri* in 1925. Thus, only 1 of 34 (3.0%) birds were not diagnosable with mtDNA sequences.

DISCUSSION

EVIDENCE FROM MORPHOLOGY AND VOCALIZATIONS

Subtle yet distinctive diagnostic differences in external morphology have long been apparent for these taxa. *Spizella taverneri* as originally described by Swarth and Brooks (1925) is slightly larger with a more slender, darker bill, and visibly darker plumage than *breweri.* In comparing their type specimens with "hundreds of skins from many localities," these authors encountered "no specimen of equivocal character." They remarked that the bill characters alone appeared to be diagnostic. These findings have recently been corroborated by Doyle (1997) who noted that in many plumage characteristics, *taverneri* appeared intermediate between *breweri* and *S. pallida.*

The morphometric analyses presented here show that, despite their general similarities, consistent and statistically significant differences in size do exist. Geography is not a mitigating factor as distinct *breweri* and *taverneri* populations occur in close proximity (e.g., Alberta), whereas similar populations of *taverneri* can be widely separated (e.g., Alberta and Yukon). Clinal variation would not produce the disjunctions seen in Table 3. These differences support the original contention of Swarth and Brooks (1925) that *taverneri* is distinct from *breweri.*

The song dissimilarities between the two taxa are substantial and given elevational and habitat differences of the two forms, song may be an important isolating mechanism serving to minimize hybridizing when stragglers of one form occur within the range of the other. Doyle (1997)

noted that playback of *taverneri* song to a *taverneri* population at Gold Hill, Alaska, evoked a more aggressive response than did playback of *breweri* song.

MOLECULAR AND THEORETICAL IMPLICATIONS

Thirty-three of 34 specimens representing several widely separated breeding localities can each be sorted to type using the diagnostic molecular marker. This decidedly nonrandom distribution of haplotypes is consistent with the view that these taxa are presently on independent evolutionary trajectories. The lone outlier, a *taverneri* specimen from northwestern British Columbia, could be an expression of gene flow, but the geographic locality of origin suggests either homoplasy or an incomplete sorting of lineages as a more likely explanation. The latter is not an unexpected result if these two forms have recently diverged (discussed below).

The level of distinctiveness between *breweri* and *taverneri* is one of the lowest observed among avian species. For comparison, the average sequence divergence between 35 species pairs hypothesized to be of recent Pleistocene origin is 5.1 ± 3.0% (Klicka and Zink 1997). Any reasonable calibration rate suggests that *breweri* and *taverneri* share an extremely recent common ancestor. The estimated time of divergence between *taverneri* and the nearest *breweri* haplotype (one bp change = 0.07% divergence) using a divergence rate of 2% change million-years^{-1} (Tarr and Fleischer 1993) is 35,000 years. In contrast, the species most closely related to *breweri/taverneri* appear to be the Field Sparrow (*S. pusilla*), at 5.9% sequence divergence (Klicka, unpubl. data), and the Clay-colored Sparrow (*S. pallida*), a species reputed to have diverged from *breweri/taverneri* during the Late Pleistocene (Hubbard 1973), differs by 6.1% (Klicka and Zink 1997).

Coalescence theory (Hudson 1990) predicts that haplotype phylogenies will be congruent with species trees on average by $4N_e$ (N_e = effective population size) generations after isolation of two lineages. The fact that the haplotypes of *breweri* do not form a monophyletic group to the exclusion of *taverneri* suggests that these taxa have diverged within this window of time. Generation times and N_e estimates are poorly known for birds. Most N_e estimates calculated to date are a fraction of present-day population sizes as estimated from census data (Avise et al. 1988). From mtDNA data, Avise et al. (1988) inferred N_e estimates with orders of magnitude in the 10^3 to 10^4 range. Passerine N_e estimates include 38,000 for Red-winged Blackbird (*Agelaius phoeniceus*; Avise et al. 1988) and 4,000–11,000 for Greenfinch (*Carduelis chloris*; Merila et al. 1997). If we assume an intermediate value of 10,000 for *breweri,* then the time since separation of *breweri* and *taverneri* is 40,000 years assuming a one-year generation time, or 80,000 years assuming two-years. Although these values are somewhat larger than that expected given a mtDNA sequence divergence rate of 2% million-year^{-1}, both are consistent with separation of the two forms during the last (Wisconsin) glaciation.

