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A phylogenetic analysis of the emberizid sparrows based on three mitochondrial genes

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Abstract

Previous molecular phylogenetic studies have examined the taxonomic relationships among a number of typical emberizid sparrow genera. To help clarify these relationships, we sequenced a 1673 base pair fragment for the complete sequence of three mitochondrial genes: adenosine triphosphatase (*Atp8* and *Atp6*) and cytochrome oxidase subunit III (COIII) for 38 sparrow species, along with *Passerina amoena* (Cardinalidae) and *Piranga ludoviciana* (Thraupidae) which were selected as the outgroups. Our analysis confirms the monophyly of traditional genera such as *Junco*, *Melospiza*, and *Zonotrichia*. Although *Calcarius* and *Plectrophenax* are often thought to be putative emberizids, all our analyses placed these genera basal to all other sparrows examined. As observed with *Calcarius*, *Spizella* did not form a monophyletic group, with *S. arborea* being the sister-taxon to *Passerella iliaca*. Our analyses also suggest that *Aimophila ruficeps* is probably more closely related to the "brown towhees" (*Pipilo aberti*, *P. crissalis*, and *P. fuscus*) than its putative congeners. The genus *Ammodramus* was also not monophyletic, since it appears that *Passerculus sandwichensis* is more closely related to *A. henslowii* and *A. leconteii* then either one is related to its congener *A. savannarum*. Finally, our analyses exhibited other unsuspected associations, such as the sister-taxon relationships between *Amphispiza bilineata* and the *Chondestes grammacus*/*Calamospiza melanocorys* clade, and *Amphispiza belli* and *Pooecetes gramineus*.

1. Introduction

The phylogenetic relationships within the family Emberizidae have presented avian systematists with a taxonomic puzzle despite recently published molecular studies. Systematic studies of "typical" emberizid sparrows (Paynter and Storer, 1970) have been particularly troublesome in part due to their suspected relationship with the cardueline finches as well as their questionable taxonomic status within Emberizidae (AOU, 1983; Patten and Fugate, 1998; Rising, 1996). Many of the mitochondrial-based analyses (e.g., restriction sites, direct sequence, etc.) focused on only a few sparrow genera (Dodge et al., 1995; Zink, 1994; Zink and Avise, 1990; Zink and Blackwell, 1996; Zink and Dittmann, 1991, 1993; Zink et al., 1991, 1998). Other studies have examined a more substantial selection of emberizid genera were based on other kinds of data, such as DNA–DNA hybridization (Bledsoe, 1988; Sibley and Ahlquist, 1990), allozymes (Avise et al., 1980; Zink, 1982), and morphology (Patten and Fugate, 1998). Although these analyses provide a better understanding of relationships among a limited number of sparrow groups, it is difficult to synthesize such disparate studies into a more complete analysis in order to tease out the evolutionary history of these sparrows. Since many genera have not been included in a single mitochondrial DNA (mtDNA) sequence data set, many of the sparrow groups currently considered monophyletic could be relicts of older taxonomic studies (e.g., Paynter, 1964; Paynter and Storer, 1970; Ridgway, 1901), which are based primarily on morphological characters.

Due to the lack of an inclusive phylogenetic analyses of emberizids, we produced a preliminary hypothesis of the relationships among these songbirds based on mtDNA sequence. Our study is based on the analysis of three mitochondrial protein-coding genes as molecular markers: two subunits of adenosine triphosphatase

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(*Atp8* and *Atp6*) and cytochrome oxidase subunit III (COIII). Previous studies have found these genes to contain sufficient information for robust species-level phylogenetic reconstruction in birds (e.g., Hughes and Baker, 1999; Lovette and Bermingham, 2000; Whittingham et al., 2002; Yuri and Mindell, 2002). We also sought to test putative monophyly for certain genera, such as *Aimophila* and *Ammodramus*, whose relationships are often ambiguous (Rising, 1996). In addition, we wanted to examine the relationships of infrequently analyzed taxa, such as *Calamospiza* and *Chondestes* to other emberizids. Finally, we compared and contrasted our results with past studies on the relationships among emberizid sparrows.

2. Methods and materials

Taxon sampling. Our analysis included 38 species, from 40 specimens, representing all genera of typical emberizid sparrows (with the exception of *Emberiza*, *Sporophila*, and *Tiaris*) occurring north of Mexico (AOU, 1998; Byers et al., 1995; Rising, 1996). In addition, *Passerina amoena* (Family Cardinalidae) and *Piranga ludoviciana* (Family Thraupidae) were chosen for the outgroups (Spicer and Dunipace, unpublished). Several samples were collected by us (San Francisco State University) and stored at −80 °F, while the remaining samples were obtained through tissue loans from several museums (Table 1). Table 1 contains a full list of all specimens, their catalogue numbers, their locality information, and their GenBank accession numbers.

DNA extraction. Total genomic DNA was extracted from frozen tissue samples by using either the phenol/chloroform method (Werman et al., 1990) or the DNeasy Tissue Kit (Qiagen, Valencia, CA) following manufacturer's protocols. Extraction with phenol/chloroform followed the procedure in Spicer (1995) with some modifications: 100 μl 0.5 M EDTA, 50 μl 0.1 M Tris (pH 8.0), 25 μl 20% SDS, and 20 μl 20 mg/ml (PCR H_2O) Proteinase K. The homogenate was incubated at 55 °C overnight and 20 μl 5 M NaCl was added to each sample before extraction with equilibrated phenol and chloroform. The DNA pellet was resuspended in 200 μl 0.1 mM Tris (pH 8.0).

PCR amplification. For each specimen, a fragment approximately 1700 base pairs (bp) in length containing the mitochondrial genes tRNA-Lys, *Atp8*, *Atp6*, COIII, and tRNA-Gly was amplified via PCR (Mullis et al., 1987; Saiki et al., 1988). Double-stranded DNA was amplified in 50 μl volume reactions using the PCR Optimizer Kit (Invitrogen, Carlsbad, CA), with some modifications: 23 μl PCR water, 10 μl 5× buffer C or D, 5 μl 10 mM dNTP, 5 μl of each primer, and 0.25 μl AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA). PCR primers for this fragment (Table 2 and Fig. 1) were designed from the published *Corvus frugilegus* mitochondrial genome (Härlid and Arnason, 1999): (1) tRNAlys-corvus, modeled after H9034 (Sorenson et al., 1999) and (2) CO3BB-corvus, which we developed. Reaction parameters were 94 °C for 45 s, 52 °C for 45 s, and 72 °C for 2 min for a total of 30 cycles.

Sequencing. Prior to cycle sequencing, PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Due to the length of the target region and variation across species, internal primers (Table 2 and Fig. 1) were designed to provide overlapping sequence coverage for the entire region. The region was sequenced in both directions. Cycle sequencing reactions were performed in 12 μl reactions: 1 μl ABI Prism BigDye (Applied Biosystems, Foster City, CA), 2 μl 2× buffer or 1.5 μl 5× buffer (buffer: 400 mM Tris at pH 9.0 and 10 mM $MgCl_2$), and 0.5 μl (10 μM) of primer. The remainder of the mixture was composed of PCR water and template to give 50–90 ng of template DNA in each reaction. Reaction parameters were 95 °C for 2 min for an initial denaturation, 95 °C for 20 s, 54 °C for 30 s, and 60 °C for 4 min for a total of 30 cycles. Excess dye terminators were removed using the isopropanol precipitation method (ABI Prism®, 1998) with some modifications. Each reaction tube received 100 μl 65% isopropanol and mixed thoroughly. Sample solutions were transferred to 1.5 ml tubes, incubated at room temperature in the dark for 15 min, and centrifuged at 14,000 rpm for 30 min. After centrifugation, isopropanol was carefully aspirated and 100 μl 75% isopropanol was added to each sample, vortexed, and centrifuged for an additional 5 min. Samples were again aspirated and DNA pellets were dried for 5 min in a vacuum concentrator. Dried reaction products were resuspended in 3 μl of a 5:1 deionized formamide (25 mM EDTA with 50 mg/ml Blue Dextran) solution. Resuspended samples were vortexed, denatured at 90 °C for 2 min and placed over ice until loading onto an ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA) using a membrane comb (The Gel Company, San Francisco, CA) and following manufacturer's protocols.

