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A Molecular Phylogeny of the New World Orioles (*Icterus*): The Importance of Dense Taxon Sampling

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We sequenced 2005 bp of the mitochondrial ND2 and cytochrome *b* genes from the 25 recognized species of New World orioles (*Icterus*). Our data confirmed the monophyly of *Icterus* and produced a well-resolved phylogeny showing three main clades of orioles. We also sequenced multiple subspecies for most polytypic taxa. Our findings demonstrated the importance of dense taxon sampling below the species level in two ways. First, we found evidence that two species are polyphyletic, *I. galbula* (Northern oriole) and *I. dominicensis* (Black-cowled oriole). Choosing different subspecies from either of these taxa would lead to different species-level phylogenies. Second, adding subspecies even to monophyletic groups produced a bootstrap tree with significantly more support. Of the two genes that we used, ND2 provided more resolution than did cytochrome *b*. ND2 evolved up to 40% faster than cytochrome *b*, yet shows a higher saturation threshold. Our findings suggest that ND2 may be superior to cytochrome *b* for resolving species-level phylogenies in passerine birds. © 1999 Academic Press

INTRODUCTION

The New World oriole genus (*Icterus*) is the most speciose genus in the blackbird family (Icteridae), with 25 species currently recognized (Sibley and Monroe, 1990). These species inhabit most of the New World, from southern Canada (e.g., Baltimore oriole, *I. g. galbula*) to northern Argentina (e.g., Epaulet oriole, *I. cayanensis*), with the greatest oriole diversity occurring in Mexico where 14 species breed (Howell and Webb, 1995). Orioles are interesting from the standpoint of plumage evolution, for the genus exhibits variation in color pattern and plumage maturation schedules and contains both dimorphic and monomorphic species. The genus also exhibits an interesting mosaic of shared and variable ecological and behavioral characteristics (e.g., Lowther, 1975; Howell and Webb, 1995).

Unfortunately, phylogenetic relationships within the genus are poorly known, with only a few previous studies providing testable phylogenetic hypotheses. Beecher (1950) studied skull characteristics and nest shape and concluded that there were two distinct lineages of orioles, each of which had evolved independently from different *Agelaius*-like ancestors. He suggested renaming one of the groups, which he characterized as having "more slender, nectar-adapted bills," as genus *Bananivorus* (Beecher, 1950, p. 61). Beecher did not use explicit tree-building methodologies, nor did he publish a character state matrix. However, two testable hypotheses are implicit in Beecher's conclusions: the hypothesis that the genus consists of two lineages and the hypothesis that the genus is not monophyletic.

No other researchers had attempted to resolve relationships within the genus until the advent of molecular data. Freeman and Zink (1995) included 11 species of *Icterus* in a study of blackbird mitochondrial DNA restriction sites. However, their data did not resolve relationships among most of the species; their well-supported tree included only one clade of more than two orioles. Their most surprising conclusion was that the Troupial (*I. icterus*) is not closely related to other *Icterus*, but perhaps is basal to all other blackbirds. Thus, their data also suggest the possibility that the oriole genus might not be monophyletic, but not in the way proposed by Beecher (1950). Lanyon and Omland (1999) included 10 species of orioles in a molecular study of the whole blackbird family (Icteridae) based on cytochrome *b* sequence data. Contrary to the suggestions of Beecher (1950) and Freeman and Zink (1995), these data identified *Icterus* as one of five well-defined monophyletic lineages. In addition, several nodes within *Icterus* were robust to a bootstrap manipulation.

Uncertainty about relationships among oriole species is compounded by uncertainty about species limits. Several species complexes within the orioles have been the subject of much debate as to whether certain taxa

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are subspecies or species. These controversies and changes in classification have taken place mostly in the absence of formal systematic studies. For example, the Baltimore (*I. g. galbula*), Bullock's (*I. g. bullockii*), and Black-backed (*I. g. abeillei*) orioles were formerly considered separate species (American Ornithologists' Union, 1957) or the latter two as subspecies of *bullockii* (Miller, 1906). In 1983, the three were lumped into a single species, the Northern oriole (*I. galbula*) (American Ornithologists' Union, 1983), but were later resplit into the three species (American Ornithologists' Union, 1995). The *Icterus dominicensis* group of the Caribbean Basin is even more confusing because more taxa are involved. Further examples of species rank challenges are provided by two South American taxa that Blake (1968) considered subspecies, but that Sibley and Monroe (1990) raised to species level. These are *I. jamacaii* within their *I.* [*icterus*] superspecies and *I. chrysocephalus* within their *I.* [*cayanensis*] superspecies (Sibley and Monroe, 1990; brackets are their notation for superspecies). Uncertainty about species limits was the most basic of several reasons that we decided to include multiple subspecies in our study.

Whereas a number of studies have addressed the importance of taxon sampling at higher taxonomic levels (e.g., Lanyon, 1985; Weller *et al.*, 1992), few studies have addressed this issue below the species level (but see Melnick *et al.*, 1993; Zink *et al.*, 1998). We conducted dense taxon sampling below the species level (45/73 subspecies) to address three further objectives. First, we tested the monophyly of as many species as possible. Paraphyletic relationships involving recognized species have been documented in many taxa (e.g., ducks, Avise *et al.*, 1990; pocket gophers, Patton and Smith, 1994; beetles, Funk *et al.*, 1995). Verifying monophyly at the species and subspecies levels is as crucial to reliable treebuilding and ancestral state reconstruction as it is at any other taxonomic level (Graybeal, 1995; Omland, 1997). If some species are polyphyletic or paraphyletic, substantially different placement of such "species" may result, depending upon which exemplar lineage is sampled (e.g., Melnick *et al.*, 1993). Second, many authors have shown that dense taxon sampling subdivides long branches that might otherwise confound treebuilding algorithms (e.g., Felsenstein, 1978; Huelsenbeck and Hillis, 1993). For this reason several authors have suggested that adding taxa may be an effective means of producing reliable and well-resolved trees (Hillis, 1996; Graybeal, 1998). Including additional subspecies may also lead to more strongly resolved nodes on a tree.

Increasing the number of taxa sampled is one way to increase phylogenetic resolution. However, the more conventional approach is to increase the number of characters; so we also elected to sequence two genes. Sequencing multiple genes offers several additional advantages that we will explore in this paper. In the best of cases, using genes with slightly different evolutionary rates can lead to complementary information (e.g., Olmstead and Sweere, 1994). More problematic, large rate differences can lead to significant conflict even between mitochondrial genes (e.g., Cummings *et al.*, 1995; Sullivan, 1996; Cunningham, 1997). The genes that we chose to sequence, cytochrome *b* and ND2 (NADH2) differ in evolutionary rate (e.g., Hackett, 1996) making them useful for exploring these issues. Furthermore, we will use this study to compare the utility of these two genes for intrageneric phylogenies.

MATERIALS AND METHODS

Taxa Sequenced

We obtained samples from 45 oriole taxa for inclusion in this study. These samples were chosen to represent variation between and within all 25 species of orioles recognized by Sibley and Monroe (1990). To ensure adequate sampling of within-species diversity, we included multiple subspecies from all species of orioles with well-marked differences in plumage pattern, as well as from most polytypic species with only subtle differences between subspecies (e.g., carotenoid hues in *I. cucullatus*). The three species with multiple subspecies for which we used only one exemplar were *I. leucopteryx*, *I. pectoralis*, and *I. wagleri*. (See Appendix for sample sources and for voucher and locality information on the included taxa.) Throughout this paper we use the nomenclature of Sibley and Monroe (1990) for species level and higher because it is the most widely available and geographically comprehensive recent checklist. However, because Sibley and Monroe (1990) do not list any subspecies, we follow Blake (1968) for all subspecies names.

For outgroup taxa we chose seven species to represent each of the four other lineages of blackbirds (Icteridae) (Lanyon and Omland, 1999). We used the one member of the monotypic cup-nesting cacique lineage (*Amblycercus holosericeus*) and chose two species from each of the other three lineages (see Lanyon and Omland, 1999). The grackles and allies lineage was represented by *Agelaius phoeniceus* and *Nesopsar nigerrimus*, the meadowlarks and allies by *Dolichonyx oryzivorus* and *Sturnella neglecta*, and the caciques and oropendolas by *Cacicus solitarius* and *Gymnostinops bifasciatus*.

Genes Chosen

We chose cytochrome *b* because it has proven useful for species-level phylogenies in other bird groups (e.g., Hackett, 1996; Moore and DeFilippis, 1997; but see Meyer, 1994) and we had sequence data for many outgroup taxa (Lanyon and Omland, 1999). We chose ND2 because it seems to evolve at a faster rate than cytochrome *b* and most other mitochondrial genes in passerine birds (e.g., Hackett, 1996; S. M. Lanyon,

unpublished data). Although we sequenced two mitochondrial genes (which are in a single linkage unit), the use of multiple genes and the analysis of data sets separately as well as in combination offer several advantages (e.g., Bull *et al.,* 1993; Omland, 1994; Cunningham, 1997). Furthermore, separate amplification of two genes that are nearly 6000 kb away from each other (Desjardins and Morais, 1990) greatly decreases the possibility that both sequences are from a continuous nuclear copy of the mitochondrial genome (e.g., see Collura and Stewart, 1996; Sorenson and Quinn, 1998).