We found no evidence to suggest that the Rocky Mountains present a barrier to gene flow or that gene flow between the northern and southernmost parts of the *breweri* range is impeded. We note, however, that a very shallow haplotype tree and a complete lack of geographic structure also is consistent with a recent and rapid range expansion (Rogers and Harpending 1992). Thus, barriers to gene flow may be in place but are not yet detectable using our methods.

The topology (Fig. 2) depicted is consistent with a speciation scenario in which one taxon (*taverneri*) has recently originated from within another (*breweri*). Many avian speciation models (Mengel 1964, Hubbard 1973) explicitly predict this result. Although such a case has not yet been demonstrated for birds, Patton and Smith (1994) provide convincing evidence that this result has occurred among pocket gopher (genus *Thomomys*) lineages. Our data are consistent with such an hypothesis, although they are not robust enough to rule out a traditional allopatric speciation model. Monophyly of *breweri* haplotypes might be achieved with additional data. However, because *breweri* haplotypes themselves are closely related, a recent population expansion (Rogers and Harpending 1992) may be a more likely explanation for the topology recovered (Fig. 2). The lack of haplotype diversity in *taverneri* also indicates a recent origin following a probable population bottleneck. Many studies (summarized by Hewitt 1996) have noted lowered haplotype diversity in species colonizing recently deglaciated areas, such as those currently occupied by these sparrows.

For birds, this effect has been demonstrated previously for North American chickadees (*Poecile* spp., Gill et al. 1993).

SPECIES LIMITS: ONE SPECIES OR TWO?

We cannot state unequivocally that *taverneri* and *breweri* are reproductively isolated and hence represent two biological species. There is, however, no evidence that they hybridize, although hybrids would be difficult to identify using morphological characters. Nevertheless, efforts (over eight years, 1984–1991, McGillivray, unpubl. data) to find zones of contact in southern Alberta, British Columbia, and Montana have failed, with the closest approach of the species being about 150 km. No mixing of *taverneri* (n = 7) and *breweri* (n = 7) mtDNA haplotypes is observed among birds sampled from this region, providing strong, but not conclusive, evidence in favor of the case for reproductive isolation. Because of mtDNA's mode of inheritance, it is not uncommon in zones of introgression for birds to display the morphological (and presumably nuclear genetic) characters of one species and have a mtDNA haplotype of the other (Zink 1994). Typically, mtDNA haplotypes of both parental species will be pooled in introgressed populations, but in some instances (Gill 1997) all members of the local population can be "pure" for the "wrong" mtDNA haplotype.

Because there are no apparent physical geographic features separating these two forms, it may be that reproductive isolation, if it does exist, is due to sharply distinct ecological preferences or perhaps vocalization differences. Analyzing larger samples from putative hybrid zones may provide a definitive answer. For example, Grinnell et al. (1930) describe a population near Lassen Peak (Manzanita Lake) in California (near our own sampling locality) in which birds having "tendencies toward the characters of *taverneri*" are found on chaparral-covered higher slopes; whereas, typical *breweri* are found in abundance in the surrounding sage flats.

We also have failed to meet the criteria established for identifying a phylogenetic species in the strict sense (McKitrick and Zink 1988) in that we have not provided a single character that unambiguously diagnoses (i.e., without homoplasy) taxon limits. However, using suites of characters, *breweri* and *taverneri* are easily and unambiguously diagnosable, thereby warranting species status under more recent formulations of the phylogenetic species concept (Nixon and Wheeler 1990, Zink and McKitrick 1995). The occupation of independent evolutionary trajectories also qualifies *breweri* and *taverneri* as evolutionary species (Mayden and Wood 1995). Zink and McKitrick (1995) point out that phylogenetic and evolutionary species concepts are more similar than has generally been appreciated.

