RESEARCH ARTICLES

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JOURNAL OF AVIAN BIOLOGY 34: 360–370, 2003

Phylogenetic relationships of Neotropical honeycreepers and the evolution of feeding morphology

Kevin J. Burns, Shannon J. Hackett and Nedra K. Klein

Burns, K. J., Hackett, S. J. and Klein, N. K. 2003. Phylogenetic relationships of Neotropical honeycreepers and the evolution of feeding morphology. – J. Avian Biol. 34: 360–370.

Phylogenetic relationships among Neotropical honeycreepers were studied by using cytochrome *b* sequence data. Although honeycreepers were once placed in their own family (Coerebidae), these species did not form a monophyletic group in any of the phylogenies in this study. Thus, our results indicate that nectar-feeding evolved independently multiple times among these birds. The Neotropical honeycreepers are best considered disparate members of a larger radiation of tanagers and finches, in which bill size and shape, and associated feeding behaviors have changed frequently to fill a variety of niches. Our phylogenies also provide specific taxonomic recommendations on the placement of each honeycreeper genus. The use of the taxon Coerebidae is no longer warranted given the well-supported relationships between different honeycreeper species and a variety of tanagers and finches.

K. J. Burns (correspondence), Department of Biology, San Diego State University, San Diego, CA 92182-4614, USA. E-mail: kburns@sunstroke.sdsu.edu. S. J. Hackett, Department of Zoology, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA. N. K. Klein, Department of Ornithology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA.

Studies of feeding adaptations have provided insight into evolutionary processes since the inception of the theory of natural selection. For example, the remarkable diversity of bill types displayed by Darwin's finches of the Galapagos Islands have been a primary example of how natural selection can shape morphologies (Darwin 1859, Lack 1947, Grant 1999). Studies of feeding adaptations in a wide variety of organisms have continued to provide new insights (e.g., Lauder 1983, Roth and Wake 1989, Meyer et al. 1990, Schwenk 2000). To study the evolution of any trait, including bill size or shape, an accurate phylogeny of the group in question is essential. Unfortunately, traditional classifications have often included information about diet and feeding morphology among the suite of traits examined. Thus, phylogenetic hypotheses are often conflated with information about the presumed importance of certain feeding specializations. This has occurred despite evidence that feeding morphology may change readily, and thus may not be a good indicator of evolutionary relationship. For example, in both Hawaiian honeycreepers and Darwin's finches, a diversity of bill types has evolved from a common ancestor over a relatively short span of time. In addition, selection can alter bill size significantly from one year to the next in a single population (Boag and Grant 1981), and dramatic bill differences can occur within a single species (Smith 1990).

One group in which dietary information has played a role in taxonomic decisions is the assemblage of birds

Table 1. Conflicting classifications of Neotropical honeycreepers and their relatives. All genera of New World honeycreepers were once placed in Coerebidae (e.g., Sclater 1886, Hellmayr 1935). The placement of each genus in more recent classifications is shown below.

	Paynter (1968, 1970)	Sibley and Monroe (1990)	Howard and Moore (1991)	AOU (1998)
Goldfinches and relatives	Fringillidae	Fringillinae	Fringillidae	Fringillidae
Blackbirds	Icteridae	Icterini	Icteridae	Icteridae
Warblers	Parulidae	Parulini	Parulidae	Parulidae
Tanagers	Thraupinae	Thraupini	Thraupinae	Thraupidae
Cardinals	Cardinalinae	Cardinalini	Cardinalinae	Cardinalidae
New World finches and sparrows	Emberizinae	Emberizini and Thraupini	Emberizinae	Emberizidae
Neotropical Honeycreepers:				
Coereba	Parulidae	Thraupini	Coerebidae	Coerebidae
Conirostrum	Parulidae	Thraupini	Parulidae	Thraupidae
Diglossa	Thraupinae	Thraupini	Thraupinae	Emberizidae
Euneornis	Thraupinae	Thraupini	Thraupinae	Emberizidae
Dacnis	Thraupinae	Thraupini	Thraupinae	Thraupidae
Cyanerpes	Thraupinae	Thraupini	Thraupinae	Thraupidae
Chlorophanes	Thraupinae	Thraupini	Thraupinae	Thraupidae
Xenodacnis	Thraupinae	Thraupini	Thraupinae	not considered
Oreomanes	Thraupinae	Thraupini	Thraupinae	not considered
Iridophanes	Thraupinae	Thraupini	Thraupinae	not considered

known as the New World, nine-primaried oscines. Traditional taxonomies (Table 1) have divided members of this group into categories that were based partly on general impressions of feeding behavior: fruit-eaters were placed in Thraupidae (tanagers), arboreal insect-eaters in Parulidae (wood-warblers), large-billed seed-eaters in Cardinalidae (cardinals and grosbeaks), smaller-billed seed-eaters in Emberizidae (New World finches and sparrows), ground-foraging insect-eaters in Icteridae (blackbirds), and nectar-feeders in the Coerebidae (the Neotropical honeycreepers). Other characters besides diet were also used to define these categories. However, general assumptions about the evolutionary significance of feeding morphology and behavior have potentially obscured phylogenetic relationships among these taxa.