Phylogenetic analysis. Sequence was aligned using the computer programs Sequencing Analysis 3.4 (ABI Prism™, 1999) and Sequencher 3.1.1 (GeneCodes, 1998), respectively. Base composition bias was calculated (Irwin et al., 1991) for the entire fragment as well as for the individual genes, *Atp8*, *Atp6*, and COIII. A value of zero indicates no bias and a value of one indicates complete bias.

To infer relationships among emberizid sparrows, several phylogenetic analyses were performed using PAUP 4.0B8 (Swofford, 2001). First, parsimony was performed using the random stepwise addition option of the heuristic search for 1000 replicates with unordered changes. Duplicating the same search parameters and replicate number, a second parsimony analysis was ex-

Table 1
Specimens used, catalogue numbers, localities, and GenBank accession numbers

Taxon	Owner Catalog or Accession* Nos.	Location state, Co.	GenBank Accession No.
Family Emberizidae:			
Aimophila aestivalis	LSU (B-2476)	LA, St. Tammany	AF468637
Aimophila cassinii	MVZ (178273)	OK, Cimarron	AF468636
Aimophila ruficeps 1	LAM (LAF5863)	CA, Los Angeles	AF468611
Aimophila ruficeps 2	MVZ (178276)	OK, Cimarron	AF468612
Ammodramus henslowii	LSU (B-16828)	LA, St. Tammany	AF468607
Ammodramus leconteii	LSU (B-21189)	LA, Cameron	AF468610
Ammodramus savannarum	MVZ (178277)	OK, Cimarron	AF468638
Amphispiza belli	MVZ (169312)	CA, Lake	AF468608
Amphispiza bilineata	MVZ (178280)	OK, Cimarron	AF468635
Calamospiza melanocorys	LSU (B-3925)	No data available	AF468634
Calcarius lapponicus	UAM (9855)	AK, Alaska Pen.	AF468640
Calcarius mccownii	MSB (NK37629)	NM, Quay	AF468642
Calcarius ornatus	MSB (NK43222)	NM, Roosevelt	AF468641
Chondestes grammacus	MVZ (169087)	CA, San Luis Obispo	AF468633
Junco hyemalis	SFSU (SL91)	CA, Monterey	AF468624
Junco phaeonotus	MSB (NK10483)	AZ, Cochise	AF468625
Melospiza georgiana	CAS 5594*	CA, Marin	AF468603
Melospiza lincolnii	CAS 5594*	CA, Marin	AF468604
Melospiza melodia	MVZ (173560)	CA, Nevada	AF468605
Passerculus sandwichensis	CAS 5594*	CA, Marin	AF468606
Passerella iliaca	SFSU (SL24)	CA, Sierra	AF468626
Plectrophenax nivalis	UAM (8477)	AK, Aleutian Is.	AF468639
Pipilo aberti	LSU (B-14281)	CA, Imperial	AF468613
Pipilo chlorurus	MVZ (165819)	CA, Nevada	AF468614
Pipilo crissalis	SFSU (SLB1)	CA, Fresno	AF468615
Pipilo erythrophthalmus	LSU (B-14281)	LA, East Baton Rouge	AF468616
Pipilo fuscus	MVZ (178298)	OK, Cimarron	AF468617
Pipilo maculates	SFSU (SL59)	CA, Monterey	AF468618
Pooecetes gramineus	MVZ (170232)	CA, Inyo	AF468609
Spizella arborea	UAM (8873)	AK, Interior	AF468627
Spizella atrogularis	MVZ (169967)	CA, San Luis Obispo	AF468628
Spizella breweri	UAM (11666)	NM, Grant	AF468629
Spizella pallida	UAM (11665)	MN, Scott	AF468630
Spizella passerina	SFSU (SL29)	CA, Sierra	AF468631
Spizella pusilla	MVZ (178302)	OK, Sequayah	AF468632
Zonotrichia albicollis	MVZ (178309)	OK, Cleveland	AF468619
Zonotrichia atricapilla 1	SFSU (SL41)	CA, Sierra	AF468620
Zonotrichia atricapilla 2	SFSU (SL81)	CA, Monterey	AF468621
Zonotrichia leucophrys	MVZ (169413)	CA, San Francisco	AF468622
Zonotrichia querula	MVZ (178366)	OK, Cleveland	AF468623
Family Cardinalidae:			
Passerina amoena	SFSU (SL13)	CA, Sierra	AF468644
Family Thraupidae:			
Piranga ludoviciana	SFSU (SL14)	CA, Sierra	AF468643

Note. CAS, California Academy of Sciences, San Francisco, California; LAM, Natural History of Museum of Los Angeles County, Los Angeles, California; LSU, Louisiana State University Museum of Natural Science Collection of Genetic Resources, Louisiana State University, Baton Rouge, Louisiana; MSB, Division of Birds, Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, California; SFSU, San Francisco State University, San Francisco, California; and UAM, University of Alaska Museum, University of Alaska, Fairbanks, Alaska.

ecuted by using only transversions (Brown et al., 1982; Swofford and Olsen, 1990). If searches produced more than one tree, a strict consensus was performed to summarize data analyses. To measure the robustness of branching patterns of the parsimony trees, bootstrap analyses (Felsenstein, 1985) were executed by using the closest stepwise addition of the heuristic search for 1000 replicates.

In order to evaluate the fit of the data to a molecular evolutionary substitution model, a maximum likelihood analysis was executed. To determine which model best fit the data, a series of nested [i.e., the null hypothesis (H_0) is a special case of the alternative hypothesis (H_1)] hypotheses were performed on various nucleotide substitution models. An initial neighbor-joining (NJ) tree based on the Jukes–Cantor distance model (JC) (Jukes

Table 2
Primer sequences used for *Atp8*, *Atp6*, and COIII

Primers		Sequence
1	tRNAlys-corvus	5′-CAGCACTAGCCCTTTTAAGCTAG-3′
2	FINCH1FD	5′-GAGCCCTAATCCTAGCATCCC-3′
3	MELO1FD	5′-CATAATCTTCCTCCTACTCATC-3′
4	SPIZ1FD	5′-CCCAACCACCCAACTATCC-3′
5	PIPILO1FD	5′-CCCAACTATCCATAAACC-3′
6	ZONO1FD	5′-CCCCTATGACTAGCAACCC-3′
7	SPARROW1FD	5′-CCTAACAGGACTACGAAACC-3′
8	SPIZ2FD	5′-GACCCATCCTAGGAGCAGCCGC-3′
9	MELO2FD	5′-GGACTAACAATATGATTCCAC-3′
10	FINCH2FD	5′-CGAGAAAGCACATTCCAAGG-3′
11	ZONO2FD	5′-CTGAGCCTTCTTCCACTCAAGCC-3′
12	FINCH2-1BK	5′-CGTAGTATTCTATGGCTTGTAG-3′
13	AIMO1BK	5′-GGCTTGGATTGCTGTTTTCGG-3′
14	ZONO1BK	5′-GCTAGGCTTGAGTGGAACAAGG-3′
15	FINCH1-1BK	5′-GGCTTCGGATGTGATGAATAGG-3′
16	CALCARIUS2BK	5′-GAATGTGCTTGGTGTGCCATT-3′
17	AIMO2BK	5′-GTTAGATGTTTTCTTGTAAG-3′
18	SPIZ2BK	5′-GTACGAAGACGTAGGCTTGG-3′
19	SNOW2BK	5′-GAATGTGCTTGGGTGCCATT-3′
20	ZONO2BK	5′-GCTGTGAGGTTGGCTGTTAGGC-3′
21	CALCARIUS3BK	5′-GACAGTTGGGTGGTGGGGTG-3′
22	SNOW3BK	5′-GGATAGTTGGGTGGTTGGGGTG-3′
23	SPIZ3BK	5′-GATTAGTGCTCATTTGTG-3′
24	ZONO3BK	5′-GGGGTGTTATTAGTTGTTTTG-3′
25	LABU3BK	5′-CTAGGTTGATGAATCATAATTG-3′
26	CO3BB-corvus	5′-GATTGGAAGTCGATTATAAT-3′

Note. All primers were designed by authors to be specific for sparrow genera, with the exception of tRNAlys-corvus, which was modeled after H9034 (Sorenson et al., 1999). Primer names were based on the genera they were designed from, their position in the fragment, and their sequence direction.