Laboratory Procedures

DNA was extracted using standard phenol/chloroform extractions (Hillis *et al.,* 1990) or using Qiaamp Tissue Extraction Kits (Qiagen). (Samples from the Smithsonian Tropical Research Institute [see Appendix] were obtained as lyophilized DNA). Specific fragments of the mitochondrial genome were amplified using two primers spanning 1074 bp of the cytochrome *b* gene and spanning 1098 bp of ND2. Primers used for cytochrome *b* were: L14990 (Kocher *et al.,* 1989), H15424 (Hackett, 1996), H15350 (J. Klicka, pers. comm. [HCBA]), H15916 (Lanyon, 1994 [B4]), and H16065 (Harshman, 1996 [H4a]; PCR only). ND2 primers used were L5215 (Hackett, 1996), H5776I (modified from primer designed by K. Johnson [TGGGARATGGAGGACAARGC]), L5758 (Johnson and Sorenson, 1998), and H6313 (Johnson and Sorenson, 1998). (All numbers refer to the 3′ base of the primer referenced to the corresponding chicken mtDNA sequence [Desjardins and Morais, 1990].) A typical amplification involved an initial cycle (3 min at 93°C, 1 min at 50°C, 45 s at 72°C) followed by 35 cycles (1 min at 93°C, 1 min at 50°C, 45 s at 72°C) and a final 10-min extension at 72°C. PCR products were cleaned using QIAquick Kits (Qiagen). We obtained double-stranded DNA for automated sequencing, typically with two sets of primers for each gene. We used a set of primers for each half of the gene, sequencing each half in both directions using cycle sequencing (10 s 96°C, 5 s 50°C, 4 min 60°C, 25 cycles). These products were cleaned using Centri-Sep columns (Princeton Separations, Adelphia, NJ) and sequenced on an ABI 377 automated sequencer. Chromatograms were aligned and confirmed using Sequencher sequence analysis software (Genecodes Corp., Inc.). We obtained 920 bp of sequence for cytochrome *b* and 1085 bp of sequence for ND2. The sequences have been deposited in GenBank under Accession Nos. AF099276–310 (cytochrome *b*) and AF099311–360 (ND2). Due to occasional ambiguous peaks and difficulty reading sequences at the ends, sequences for about half the taxa include some ambiguous base calls (average 3 Ns for ND2, 17 Ns for cytochrome *b*). Text files from each of the taxa were aligned using Sequencher. We obtained cytochrome *b* sequences for several *Icterus* species and the outgroup taxa using manual sequencing and other protocols detailed in Lanyon and Omland (1999) (sequences deposited under GenBank Accession Nos. AF089004–068). For one species, *I. auricapillus,* we extracted DNA from a toepad of a museum specimen and amplified and sequenced DNA using only the first cytochrome *b* primer pair. Also, we did not successfully sequence the ND2 gene for *I. dominicensis dominicensis.* These two taxa are included only in the cytochrome *b* tree.

Phylogenetic Analysis

Aligned sequences were imported into PAUP* for phylogenetic analyses (test versions 4.0d60-63, written by David L. Swofford). Using PAUP*, we computed uncorrected pairwise divergence values for each gene for several classes of sequence changes (overall, transitions, transversions, codon positions). Unless otherwise stated, all genetic distances reported are overall uncorrected divergence values for both genes combined. We then plotted a number of these classes of change to test for evidence of sequence saturation and to evaluate the utility of cytochrome *b* and ND2. We first conducted phylogenetic analysis on the data sets from the two genes separately and present trees based on each gene individually. We examined the topologies for conflicts between the data sets, especially involving nodes supported by bootstrap analyses. We then used the partition homogeneity test in PAUP* to test for significant incongruence (Farris *et al.,* 1994) before combining the data sets. We used a two-tailed binomial test to compare levels of bootstrap support among the two individual data sets and the combined data set.

Because different mutations accumulate at different rates, especially due to differences between transitions and transversions as well as base composition biases, we chose six-parameter weighting (Williams and Fitch, 1989) as our primary weighting scheme. Six-parameter weighting accounts for both of these main sources of mutation rate differences, recovers "known phylogenies" at a range of taxonomic levels, and does not seem highly dependent on the initial tree (Cunningham, 1997). We obtained the weighting values by finding a shortest combined data tree using equally weighted parsimony and then reconstructing transformations onto that tree using MacClade (Maddison and Maddison, 1992). Following Cunningham (1997), we computed the frequency of the six types of substitutions (without regard to polarity) and derived weights by computing the negative natural log (-LN). These weights were used for one set of six-parameter searches only and not recomputed for multiple iterative searches. We examined the impact that this a priori weighting strategy had on our analyses by using two other weighting schemes, equally weighted parsimony and transversions given three times the weight of transitions (3× weighting). We obtained the weighting factor

of three by computing the ratio of the frequency of all reconstructed transitions to transversions on the equally weighted tree (see above).

We conducted 10 heuristic searches using the TBR algorithm (trees were rooted using the seven outgroup taxa). When 1 or more of the 10 replicates failed to find shortest trees, we then conducted an additional 100 searches. All equally parsimonious trees resulting from the analyses were combined to produce a majority rule consensus tree. To estimate the degree to which the topology resulting from these parsimony analyses was dependent upon character and taxon composition, we used two statistical resampling procedures. Bootstrapping characters (Felsenstein, 1985) examines the degree to which topology is dependent on the character composition of the data set. Jackknifing taxa (Lanyon, 1985) determines the degree to which topology is dependent on the taxonomic composition of the data set. We conducted 100 bootstrap replications with heuristic searches and random addition of taxa. We jackknifed taxa by deleting each taxon one at a time using commands lines (available upon request) in PAUP*. The output from these 50 jackknife pseudoreplicates was then synthesized using two programs written by S. M. L. for DOS (available upon request). We conducted heuristic, bootstrap, and jackknife searches using each of the three weighting schemes. To estimate the relative timing of key speciation events, we used Kimura's two-parameter model to correct for multiple substitutions and mapped those distances onto the majority rule consensus of the equal and 3× weighted heuristic trees.

With 50 taxa and 2005 bp, searching tree space using maximum likelihood analysis is difficult (e.g., Huelsenbeck, 1998). However, we conducted some exploratory analyses following the general protocol outlined by Huelsenbeck (1998). We started with the equally weighted parsimony tree, estimated parameters on that tree, fixed those parameters, found a new tree using NNI or TBR algorithms, and then conducted several iterations until a stable tree was obtained. We focused on using the general time-reversible model with gamma-distributed rate heterogeneity because the complex models that account for rate heterogeneity may be more likely to yield trees different from those produced by parsimony approaches (e.g., Sullivan, 1996).

Taxon Sampling

We explored the impact of dense taxon sampling on the combined data tree using a series of bootstrap analyses in which we subsampled from among the multiple subspecies. First we excluded two species that were polyphyletic with respect to mtDNA. We then subsampled from all the other species with multiple subspecies and two taxa labeled as superspecies by Sibley and Monroe (1990), *I.* [*cayanensis*] and *I.* [*icterus*]. For all these polytypic taxa, we randomly chose one subspecies to retain in the analysis and then conducted 500 bootstrap replications on the subsample of taxa (sparse taxon sample). We repeated this randomized taxon subsampling four times with replacement. We computed the average bootstrap value across the four replications for each of the supraspecific nodes in the complete tree. We then compared the average value for the sparse taxon analyses to the corresponding values from 500 bootstrap replications on the complete taxon data set (dense taxon sample). For all values that differed between the sparse and the dense sample, we tested for significant deviation from equal bootstrap values using a two-tailed binomial test. We conducted these comparisons using equally weighted analyses because such analyses are much faster than analyses with other weighting schemes, given the large number of bootstrap replications needed.

RESULTS

Sequence Data

Of the 920 bp of cytochrome *b* sequence, 419 (46%) sites were variable and 256 (28%) were parsimony informative. For the 1085 bp of ND2, 535 (49%) were variable and 382 (35%) were parsimony informative. Substitution proportions and calculated six-parameter weights are shown in Table 1. The two genes evolved at similar rates for closely related taxa (<4% divergent), but at greater divergences, ND2 evolved about 40% faster than cytochrome *b* for most ingroup comparisons (Fig. 1). We plotted transitions as a function of overall divergence for cytochrome *b* and found that the plot was linear within the ingroup (to about 9% divergence), but showed evidence of saturation to and among the outgroup taxa (Fig. 2). Further analysis revealed that this saturation was due to third position transitions. In contrast, ND2 showed no evidence of saturation, even for the outgroup taxa, for which pairwise comparisons are up to 16% divergent (Fig. 3). Ingroup comparisons for ND2 revealed divergences extending to over 13%.

Individual Gene Trees and Combinability

The cytochrome *b* data, when subjected to six-parameter weighting, produced a 50% bootstrap tree that resolved 26 of the possible 42 nodes (62%) (Fig. 4a). The tree contains an unresolved basal polytomy involving five lineages. Two of those lineages contain more than five taxa (labeled Clades A and C). The cytochrome *b* data suggest that three recognized species are not monophyletic: *I. galbula* (Northern oriole), *I. dominicensis* (Black-cowled oriole), and *I. cayanensis* (Epaulet oriole). *I. auricapillus* was revealed as sister to the four-taxon cayanensis clade and *I. dominicensis dominicensis* as sister to those five taxa in all heuristic searches.

