The Auk 119(3):695–714, 2002

WHAT IS A WOOD-WARBLER? MOLECULAR CHARACTERIZATION OF A MONOPHYLETIC PARULIDAE

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ABSTRACT.—The wood-warblers (family Parulidae) fall within a radiation of passerine birds commonly known as the New World nine-primaried oscines. Defining familial relationships within that radiation has previously been challenging because of its extremely high diversity, a paucity of phylogenetically informative morphological characters, and an apparent high rate of cladogenesis early in the radiation's history. Here, analyses of mitochondrial and nuclear DNA sequences demonstrate that the 25 extant genera traditionally placed in the Parulidae do not form a monophyletic group. Instead, all reconstructions identify a well-resolved clade of 19 genera (*Vermivora*, *Parula*, *Dendroica*, *Catharopeza*, *Mniotilta*, *Setophaga*, *Protonotaria*, *Helmitheros*, *Limnothlypis*, *Seiurus*, *Oporornis*, *Geothlypis*, *Wilsonia*, *Cardellina*, *Ergaticus*, *Myioborus*, *Euthlypis*, *Basileuterus*, and *Phaeothlypis*) that are all morphologically typical wood-warblers traditionally placed in the Parulidae. Six genera traditionally assigned to the Parulidae—*Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus*, and *Xenoligea*—fall outside this highly supported clade in all mtDNA-based and nuclear DNA-based reconstructions, and each is probably more closely allied to taxa traditionally placed in other nine-primaried oscine families. The long, well-supported, and independently confirmed internode at the base of this wood-warbler clade provides the opportunity to define a monophyletic Parulidae using several complementary molecular phylogenetic criteria. Support for those relationships comes from reconstructions based on a range of nucleotide-intensive (from 894 to 3,638 nucleotides per taxon) and taxon-intensive (45 to 128 species) analyses of mtDNA sequences, as well as independent reconstructions based on nucleotide substitutions in the nuclear-encoded *c-mos* gene. Furthermore, the 19 typical wood-warbler genera share a synapomorphic one-codon *c-mos* deletion not found in other passerines. At a slightly deeper phylogenetic level, our mtDNA-based reconstructions are consistent with previous morphologic and genetic studies in suggesting that many nine-primaried oscine taxa have unanticipated affinities, that many lineages arose during an early and explosive period of cladogenesis, and that the generation of a robust nine-primaried oscine phylogeny will require robust taxonomic sampling and extensive phylogenetic information. *Received 29 June 2001, accepted 15 March 2002.*

RESUMEN.—Las reinitas (familia Parulidae) forman parte de una radiación de aves paserinas conocida comúnmente como los oscines de nueve primarias del Nuevo Mundo. La definición de las relaciones entre las familias de esta radiación ha sido difícil debido a su gran diversidad, a la carencia de caracteres morfológicos filogenéticamente informativos y a una tasa de cladogénesis aparentemente alta al inicio de la radiación. En este artículo, análisis de secuencias de ADN mitocondrial (ADNmt) y nuclear demuestran que los 25 géneros vivientes que tradicionalmente se han incluido en Parulidae, no forman un grupo monofilético. En cambio, todas las reconstrucciones identifican un clado bien resuelto de 19 géneros (*Vermivora*, *Parula*, *Dendroica*, *Catharopeza*, *Mniotilta*, *Setophaga*, *Protonotaria*, *Helmitheros*, *Limnothlypis*, *Seiurus*, *Oporornis*, *Geothlypis*, *Wilsonia*, *Cardellina*, *Ergaticus*, *Myioborus*, *Euthlypis*, *Basileuterus* y *Phaeothlypis*) correspondientes a reinitas morfológicamente típicas que tradicionalmente han sido incluidas en Parulidae. En todas las reconstrucciones basadas en ADNmt y ADN nuclear, seis géneros tradicionalmente asignados a Parulidae (*Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus* y *Xenoligea*) aparecen por fuera de este clado que posee buen respaldo. Probablemente, cada uno de estos géneros tiene una relación más cercana con taxa tradicionalmente ubicados en otras familias de oscines de nueve primarias. El largo

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internodo de la base del clado de las reinitas, que está bien respaldado y ha sido confirmado independientemente, permite definir una familia Parulidae monofilética con base en varios criterios moleculares complementarios. El respaldo para esas relaciones se deriva de reconstrucciones basadas en una variedad de análisis de secuencias de ADNmt intensivos en términos de nucleótidos (de 894 a 3,638 por taxón) y taxa (45 a 128 especies), y de reconstrucciones independientes basadas en sustituciones de nucleótidos en el gen nuclear *c-mos*. Más aún, los 19 géneros típicos de Parulidae comparten una deleción sinapomórfica en un codón de *c-mos* que no se encuentra en otras aves paserinas. A un nivel filogenético un poco más profundo, nuestras reconstrucciones basadas en ADNmt son consistentes con estudios morfológicos y genéticos anteriores que sugieren que muchos taxa de los oscines de nueve primarias tienen afinidades inesperadas, que muchos linajes surgieron durante un período temprano de cladogénesis explosiva, y que la generación de una filogenia robusta para los oscines de nueve primarias requerirá un muestreo taxonómico robusto y una gran cantidad de información filogenética.