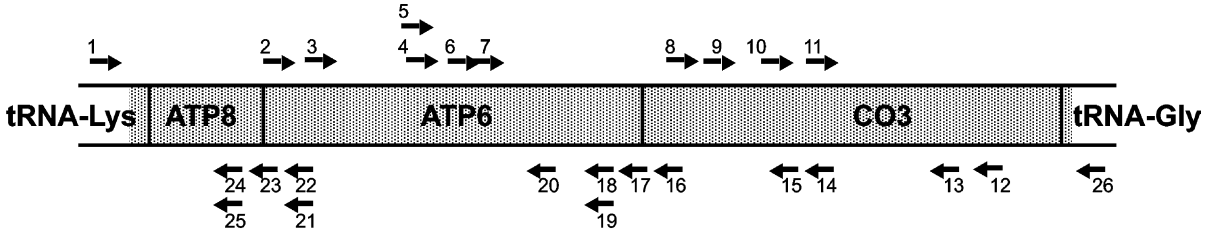

Fig. 1. Primers used to amplify and sequence the 1673 aligned base pairs of mtDNA. The primers (1) tRNAlys-corvus and (26) CO3BB-corvus were used for the initial amplification. Emberizid specific sequencing primers were designed for internal sequencing (see Table 2 for primer sequences).

and Cantor, 1969) was generated and a likelihood ratio test (LRT) was performed (Goldman, 1993) to test the models. The test statistic was calculated as $2(\ln L_0 - \ln L_1) = -2\ln \Lambda$, where L_0 and L_1 are the likelihood values under the null and alternative hypotheses, respectively. The associated probability was calculated by using a χ^2 distribution with the degrees of freedom equal to the difference in the number of free parameters between the two models. The models tested included the simplest substitution model, the JC model, which assumes that all nucleotide substitutions are equally probable and that the nucleotides occur in equal frequencies. The more complicated Hasegawa, Kishino, and Yano model (HKY85) (Hasegawa et al., 1985) allows the transition and transversion rate to differ and incorporates observed average nucleotide frequencies. Finally, the most parameter rich model tested was the general time-reversible model (GTR) (Lanave et al., 1984; Rodriguez et al., 1990; Tavare, 1986), which incorporates observed average base frequencies and allows for rate variation among six substitution types. In addition to the nucleotide models other parameters were investigated. These included the extent of the among site rate variation (α value of the Γ-distribution) estimated with eight rate categories along with the number of invariable sites (I). After the best-fit model was found a heuristic search was begun using the initial parameter estimates obtained from the NJ tree. Once a better tree was found the parameters were re-estimated and the search was repeated. This process was continued until a

tree converged on the same maximum likelihood tree. To test the robustness of the final maximum likelihood tree a bootstrap analysis was generated by using the closest stepwise addition option of the heuristic search for 100 replicates.

In addition, tree topologies based on the assumption that certain genera are monophyletic were compared using the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) to test for significant difference in tree lengths. This test was performed using the resampling estimated log-likelihood (RELL) (Kishino et al., 1990) method with 1000 bootstrap replicates and the results evaluated as a one-tailed test. The monophyly trees were generated by creating a monophyletic backbone tree for each of the genera tested. This tree was then used as a constraint tree for a maximum likelihood search using the parameters from the unconstrained maximum likelihood tree.

The maximum likelihood model was used to determine whether the sequence among taxa was evolving at a constant rate and fit a molecular clock (Felsenstein, 1993). We used a procedure proposed by Felsenstein (1993) to test the H_0 of a molecular clock. This test uses a LRT to determine if there are significant differences between the likelihood scores obtained from an analysis where the branch lengths are unconstrained as compared to an analysis where the branch lengths are constrained so terminal ends are contemporaneous. The likelihood test statistic was assumed to be approximately equal to a χ^2 distribution with $n-2$ degrees of freedom, where n equals the number of taxa sampled (Felsenstein, 1981).

3. Results

Our study produced a final 1673 base pair mtDNA fragment, for each taxon, comprising the complete sequence of *Atp8*, *Atp6*, and COIII, partial sequence of tRNA-Lys and tRNA-Gly, and a seven base pair spacer between *Atp6* and COIII. For the complete sequence 652 sites were variable (39.0%) and 551 of those sites were phylogenetically informative (84.5%). For *Atp8* 55 out of 168 (32.7%), *Atp6* 256 out of 684 (37.4%), and COIII 228 out of 784 (29.1%) sites were phylogenetically informative. The partial sequences of tRNA-Lys (32 bp) and tRNA-Gly (8 bp) had nine and one phylogenetically informative characters, respectively. Due to the 10 base pair overlap between *Atp8* and *Atp6*, two variable sites were counted twice (positions 197 and 200), but this had no effect on the resulting analyses. Base composition and base composition bias are shown in Table 3. This data set exhibited low to relatively low base composition bias for all characters. The homogeneity test among taxa for base frequencies for the complete sequence as well as for the individual genes indicated that none of the sequence was heterogeneous ($P = 1.00$).

We calculated uncorrected pairwise divergences (*p* distance) among our ingroup and outgroup taxa, and Table 4 shows the percentages discussed below. For the ingroup, the divergence range was 0.0% (*Zonotrichia atricapilla* 1 vs *Z. leucophrys*) to 14.1% (*Chondestes grammacus* vs *Calcarius mccownii*) for all genes, 0.0% (*Aimophila ruficeps* 1 vs *A. ruficeps* 2, *Junco hyemalis* vs *J. phaeonotus*, and *Z. atricapilla* 2 vs *Z. leucophrys*) to 19.6% (*Chondestes grammacus* vs *Plectrophenax nivalis*/*Spizella arborea*) for *Atp8*, 0.0% (*Z. atricapilla* 1 vs *Z. leucophrys*) to 15.4% (*C. mccownii* vs *Aimophila aestivalis*) for *Atp6*, and 0.0% (*J. hyemalis* vs *J. phaeonotus* and *Z. atricapilla* 1/2 vs *Z. leucophrys*) to 13.3% (*P. nivalis* vs *C. grammacus*) for COIII. Between the ingroup and outgroup taxa the divergence range was 10.7% (*Piranga ludoviciana* vs *Ammodramus henslowii*) to 14.1% (*Passerina amoena* vs *C. grammacus*) for all genes, 10.7% (*P. ludoviciana* vs *P. amoena*/*Pipilo erythrophthalmus*/*P. maculates*) to 18.5% (*P. ludoviciana* vs *S. arborea*, and *P. amoena* vs *C. grammacus*) for *Atp8*, 10.7% (*P. ludoviciana* vs *Spizella atrogularis*/*S. pallida*) to 14.6% (*P. amoena* vs *A. aestivalis*) for *Atp6*, and 9.8% (*P. ludoviciana* vs *C. mccownii*) to 13.5% (*P. ludoviciana*/*P. amoena* vs *C. grammacus*) for COIII.