In sum, the information that we have gathered does not provide incontrovertible evidence in support of *S. taverneri* being either a "strict sense" phylogenetic species (sensu McKitrick and Zink 1988) or a biological species. Paradoxically, we find it compelling enough to lead us to believe that *taverneri* may in fact be both of these. *Spizella breweri* and *taverneri* are diagnosable evolutionary units that are evolving independently of each other. The morphometric and molecular data presented, taken together with the distributional, vocal, morphological, and ecological evidence, support an hypothesis of reproductive isolation between these taxa. *Spizella taverneri* exhibits the genetic signatures of a very recently evolved, that is, incipient species. Our study demonstrates the difficulties of accommodating such species under any species definition. However, young species (if diagnosable) are as valid as those possessing longer evolutionary histories. We concur with Swarth and Brooks' (1925) original assessment and advocate recognition of *Spizella taverneri* as a species.

ACKNOWLEDGMENTS

We thank C. Cicero for providing tissue specimens and for reviewing a draft of this manuscript. A. Kessen also provided helpful comments on the manuscript. JCB and WBM thank J. A. Dick, G. Erickson, and M. Luchanski for expert field assistance, H. Ardes for help with sound spectrograph work, and S. Pernanen for her discussions of *Spizella breweri*. JCB is grateful for research funds from NSERC grant A 3472. TJD acknowledges the U.S. Fish and Wildlife Service and National Park Service for providing the funds for fieldwork in Alaska. D. Gibson provided valuable assistance in the synthesis of Alaska information and we thank both he and the University of Alaska Museum at Fairbanks for tissue specimens. Funding for laboratory work was provided by RMZ's NSF grant DEB-9317945.

LITERATURE CITED

AMERICAN ORNITHOLOGISTS' UNION. 1957. Check-list of North American birds. 5th ed. American Ornithologists' Union, Washington, DC.

AMERICAN ORNITHOLOGISTS' UNION. 1998. Check-list of North American birds. 7th ed. American Ornithologists' Union, Washington, DC.

ANDERSON, E. 1973. Ferret from the Pleistocene of central Alaska. J. Mammal. 54:778–779.

AVISE, J. C., R. M. BALL, AND J. ARNOLD. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Mol. Biol. Evol. 5: 331–344.

BAKER, A. J., AND D. MARSHALL. 1997. Mitochondrial control-region sequences as tools for understanding the evolution of avian taxa, p. 51–82. *In* D. P. Mindell [ed.], Avian molecular evolution and systematics. Academic Press, New York.

BIERMANN, G. C., W. B. MCGILLIVRAY, AND K. E. NORDIN. 1987. The effect of cowbird parasitism on Brewer's Sparrow productivity in Alberta. J. Field Ornithol. 58:350–354.

BURNS, J. A. 1996. Vertebrate paleontology and the alleged ice-free corridor: the meat of the matter. Quaternary Int. 32:107–112.

DELORME, L. D., S. C. ZOLTAI, AND L. L. KALAS. 1977. Freshwater shelled invertebrate indicators of paleoclimate in northwestern Canada during late glacial times. Can. J. Earth Sci. 14:2029–2046.

DODGE, A. G., A. J. FRY, R. C. BLACKWELL, AND R. M. ZINK. 1995. Comparison of phylogenies derived from two molecular data sets in the avian genera *Pipilo* and *Spizella*. Wilson Bull. 107:641–654.

DOYLE, T. J. 1997. The Timberline Sparrow, *Spizella (breweri) taverneri,* in Alaska, with notes on breeding habitat and vocalizations. Western Birds 28:1–12.

ELLEGREN, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites—a new approach to studies of genetic relationships in birds. Auk 109: 886–895.

GILL, F. B. 1997. Local cytonuclear extinction of the Golden-winged Warbler. Evolution 51:519–525.

GILL, F. B., A. M. MOSTROM, AND A. L. MACK. 1993. Speciation in North American chickadees: 1. Patterns of mtDNA genetic divergence. Evolution 47: 195–212.