In this study, we assess the phylogenetic relationships of birds that historically were placed in the group of nectar-feeding passerines described as the family Coerebidae. Early workers (e.g., Sclater 1886, Hellmayr 1936) placed several genera of passerines in this family based on the shared behavior of feeding on nectar. Genera included in this family were *Diglossa*, *Cyanerpes*, *Chlorophanes*, *Iridophanes* (= *Tangara pulcherrima*), *Dacnis* (including *Hemidacnis*), *Euneornis*, *Xenodacnis*, *Oreomanes*, *Coereba* and *Conirostrum* (including *Ateleodacnis*). Species in these genera have slender bills that are decurved in some species and sharply-pointed or hooked in others. In addition, many species also have highly modified tongues that are bifurcated, tubular, and lined with fringes (Bock 1985, Gardner 1925). A study of the anatomy of coerebid species (Beecher 1951) suggested that many of the similarities were convergent adaptations for nectar-feeding. Based on his study, Beecher (1951) argued for the dissolution of Coerebidae and placed some of the honeycreeper genera in a separate tribe within Thraupidae and some of the genera in a separate tribe within Parulidae. This recommendation was largely adopted in subsequent systematic treatments (e.g., Tordoff 1954, Paynter 1968, 1970; Table 1). Raikow (1978) studied the appendicular musculature of nine-primaried oscines and tentatively agreed with Beecher's assessment. Other authors (e.g., AOU 1998, Table 1) have disagreed, and split the genera among the families Coerebidae, Emberizidae and Thraupidae. Sibley and Ahlquist (1990) included representatives of three genera formerly considered members of Coerebidae (*Coereba*, *Diglossa* and *Cyanerpes*) in their DNA-DNA hybridization studies. Based on these findings, Sibley and Monroe (1990) placed all the former members of Coerebidae into their tribe Thraupini, a large complex of tanagers and finches. Thus, there has been little consensus on the phylogenetic relationships of the Neotropical honeycreepers either to one another or to other nine-primaried oscines (Table 1).

Recent studies using DNA sequence data (Burns 1997, Klicka et al. 2000, Burns et al. 2002, Lovette and Bermingham 2002, Yuri and Mindell 2002) have included some members of Coerebidae. In general, these studies showed that several species of Coerebidae are not closely related to each other. However, none of these DNA studies included representatives of all genera within Coerebidae. To assess the monophyly of Coerebidae and to investigate the relationships of these taxa, we sequenced the mitochondrial cytochrome *b* gene from representatives of all genera that historically were placed in the family Coerebidae. We constructed phylogenetic hypotheses using these sequences and sequences of several tanagers, wood-warblers and finches. The resulting phylogenies are used to interpret the evolution of different feeding morphologies and behaviors in these birds.

Materials and methods

Taxon sampling and outgroup choice

For ease of discussion, the taxonomy used below follows Sibley and Monroe (1990), with the exception that Neotropical honeycreepers will be referred to as Coerebidae. Representatives of all 10 genera that were historically considered part of Coerebidae were included in this study. Also included were representatives of three genera (*Dendroica*, *Basileuterus* and *Vermivora*) of Parulini, representatives of two genera (*Pheucticus* and *Passerina*) of Cardinalini, and representatives of 61 genera of tanager-finches (Thraupini). Of the representatives of Thraupini, 42 genera were traditionally considered tanagers and 19 genera were traditionally considered New World finches and sparrows. All of the above mentioned taxa are members of Sibley and Monroe's (1990) Emberizinae. Because of the close relationship of Fringillinae to Emberizinae (Sibley and Ahlquist 1990, Klicka et al. 2000, Yuri and Mindell 2002), a representative of Fringillinae (*Carduelis pinus*) was also included to help root relationships among the taxa. However because of recently identified close relationships of some of the Thraupini taxa to Fringillinae (Klicka et al. 2000, Yuri and Mindell 2002) we used a non-emberizine, non-fringilline passerine, *Cyanocitta cristata* (Helm-Bychowski and Cracraft 1993; GenBank accession number X74258), as an outgroup for the sequences used in this study. In all, 95 sequences representing 90 species were used in our analyses. Some of these sequences were obtained from GenBank (Hackett 1996: U15717, U15718; Burns 1997: AF006211–AF006258; Sato et al. 1999: AF108772, AF108796; Klicka et al. 2000: AF290142, AF290145, AF290156, AF301458; Lougheed et al. 2000: AY005206, AY005218–AY005221; Sato et al. 2001: AF310041, AF310043, AF310049, AF310053, AF310055). Other individuals were sequenced for use in this study (GenBank Accession Nos. AF489878–AF489903, AY190166–AY190169). Locality and voucher information for these sequences is reported with the GenBank records.

DNA isolation and sequencing

DNA extracts were prepared from liver or muscle tissue preserved in 95% ethanol, stored in "Queen's" tissue buffer (Seutin et al. 1991), or frozen at −80°C. For most samples, extractions were performed using a 5% Chelex solution (Walsh et al. 1991), or a PureGene DNA extraction kit (Gentra Systems). For samples of *Coereba flaveola*, *Conirostrum bicolor* and *Dendroica pensylvanica*, purified mtDNA was prepared following standard protocols (Dowling et al. 1990, Klein and Brown 1994). Specific fragments of the cytochrome *b* gene were then amplified using the polymerase chain reaction (PCR) and an assortment of primers (Hackett 1996, Groth 1998). Double-stranded amplifications were performed in capillary tubes in 10 µl volumes. A typical reaction involved 30–40 amplification cycles (3 s at 94°C, 1 s at 43°C, 30 s at 71°C). Agarose plugs of double-stranded products were taken using Pasteur pipets and diluted in 250 µl of water. Plugs were then melted and 3 µl of this solution was re-amplified in a 40 µl total reaction volume. Typical re-amplification involved 41 cycles (12 s at 94°C, 4 s at 52°C, 26 s at 71°C). Double-stranded products were cleaned and sequenced (ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq® DNA Polymerase; Perkin Elmer) for 32 cycles under the following conditions: 10 s at 96°C, 5 s at 50°C, and 3 min at 60°C. Amplified DNA was isolated by ethanol precipitation or by using spin columns containing Sephadex beads. Samples were run on polyacrylamide gels for 3–7 h on an ABI Prism™ 377. Sequence Navigator Version 1.0.1 (Applied Biosystems, Perkin Elmer) or Sequencher (Gene Codes, Ann Arbor, MI) was used to reverse complement opposing directions, to align different fragments from the same individual, and to translate complete sequences into amino acids.