THE RECONSTRUCTION OF phylogenetic relationships among lineages that radiated rapidly and extensively presents formidable systematic challenges. This situation is typified by a songbird assemblage commonly termed the ''New World nine-primaried oscine passerines,'' which includes >8% of the world's extant avian species (Sibley and Monroe 1990). That assemblage comprises a number of groups traditionally given family rank (e.g. American Ornithologists' Union [AOU] 1998), including the wood-warblers (Parulidae), Bananaquit (Coerebidae: *Coereba flaveola*), tanagers (Thraupidae), sparrows (Emberizidae), cardinals and allies (Cardinalidae), and orioles and blackbirds (Icteridae). Phylogenetic reconstructions based on morphological characters (Beecher 1953, Raikow 1978), allozymes (Barrowclough and Corbin 1978, Avise et al. 1980), DNA–DNA hybridization distances (Sibley and Ahlquist 1985, Bledsoe 1988, Sibley and Ahlquist 1990), and mtDNA sequences (e.g. Burns 1997, Groth 1998, Klicka et al. 2000) have repeatedly emphasized the difficulty of defining strongly supported subclades within the nine-primaried oscines that correspond to this and other classifications. Those difficulties are usually ascribed to the high lineage diversity of the nine-primaried oscine assemblage coupled with the general absence of morphological or genetic synapomorphies that define clades within it (Beecher 1953, Raikow 1978), phylogenetic features typical of groups that have undergone rapid and extensive radiations (e.g. Lovette and Bermingham 1999).

Recent studies based on mitochondrial DNA (mtDNA) sequences have indicated that several nine-primaried oscine families, as they are traditionally defined, are not monophyletic (Burns 1997, Klicka et al. 2000). Here, we use both mtDNA and nuclear-encoded DNA sequences to test whether the 26 genera currently placed in the wood-warbler family Parulidae (AOU 1998) form a monophyletic group and to examine the relationship of those taxa to other nine-primaried oscine lineages. The phylogenetic boundaries of the wood-warblers are one of the many long-standing controversies in nine-primaried oscine systematics because some taxa traditionally placed in the Parulidae may be more closely allied to other groups within the nine-primaried oscine radiation (Sclater 1886; Hellmayr 1935, 1936; Mayr and Amadon 1951; Beecher 1953; Storer 1969; Sibley 1970; Paynter and Storer 1970). The importance of assessing parulid relationships extends beyond avian systematics, because the Parulidae have long served as a model group for the study of comparative ecology and behavior (e.g. MacArthur 1958, Robinson and Holmes 1982, Morse 1989, Shutler and Weatherhead 1990, Price et al. 2000) and future studies of this type will require a phylogenetic context.

Determining affinities of the most morphologically aberrant parulid taxa has proven to be particularly problematic because there has been no comprehensive analysis of wood-warbler taxa using morphological or molecular markers. In addition, a number of nine-primaried oscine families contain small, brightly colored, frugivorous or insectivorous genera that show a remarkable degree of variation in plumage patterns and behavior (Storer 1969, Morse 1989, Burns 1997), have high species diversities (e.g. Sibley and Monroe 1990), and are linked by no known synapomorphies (Beecher 1953, Storer 1969, Raikow 1978). Parulids of particular systematic interest include the morpholog-

ically aberrant genera *Zeledonia*, *Icteria*, *Granatellus*, *Microligea*, and *Xenoligea*; conversely, nonparulids that particularly resemble wood-warblers include *Coereba*, *Conirostrum*, and *Hemispingus*.