GODFREY, W. E. 1986. The birds of Canada. Rev. ed. Natl. Mus. Nat. Sci. Ottawa, Ontario, Canada.

GRINNELL, J. 1932. An United States record of the Timberline Sparrow. Condor 34:231–232.

GRINNELL, J., J. DIXON, AND J. LINSDALE. 1930. Vertebrate natural history of a section of northern California through the Lassen Peak region. Univ. Calif. Publ. Zool. 35:1–594.

HACKETT, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Mol. Phylo. Evol. 5:368–382.

HARSHMAN, J. 1996. Phylogeny, evolutionary rates, and ducks. Ph.D. diss., Univ. Chicago, Chicago.

HEWITT, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58:247–276.

HUBBARD, J. P. 1973. Avian evolution in the aridlands of North America. Living Bird 12:155–196.

HUDSON, R. R. 1990 Gene genealogies and the coalescent process. Oxford Surv. Evol. Biol. 7:1–44.

JEWETT, S. G., W. P. TAYLOR, W. T. SHAW, AND J. W. ALDRICH. 1953. Birds of Washington State. Univ. Washington Press, Seattle.

JOHNSTON, R. F., AND R. K. SELANDER. 1971. Evolution in the House Sparrow, II. Adaptive differentiation in North American populations. Evolution 25:1–28.

KLICKA, J. K., AND R. M. ZINK. 1997. The importance of recent ice ages in speciation: a failed paradigm. Science 277:1666–1669.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. 86:6196–6200.

KUMAR, S., K. TAMURA, M. NEI, AND PENNSYLVANIA STATE UNIV. 1993. MEGA, Molecular evolutionary genetic analysis, version 1.01. Pennsylvania State Univ., University Park, PA.

LUCKMAN, B. H., AND M. S. KEARNEY. 1986. Reconstruction of Holocene changes in alpine vegetation and climate in the Maligne Range, Jasper National Park, Alberta. Quaternary Res. 26:244–261.

MAYDEN, R. L., AND R. M. WOOD. 1995. Systematics, species concepts, and the evolutionarily significant unit in biodiversity and conservation biology. Am. Fish. Soc. Symposium 17:58–113.

MCGILLIVRAY, W. B., AND R. F. JOHNSTON. 1987. Differences in sexual size dimorphism and body proportions between adult and subadult House Sparrows in North America. Auk 104:681–687.

MCKITRICK, M. C., AND R. M. ZINK. 1988. Species concepts in ornithology. Condor 90:1–14.

MCTAGGART-COWAN, I. 1946. Notes on the distribution of *Spizella breweri taverneri.* Condor 48:93–94.

MENGEL, R. M. 1964. The probable history of species formation in some northern wood warblers (Parulidae). Living Bird 3:9–43.

MERILA, J., M. BJORKLAND, AND A. J. BAKER. 1997. Historical demography and present day population structure of the Greenfinch, *Carduelis chloris*—An analysis of mtDNA control-region sequences. Evolution 51:946–956.

MURRAY, B. M. 1992. Bryophyte flora of Alaskan steppes. Bryobrothera 1:9–33.

NIXON, K. C., AND Q. D. WHEELER. 1990. An amplification of the phylogenetic species concept. Cladistics 6:211–223.

NORDIN K. E., W. B. MCGILLIVRAY, AND G. C. BIERMANN. 1988. Courtship feeding in Brewer's Sparrows (*Spizella breweri*). J. Field Ornithol. 59:33–36.

OBERHOLSER, H. C. 1974. The bird life of Texas. Univ. Texas Press, Austin, TX.

PATTON, J. L., AND M. F. SMITH. 1994. Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (Genus *Thomomys*). Syst. Biol. 43:11–26.

PERNANEN, S. K. 1994. Behavioral and ecological interactions of Brewer's (*Spizella breweri*) and Clay-colored (*S. pallida*) sparrows. MSc. thesis, Univ. Toronto, Toronto.