All phylogenetic analyses were performed with all 40 sparrow taxa and both outgroup samples. The unordered parsimony analysis produced two equally parsimonious tress with tree lengths $(L) = 2619$ steps, a consistency index (CI) = 0.3559, and a retention index (RI) = 0.4945. The only difference between the two trees was the reversed positions of *Spizella pallida* and *S. pusilla*. The strict consensus tree for both trees is shown in Fig. 2, with bootstrap values of 50% or greater at branch nodes. In the unordered parsimony analysis several unusual branching patterns were observed. Though *Calcarius* and *Plectrophenax* are often referred to as putative emberizids, their placement is basal and

Table 3
Base composition and base composition bias for *Atp8*, *Atp6*, and COIII

Bases	A	C	G	T	Bias
Complete sequence	0.287	0.355	0.121	0.237	0.190
Atp8	0.302	0.380	0.075	0.244	0.242
Atp6	0.304	0.365	0.096	0.234	0.226
COIII	0.273	0.338	0.152	0.237	0.148

Table 4
Uncorrected pairwise divergences (p distance) among selected pairs of species

Taxon		Taxon	All data	*Atp8*	*Atp6*	COIII
Ingroup vs ingroup						
Aimophila aestivalis	vs	*Calcarius mccownii*	0.12232	0.11905	0.15351	0.09694
Aimophila ruficeps 1	vs	*Aimophila ruficeps* 2	0.00120	0.00000	0.00146	0.00128
Chondestes grammacus	vs	*Calcarius mccownii*	0.14063	0.19048	0.14912	0.12117
Chondestes grammacus	vs	*Plectrophenax nivalis*	0.13953	0.19643	0.12865	0.13265
Chondestes grammacus	vs	*Spizella arborea*	0.12276	0.19643	0.11111	0.11607
Junco hyemalis	vs	*Junco phaeonotus*	0.00120	0.00000	0.00292	0.00000
Zonotrichia atricapilla 1/2	vs	*Zonotrichia leucophrys*	0.00000	0.00000	0.00000	0.00000
Ingroup vs outgroup						
Passerina amoena	vs	*Aimophila aestivalis*	0.13611	0.14881	0.14620	0.12628
Passerina amoena	vs	*Chondestes grammacus*	0.14063	0.18452	0.13596	0.13520
Passerina amoena	vs	*Piranga ludoviciana*	0.11131	0.10714	0.12135	0.10204
Piranga ludoviciana	vs	*Ammodramus henslowii*	0.10657	0.13095	0.11257	0.09694
Piranga ludoviciana	vs	*Calcarius mccownii*	0.11550	0.14881	0.12281	0.09821
Piranga ludoviciana	vs	*Chondestes grammacus*	0.13645	0.17262	0.12719	0.13520
Piranga ludoviciana	vs	*Pipilo erythrophthalmus*	0.11730	0.10714	0.11842	0.12771
Piranga ludoviciana	vs	*Pipilo maculates*	0.11490	0.10714	0.11404	0.11990
Piranga ludoviciana	vs	*Spizella arborea*	0.12276	0.18452	0.11696	0.11224
Piranga ludoviciana	vs	*Spizella atrogularis*	0.11443	0.11905	0.10673	0.11990
Piranga ludoviciana	vs	*Spizella pallida*	0.11610	0.12500	0.10673	0.11990

quite distant from the other sparrows examined. In addition, *Calcarius* does not form a monophyletic group, since *C.* mccownii always appears outside the *C. ornetus*/*C. lapponicus*/*Plectrophenax nivalis* clade. This analysis also demonstrated that four other genera are not monophyletic: (1) *Aimophila* (*A. ruficeps* sister to the brown towhees), (2) *Ammodramus* (*A. savannarum* sister to the *Aimophila cassinii*/*A. aestivalis* clade, *A. leconteii* basal to the *Amphispiza belli*/*Pooecetes gramineus* clade, and *A. henslowii* sister to *Passerculus sandwichensis*), (3) *Amphispiza* (*A. bilineata* sister to the *Chondestes grammacus*/*Calamospiza melanocorys* clade), and (4) *Spizella* (*S. arborea* sister to *Passerella iliaca*).

The transversion parsimony analysis produced 12 equally parsimonious trees with $L = 526$, CI = 0.5038, and RI = 0.7003. The strict consensus for all 12 trees is shown in Fig. 3, with bootstrap values of 50% or greater at branch nodes. The transversion tree differed from the unordered parsimony tree: (1) the *C. ornatus*/*C. lapponicus*/*Plectrophenax nivalis* clade is basal to *Calcarius mccownii*, (2) the *Amphispiza belli*/*Pooecetes gramineus* clade is basal to *Ammodramus leconteii*, which is basal to *Passerculus sandwichensis*, which is basal to *A. henslowii*, and (3) *Passerella iliaca* and *Spizella arborea* are unresolved but appear in the same clade. Due to the nature of a transversion analysis (reduction of characters) many of the larger clades were unresolved.

For the maximum likelihood analysis, the most appropriate model for this data set, GTR + I + Γ, was determined by using the LRT. The likelihood search using this model resulted in one maximum likelihood tree (Fig. 4) with a $-\ln L = 13,704.62514$, which was considered to be the best estimate of the phylogeny. The parameter values estimated from this tree were: (1) AC: 3.3158103, AG: 52.475227, AT: 1.8739897, CG: 4.4461583, CT: 46.378593, and GT: 1.0 for the GTR model, (2) A = 0.320742, C = 0.387783, G = 0.099895, and T = 0.191581 were the estimated base compositions, (3) $\alpha = 1.419215$ for the Γ distribution, and (4) $I = 0.567824$ for the proportion of invariable sites. The maximum likelihood model was also used to test for a molecular clock. A molecular clock was generated using the same parameters given above, producing a likelihood score of $-\ln L = 13741.63629$ ($\chi^2 = 74.02$, df = 40, and $P = 0.0009$). Based on this calculation, the use of a molecular clock had to be rejected.

The maximum likelihood tree is shown in Fig. 4, with bootstrap values of 50% or greater at branch nodes. This tree was most similar to the transversion tree, but differed from the latter: (1) *Spizella arborea* and *Passerella iliaca* are sister taxa, (2) all *Spizella* species are resolved, and (3) *Z. leucophrys* and *Z. atricapilla* are unresolved. When compared to the unordered parsimony tree the likelihood tree differed slightly: (1) *Calcarius mccownii* is basal to all of the sparrows, except for *C. ornatus*, *C. lapponicus*, and *Plectrophenax nivalis*, (2) all *Spizella* species are resolved, and (3) the *Amphispiza belli*/*Pooecetes gramineus* clade is basal to *Ammodramus leconteii*, which is basal to *Passerculus sandwichensis*, which is basal to *Ammodramus henslowii*. Though the results from the different phylogenetic techniques exhibit some differences, overall tree topology is retained for all analyses performed.