Accuracy of DNA sequencing was verified in five ways: 1) sequencing both heavy and light strands of most PCR fragments, 2) using overlapping fragments of cytochrome *b* (approximately 12% of the total sequence is overlapped by two fragments), 3) checking that amino acid translation is possible without stop codons or gaps, 4) comparing levels of sequence divergence separately for the three fragments sequenced in each individual (as suggested by Hackett et al. 1995), and 5) for some samples, purified mitochondrial DNA was used.

The resulting sequences include the entire cytochrome *b* gene as well as portions of ND 5 and the Threonine t-RNA with the intervening spacer region. To make the sequences obtained for the current study directly comparable to sequences from previous studies, only 1045 base pairs of cytochrome *b* were used (from base 14 991 to base 16 035 relative to the published sequence of *Gallus gallus*; Desjardins and Morais 1990).

Phylogenetic analyses

Phylogenetic analyses were carried out using both Bayesian and parsimony approaches. For the Bayesian analyses, we used ModelTest, ver. 3.06 (Posada and Crandall 1998) to choose the best fit model for the data set containing all individuals. We used an iterative approach whereby a starting tree (determined initially by neighbor joining based on Kimura 2-parameter

distances) was entered into ModelTest and a model was chosen via a likelihood ratio test. We then used the chosen model (GTR+I+gamma) in conjunction with MrBayes 2.0 (Huelsenbeck and Ronquist 2001) to perform Bayesian analyses on the data set. Our analyses did not specify values for specific nucleotide substitution model parameters. Thus, parameters were treated as unknown variables with uniform prior values and estimated as part of the analysis. All Bayesian analyses were run for two million generations and sampled every 100 generations. Thus, each analysis resulted in 20 000 samples. Four Markov Chain Monte Carlo chains were run for each analysis. Resulting log likelihood scores were plotted against generation time to identify the point at which log likelihood values reached a stable equilibrium value. Sample points prior to this point of stationarity were discarded as "burn-in" samples. The remaining samples were used to produce a majority rule consensus tree with the percentage values indicating the percentage of samples that identified a particular clade (the clade's posterior probability). In Bayesian analyses, posterior probabilities are true probabilities of clades such that values of 95% or greater deemed significantly supported. We repeated the analyses several times to ensure that results were not dependent on the initial random starting tree used. For these repeated analyses, we compared log likelihood values and posterior probabilities of each repeated analysis to confirm that using a different starting tree did not alter our results significantly.

We also analyzed the data using parsimony as implemented in PAUP* 4.0b8 (Swofford 2001). We used the heuristic search option with 1000 random addition replicates and the tree-bisection-reconnection branch swapping algorithm. A previous analysis of the tanager sequences used in this study (Burns 1997) revealed that third position sites were saturated for transitions. Therefore, in addition to equal weighted analyses, we performed additional parsimony analyses in which third position transitions were downweighted relative to the other sites. To explore the sensitivity of the data to different degrees of downweighting, we downweighted third position transitions by a factor of 6, 20 and 50. The ratio of 6:1 was obtained empirically from examining multiple sequences from each of two of the genera used in this study: *Piranga* (Burns 1998) and *Ramphocelus* (Hackett 1996). Because close relatives will have fewer multiple substitutions at a given site, examining the transition bias among closely related individuals is an accurate way of determining actual transition bias (Edwards 1997). All of these parsimony analyses were bootstrapped with 10 random addition replicates for each of 100 bootstrap replicates (Felsenstein 1985).

Results

Sequence variation

As expected for a protein-coding mitochondrial gene, all sequences aligned without gaps or insertions. Of the 1045 sites, 485 (46%) were variable. Levels of uncorrected sequence divergence ("p"-distance of Nei (1987)) within the ingroup varied from 0.2% (between two individuals of *Phaenicophilus palmarum*) to 17.2% (between *Conirostrum* and *Chlorophonia*). Base composition (guanine 13.5%, adenine 26.7%, thymine 24.6%, cytosine 35.3%) was similar to that reported in other studies of cytochrome *b* in birds (Edwards et al. 1991, Helm-Bychowski and Cracraft 1993, Kornegay et al. 1993). Changes at third position sites were more common than changes at second and first position sites. Of the 485 variable sites, 113 occurred at the first codon position, 32 occurred at the second position, and 340 at the third position of a codon. Transitions between individual sequences were approximately twice as common as transversions.

Phylogenetics

In the Bayesian analyses, log-likelihood values reached a stable equilibrium well before 500 000 generations. Thus, we chose a burn-in value of 5000 samples and constructed a majority rule consensus tree using the remaining 15 000 samples (Fig. 1). The repeated analyses had similar posterior probabilities and likelihood values, indicating insensitivity to initial starting tree. In the Bayesian tree (Fig. 1), species for which more than one individual was included (*Phaenicophilus palmarum* and *Coereba flaveola*) were monophyletic and showed strong support for monophyly. Some genera for which more than one individual of a species was included were monophyletic (*Creurgops*, *Cyanerpes*, *Piranga, Ramphocelus, Sporophila, Spindalis* and *Oryzoborus*), while others did not form monophyletic groups (*Conirostrum*, *Loxigilla*, *Tangara* and *Tiaris*).