The existing molecular evidence provides conflicting perspectives on relationships among wood-warblers and other nine-primaried oscines. Sibley and Ahlquist (1990) included representatives of nine wood-warblers in their DNA–DNA hybridization-based phylogeny; based on that reconstruction, they placed wood-warblers as a tribe (Parulini) within the subfamily Emberizinae, along with the four other tribes (Thraupini, Emberizini, Cardinalini, and Icterini) that consititue the New World nine-primaried oscine passerines in their classification. Inspection of their phylogeny (Sibley and Ahlquist 1990), however, demonstrates that the basal lineages in each of those putative tribes are separated by very short internodes from lineages assigned to other tribes, raising doubts about the strength of support for those relationships. The parallel DNA–DNA hybridization study of Bledsoe (1988) grouped three representative wood-warbler genera as a sister clade to the Icteridae and identified a larger tanager clade as the sister taxon to the Cardinalidae. Burns (1997) included 3 wood-warbler genera and 47 tanager genera in a cytochrome-*b*-based survey of the Thraupidae and found that his Thraupidae constitute a paraphyletic group, with the three representative wood-warblers as the sister taxon to a clade consisting of most tanager genera, but with the traditionally thraupid genera *Euphonia* and *Chlorophonia* falling as the distant sister taxon of that tanager–wood-warbler group. Recently, Klicka et al. (2000) included three representative wood-warblers in an mtDNA-based survey of nine-primaried oscines and concluded that the lineage represented by the wood-warbler genera *Dendroica* and *Geothlypis* is the sister taxon to a clade of emberizid sparrows, but that those two genera and the parulid genus *Icteria* do not form a monophyletic group. Further inferences about the general affinities of wood-warblers are difficult to derive from those molecular studies because of the weak topological support at many nodes in most reconstructions and their sparse taxonomic sampling, especially the absence of most of the morphologically aberrant wood-warbler taxa.

As part of a taxonomically nested investigation of phylogenetic relationships of the Parulidae, we have obtained long protein-coding mtDNA sequences from a large number of oscine passerine birds, including representatives of all 25 extant wood-warbler genera and a variety of other nine-primaried oscine genera. In conjunction with sequences previously reported by Burns (1997) and Klicka et al. (2000), those novel sequences allow us to investigate relationships within the nine-primaried oscines using several complementary data sets that span a broad range of taxonomic and nucleotide sampling, from a partial cytochrome-*b* data set of 894 nucleotides from 128 taxa to a combined-gene data set of 3,638 bases from 45 taxa. We also present reconstructions based on a slowly evolving nuclear locus, the *c-mos* proto-oncogene, that are congruent with the mtDNA reconstructions in that they provide independent confirmation of an important basal internode that defines a monophyletic wood-warbler assemblage. Those analyses allow us to investigate parulid relationships with complete genus-level taxonomic sampling of the Parulidae, whereas the inclusion of sequences representing many taxa in other nine-primaried oscine groups allows us to consider the parulid radiation in a broader phylogenetic context.

Our primary objectives in this study were to use those complementary mtDNA- and nuclear-based analyses to test whether the genera traditionally placed in the Parulidae, or a subset of those genera, form a well-supported monophyletic group and to investigate the phylogenetic affinities of the most morphologically aberrant wood-warbler genera and the most parulid-like tanager genera.

Methods

We conducted analyses based on three mtDNA data sets spanning a broad range of nucleotide and taxonomic sampling. All analyses included representatives of all 25 extant genera currently placed in the Parulidae (AOU 1998) as well as several representative species from the most diverse parulid genera we sequenced, and hence the primary difference in taxonomic sampling among the three data sets is in their proportional representation of taxa traditionally placed in other nine-primaried oscine families. Sample sources and GenBank accession information for the novel sequences used to generate the phylogenetic reconstructions reported here are given in the Appendix.

Partial cytochrome-b *sequences.*—Our most taxonomically intensive sample initially involved 157 partial cytochrome-*b* sequences representing a total of 128 species, of which 47 were obtained from Burns (1997, 1998; GenBank accession numbers AF006211–AF006246, AF006249–AF006258, and AF011777), 1 from Hackett (1996; U15717), 40 from Klicka et al. (2000; AF290137–AF290176), and 71 from our laboratory. Analyses were based on the homologous 894-nucleotide region corresponding to bases 15013–15906 in the chicken (*Gallus gallus domesticus*) mitochondrial genome (GenBank X52392; Desjardins and Morais 1990) available for most individuals.