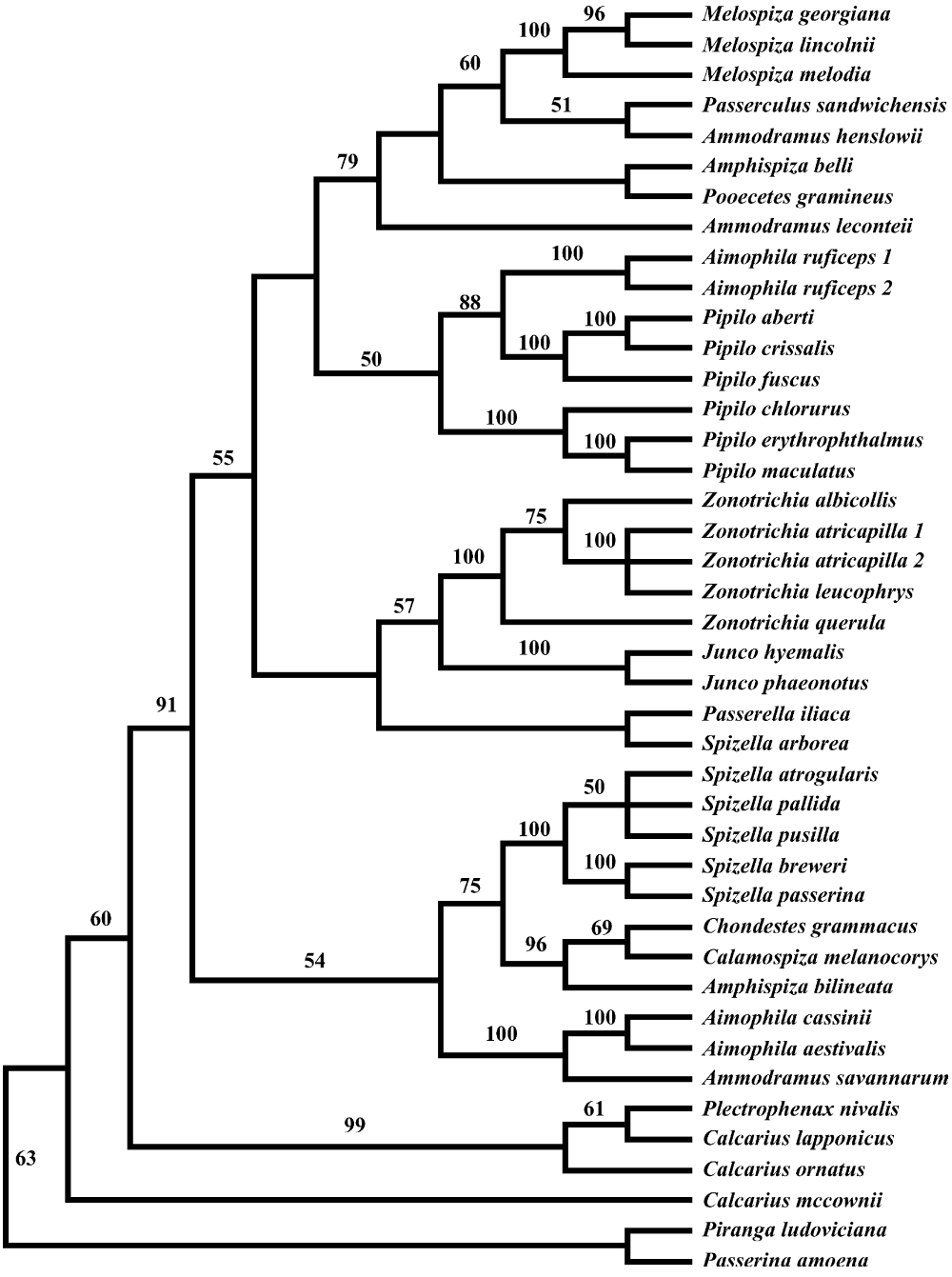

Fig. 2. Strict consensus tree based on two unordered parsimony trees with bootstrap values of 50% or greater at branch nodes.

Results of the Shimodaira–Hasegawa tests based on the GTR + I + Γ model with the parameters specified above are presented in Table 5. These results reveal that the maximum likelihood tree based on the sequence of *Atp8*, *Atp6*, and COIII statistically conflicts with several taxonomically proposed monophyletic genera: *Aimophila*, *Ammodramus*, *Amphispiza*, and *Calcarius*. However, our results suggest that *Spizella* is not monophyletic, but we cannot statistically distinguish this from our tree by using the Shimodaira–Hasegawa test.

4. Discussion

Despite the efforts of previous analyses, the taxonomic organization is still quite incomplete for the majority of emberizid sparrows. We sought to produce an inclusive phylogenetic study of New World sparrows based on mtDNA sequence. Our analyses bring into question the relationships of traditional monophyletic groups, such as *Ammodramus*, *Amphispiza*, *Calcarius, Pipilo*, and *Spizella*. They also revealed unsuspected phylogenetic placements among certain genera, such as

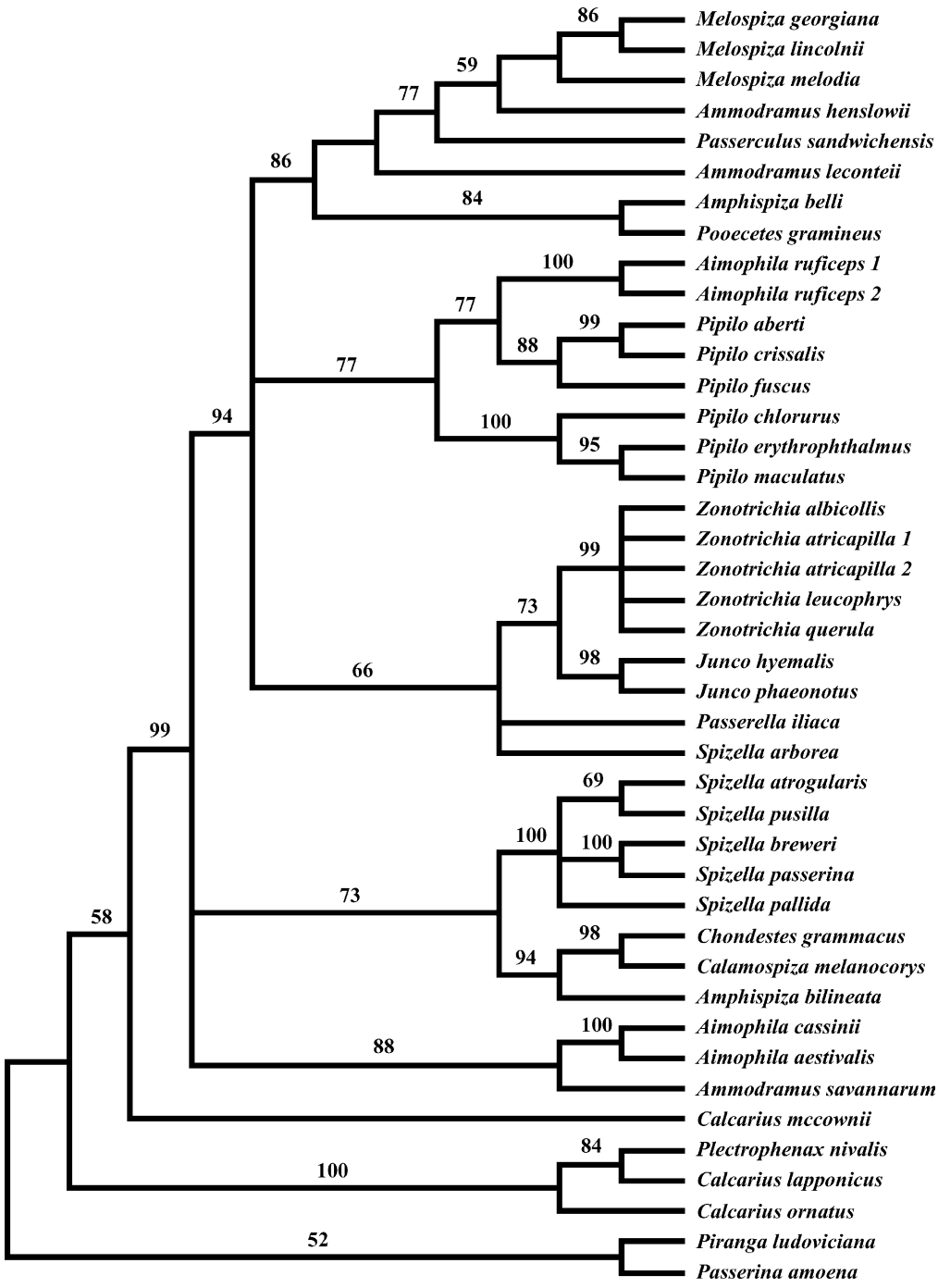

Fig. 3. Strict consensus tree based on 12 transversion only parsimonious trees with bootstrap values of 50% or greater at branch nodes.

Aimophila, *Passerculus*, and *Passerella*. This investigation provides some clarity for relationships among certain species in ambiguous and infrequently analyzed genera, such as *Chondestes* and *Calamospiza*. Finally, this study supports putative monophyly of conventionally recognized groups, such as *Junco*, *Melospiza*, and *Zonotrichia*.