The ND2 data subjected to six-parameter weighting

TABLE 1

Proportions of Substitution Types and Six-Parameter Weights

Bases	A	C	G	T
A	—	14%	**24%**	7%
C	2.0	—	3%	**50%**
G	**1.4**	3.4	—	2%
T	2.7	**0.7**	3.9	—

Note. Proportions of substitution types are shown above the diagonal. Six-parameter weights calculated from these proportions are shown below the diagonal. Values in boldface pertain to transition substitutions, whereas values in plain font pertain to transversion substitutions. All values are based on the combined cytochrome *b* plus ND2 data set reconstructed onto the shortest equally weighted tree (see Materials and Methods).

produced a 50% bootstrap tree (Fig. 4b) that is much-more resolved than the cytochrome *b* tree. The ND2 bootstrap tree resolved 33 of the possible 42 nodes (79%). The ND2 tree has higher bootstrap values for 11 of 12 nodes that are comparable with the cytochrome *b* bootstrap tree (binomial test, $P = 0.01$) (11 nodes had equal values and 10 were not shared). The ND2 tree is split into two basal lineages. Figure 4b shows that the group that we have labeled Clade C is sister to a group containing Clade A, Clade B, and *I. maculialatus*. ND2 and cytochrome *b* each provided 99% bootstrap support for oriole monophyly. Like cytochrome *b*, the ND2 data show that three recognized species are not monophyletic.

The partition homogeneity test in PAUP* detected no significant incongruence between the cytochrome *b* and the ND-2 data sets ($P = 0.53$). Furthermore, there are no nodes that conflict between the two bootstrap trees. Therefore we combined the two data sets for all subsequent analyses (e.g., see Bull *et al.*, 1993).

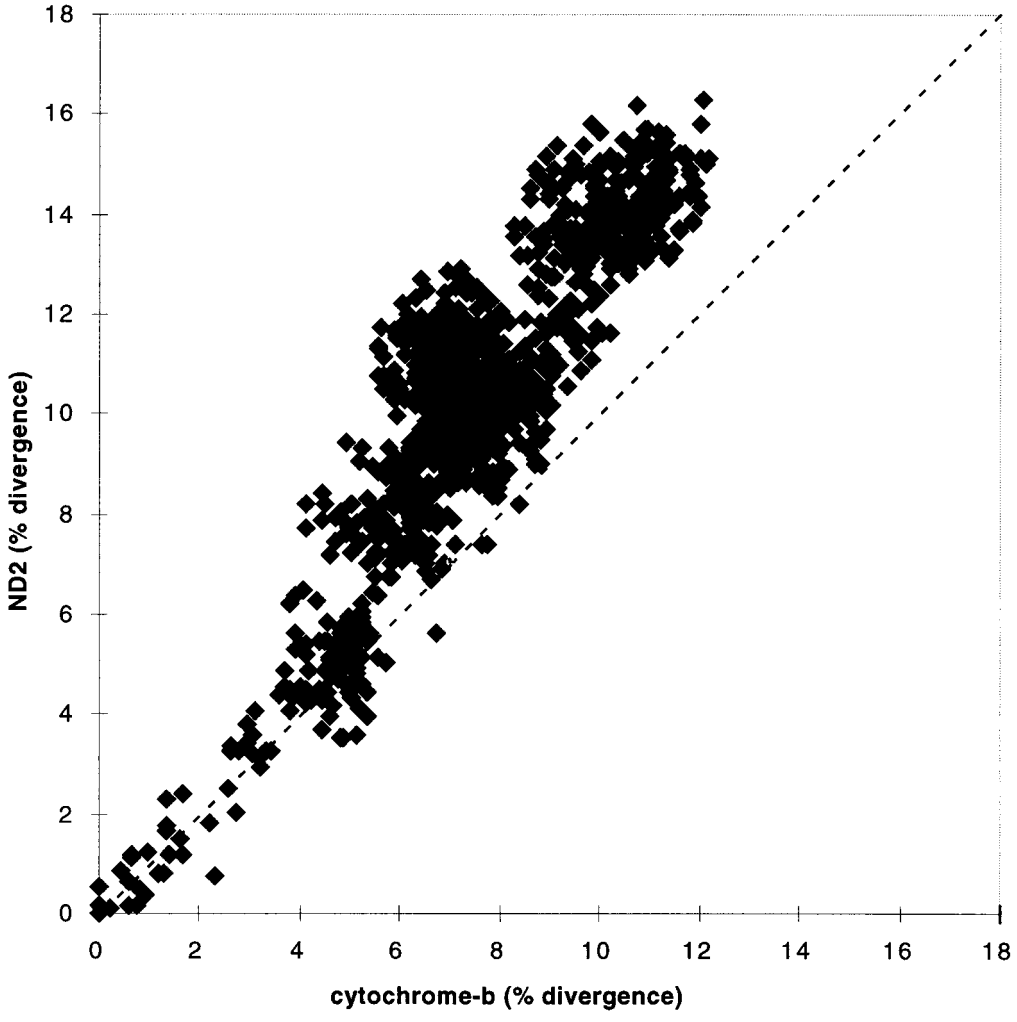

FIG. 1. Scatterplot of uncorrected pairwise sequence divergence for cytochrome *b* versus ND2. The dashed line corresponds to $x = y$, for which pairwise divergence values are equal for the two genes. ND2 accumulates changes more rapidly that cytochrome *b* beyond about 5% divergence.

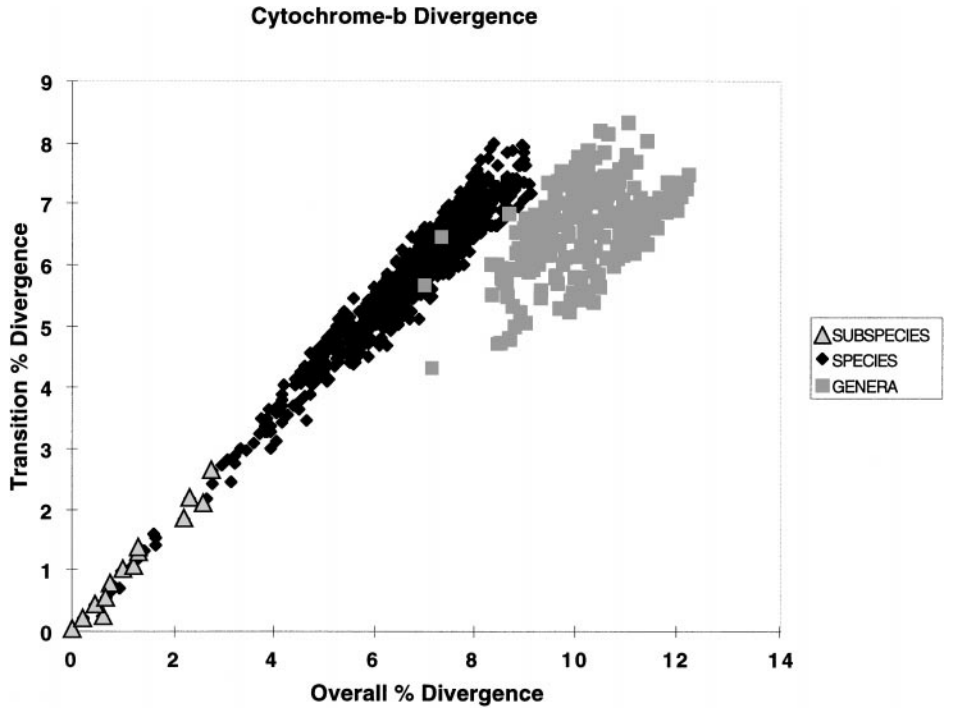

FIG. 2. Transition divergence for the cytochrome *b* gene. Percentage transition divergence is plotted as a function of overall percentage divergence (uncorrected "p" distance) for all pairs of taxa. Ingroup comparisons (diamonds and triangles) show no evidence of saturation, but comparisons involving outgroup taxa (squares) show strong evidence of saturation (beyond about 9% overall divergence).

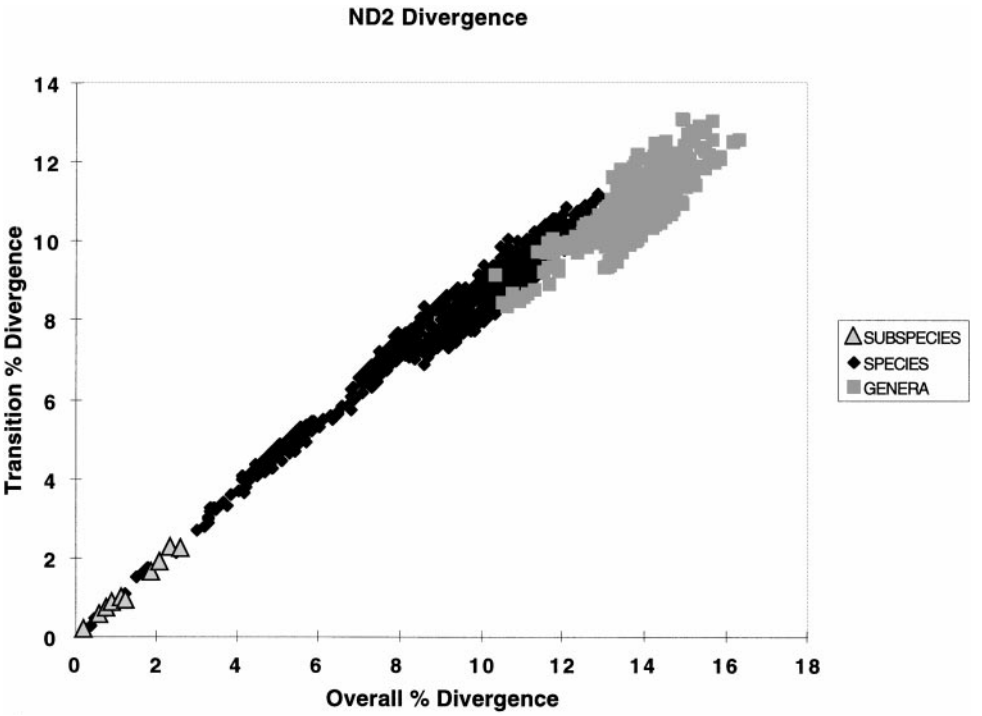

FIG. 3. Transition divergence for the ND2 gene. Percentage transition divergence is plotted as a function of overall percentage divergence (uncorrected "p" distance) for all pairs of taxa. The plot is linear for all comparisons, even for those involving outgroup divergences (up to over 16% overall divergence).