Thraupidae, as traditionally defined (Storer 1970), did not form a monophyletic group. As found in previous studies (Bledsoe 1988, Sibley and Ahlquist 1990, Lougheed et al. 2000, Yuri and Mindell 2002), many genera of Neotropical finches (traditionally considered members of Emberizidae) are closely related to genera of tanagers. That is, 60 genera formed a monophyletic group similar in composition to Sibley and Monroe's (1990) Thraupini, with 52% posterior probability. However, some exceptions to this tanager-finch clade were also identified. Some genera of traditional tanagers are more closely related to non-Thraupini taxa. In agreement with Yuri and Mindell (2002) and Klicka et al. (2000), the "tanager" genera *Euphonia* and *Chlorophonia* are more closely related to the representative of Fringillinae

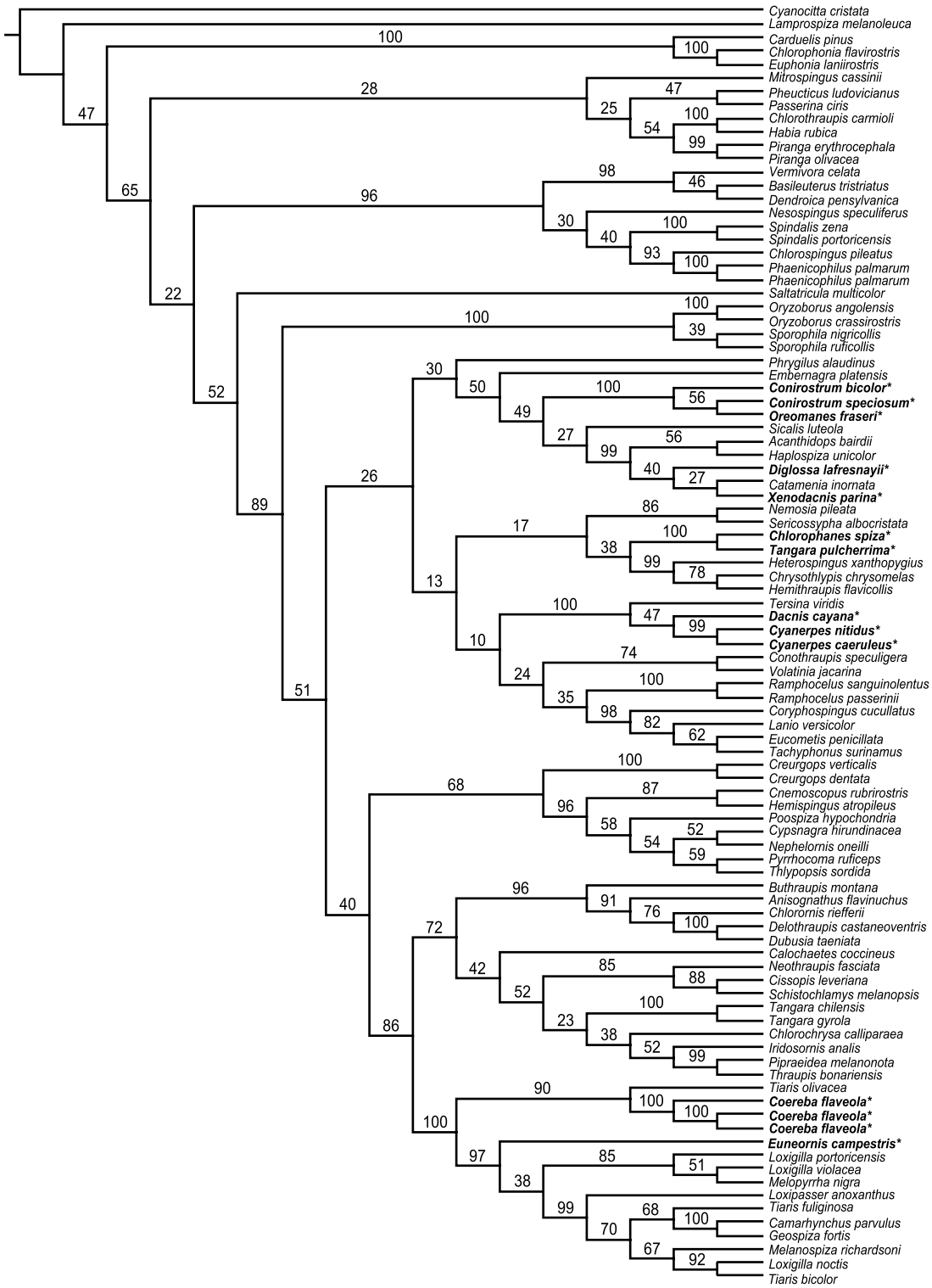

Fig. 1. Majority rule consensus tree of the 15 000 trees resulting from the Bayesian analysis. Numbers on nodes indicate the posterior probability of a particular clade. Neotropical honeycreepers are indicated in bold and are marked by an asterisk.