All topologies in our study exhibited a basal position of *Calcarius* and *Plectrophenax nivalis* to all other sparrows examined. Our study did not support monophyly of *Calcarius* (see Table 5), but it did provide evidence for a close relationship between *Calcarius* and *Plectrophenax*. Though, this basal position of these two genera did not receive high bootstrap support, this basal position has been demonstrated in previous studies based on allozymes (Avise et al., 1980), morphology (Patten and Fugate, 1998; Tordoff, 1954), and mtDNA sequence (Grapputo et al., 2001; Klicka et al., 2000;

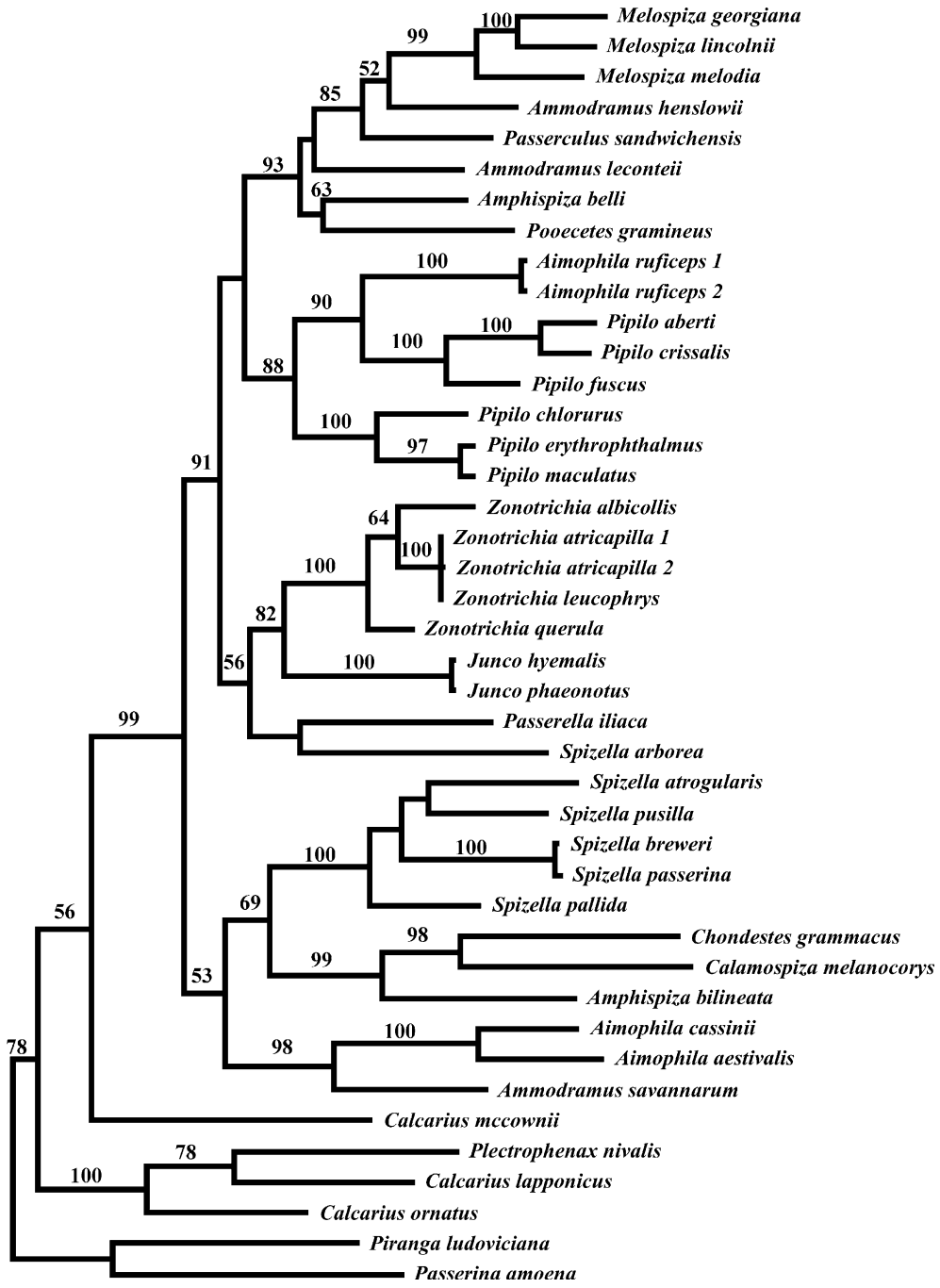

Fig. 4. Estimated maximum likelihood tree based on GTR $+I+\Gamma$ model (see text for parameter values), with bootstrap values of 50% or greater at branch nodes.

Yuri and Mindell, 2002). Traditionally, *Calcarius* and *Plectrophenax* are thought to be sister taxa as well as being the closest relatives to the Old World buntings (*Emberiza*) (Byers et al., 1995; Greenlaw, 1977; Harrison, 1967; Jehl, 1968; Paynter and Storer, 1970). In Klicka et al.'s (2000) study on New World oscine relationships, based on mtDNA cytochrome *b* and NADH dehydrogenase subunit 2, they found that when *C. lapponicus* was subjected to different analytical techniques (e.g., weighting schemes) its position shifted greatly in their trees. However, it not only remained basal to the typical North American sparrows (e.g., *Melospiza* and *Spizella*), but also remained basal to other emberizid genera, such as *Emberiza*, *Tiaris*, and *Sporophila*. Thus, Klicka et al. (2000) postulated that *Calcarius* "represents a previously unrecognized early radiation event and convergence upon sparrow morphology" (Klicka et al., 2000: 331). Grapputo et al. (2001) and Yuri and Mindell

Table 5
Shimodaira–Hasegawa tests comparing the maximum likelihood tree to the generic monophyly constraint trees

Tree	− ln Length	Difference − ln Length	P^*
Present study	13704.62514	(Best)	
Aimophila monophylic	13775.39176	70.76662	0.000*
Ammodramus monophylic	13795.41937	90.79423	0.000*
Amphispiza monophylic	13781.83405	77.20891	0.000*
Calcarius monophylic	13753.23756	48.61242	0.030*
Spizella monophylic	13733.34804	28.72289	0.176

Note. Probability of getting a more extreme t value under the null hypothesis of no difference between the two trees (one-tailed test). Asterisked values in the table indicate significant difference at $P < 0.05$.

(2002) found similar results, and Yuri and Mindell (2002) speculate that the group Emberizinae was derived from a "*Calcarius*-like" ancestor that radiated from the north to the south in the New World. These latter findings strengthen the hypothesis that *Calcarius* and *Plectrophenax* are not particularly close to either *Emberiza* or other sparrows within Emberizini. Among the longspurs, *Calcarius mccownii* is distinct from its congeners by having a larger bill as well as a sorter tail and spur (Rising, 1996). These differences may explain its position relative to the other longspurs examined in our analyses.

Avian systematists previously have suspected that the species within the genus *Aimophila* (Storer, 1955; Ridgeway, 1901; Wolf, 1977) do not form a natural group. These observations are substantiated in our analyses (see Table 5). Wolf (1977) divided *Aimophila* into three complexes plus *A. quinquestriata*, which is occasionally placed in *Amphispiza* (Byers et al., 1995; Rising, 1996). These complexes include: (1) *botterii* complex (*A. aestivalis*, *A. botterii*, and *A. cassinii*), (2) *ruficeps* complex (*A. notostricta*, *A. rufescens*, and *A. ruficeps*), and (3) *haemophila* complex (*A. carpalis*, *A. humeralis*, *A. mystacalis*, *A. ruficauda*, and *A. sumichrasti*). However, Wolf (1977) concluded the *Aimophila* complexes may be related to different emberizid genera.