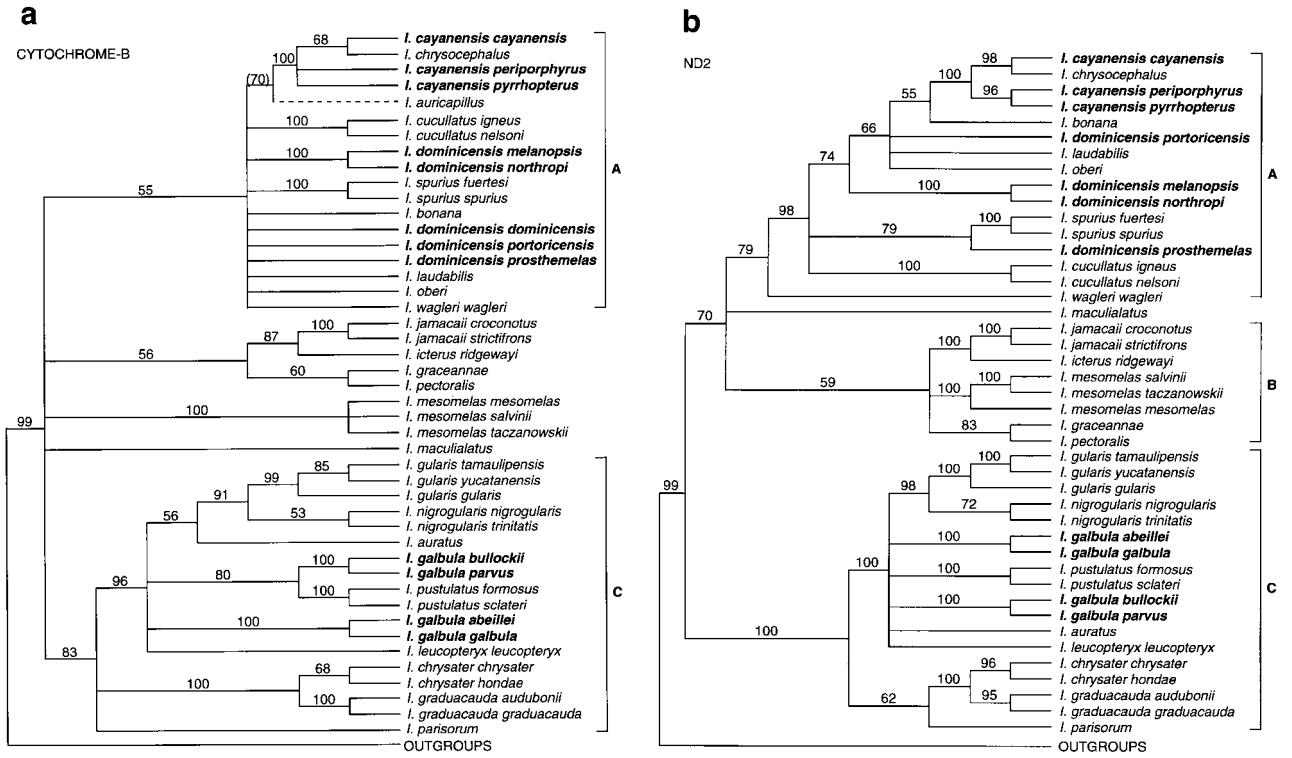

FIG. 4. (a) 50% bootstrap topology for cytochrome *b* based on six-parameter weighting (100 bootstrap replications). Two well-defined large clades are labeled as Clade A and Clade C. Names of nonmonophyletic taxa are printed in bold. All trees are rooted with outgroups representing the four other lineages of Icterines (see Materials and Methods). The dashed line to *I. auricapillus* signifies that its position is based on only 346 bp from beginning of cytochrome *b*. (b) 50% bootstrap topology for ND2 based on six-parameter weighting (100 bootstrap replications). Three well defined large clades are labeled. Clade A and Clade C include the same taxa as in (a) and ND2 identifies a third clade, Clade B. Names of nonmonophyletic taxa are printed in bold.

Combined Data Analyses

The topology of the combined-data bootstrap tree based on six-parameter weighting (bootstrap values shown in Fig. 5) is very similar to that of the ND2 bootstrap tree. There are no nodes that conflict between the combined data and the ND2 bootstrap trees. The combined-data bootstrap analysis resolved three clades of orioles, with *I. maculialatus* as sister to Clade A. The combined data provide 100% bootstrap support for *Icterus* monophyly.

The combined-data bootstrap tree resolved 37 of 42 possible nodes (88%), which is an increase in resolution compared to the ND2 tree alone. Of the 38 nodes resolved by either or both data sets, the combined tree has higher bootstrap values for 18 nodes, whereas the ND2 tree has higher values for only 3 nodes ($P = 0.001$, binomial test) (17 nodes have equal bootstrap values). The combined bootstrap tree recovers all 27 of the resolved nodes in the cytochrome *b* tree and has equal or higher bootstrap values for each of those nodes (higher values, 16 vs 0; $P < 0.0001$, binomial test).

A heuristic six-parameter weighted search of the combined data produced two islands of nearly equally parsimonious trees. The island containing the shortest heuristic trees (4 trees, score 2917.4, Fig. 5) was found in 38 of 100 random addition replications. They identify *I. maculialatus* as sister to all other *Icterus* and Clade B as sister to Clade C. The remaining 62 replications found a second island containing trees only 0.03% longer (5 trees, score 2918.3). Like the 50% bootstrap tree, the trees from this second island place *I. maculialatus* as sister to Clade A.

Alternative Weighting Schemes

We employed alternative weighting schemes to determine the sensitivity of our results to our choice of analytical methods. Other weighting schemes produce 50% bootstrap topologies nearly identical to the six-parameter 50% bootstrap tree (see Fig. 6), and bootstrap support values are similar. The only topological difference is that two nodes that are resolved in the equal and 3× bootstrap tree are not resolved in the six-parameter bootstrap tree. First, the alternative weighting schemes both identify *I. maculialatus* as sister to Clade A. *I. maculialatus* is placed in this same position in most trees resulting from all three weight-

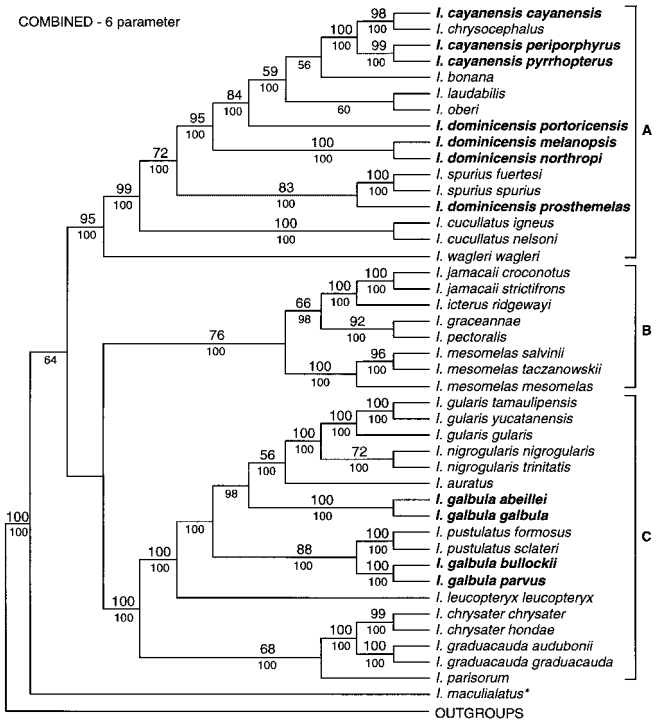

FIG. 5. Statistical support for the single most parsimonious tree found by the six-parameter weighted heuristic search. Bootstrap values are shown above branches (100 replications), and jackknife taxon values (Lanyon, 1985) are shown below branches. (Branches supported by less than 50% of the replications are shown with no value.) *The bootstrap analysis supported a different position for *I. maculialatus:* 59% of the replications found *maculialatus* to be sister to Clade A. Other labeling follows Fig. 4b.

ing schemes applied to either the combined or the separate data sets. Second, the equal and 3× weighted trees both resolve the position of *I. auratus* as sister to the *gularis/nigrogularis* clade. In addition to these two areas, there is a third area also characterized by uncertainty. Heuristic searches with these two weighting schemes each found the same three shortest trees (in all 10 random addition replicates) that differed among each other only in minor rearrangements of *I. oberi, I. laudabilis,* and *I. d. portoricensis.*

Additional weighted analyses with 10× and 20× transversion weighting and with separate transversion and six-parameter weights calculated for each gene resulted in very similar trees. Generally, the changes affected nodes with <50% bootstrap support in the same three areas just discussed. Corrected branch lengths (Fig. 7) show that all three of these areas that differ between and within weighting schemes involve short internodes.