(*Carduelis*) included in this study. Support for this relationship was high (100% posterior probability). We also found that *Habia*, *Chlorothraupis* and *Piranga* formed a monophyletic group with the representatives of Cardinalini (cardinals and grosbeaks) included in this study. However, the posterior probability for this clade was only 25%. The association between these three traditional tanager genera and the cardinals and grosbeaks was also found in other studies (Klicka et al. 2000, Yuri and Mindell 2002) in which the taxon sampling was more complete in regards to this question. As found in Yuri and Mindell (2002), two genera of "tanagers", *Lamprospiza* and *Mitrospingus*, do not appear to be closely related to other Thraupini, but their relationship with regards to other nine-primaried oscines remains enigmatic. More nine-primaried oscines need to be included to fully address their relationships. In agreement with Burns (1997), the "tanager" genera *Phaenicophilus*, *Nesospingus*, *Spindalis* and *Chlorospingus* form a close relationship. In the Bayesian tree, they form a monophyletic clade that is sister to the warbler taxa included in this study (with 96% posterior probability).

Taxa once considered part of Coerebidae do not form a monophyletic group (Fig. 1). Also, none of the former Coerebidae is closely related to the wood-warblers (Parulini). Beecher (1951), Lowery and Monroe (1968), and Raikow (1978) considered *Coereba* and *Conirostrum* to be nectar-adapted wood-warblers. However, neither of these two taxa are closely related to the members of Parulini included in this study (see also Lovette and Bermingham (2002)). All of the Neotropical honeycreepers included in this study are instead closely related to species classified in Sibley and Monroe's (1990) Thraupini. Support for many of these relationships is strong (Fig. 1).

For the parsimony analyses, 428 (88%) of the 485 variable sites were phylogenetically informative. Number of trees found, consistency indices excluding uninformative characters, and number of bootstrap nodes above 50% for each of the four analyses are as follows: equal weighting (673 trees, 0.15, 29 nodes), 6:1 weighting (13 trees, 0.17, 33 nodes), 20:1 weighting (15 trees, 0.18, 32 nodes), 50:1 weighting (9 trees, 0.18, 37 nodes). In general, the consistency index increased and fewer most parsimonious trees were found in the analyses that downweighted third position transitions to a greater degree. Strict consensus trees show many unresolved nodes due to conflict among most equally parsimonious trees (e.g., strict consensus of 50:1 tree; Fig. 2). However, for the nodes that are resolved and show bootstrap support, results of the parsimony analyses are in general consistent with the Bayesian analysis (Table 2). Importantly, the Coerebidae do not form a monophyletic group in any of the most parsimonious trees. In addition, in agreement with the Bayesian tree, none of the Coerebidae taxa are closely related to the warblers included in the analyses. As in the Bayesian analyses, many of the Neotropical honeycreepers form well-supported clades with specific tanagers and finches (Table 2, Fig. 2).

Discussion

Evolution of feeding morphologies

Because Coerebidae is not monophyletic in any of the phylogenies in this study (Figs. 1 and 2), nectar-feeding must have arisen multiple times during the evolutionary history of tanagers and their relatives. Thus, similarities in the characters associated with nectar-feeding are best interpreted as the result of convergent evolution. In our phylogenies (Figs. 1 and 2), species in general do not segregate into monophyletic groups according to their bill shape and associated feeding behaviors. Instead, species with vastly different bill types are closely related to each other. For example, *Coereba flaveola*, a species with a thin decurved bill, is the sister taxon of *Tiaris olivacea*, a species with a relatively conical bill.

In their DNA-DNA hybridization studies of birds of the world, Sibley and Ahlquist (1990) reached similar conclusions regarding bill shape. In their study, they identified a group of "tanager-finches" (tribe Thraupini) which contained species with a variety of bill sizes and shapes. Traditional members of Emberizidae, Coerebidae and Thraupidae were placed in this group. The current study uses character-state data and samples over twice the number of species from members of these groups as the Sibley and Ahlquist study and confirms the general idea of a clade of "tanager-finches". Within this clade, bill morphology has changed numerous times resulting in a diversity of sizes and shapes.

As noted above, some of the taxa (*Habia*, *Piranga*, *Chlorothraupis*, *Chlorospingus*, *Phaenicophilus*, *Spindalis*, *Nesospingus*, *Euphonia*, *Chlorophonia*, *Lamprospiza* and *Mitrospingus*) included in Sibley and Monroe's (1990) Thraupini do not have close relationships to members of this group. However, the 60 other genera included from Sibley and Monroe's (1990) Thraupini are monophyletic in our Bayesian tree (Fig. 1). Thus, of the current classifications available, the taxonomy of Sibley and Monroe (1990) is most consistent with the results of our study. However, many details of the AOU's (1998) classification are reflected in our results. For example, in the AOU classification, the genus *Euneornis* was placed near *Loxigilla* and *Diglossa* was placed near *Haplospiza* and *Acanthidops*. This agrees with the position of these taxa in our phylogenies.