Our topologies demonstrated a sister-taxon relationship between *A. aestivalis* and *A. cassinii*, which was predicted by the *botterii* complex. The sister taxa relationship between *A. ruficeps* and the brown towhees also supported the Wolf (1977) study. Wolf (1977) based this suspected relationship on several factors, but with an emphasis on the pair reunion and the display behaviors between *A. rufescens* and towhees. Johnson and Haight (1996) also suggested a close relationship between *Pipilo* and *Aimophila* based on: (1) similarities in vocalization and habitat selection between *P. fuscus* and the Rufous-winged Sparrow (*A. carpalis*), (2) the ground-dwelling behavior of *A. ruficeps*, and (3) the calls and behaviors of the Rusty Sparrow (*A. rufescens*). The *ruficeps* complex has been further differentiated from its congeners by its probable evolution in the pine-oak woodlands of the Mexican southwest, while the remaining *Aimophila* species evolved in the grasslands (Rising, 1996; Wolf, 1977). This scenario may explain why *Ammodramus savannarum*, a grassland species, appears to be more closely related to *A. aestivalis* and *A. cassinii* than *A. ruficeps* in our analyses. Some morphological evidence (Patten and Fugate, 1998) suggests that the genus is monophyletic, however, our genetic data corroborate previous non-genetic studies (Storer, 1955; Wolf, 1977) that *Aimophila* does not constitute a monophyletic group.

Though the species within the genus *Ammodramus* are collectively known as the grassland sparrows their relationships in our analyses suggest that the genus is not a monophyletic group (see Table 5). This genus is quite diverse and as a result some of its members have been assigned to different genera in the past (Rising, 1996). Additionally, the classification of *Ammodramus* has been referred to as an ambiguous genus (Murray, 1968; cf. AOU, 1957, 1983) and may exhibit polyphenetic structure (Avise et al., 1980; Vickery, 1996; Zink and Avise, 1990). Robins and Schnell (1971) suggested that the genus be divided into two separate genera: (1) *Ammodramus*, grassland species (*A. aurifrons*, *A. bairdii*, *A. savannarum*, and *Passerculus sandwichensis*), and (2) *Ammospiza*, marshland species (*A. caudacutus*, *A. henslowi*, *A. leconteii*, and *A. maritimus*). In contrast, Zink and Avise's (1990) study, based on mitochondrial restriction fragments, showed that *A. bairdii* was sister to *A. henslowii* and occurred in the same clade with *A. caudacutus*, *A. leconteii*, and *A. maritimus*. Their data set detected the distinctness of the clade containing *A. maritimus*, *A. caudacutus*, and *A. leconteii*; however, they concluded the relationships within the genus remained unclear (Zink and Avise, 1990). Rising (1996) split the genus into two ecological groups: the dry grassland species (*A. bairdii*, *A. henslowi*, and *A. savannarum*) and the wet grassland species (*A. caudacutus*, *A. leconteii*, and *A. maritimus*). Nevertheless, when *A. henslowii*, *A. leconteii*, and *A. savannarum* are analyzed with other emberizid species, based on *Atp8*, *Atp6*, and COIII mtDNA sequence, none of these previously suggested groupings of *Ammodramus* are reproduced.

However, we only examined three species in the genus and this limited scope precludes a true comparison of taxonomic relationships within the group as suggested in these earlier studies.

In all of our analyses, *A. savannarum* was sister to the *Aimophila aestivalis*/*A. cassinii* clade, where its position was quite distant from the other two *Ammodramus* congeners examined. As mentioned previously, the relationship between *A. savannarum* and the *Aimophila aestivalis*/*A. cassinii* clade may reflect a shared evolutionary history, as the *botterii* complex is believed to have evolved in the grasslands (Rising, 1996; Wolf, 1977). Previous studies corroborate this basal position of *A. savannarum* (Avise et al., 1980; Grapputo et al., 2001; Klicka et al., 2000). Although *A. savannarum* did not cluster with its congeners, *A. henslowii* and *A. leconteii* did consistently appear together in the same clade in all of our analyses, though never as sister taxa. Zink and Avise (1990) found that the species within the genus *Ammodramus* displayed greater genetic differentiation than what was found in most other avian congeners. As demonstrated in our study, this genetic differentiation may become amplified when members within the genus are subjected to a phylogenetic analysis involving many other emberizid genera, resulting in a polyphyletic distribution of the genus *Ammodramus*.

Taxonomists traditionally have suggested that *Passerculus sandwichensis* be placed within the genus *Ammodramus* and that it is probably most closely related to *A. bairdii* and *A. savannarum* (Rising, 1996). *Passerculus sandwichensis* was sister to *A. henslowii* in the unordered parsimony tree in our study; however, in the transversion and maximum likelihood analyses, *A. leconteii* was basal to *P. sandwichensis*, which in turn was basal to *A. henslowii*. Though our analyses do not provide a clear picture of the relationship between *P. sandwichensis* and the dry (e.g., *A. henslowii*) and wet grassland (e.g., *A. leconteii*) species groups as described by Rising (1996), our study does suggest that *P. sandwichensis* may belong within the genus *Ammodramus* when *A. savannarum* is excluded.

The relationship of *Pooecetes gramineus* to other emberizids has not been investigated using mitochondrial sequence. Rising (1996) suggested the genus maybe closely related to the grassland sparrows, *Ammodramus*. Patten and Fugate (1998) presented a sister-taxon relationship between *Pooecetes* and *Chondestes*, which was never demonstrated in any of our analyses. Though *Pooecetes* and *Amphispiza belli* are sister taxa in our study, *Pooecetes* consistently appears in the same clade with *Ammodramus leconteii*, *A. henslowii*, *Passerculus sandwichensis*, and *Melospiza*. The latter results, therefore, provide some support for a possible relationship between *Pooecetes* and the grassland sparrows, *Ammodramus*, when *A. savannarum* is excluded. However, the placement of the *Pooecetes*/*A. belli* clade among *A. henslowii* and *A. leconteii* changed depending on the analysis method. Few studies have examined the relationship between *Pooecetes* and other sparrows and our analysis thus provides a genetic starting point for future investigations.

Our study demonstrates that *Amphispiza belli* and *A. bilineata* are not sister taxa, but are relatively distant and consequently the genus is not monophyletic (see Table 5). After analyzing a 288 bp fragment of cytochrome *b*, Johnson and Cicero's (1991) study calculated a 10.9% average nucleotide difference between *A. belli* and *A. bilineata*. This detected genetic distance may explain why these two congeners appear so spread apart in our analyses. The closest relatives to *Amphispiza* are thought to be *Aimophila* and *Chondestes* (Rising, 1996). The sister-taxon relationship between *A. bilineata* and the *Calamospiza*/*Chondestes* clade shown in our study may imply a possible association between *Amphispiza* and *Chondestes*; however, the suggested relationship between *Amphispiza* and *Aimophila* is somewhat more difficult to ascertain. In the unordered parsimony and maximum likelihood analyses, *A. bilineata* does appear in the same clade with *A. aestivalis* and *A. cassinii*, but never as a sister-taxon. Due to the ambiguous relationship between *A. bilineata* and *Aimophila* it is not possible to discern whether an association exists between these two genera. Monophyly of *Amphispiza* cannot be established based on our current findings. More studies are necessary to determine the relationships between it and other emberizids.

The sister-taxon relationship between *Chondestes grammacus* and *Calamospiza melanocorys* has challenged previous opinions that *C. melanocorys* is closely related to *Calcarius* and *Plectrophenax* (Byers et al., 1995; Rising, 1996). Patten and Fugate (1998) presented an unresolved relationship among *Emberiza*, the *Calcarius*/*Plectrophenax* clade, and *C. melanocorys*, though all these genera remained basal to all other emberizids they examined. Several authors (Graber, 1955; Patten and Fugate, 1998) have concluded that *C. melanocorys* has no close allies (Shane, 2000). Our study, however, clearly demonstrates that *C. melanocorys* belongs within the major emberizid clade (which received high bootstrap support) instead of a more basal position like that of *Calcarius*.