Our exploratory maximum likelihood analyses also produced trees that differ from parsimony in the same three areas weakly supported by bootstrap analyses. The likelihood trees are similar to the equal and 3× parsimony trees including the placement of *maculialatus* as sister to Clade A. We also used maximum likelihood with the Kishino–Hasegawa test in PAUP* to test alternative tree topologies. Using a range of likelihood models, the equal and 3× parsimony trees are a better fit to the data than the shortest six-parameter trees, although not significantly better.

Species Limits

We found evidence that two species of orioles are polyphyletic: *I. dominicensis,* the Black-cowled oriole of the Caribbean basin, and *I. galbula,* the Northern oriole. These sequence data show that *I. g. galbula* (the Baltimore oriole) is not the sister taxon of *I. g. bullockii* (Bullock's oriole). These two taxa are 4.9% divergent from each other in the combined data set. Two other subspecies, *I. g. galbula* and *I. g. abeillei,* were found to be sister taxa and are about 0.5% divergent from each other (Table 2).

I. dominicensis consists of at least three separate lineages; for example, the position of *I. d. prosthemelas* (the Central American subspecies) is very different from that of *I. d. portoricensis* (the Puerto Rican subspecies). Furthermore, most described subspecies of *I. dominicensis* are from 4 to 7% divergent from each other. Three other island taxa (*I. bonana, laudabilis,* and *oberi*) are all from 3 to 7% divergent from each other and the subspecies of *I. dominicensis* (Table 2).

Our data also show that the three subspecies of *I. cayanensis* are paraphyletic with respect to the monotypic *I. chrysocephalus. I. cayanensis cayanensis* groups with *I. chrysocephalus* with 98% bootstrap support in the combined six-parameter tree. These two taxa are 0.6% divergent from each other, and all distances among the four taxa sampled in the *cayanensis* superspecies are between 0.4 and 1.4% sequence divergence.

Figure 7 also shows that there is a wide range of distances among subspecies in monophyletic species. Species with large divergences among subspecies include *I. nigrogularis* (2.5%) and *I. mesomelas* (1.4–2.3%). Pairs of subspecies with very low divergences include *I. galbula bullockii* to *I. g. parvus* (0.3%) and *I. graduacauda graduacauda* to *I. g. audubonii* (0.1%). The between-species comparison with the lowest divergence value was *I. audubonii* to *I. chrysater,* whose subspecies are 1.7% different from each other on average (Table 2).

Taxon Sampling

We also explored the effect that dense taxon sampling might have on tree topology and support. When we randomly deleted all but one subspecies for each of the nonpolyphyletic polytypic taxa, this subsampling did not cause changes in tree topology. However, the degree

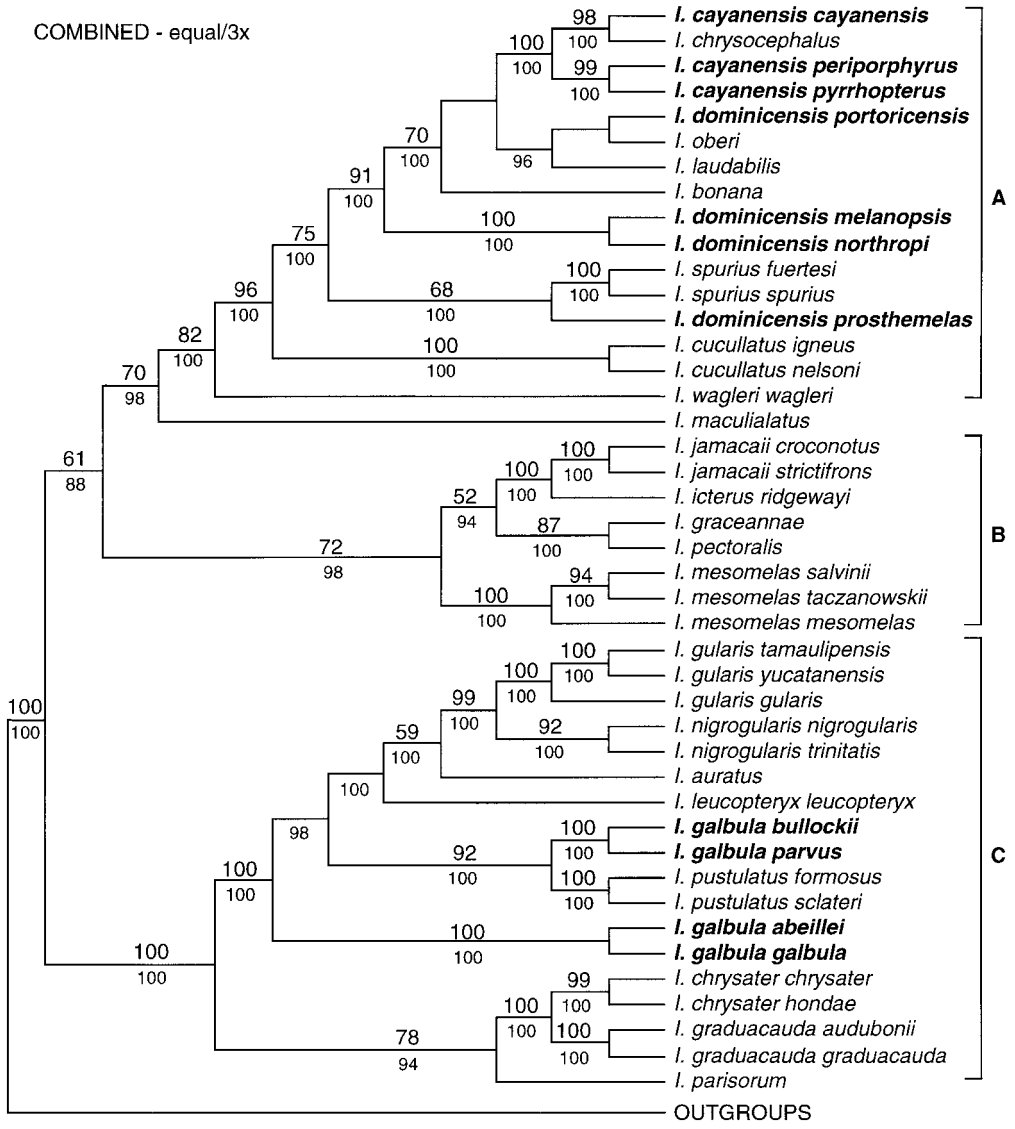

FIG. 6. Statistical support for the majority rule consensus resulting from both equal and 3× weighted heuristic searches. Bootstrap values (above branches) and jackknife taxon values (below branches) pertain to the equally weighted data. (Branches supported by less than 50% of the replications are shown with no value.) The three shortest equally weighted trees all were 2643 steps (CI = 0.36, RI = 0.64). Other labeling follows Fig. 4b.

of bootstrap support for supraspecific nodes did differ. The average bootstrap values for the dense sample tree were higher for 12 nodes, whereas the values from the sparse sample trees were higher for only 4 nodes. This difference is significant (binomial test, $P = 0.05$). (Five other nodes that had 100% bootstrap support in the dense sample tree also had 100% bootstrap support in all sparse sample trees.)

DISCUSSION

Our data reveal three major clades of orioles (Fig. 5): Clade A with 95% bootstrap support, Clade B with 76% bootstrap support, and Clade C with 100% bootstrap support. Most data sets and weighting schemes provide evidence of a sister relationship between Clades A and B. According to most data sets and weighting schemes, *I. maculialatus* (the Bar-winged oriole) comes out as sister to clade A (but not in the six-parameter heuristic tree). Our Clade A (Fig. 5) corresponds well with Beecher's (1950) "Bananivorus" group. Of the 12 taxa in his grouping, only *I. parisorum* is not shared with Clade A. However, his other group, "Icterus," is not supported by sequence data; our results indicate that this group was a grade that includes a random mixture of taxa in our Clades B and C.

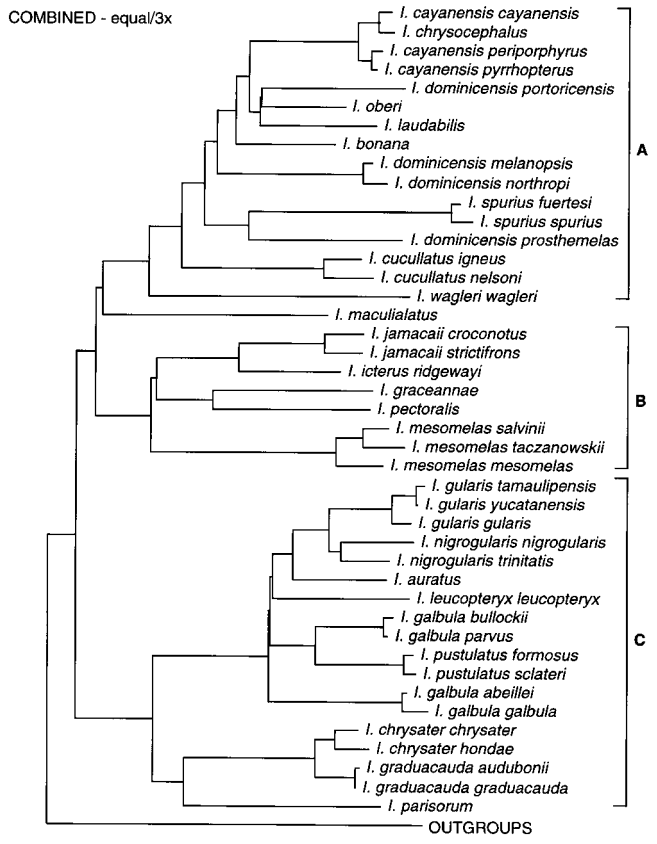

FIG. 7. Corrected distance tree based on majority rule consensus resulting from both equal and 3× weighted heuristic searches. Branch lengths were corrected using the Kimura two-parameter model in PAUP*. Clades A, B, and C are labeled as in Fig. 4b.