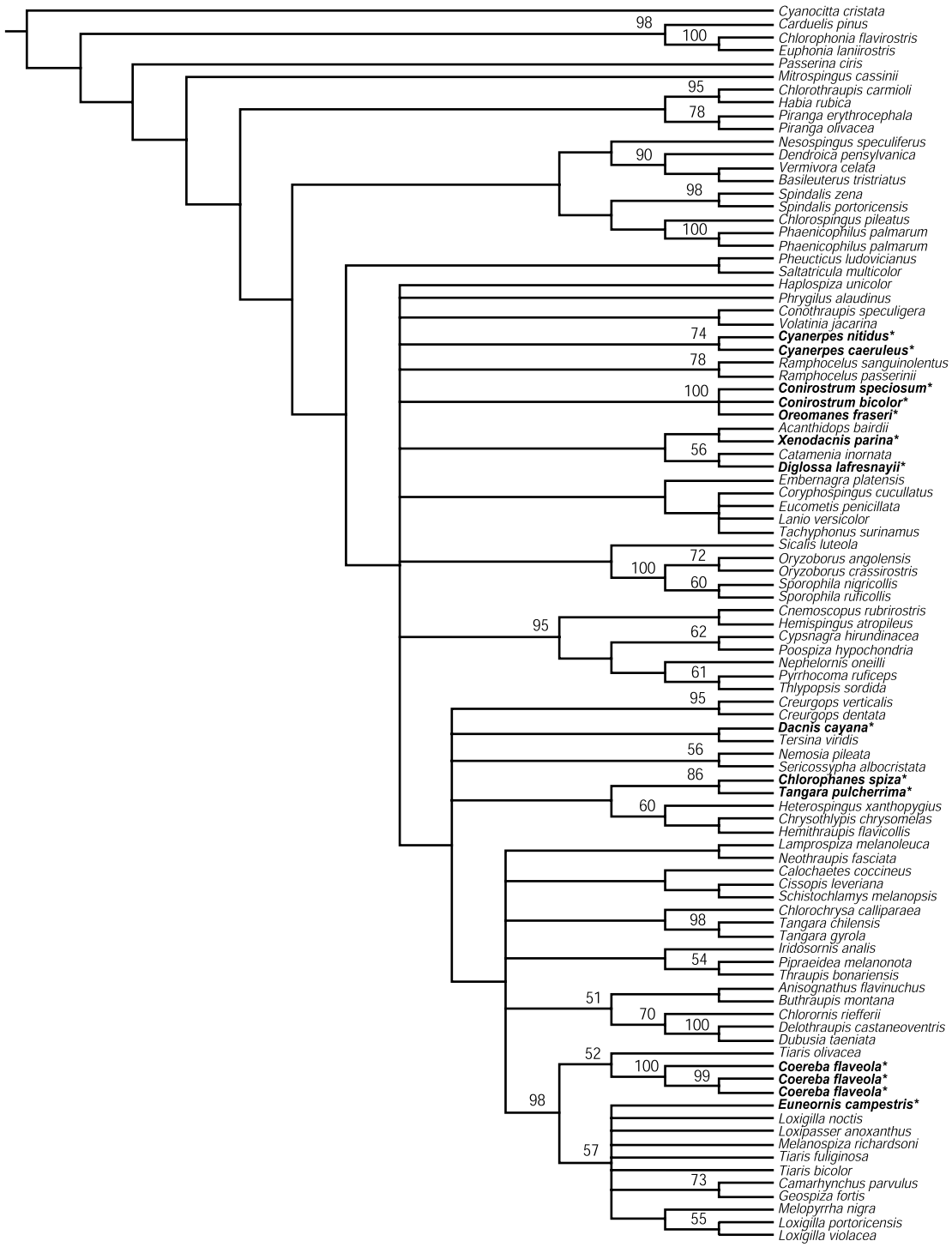

Fig. 2. Strict consensus tree of the nine most parsimonious trees resulting from downweighting third position transitions by a factor of 50. Numbers on nodes indicate bootstrap support for those nodes retained in greater than 50% of replicates. Asterisks and bold type indicate Neotropical honeycreepers.

Table 2. Level of support for particular clades in Bayesian and parsimony (equal weight, 6:1, 20:1, and 50:1) analyses. All clades with either > 50% bootstrap support or > 95% posterior probability are shown. M = clade is monophyletic, but not recovered in > 50% of bootstrap replicates. Dash indicates the clade was not recovered in the analysis.

Node	Bayesian	Equal weight	6:1	20:1	50:1
Nodes involving Neotropical honeycreepers:					
Chlorophanes, *Tangara pulcherrima*	100%	94%	94%	94%	86%
Cyanerpes, *Dacnis*, *Tersina*	100%	–	–	–	–
Cyanerpes	99%	M	72%	70%	74%
Conirostrum, *Oreomanes*	100%	100%	100%	100%	100%
Acanthidops, *Diglossa*, *Catamenia*, *Xenodacnis*, *Haplospiza*	99%	–	–	–	–
Diglossa, *Catamenia*	–	M	M	54%	56%
Domed nest clade (Tholospiza)	100%	82%	98%	99%	98%
Coereba, *Tiaris olivacea*	90%	M	M	53%	52%
Coereba	100%	100%	100%	100%	100%
Camarhynchus, *Geospiza*, *Tiaris fuliginosa*, *T. bicolor*, *Euneornis*, *Loxigilla*, *Loxipasser*, *Melanospiza*, *Melopyrrha*	97%	82%	62%	63%	57%
Other nodes:					
Carduelis, *Chlorophonia*, *Euphonia*	100%	64%	96%	97%	98%
Chlorophonia, *Euphonia*	100%	99%	100%	100%	100%
All ingroup taxa excluding *Chlorophonia*, *Euphonia*, *Carduelis*	–	71%	94%	92%	90%
Chlorothraupis, *Habia*	100%	98%	98%	96%	95%
Piranga	99%	93%	82%	77%	78%
Parulini (*Dendroica*, *Vermivora*, *Basileuterus*)	98%	93%	86%	93%	90%
Parulini, *Nesospingus*, *Spindalis*, *Chlorospingus*, *Phaenicophilus*	96%	–	–	M	M
Anisognathus, *Buthraupis*, *Chlorornis*, *Delothraupis*, *Dubusia*	96%	M	M	M	51%
Chlorornis, *Delothraupis*, *Dubusia*	76%	51%	67%	69%	70%
Delothrauis, *Dubusia*	100%	81%	100%	100%	100%
Camarhynchus, *Geospiza*, *Tiaris fuliginosa*, *T. bicolor*, *Loxigilla noctis*, *Loxipasser*, *Melanospiza*	99%	–	–	–	M
Loxigilla portoricensis, *L. violacea*	–	–	M	61%	55%
Tiaris fuliginosa, *Camarhynchus*, *Geospiza*	68%	M	53%	60%	52%
Camarhynchus, *Geospiza*	100%	100%	91%	68%	73%
Chrysothlypis, *Hemithraupis*, *Heterospingus*	99%	–	52%	M	60%
Chrysothlypis, *Hemithraupis*	78%	–	54%	M	M
Cissopis, *Schistochlamys*	88%	84%	82%	79%	71%
Cnemoscopus, *Cypsnagra*, *Poospiza*, *Hemispingus*, *Nephelornis*, *Pyrrhocoma*, *Thlypopsis*	96%	60%	98%	96%	95%
Cypsnagra, *Poospiza*	–	75%	60%	68%	62%
Pyrrhocoma, *Thlypopsis*	59%	90%	73%	61%	61%
Nemosia, *Sericossypha*	86%	M	56%	M	56%
Pipraeidea, *Thraupis*	99%	–	M	M	54%
Coryphospingus, *Lanio*, *Eucometis*, *Tachyphonus*	98%	–	M	M	M
Lanio, *Eucometis*, *Tachyphonus*	82%	56%	–	–	–
Eucometis, *Tachyphonus*	62%	65%	–	–	–
Sporophila, *Oryzoborus*	100%	100%	99%	100%	100%
Sporophila	39%	58%	58%	64%	60%
Oryzoborus	100%	63%	68%	70%	72%
Tangara chilensis, *Tangara gyrola*	100%	79%	99%	95%	98%
Ramphocelus	100%	M	81%	83%	78%
Creurgops	100%	70%	96%	96%	95%
Spindalis	100%	94%	99%	100%	98%
Phaenicophilus	100%	100%	100%	100%	100%