REA, A. M. 1967. Some bird records from San Diego County, California. Condor 69:316–318.

RISING, J. D. 1996. The sparrows of the United States and Canada. Academic Press, San Diego, CA.

RISING, J. D., AND K. M. SOMERS. 1989. The measurement of overall body size in birds. Auk 106:666–674.

RITCHIE, J. C. 1976. The late Quaternary vegetational history of the western interior of Canada. Can J. Bot. 54:1793–1818.

ROGERS, A. R., AND H. C. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9: 552–569.

SAS INSTITUTE INC. 1989. SAS/STAT user's guide. Version 6, 4th ed. Vol. 1. SAS Institute Inc., Cary, NC.

SIBLEY, C. G., AND B. L. MONROE JR. 1990. Distribution and taxonomy of birds of the world. Yale Univ. Press, New Haven, CT.

SWARTH, H. S., AND A. BROOKS. 1925. The Timberline Sparrow, a new species from Northwestern Canada. Condor 27:67–69.

SWOFFORD, D. L. 1993. "PAUP: Phylogenetic Analysis Using Parsimony, ver. 3.1.3." Lab. Mol. Systematics, Smithson. Inst., Washington, DC.

TARR, C. L. 1995. Primers for amplification and determination of mitochondrial control-region sequences in oscine passerines. Mol. Ecol. 4:527–529.

TARR, C. L., AND R. C. FLEISCHER. 1993. Mitochondrial-DNA variation and evolutionary relationships in the Amakihi complex. Auk 110:825–831.

VANCE, R. E., A. B. BEAUDOIN, AND B. H. LUCKMAN 1995. The paleoecological record of 6 ka bp climate in the Canadian Prairie Provinces. Geographie Physique et Quaternaire 49:81–98.

VERESCHAGIN, N. K., AND G. F. BARYSHNIKOV 1982. Paleoecology of the Mammoth fauna in the Eurasian arctic, p. 267–279. *In* D. Hopkins, J. Matthews Jr., C. Schweger, and S. Young [eds.], Paleoecology of Beringia. Academic Press, New York.

VITT, D. H., AND D. G. HORTON. 1979. Mosses of the Nahanni and Liard ranges area, southwestern Northwest Territories. Can. J. Bot. 57:269–283.

ZINK, R. M. 1994. The geography of mitochondrial DNA variation, population structure, hybridization, and species limits in the Fox Sparrow (*Passerella iliaca*). Evolution 48:96–111.

ZINK, R. M., AND M. C. MCKITRICK. 1995. The debate over species concepts and its implications for ornithology. Auk 112:701–719.

APPENDIX. Museum[a] voucher and identification numbers and localities of origin for taxa sequenced. Bold numbers indicate those individuals sequenced for complete 1,413 bp mtDNA segment (Fig. 2). All others were surveyed only for the small region of cyt *b* containing the diagnostic marker. Samples for which study skins were used as mtDNA source are denoted by asterisks.

Voucher/tissue number	Code	Locality of origin	Collecting date
taverneri:			
UAM6670/AF11917	AKSC	southern Alaska, Gold Hill, Chisana, 10.4 km E	26 June 1995
UAM6669/AF11916	AKSC	southern Alaska, Gold Hill, Chisana, 10.4 km E	25 June 1995
UAM6939/AF14338	AKSE	Southeast Alaska, Ketchikan Quadrangle, Hyder	5 June 1996
UAM7048	AKSE	Southeast Alaska, Ketchikan Quadrangle, Hyder	7 June 1997
PMA84·40·1/ROM84-41	ABHB	southwest Alberta, Calgary, 80 km SSW, Hailstone Butte	18 July 1984
PMA84·40·2/ROM84-42	ABHB	southwest Alberta, Calgary, 80 km SSW, Hailstone Butte	18 July 1984
PMA84·40·4/ROM84-44	ABHB	southwest Alberta, Calgary, 80 km SSW, Hailstone Butte	18 July 1984
PMA84·40·3/ROM84-43	ABHB	southwest Alberta, Calgary, 80 km SSW, Hailstone Butte	18 July 1984
PMA84·40·6/ROM84-46	ABHB	southwest Alberta, Calgary, 80 km SSW, Hailstone Butte	18 July 1984
PMA86·36·39*	ABBM	southwest Alberta, Beaver Mines, 17.6 km S, 4.8 km W	30 May 1986
PMA86·36·40*	ABBM	southwest Alberta, Beaver Mines, 17.6 km S, 4.8 km W	30 May 1986
PMA84·39·64*	YUBC	southwest Yukon, Dezadeash Lake, 35.2 km S, Haines Rd.	26 June 1984
PMA84·39·73*	YUBC	northwest Br. Columbia, Dezadeash Lake, 56 km S, Haines Rd.	27 June 1984
PMA84·39·75*	YUBC	northwest Br. Columbia, Dezadeash Lake, 56 km S, Haines Rd.	27 June 1984