Our mtDNA sequence data corroborate the monophyly of *Icterus* with 100% bootstrap support in all three weighting schemes. In an earlier cytochrome *b* study, Lanyon and Omland (1999) found evidence of *Icterus* monophyly with dense taxon sampling from among all other groups of blackbirds. The present study confirms that finding with nearly complete taxon sampling within the genus and an additional gene. We find no support for two independently derived lineages of orioles, as suggested by Beecher (1950). Our findings also conflict with those of Freeman and Zink (1995), whose data suggested placing *I. icterus* outside the genus. Our data identify the *I. icterus* group as belonging to clade C. Other aspects of Freeman and Zink's (1995) tree generally agree with our findings, with the other exception being their placement of *I. parisorum* (as sister to a *I. mesomelas* and *I. graceannae* clade).

As might be expected in a gene tree for 45 taxa, we found regions in both clades A and C that are poorly resolved, as indicated by several nodes with less than 50% bootstrap support (Fig. 5). Within Clade A there is weak resolution involving the *I. cayanensis* superspecies and the three Lesser Antillean orioles (*I. bonana*, *I. laudabilis*, and *I. oberi*). Within Clade C there is a weak resolution at the base of that clade involving four lineages including *I. leucopteryx*. Both of these poorly resolved areas correspond to approximately 5% uncorrected sequence divergence. The corrected distance tree (Fig. 7) suggests that these two polytomies may have occurred contemporaneously. Such areas of poor resolution suggest rapid radiations. That these unresolved areas are evident in both genes separately (Figs. 4a and 4b), and seem to have occurred at similar times in both clades, provides further support for such an explanation in this case (also see Zink *et al.*, 1998). Five percent sequence divergence approximates the average divergence time among many North American songbirds and may correspond to the onset of glaciation near the end of the Pliocene (Klicka and Zink, 1997).

Weighting Methods

We chose six-parameter weighting (Williams and Fitch, 1989; Cunningham, 1997) as our primary weighting scheme. However, our results appear to be relatively robust across the various weighting schemes we employed. Six-parameter weighting produced bootstrap trees that are similar in topology and support values to trees produced by other weighting schemes. (Our results thus highlight the value of statistical resampling procedures because the trees that result seem robust to a range of weighting schemes.) Six-parameter analysis of the combined data resulted in two islands of trees that differed in score by only 0.03%. The shortest trees resulting from the six-parameter weighting were recovered only by this analysis. Trees in the second island did not differ significantly from those in the shortest island according to the Kishino–Hasegawa test. This island was supported in all the equally weighted and transversion weighted parsimony analyses and had slightly higher likelihood scores. Thus, the majority rule consensus that resulted from both the 3× and the equally weighted analyses (Fig. 6) may be the single best estimate of the oriole tree. This topology is supported by a wide range of weighting and resampling schemes. We plan to base our primary reconstructions of ancestral plumage characteristics on this equal/3× tree, although the shortest six-parameter tree remains a viable alternative hypothesis that we will also use. More work is needed with "known phylogenies" (e.g., Sullivan, 1996; Cunningham, 1997) and simulation studies (e.g., Hillis, 1996) to test six-parameter weighting and other weighting schemes further.

Species Limits

Our results show that the Northern oriole group (*I. galbula*) is polyphyletic. The Baltimore oriole (*I. g. galbula*, eastern United States) and Bullock's oriole (*I.*

TABLE 2

Combined Genetic Distances among Oriole Taxa (Uncorrected Percentages for Cytochrome *b* Plus ND2)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1. *I. galbula abeillei*																																										
2. *I. auratus*	4.5																																									
3. *I. bonana*	8.7	8.9																																								
4. *I. galbula bullockii*	4.7	4.3	8.7																																							
5. *I. galbula parvus*	4.5	4.2	8.6	0.3																																						
6. *I. cayanensis cayanensis*	8.7	8.7	4.3	8.8	8.7																																					
7. *I. chrysocephalus*	8.8	8.9	4.6	8.9	8.8	0.6																																				
8. *I. cayanensis periporphyrus*	8.8	8.8	4.1	8.6	8.5	1.0	1.4																																			
9. *I. cayanensis pyrrhopterus*	8.7	8.7	4.1	8.5	8.4	1.0	1.3	0.4																																		
10. *I. chrysater chrysater*	7.3	7.1	8.6	7.4	7.3	8.5	8.5	8.3	8.2																																	
11. *I. chrysater hondae*	7.4	7.3	8.7	7.3	7.2	8.6	8.6	8.4	8.3	1.1																																
12. *I. cucullatus igneus*	9.3	8.9	6.4	9.0	8.8	6.5	6.6	6.8	6.8	8.9	9.0																															
13. *I. cucullatus nelsoni*	9.3	8.8	6.4	9.0	8.8	6.5	6.7	6.8	6.8	8.5	8.7	1.9																														
14. *I. dominicensis melanopsis*	8.9	8.7	4.7	8.9	8.7	5.4	5.6	5.5	5.4	9.1	9.1	6.2	6.4																													
15. *I. dominicensis northropi*	9.0	9.1	4.8	9.2	9.1	5.6	5.8	5.7	5.5	9.2	9.3	6.5	6.7	0.7																												
16. *I. dominicensis portoricensis*	9.1	9.1	4.6	9.1	9.0	5.2	5.4	5.2	5.0	9.1	9.2	6.5	6.7	5.1	5.4																												
17. *I. dominicensis prosthemelas*	9.2	9.2	6.3	9.4	9.4	6.8	7.1	6.9	6.8	9.1	9.3	6.7	6.3	6.1	6.4	6.6																												
18. *I. galbula galbula*	0.6	4.9	8.8	4.9	4.7	8.9	9.1	9.0	8.9	7.4	7.4	9.3	9.6	9.1	9.2	9.4	9.3																											
19. *I. graceannae*	9.5	9.7	8.2	9.3	9.2	9.0	9.2	9.0	9.2	9.4	9.9	8.9	8.9	8.6	8.8	8.4	8.8	10.0																										
20. *I. graduacauda audubonii*	7.5	7.4	8.7	7.3	7.1	8.7	8.7	8.4	8.2	1.5	2.1	8.7	8.6	9.0	9.3	8.9	9.1	7.5	9.7																									
21. *I. graduacauda graduacauda*	7.4	7.3	8.6	7.1	7.0	8.6	8.6	8.3	8.2	1.6	2.1	8.7	8.7	8.9	9.2	8.8	9.1	7.4	9.6	0.1																						
22. *I. gularis gularis*	5.1	3.9	8.8	5.2	5.0	8.8	9.0	8.9	8.8	7.2	7.4	9.3	9.1	8.8	9.1	9.3	9.5	5.3	9.7	7.1	7.0																					
23. *I. gularis tamaulipensis*	5.1	4.2	9.1	5.3	5.1	9.1	9.1	9.2	9.1	7.3	7.4	9.4	9.1	9.0	9.3	9.3	9.6	5.4	9.7	7.4	7.2	0.9																				
24. *I. gularis yucatanensis*	5.0	4.1	9.0	5.2	5.0	9.0	8.9	9.0	8.9	7.1	7.2	9.2	9.1	8.9	9.3	9.2	9.5	5.4	9.6	7.2	7.1	0.9	0.1																			
25. *I. jamacaii croconotus*	9.2	9.1	7.7	9.2	9.2	8.4	8.3	8.4	8.3	8.2	8.6	8.7	8.8	7.9	8.0	8.6	8.6	9.5	7.3	8.5	8.5	9.4	9.6	9.4																		
26. *I. icterus ridgewayi*	9.2	9.2	7.5	9.0	9.0	8.0	8.2	8.1	8.0	8.4	9.0	8.1	8.4	8.0	8.1	8.4	8.4	9.5	7.6	8.4	8.5	9.7	9.7	9.6	4.3																	
27. *I. jamacaii strictifrons*	9.3	8.8	7.6	9.0	9.0	8.1	8.2	8.2	8.1	8.5	8.7	8.4	8.6	7.8	7.9	8.5	8.6	9.6	7.4	8.7	8.7	9.3	9.5	9.3	1.5	4.3																
28. *I. laudabilis*	9.0	9.0	4.1	9.3	9.2	4.7	4.7	4.8	4.8	8.7	9.0	6.7	6.3	5.4	5.2	5.0	6.9	9.4	8.7	8.8	8.7	8.7	8.9	8.7	8.4	8.5	8.2															
29. *I. leucopteryx leucopteryx*	5.2	4.5	9.5	5.0	4.8	9.5	9.5	9.5	9.4	7.7	7.6	9.3	9.1	9.2	9.6	9.7	9.8	5.5	9.3	7.7	7.7	5.0	5.3	5.3	9.2	9.1	9.2	9.6														
30. *I. maculialatus*	9.3	9.3	8.1	9.2	9.0	8.4	8.5	8.4	8.3	8.7	8.9	7.9	8.1	8.0	8.0	8.1	8.3	9.5	8.2	8.5	8.4	9.5	9.6	9.5	7.7	7.6	7.4	8.3	9.3													
31. *I. mesomelas mesomelas*	9.3	9.8	8.2	9.4	9.3	8.4	9.0	8.6	8.6	8.5	9.0	8.4	8.3	9.4	9.5	8.7	9.0	9.5	7.5	8.3	8.3	9.6	9.7	9.7	7.3	7.6	7.5	8.9	9.5	8.2												
32. *I. mesomelas salvinii*	9.2	9.6	8.3	9.3	9.1	8.6	8.8	8.5	8.6	8.7	9.1	8.7	8.7	9.3	9.5	9.3	9.2	9.5	7.4	8.4	8.5	9.5	9.6	9.6	7.2	7.7	7.2	9.2	9.4	8.2	2.0											
33. *I. mesomelas taczanowskii*	9.3	9.7	8.4	9.4	9.1	8.8	9.0	8.8	8.8	8.9	9.3	8.7	8.7	9.6	9.6	9.1	9.3	9.5	7.5	8.6	8.7	9.7	9.8	9.8	7.3	7.7	7.3	9.1	9.7	8.2	2.3	1.4										
34. *I. nigrogularis nigrogularis*	4.7	4.0	9.1	5.0	4.8	9.1	9.2	9.2	9.1	7.5	7.9	9.3	9.0	9.3	9.4	9.6	9.9	5.3	9.0	7.8	7.7	3.0	3.0	3.0	9.5	9.5	9.4	9.1	4.9	9.4	9.1	9.0	9.0									
35. *I. nigrogularis trinitatis*	5.2	4.1	8.8	5.0	4.8	9.1	9.2	8.9	9.0	7.4	7.3	9.0	8.7	9.0	9.1	9.2	9.4	5.5	9.6	7.3	7.2	3.1	3.3	3.2	9.5	9.5	9.3	8.8	5.3	9.4	9.3	9.4	9.5	2.5								
36. *I. oberi*	8.7	8.6	3.3	8.4	8.4	4.3	4.4	4.4	4.3	8.3	8.5	6.5	6.2	4.7	4.8	4.2	6.0	8.9	8.3	8.4	8.3	8.4	8.6	8.5	7.7	7.7	7.8	3.7	9.1	7.9	7.8	8.1	8.2	8.8	8.4							
37. *I. parisorum*	7.7	8.0	9.1	8.0	8.0	9.5	9.7	9.5	9.4	6.5	6.9	9.0	9.3	9.5	9.9	9.4	9.8	7.9	9.3	6.5	6.4	8.3	8.5	8.3	9.1	8.7	9.1	9.4	8.0	9.0	8.9	9.0	9.1	8.0	8.0	8.5	9.2					
38. *I. pectoralis*	9.5	9.7	8.3	9.7	9.6	8.9	9.0	8.9	8.9	9.0	9.1	8.7	9.2	9.0	9.2	8.9	9.2	9.7	6.1	9.0	8.9	10.2	10.4	10.2	7.0	6.9	6.9	9.3	9.5	8.5	7.6	7.3	7.5	9.6	9.8	8.5	9.3					
39. *I. pustulatus formosus*	4.6	3.9	9.2	3.3	3.1	9.2	9.3	9.0	9.0	6.8	7.0	9.2	9.3	9.3	9.5	9.5	9.6	4.8	9.5	6.8	6.7	4.7	4.8	4.9	10.0	9.4	9.7	9.6	4.8	9.4	9.2	9.3	9.2	4.8	4.8	9.0	8.2	9.6				
40. *I. pustulatus sclateri*	4.7	4.2	9.0	3.4	3.2	9.2	9.2	8.9	8.9	6.8	6.9	9.2	9.3	9.3	9.6	9.7	9.8	4.9	9.4	6.9	6.8	4.9	5.1	5.1	9.8	9.3	9.6	9.5	4.9	9.3	9.4	9.5	9.4	5.0	4.9	9.1	8.1	9.5	0.4			
41. *I. spurius fuertesi*	10.3	10.2	6.3	9.9	9.8	6.9	7.0	6.9	6.8	9.9	9.9	6.3	6.6	6.6	6.8	6.7	6.4	10.3	9.7	9.9	9.9	10.2	10.3	10.1	9.1	9.2	9.0	6.7	10.2	8.8	9.3	9.4	9.8	10.2	9.8	6.0	10.1	9.7	10.3	10.3		
42. *I. spurius spurius*	10.1	10.3	6.4	9.8	9.7	6.8	7.0	6.9	6.9	10.2	10.1	6.2	6.6	6.7	6.9	6.8	6.5	9.9	9.5	10.1	10.0	10.3	10.4	10.3	9.1	9.1	8.9	6.7	10.1	9.0	9.3	9.4	9.8	10.2	9.8	6.0	10.2	9.4	10.3	10.3	0.6	
43. *I. wagleri wagleri*	10.0	9.8	7.5	10.2	10.0	8.2	8.1	8.2	8.2	9.7	9.8	7.6	7.9	7.9	8.2	7.3	8.6	10.0	9.3	9.5	9.5	9.7	9.8	9.7	8.5	8.4	8.4	7.9	10.1	7.8	9.7	9.5	9.7	10.2	9.9	7.9	9.9	9.3	10.0	10.0	8.7	8.7