Phylogenetic conclusions and taxonomic recommendations

Even though Beecher (1951) realized that Coerebidae was not monophyletic, he still argued that the genera moved to the tanager subfamily Thraupinae (*Diglossa*, *Cyanerpes*, *Chlorophanes*, *Iridophanes* = *Tangara pulcherrima*, *Dacnis*, *Euneornis*, *Xenodacnis* and *Oreomanes*) formed a monophyletic group. The cytochrome *b* phylogenies (Fig. 1 and 2) contradict these results. The former Coerebidae are found in at least five different clades that are scattered throughout the phylogeny. Thus, most "Coerebidae" are more closely related to a variety of tanagers and finches than they are to other Neotropical honeycreepers. The support for and significance of these five clades is discussed below.

Tangara pulcherrima and Chlorophanes

Tangara pulcherrima was formerly considered the sole member of the monotypic genus *Iridophanes*. Based on plumage similarities to some members of the genus *Tangara*, especially *T. cyanoptera*, this species was included in the genus *Tangara* by Storer (1970). Some recent taxonomic treatments (e.g., Howard and Moore

1991) of tanagers agreed with this assessment and include this species with the other *Tangara*. However, other reference works (e.g., Hilty and Brown 1986, Ridgely and Tudor 1989) did not include this species in *Tangara* because of its different bill color and shape, its red-colored iris, and behavioral similarities to *Chlorophanes*.

The molecular phylogenies of this study show that *Tangara pulcherrima* does not form a monophyletic group with the other *Tangara* included in this study. In contrast, *T. pulcherrima* is the sister taxon to the monotypic genus *Chlorophanes* (Fig. 1 and 2). Support for this relationship is strong (Table 2). This relationship is consistent with the characters identified by Ridgely and Tudor (1989). Because of the close relationship between these two taxa, we recommend either reassigning *Tangara pulcherrima* to its own genus *Iridophanes*, or preferably, assigning both of these monotypic genera to *Chlorophanes* (which has taxonomic precedence).

Dacnis, Tersina and Cyanerpes

A previous study of tanager phylogeny (Burns 1997) identified a close relationship among the genera *Cyanerpes*, *Dacnis* and *Tersina*. This relationship was unexpected due to morphological differences between *Tersina* and the other two genera. Foraging behaviors and associated feeding morphologies of these genera are quite different. Both *Cyanerpes* and *Dacnis* have slender bills, with *Dacnis* having a shorter bill and *Cyanerpes* having a longer, decurved bill. *Tersina* has a uniquely flattened bill that is broad at its base. The morphological distinctiveness of *Tersina* led some classifications (e.g., Hellmayr 1936, Storer 1970, Howard and Moore 1991) to place this genus in its own subfamily or family (Tersininae or Tersinidae). In contrast to these differences in feeding morphology, the three genera share plumage similarities that are consistent with their close, phylogenetic relationship. All of these taxa are sexually dimorphic, with males having bright blue plumage and females having greenish plumage. The current study again suggests a close relationship among these taxa. Although bootstrap support is lacking, *Dacnis* and *Tersina* form a monophyletic group in all most parsimonious trees of the equal weight, 20:1, and 50:1 parsimony analyses. *Dacnis*, *Tersina* and *Cyanerpes* form a monophyletic group in the Bayesian tree (Fig. 1) with strong support (100% posterior probability).