The taxonomic relationship between *Spizella* and other sparrows is not well understood (Rising, 1996; Rotenberry et al., 1999). Previous analyses based on morphological characters suggest the genus is closely associated with *Aimophila*, *Junco*, and *Zonotrichia* (Patten and Fugate, 1998; Rising, 1996). Our study shows that *Spizella* (except *S. arborea*) appears to be more closely related to *Amphispiza bilineata*, *Calamospiza*, and *Chondestes*. *Spizella* does appear within the same larger clade containing *Aimophila aestivalis* and *A. cassinii* in the unordered parsimony and

maximum likelihood analyses. However, this larger clade received low bootstrap support, thus implying the relationship between *Spizella* and *Aimophila* as tentative. Our study also showed that the genus *Spizella* is not monophyletic, but the Shimodaira–Hasegawa test did not show a statistically significant difference (see Table 5). *Spizella arborea* was most often sister to *Passerella iliaca* as well as appearing in the same clade with *Junco* and *Zonotrichia*. This distant relationship between *S. arborea* and its congeners has also been recognized in past taxonomic studies (Dodge et al., 1995; Mayer and Short, 1970; Patten and Fugate, 1998; Zink and Dittmann, 1993). Mayer and Short (1970) suggested *S. arborea* had no close allies. Zink and Dittmann (1993) reported that *S. arborea* had an average genetic distance of 10.5% from its congeners, which was more than the genetic distance between the other *Spizella* species and their outgroup *Junco hyemalis*. Cytochrome *b* sequence data exhibited a similar degree of genetic distance in Dodge et al. (1995). Dodge et al. (1995) combined both mtDNA sequence and restriction enzyme (Zink and Dittmann, 1993) data sets and found the same distant relationship between *S. arborea* and its congeners as previously observed in both uncombined analyses. To help clarify the position of *S. arborea*, Dodge et al. (1995) added mtDNA sequence from several other emberizid species, and again, *S. arborea* continued to fall outside of the *Spizella* clade. Despite these results, Dodge et al. (1995) supported monophyly of the genus based on their uncertainty as to which outgroup was appropriate and the possibility of long-branch attraction (Felsenstein, 1978). However, Zink and Dittmann (1993) concluded *Spizella* should be tested for monophyly. Our study lends additional support that *Spizella* is not a natural group and *S. arborea* is quite distinct from its congeners.

Based on morphological similarities Mayer and Short (1970) advocated a "*passerina*" complex (*S. breweri*, *S. passerina*, and *S. pallida*) within the genus *Spizella* and that *S. atrogularis* was closely related to the complex. However, those proposed relationships were not corroborated by phylogenetic analyses based on mitochondrial restriction enzyme or cytochrome *b* sequence data (Dodge et al., 1995; Zink and Dittmann, 1993). In these earlier studies, the positions of *S. atrogularis*, *S. breweri*, *S. passerina*, and *S. pusilla* changed depending on what analyses were performed. In our analyses, *S. breweri* and *S. passerina* were consistently sister taxa, but the relationships of the remaining taxa varied depending on the phylogenetic technique.

The genera *Junco*, *Melospiza*, *Passerella*, and *Zonotrichia* are often thought to be closely related based on their morphological characters. Systematists previously have advocated various combinations and merges of these genera (Graber, 1955; Mayer and Short, 1970; Paynter, 1964; Paynter and Storer, 1970; Short and Simon, 1965). In contrast, molecular evidence suggests that all four genera are sufficiently distinct from each other, and Zink (1982), and Rising (1996) recommends generic separation is warranted. Though *P. iliaca* consistently appears in the same clade with *Junco* and *Zonotrichia*, it appears to have a closer relationship with *Spizella arborea*. *Melospiza* appears to be quite divergent from its suggested closest relatives. The genus repeatedly appears in the same clade with *Ammodramus henslowii*, *A. leconteii*, *Amphispiza belli*, and *Pooecetes* with high bootstrap support. Within the genus *Melospiza*, the branching pattern (*M. melodia* basal to the *M. georgiana*/*M. lincolnii* clade) was consistent with Zink (1982), and Zink and Blackwell (1996). Interestingly, a codon deletion was observed in the *Atp8* gene for *M. georgiana* and *M. lincolnii*, but not in *M. melodia*.

Mayer and Short (1970) suggested *Z. albicollis* and *Z. atricapilla* were sister species. However, previous studies (Zink, 1982; Zink and Blackwell, 1996; Zink et al., 1991) clearly indicated that the sister-taxon to *Z. atricapilla* is *Z. leucophrys*. In our study, all three topologies exhibit an unresolved relationship between both samples of *Z. atricapilla* and *Z. leucophrys*, but both taxa routinely clustered with the other *Zonotrichia* species examined. *Zonotrichia atricapilla* 1 and *Z. leucophrys* shared the same haplotype, and *Z. atricapilla* 2 exhibited a single base pair difference in the *Atp6* gene from the other two sequences. This low variation between these sister taxa has also been observed in mitochondrial restriction sites, cytochrome *b*, and control region sequence data (Weckstein et al., 2001; Zink and Blackwell, 1996; Zink et al., 1991). Weckstein et al. (2001) observed haplotype sharing between certain subspecies of *Z. atricapilla* and *Z. leucophrys*. They offered two alternative explanations for the extremely low observed variation. In view of haplotype sharing, they first suggested recent speciation and insufficient time for lineage sorting. However, this hypothesis was not favored due to the large amount of morphological and allozymic differences existing between these two species. In conclusion, Weckstein et al. (2001) proposed that the low variation observed in their study was best explained by a past hybridization event involving the capture, introgression, and replacement of the mitochondrial genome of either *Z. atricapilla* or *Z. leucophrys*.

Traditionally, the genus *Pipilo* has been divided into two groups: (1) the "brown towhees" (*P. aberti*, *P. albicollis*, *P. crissalis*, and *P. fuscus*) and (2) the "rufous-sided towhees" (*P. chlorurus*, *P. erythrophthalmus*, *P. maculates*, and *P. ocai*) (Davis, 1951; Zink et al., 1998). Several ornithologists have debated whether the brown towhees belong within the genus *Pipilo* or to a group that is morphologically more similar to tropical genera, such as *Arremonops* or *Melozone* (Byers et al., 1995; Davis, 1951; Parkes, 1957, 1974; Rising, 1996; Sibley, 1950, 1954, 1955; Zink et al., 1998). As previously

mentioned, the brown towhees may also be closely related to some species of *Aimophila* (e.g., *A. ruficeps* as seen in this study) (Sibley and Ahlquist, 1990; Storer, 1955; Wolf, 1977). These debates have called into question the monophyletic status of *Pipilo* as well as the two major towhee groups. Zink et al. (1998) was able to show monophyly of the brown and rufous-sided towhees, but was unable to demonstrate monophyly of the genus. When *Melozone kieneri* was added for their outgroup, its placement varied depending on which phylogenetic technique was used (Zink et al., 1998). Zink et al. (1998) suggested long-branch attraction as one possible factor for the changing positions of *Melozone*, due to the large genetic distance of this genus from the towhees they examined in their study. We were able to demonstrate monophyly of the two major towhee complexes, though the genus appears to be paraphyletic due to the relationship between the brown towhees and *Aimophila ruficeps*.

In conclusion, our study has provided a phylogenetic analysis of most of the North American emberizids. As revealed in other analyses (e.g., Grapputo et al., 2001; Klicka et al., 2000) *Calcarius* and *Plectrophenax* are basal and distant to other emberizines. This investigation also demonstrated the paraphyly of commonly viewed natural groups, such as *Aimophila*, *Ammodramus*, *Amphispiza*, *Pipilo*, and *Spizella*. We also presented relationships among uncommonly investigated genera, such as *Calamospiza*, *Chondestes*, and *Pooecetes*. Lastly, we confirmed monophyly of traditional groups, such as *Junco* and *Zonotrichia*. Because our analyses exhibited sufficient bootstrap support for both basal and terminal end branching, it appears that the mitochondrial genes *Atp8*, *Atp6*, and COIII carry enough variation for significant phylogenetic reconstruction at least among these avian taxa. Further investigations should involve adding more taxa from underrepresented genera used in this study, such as *Ammodramus* and *Aimophila* to obtain a clearer picture of the relationships among these genera and other sparrows. Additionally, nuclear DNA markers should be explored to confirm or provide resolution for emberizid phylogenetic reconstruction based on mtDNA.

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