g. bullockii, western United States) are not sister taxa and are nearly 5% different in mitochondrial DNA sequence. These findings thus corroborate Freeman and Zink's (1995) restriction site study based on a limited number of taxa. Our conclusions are bolstered by the fact that we included both races of Bullock's oriole (*I. g. bullockii* and *parvus*) and that we sequenced a second Baltimore oriole that yielded sequence data similar to that of the first (unpublished data). These findings are especially surprising given the extensive hybridization between *I. g. galbula* and *I. g. bullockii* (e.g., Sibley and Short, 1964), which is why these taxa were at one time lumped into a single species (Blake, 1968; American Ornithologists' Union, 1983). However, these taxa were recently resplit (American Ornithologists' Union, 1995) and our data support this decision. Our findings add to the mounting evidence that the ability to hybridize, at least in birds, may be a retained ancestral trait and should not be used as the main criterion for defining species boundaries (e.g., Cracraft, 1989; Gill *et al.*, 1993; Zink and McKitrick, 1995).

The placement of *I. galbula abeillei* (the Black-backed oriole) of Mexico as sister to the Baltimore oriole was unexpected. This grouping received 100% bootstrap support and is confirmed by sequences from additional individuals from each of the taxa involved (unpublished data). The two taxa are only about 0.5% divergent from each other. The Black-backed oriole has often been considered closely related to Bullock's oriole because of plumage similarity and limited hybridization (Miller, 1906), but was also involved in the recent lumping and subsequent resplitting of the Northern oriole (American Ornithologists' Union, 1983, 1995). Our findings suggest rapid morphological change in the Black-backed and/or the Baltimore oriole.

The other species that we found to be polyphyletic was *I. dominicensis* (the Black-cowled oriole) of the Caribbean Basin. Most of the recognized subspecies of *dominicensis* are at least 4% divergent from each other, which is typical of divergences among many other species of orioles. Most of these taxa also have diagnostic plumage differences (K. E. Omland and S. M. Lanyon, unpublished data) and are allopatric, with most being island endemics. The only subspecies within *dominicensis* that are sister taxa and have genetic distances typical of other subspecies of orioles are *I. d. melanopsis* and *I. d. northropi* (from Cuba and the Bahamas, respectively). An extreme manifestation of polyphyly in *dominicensis* is the sister taxon relationship of *I. dominicensis prosthemelas* and *I. spurius* (*prosthemelas* is the Mexican mainland subspecies of *dominicensis* and *I. spurius* includes the Orchard oriole of the eastern United States). Our findings show that *I. dominicensis* is a taxon into which many Caribbean Basin taxa were lumped, perhaps because of shared ancestral plumage characteristics.

In the *I. cayanensis* group, our data do not support the species limits suggested by Sibley and Monroe (1990). *I. cayanensis,* as represented in the three subspecies in this study, is paraphyletic with respect to *I. chrysocephalus.* Our data do not suggest that any one or two subspecies within this group are markedly distinctive. However, the remaining two subspecies should be sequenced as well as multiple individuals from each subspecies. In the Troupial group, our data do provide support for designation of *I. jamacaii* as a separate species by Sibley and Monroe (1990). *I. i. ridgewayi* is sister to the two *I. jamacaii* taxa and over 4% divergent from both. *I. i. ridgewayi* and other taxa within the Troupial group may indeed warrant recognition as separate species, but more intensive sampling of taxa (three more subspecies in the group), populations, and other character systems is needed.

More generally, our study shows that existing species limits do not correspond well to phylogenetic relationships suggested by mitochondrial gene trees. In the case of the Northern oriole complex, the polyphyly of *I. g. galbula* and *I. g. bullockii* resulted from two taxa that hybridize being lumped despite considerable male plumage differences. In contrast, the polyphyly of *I. dominicensis* probably resulted from an excessive lumping of species with retained similar ancestral plumage characteristics. The paraphyly of *I. cayanensis* was likely caused by recent changes in classification made in the absence of explicit argumentation and supporting data. Therefore, all of these cases of nonmonophyly were likely caused by inadequate classifications, not retained ancestral polymorphism in currently interbreeding homogeneous populations (e.g., Avise *et al.,* 1990; Omland, 1997).