Conirostrum and Oreomanes

The relationship of *Conirostrum* to other avian taxa has been enigmatic. Different authors have considered this genus to belong to Coerebidae, Parulidae, or Thraupidae (Table 1). Schulenberg (1985) argued for a close relationship between *Conirostrum* and *Oreomanes* based on morphological similarities (plumage color, plumage pattern and body shape) and the occurrence of hybridization between *Oreomanes* and *Conirostrum ferrugineiventre*. The current study includes two species of *Conirostrum* (*C. speciosum* and *C. bicolor*), and the phylogenies agree with Schulenberg's (1985) conclusions. In all trees, *Oreomanes* and *Conirostrum* form a monophyletic group with both 100% bootstrap support and 100% posterior probability (Table 2). In all of our analyses, the genus *Conirostrum* is not monophyletic with respect to *Oreomanes*, however, we feel the additional seven species of *Conirostrum* will need to be sampled before strong conclusions about the paraphyly of *Conirostrum* can be reached.

Diglossa and Xenodacnis

In the Bayesian tree, a strongly supported clade (99% posterior probability) is identified that contains *Diglossa* and *Xenodacnis* as well as representatives of the genera *Acanthidops*, *Haplospiza* and *Catamenia*. With the exception of *Haplospiza*, this clade is also identified in the 6:1, 20:1, and 50:1 parsimony analyses, albeit without bootstrap support. A close relationship among all these species would not be predicted based on bill shape and feeding morphology. The clade includes finch-billed and seed-eating species (*Catamenia*, *Haplospiza*), a bamboo specialist (*Acanthidops*), nectar specialists (*Diglossa*), and a partial nectar-feeder (*Xenodacnis*). Species in the genus *Diglossa*, known as flowerpiercers, have a particularly unusual method of obtaining nectar. In most species of *Diglossa*, a hook on the end of the upper mandible is used to hold the corolla of a flower while the bird pierces the flower with its lower bill. The bird is then able to feed on nectar from the flower. *Xenodacnis* has a sharply-pointed, short bill that is used to pick insects off the underside of leaves and probe flowers for nectar (Isler and Isler 1999).

Members of this clade may also be closely related to the *Oreomanes*/*Conirostrum* clade described above. Burns (1997) identified a close relationship among the genera *Diglossa*, *Xenodacnis* and *Oreomanes* (*Catamenia*, *Haplospiza* and *Acanthidops* were not sampled by Burns 1997). In the Bayesian analyses of the current study, *Oreomanes*, *Conirostrum*, *Sicalis*, *Acanthidops*, *Haplospiza*, *Xenodacnis*, *Diglossa* and *Catamenia* form a clade. However, support for this clade is relatively weak (only 49% posterior probability), and this clade was not retained in the parsimony analyses. Common plumage colors of these species supports the idea of a close relationship among them. For example, *Xenodacnis*, *Oreomanes*, some *Conirostrum* and some *Diglossa* have underparts that are reddish brown; other common colors of species in these genera include blues, grays, and black.

Additional studies, with more complete sampling of all species in these genera, should be pursued to clarify the nature of the relationship among these taxa.

Coereba and Euneornis

Two species of Coerebidae (*Coereba flaveola* and *Euneornis campestris*) belong to a clade of birds that all build covered, dome-shaped nests (Fig. 1 and 2). This clade was identified in previous studies (Sato et al. 2001, Burns et al. 2002), and Burns et al. (2002) referred to it as the domed nest clade or Tholospiza. This domed nest clade provides another example of a case in which bill size and shape differ dramatically among close relatives. In addition to nectar-feeders, this clade includes a number of birds with large conical seed-eating bills such as some of the Darwin's finches, species in the genus *Loxigilla*, and species in the genus *Tiaris*. These dramatic changes in bill morphology have occurred with little genetic divergence among the species (Burns et al. 2002). In fact, levels of genetic variation between species in this clade are comparable to that observed within genera of most birds (Burns et al. 2002). However, at one time species in this clade were classified into three separate families because of their morphological differences.

Status of "Coerebidae"

The current study confirms that members of the historically-recognized group Coerebidae do not form a monophyletic group in any of the phylogenies of this study (Fig. 1 and 2). Thus, we recommend that future taxonomies do not use Coerebidae or consider the Neotropical honeycreepers as a whole to be closely related. Even the retention of Coerebidae in classifications (Howard and Moore 1991, AOU 1998) and reference works (Ridgely and Tudor 1989, Howell and Webb 1995) as a monotypic taxon for the bananaquit, *Coereba flaveola*, is no longer warranted. The current study and other recent studies (Sato et al. 2001, Burns et al. 2002) clearly shows that *Coereba flaveola* is closely related to other Thraupini, specifically the New World finch *Tiaris olivacea*. As outlined above, Neotropical honeycreepers are best considered disparate members of a larger radiation of tanagers and finches, in which bill size and associated feeding behaviors have evolved to fill a variety of niches over a relatively short period of time.

Acknowledgements – The following collections provided tissues and specimens used in this study: American Museum of Natural History, Louisiana State University Museum of Natural Science, University of Michigan Museum of Zoology, the Field Museum of Natural History, the California Academy of Sciences, Museu Paraense Emílio Goeldi (Belem, Brazil), and the Museum of Vertebrate Zoology at UC Berkeley. For financial support, we thank the National Science Foundation, San Diego State University (Grant-in-Aid), the Frank M. Chapman Memorial Fund of the American Museum of Natural History, and the Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Pritzker Foundation. This project was also supported by the Ambrose Monell Molecular Laboratory and the Lewis B. and Dorothy Cullman Program for Molecular Systematic Studies, a joint initiative of the New York Botanical Garden and the American Museum of Natural History. For comments on the manuscript we thank J. M. Bates and three anonymous reviewers.

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(*Received 27 December 2002, revised 5 May 2003, accepted 8 May 2003.*)