APPENDIX. Continued.

Voucher/tissue number	Code	Locality of origin	Collecting date
PMA84·39·92*	BCNW	northwest British Columbia, Surprise, 8 km NE	30 June 1984
PMA84·39·93*	BCNW	northwest British Columbia, Surprise, 8 km NE	30 June 1984
breweri:			
PMA55840/86·28·30*	WABC	Washington, Okanogan County, Tonsaket, 5.6 km E	14 May 1986
PMA55869/86·28·33*	WABC	southcentral British Columbia, Osoyoos, 8 km N, 1.6 km W	15 May 1986
PMA55842/86·28·31*	WABC	Washington, Okanogan County, Tonasket, 5.6 km E	14 May 1986
BMNH42284/JK9466	MTNC	Montana, Chouteau County, Fort Benton, 16 km W, 25.6 km N	19 June 1994
BMNH42285/JK9465	MTNC	Montana, Chouteau County, Fort Benton, 16 km W, 25.6 km N	19 June 1994
BMNH/JK9460	MTNC	Montana, Chouteau County, Fort Benton, 8 km W, 6.4 km N	19 June 1994
BMNH/JK9462	MTNC	Montana, Chouteau County, Fort Benton, 8 km W, 6.4 km N	19 June 1994
BMNH/JK96024	MTNC	Montana, Hill County, Wild Horse Lake	21 June 1994
PMA84·35·43/ROM84-110	ABSE	southeast Alberta, 40 Mile County, Manyberries, 20 km SW	19 May 1984
PMA84·35·44/ROM84-111	ABSE	southeast Alberta, 40 Mile County, Manyberries, 20 km SW	19 May 1984
PMA85·15·39/ROM85-40	IDSE	southeast Idaho, Bear Lake County, Bear Lake, 4.8 km E	20 May 1985
PMA85·15·40/ROM85-41	IDSE	southeast Idaho, Bear Lake County, Bear Lake, 4.8 km E	20 May 1985
PMA85·15·41/ROM85-42	IDSE	southeast Idaho, Bear Lake County, Bear Lake, 4.8 km E	20 May 1985
MVZ177463/CC1026	CANE	northeast California, Modoc County, Eagleville, 6.4 km S, 1 km E	20 June 1996
MVZ177464/CC1033	CANE	northeast California, Modoc County, Eagleville, 8 km S, 1.6 km E	22 June 1996
UOK17617/MVZ500022	OKNW	northwest Oklahoma, Cimarron County, Kenton, 4.8 km N	2 May 1982
UOK17615/MVZ500075	OKNW	northwest Oklahoma, Cimarron County, Kenton, 4.8 km N	2 May 1982
UOK17616/MVZ500076	OKNW	northwest Oklahoma, Cimarron County, Kenton, 4.8 km N	2 May 1982

[a] UAM=University of Alaska Fairbanks, Museum of Natural History; UOK = University of Oklahoma, Stovall Museum; MVZ = University of California Berkeley, Museum of Vertebrate Zoology; ROM = Royal Ontario Museum; PMA = Provincial Museum of Alberta; BMNH = University of Minnesota, Bell Museum of Natural History.