Taxon Sampling

Taxon sampling below the species level can influence the species-level tree. For example, in the Northern oriole group, including just *I. galbula bullockii* would have suggested that *I. galbula* is sister to *I. pustulatus,* whereas including just *I. g. galbula* would not have suggested a close relationship to *I. pustulatus.* In neither *I. galbula* nor *I. dominicensis* was there strong a priori reason to predict polyphyly (but see Freeman and Zink, 1995). The possibility of polyphyletic or paraphyletic species is the most compelling reason to include multiple subspecies or populations in species-level phylogenetic studies (see also Melnick *et al.,* 1993; Omland, 1997; Zink *et al.,* 1998). Phylogenetic studies at all levels from kingdom to population should ideally include multiple exemplars below the level being studied to assess the monophyly of the taxa at the target level (also see Weller *et al.,* 1992; Purvis and Quicke, 1997).

To investigate the impact of taxon sampling within monophyletic groups, we randomly subsampled subspecies. The resultant sparse taxon trees had significantly lower bootstrap support than the full dense sample bootstrap tree. This is an especially surprising result because when one adds taxa, one increases homoplasy (e.g., lower consistency index; Sanderson and Donoghue, 1989) and exponentially increases the number of possible trees. In this case, increasing the number of subspecies may have helped recover unobserved substitutions that provided more support for supraspecific nodes (also see Hillis, 1996; Graybeal, 1998). However, varying the density of subspecies sampling did not change the topology, as might have been the case if additional subspecies had broken up long branches that had been spuriously attracted to each other (e.g., Felsenstein, 1978).

Gene Utility

ND2 proved to be an extremely useful gene for resolving relationships among orioles. Our results show that ND2 evolves approximately 40% faster than cytochrome *b* (based on most ingroup comparisons) and showed no sign of transition saturation, even among the outgroup taxa at over 16% divergence (see also Hackett, 1996). These observations and the extra 165 bp we sequenced for ND2 may be sufficient to explain the superior resolving power of that gene over cytochrome *b*. In addition, ND2 may have less among-site rate variation compared to cytochrome *b*, which is well known for strong functional constraints in certain parts of the molecule (e.g., Meyer, 1994; Griffiths, 1997; also see Graybeal, 1994). Although cytochrome *b* has been criticized for these reasons (e.g., Meyer, 1994) it is the most widely used gene for sequence-based studies of birds (Moore and DeFilippis, 1997) and other vertebrates (Meyer, 1994). Moore and DeFilippis (1997) argued that cytochrome *b* should be as good as any other mitochondrial gene at lower taxonomic levels (below the subfamily level) ". . . because most of the variation is synonymous, and cyt b should accrue this type of variation as rapidly as any other gene." (Moore and DeFilippis, 1997, p. 107; also see Meyer, 1994). Surprisingly, this is not the case in orioles, nor in the grackles and allies lineage (K. Johnson and S. M. Lanyon, unpublished data). However, among species and genera of waterfowl, ND2 and cytochrome *b* evolve at the same rate and show no differences in saturation pattern (Johnson and Sorenson, 1998). We suggest that for lower level studies in other nine-primaried oscine birds (and perhaps other vertebrates), researchers look first to ND2 or other genes with the same desirable properties.

Cytochrome *b* still does offer some advantages, in particular the ease of sequencing because of conserved universal primer sites (Kocher *et al.*, 1989; Meyer, 1994) and the extensive database of published cytochrome *b* sequences (also see Griffiths, 1997). Furthermore, our combined-data bootstrap tree showed more resolution than the bootstrap tree based on ND2 alone (e.g., also see Olmstead and Sweere, 1994). Also, having sequence from two distinct genes, even when they are both from the mitochondrial genome, provides additional benefits. The complete congruence between the bootstrap trees based on the two genes separately strengthens confidence in our estimate of the mitochondrial gene tree for orioles (e.g., Bull *et al.*, 1993; Lanyon, 1993; Omland, 1994; Miyamoto and Fitch, 1995).

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APPENDIX 1

Genus/species	Subspecies	Museum	Catalogue/field #	Locality
I. auratus	[Monotypic]	UAM	7222	Mex. Yucatan, El Coyo
I. auricapillus	[Monotypic]	ANSP	173534	Columbia, Meta
I. bonana	[Monotypic]	STRI	MA-IBO2	Martinique, Fond Baron
I. cayanensis	*cayanensis*	MPEG	40.357	Brazil, Rondonia, Rio Ji Parana
I. cayanensis	*periporphyrus*	FMNH	334609	Bolivia, Santa Cruz, Chiquitos
I. cayanensis	*pyrrhopterus*	FMNH	334608	Bolivia, Santa Cruz, Chiquitos
I. chrysater	*chrysater*	UWBM	DAB-1573	Nicaragua, Casitta
I. chrysater	*hondae*	STRI	PA-ICHPP4	Panama, Farfan-Antenna
I. chrysocephalus	[Monotypic]	FMNH	339734	Venezuela, Sucre, Guanoco
I. cucullatus	*igneus*	MZFC	KEO-011	Mex. Campeche, Xpujil
I. cucullatus	*nelsoni*	FMNH	ATP88-081	USA, CA, Riverside Co.
I. dominicensis	*dominicensis*	LSUMZ	9897	Haiti
I. dominicensis	*melanopsis*	MHNC	4/8/92	Cuba
I. dominicensis	*northropi*	BNT	REF024	Bahamas, Andros
I. dominicensis	*portoricensis*	STRI	PR-IDO1	Puerto Rico, Maricao
I. dominicensis	*prosthemelas*	BMNH	42543	Mex. Campeche, Xpujil
I. galbula	*abeillei*	MZFC	9657	Mex. Queretaro
I. galbula	*bullockii*	FMNH	341938	USA, CA, Monterey Co.
I. galbula	*galbula*	BMNH	42547	USA, Minnesota, Becker Co.
I. galbula	*parvus*	FMNH	341933	USA, CA, Imperial Co.
I. graceannae	[Monotypic]	ANSP	181810	Ecuador, Loja, Celica
I. graduacauda	*audubonii*	LSUMZ	4023	USA, TX, Atascosa Co.
I. graduacauda	*graduacauda*	MZFC	8770	Mex. Oaxaca, Rio Salado
I. gularis	*gularis*	FMNH	ATP91-093	Mex. Oaxaca, Zantapec
I. gularis	*tamaulipensis*	MZFC	KEO-003	Mex. Veracruz, Tlacotalpan
I. gularis	*yucatanensis*	BMNH	42540	Mex. Campeche, Xpujil
I. icterus	*ridgewayi*	LSUMZ	11328	Puerto Rico, Cabo Rojo
I. jamacaii	*stricifrons*	LSUMZ	6700	Bolivia, Santa Cruz
I. jamacaii	*croconotus*	FMNH	324092	Peru, Madre de Dios
I. laudabilis	[Monotypic]	STRI	SL-ILA4	St. Lucia, Anse la Sorciere
I. leucopteryx	*leucopteryx*	FMNH	331144	Jamaica, Cornwall
I. maculialatus	[Monotypic]	INIREB	SRF-387	Mex. Chiapas, Tuxtla Gut.
I. mesomelas	*mesomelas*	UWBM	52153	Mex. Chiapas, Estacion Juarez
I. mesomelas	*salvinii*	STRI	PAIMEPC98	Panama, French Canal
I. mesomelas	*taczanowski*	ANSP	181806	Ecuador, Loja, Cruzpamba
I. nigrogularis	*nigrogularis*	FMNH	339739	Venezuela, Falcon, Boca de Aroa
I. nigrogularis	*trinitatis*	STRI	TR-INI1	Trinidad, Livestock Station
I. oberi	[Monotypic]	STRI	MO-IOB4	Monserrat, Soufriere
I. parisorum	[Monotypic]	FMNH	341943	USA, CA, San Bernardino Co.
I. pectoralis	(Florida)	BMNH	42544	USA, FL, Dade Co.
I. pustulatus	*formosus*	UWBM	52129	Mex. Chiapas, Ocozucualta
I. pustulatus	*sclateri*	UWBM	DAB-1670	Nicaragua, La Flor
I. spurius	*fuertesi*	BMNH	42538	Mex. Veracruz, Tlacotalpan
I. spurius	*spurius*	NCSM	DLD-2538	USA, CO, Weld Co.
I. wagleri	*wagleri*	MZFC	QRO-216	Mex. Queretaro
Agelaius	*phoeniceus**	FMNH	341893	USA, LA, Cameron Par.
Amblycercus	*holosericeus*	FMNH	334662	Bolivia, El Beni, Laguna Suarez
Cacicus	*solitarius*	FMNH	324091	Peru, Madre de Dios
Dolichonyx	*oryzivorus*	FMNH	334721	Bolivia, Santa Cruz, Purubi
Gymnostinops	*bifasciatus*	FMNH	324076	Peru, Madre de Dios
Nesopsar	*nigerrimus*	FMNH	331150	Jamaica, Surrey, Portland
Sturnella	*neglecta*	FMNH	330039	USA, CA, Riverside Co.

Note. * Only genus and species are listed for outgroup taxa. Museums are abbreviated as follows: ANSP, Academy of Natural Sciences Philadelphia; BMNH, Bell Museum of Natural History; BNT, Bahamas National Trust; FMNH, Field Museum of Natural History; INREB, Instituto de Historia Natural, San Cristobal de las Casas, Chiapas, Mexico; LSUMZ, Louisiana State University Museum of Zoology; MHNC, Museo de Historie Natural Cuba; MPEG, Museu Paraense Emílio Goeldi, Belem, Brazil; MZFC, Museo de Zoologia, Facultad de Ciencias, Mexico, D. F.; NCSM, North Carolina State Museum; STRI, Smithsonian Tropical Research Institute (lyophilized DNA samples); UAM, University of Alaska Museum; UWBM, University of Washington Burke Museum.