The initial set of 157 sequences included multiple individuals representing many species and genera. To render subsequent analyses more tractable, we excluded all but one representative sequence per species. The 71 sequences we generated included sequences from two individuals each of 26 species. Because the two members of all those conspecific pairs had nearly identical partial cytochrome-*b* sequences (mean difference = 3.6 nucleotide substitutions) and were each far more similar to one another than they were to any nonconspecific sequence, we used a random-number table to select a single representative sequence from each replicated species for inclusion in subsequent phylogenetic analyses. Using samples from different sources and different PCR primers, we also obtained cytochrome-*b* sequences from two species (*Hemispingus atropileus* and *Spindalis zena*) and two additional genera (*Vermivora* and *Euphonia*) sequenced by Burns (1997), and from three species (*Coereba flaveola*, *Icteria virens*, *Geothlypis trichas*) and three additional genera (*Euphonia*, *Saltator*, and *Dendroica*) included by Klicka et al. (2000). Those sequences replicated across laboratories all showed the anticipated level of similarity: the congeneric replicates grouped together as expected in phylogenetic reconstructions, as did the conspecific replicates, which with one exception had pairwise divergences of 0–10 nucleotide substitutions (0–1.1% uncorrected sequence divergence). The sole taxon with high intraspecific divergence was the *C. flaveola*, in which the sequence selected at random from the many available from our laboratory differed from the Klicka et al. (2000) sequence at 49 nucleotide sites (5.5%). As part of a separate study, however, we subjected *C. flaveola* to intensive phylogeographic analysis (e.g. Bermingham et al. 1996) and found that this species shows a high magnitude of geographically structured mtDNA variation, particularly among islands in the West Indies. Because the Klicka et al. (2000) sequence is identical to haplotypes we have obtained from the Puerto Rico *C. flaveola* population, the high divergence between the two presently included individuals is unlikely to stem from laboratory or other errors.

Because the individuals we sequenced are also represented in the more nucleotide-intensive data sets described below, we similarly excluded from subsequent analyses the five conspecific cytochrome-*b* sequences generated by other laboratories. Our phylogenetic reconstructions based on partial cytochrome-*b* sequences therefore included 128 sequences representing an equal number of species.

Cytochrome-b *plus ND2 sequences.*—Our intermediate mitochondrial data set was derived from the combined cytochrome-*b* and NADH dehydrogenase subunit II (ND2) sequences reported for 40 taxa by Klicka et al. (2000; AF290100–AF290176, AF109953, AF109958, and AF109931) and for the 71 individuals sequenced in our laboratory, and it therefore initially included 109 sequences representing 82 species. As for cytochrome-*b*, conspecific and congeneric ND2 replicates had the expected degrees of sequence similarity, and we included in subsequent analyses one individual per species. Each of those 82 species was represented by the same 894 nucleotides of cytochrome-*b* sequence described above, plus the 1041 nucleotides of the complete ND2 coding sequence, for a total of 1935 nucleotides per taxon.

Combined-gene sequences.—Our most nucleotide-intensive data set initially included 71 individuals representing 45 species sequenced. From each individual, we obtained the complete sequences of the cytochrome-*b* (1,143 bp), ND2 (1041 bp), ATP-synthase 6 (684 bp), and ATP-synthase 8 (168 bp) genes and 613 bp of the cytochrome oxidase subunit I (COI) gene corresponding to nucleotides 732–7,954 in the chicken mtDNA genome (GenBank X52392; Desjardins and Morais 1990). Because the ATPase 6 and 8 coding regions overlap by 10 nucleotides, each individual was represented by a total of 3639 nucleotides of mitochondrial protein-coding sequence. As for cytochrome-*b*, all conspecific replicates had highly similar sequences in all gene-specific comparisons, and we excluded from analyses the same replicated conspecifics as described above, leaving a total of 45 individuals representing an equal number of species.

The mitochondrial sequences we generated were obtained using standard laboratory protocols, primers, and amplification conditions that we have described previously (Lovette et al. 1998, 1999; Lovette and Bermingham 1999; Hunt et al. 2001). In brief, genomic DNAs were extracted from muscle tissue, followed by gene-specific amplifications via the polymerase chain reaction (PCR). Sequences were generated using Dyedeoxy terminator cycle sequencing reactions (Applied Biosystems Division of Perkin Elmer Inc.) followed by electrophoresis in an Applied Biosystems 377 automated DNA sequencer. No insertions or deletions were present among any of the mitochondrial sequences included in this study, and therefore sequence alignments were unambiguous. All of the DNAs used for this study were extracted from avian muscle tissue. Muscle tissue has a very high concentration of mitochondria in com-

parison to avian blood, which decreases the likelihood of amplifying mtDNA pseudogenes translocated to the nucleus. We previously reported wood-warbler pseudogenes (Lovette and Bermingham 1999, Price et al. 2000, Lovette and Bermingham 2001), but those were amplified from DNAs isolated from avian blood. In this study, we found no evidence of mtDNA pseudogenes using the methods of detection presented in Lovette and Bermingham (2001).

mtDNA-Based Phylogenetic Analyses

All sequence comparisons and phylogenetic analyses were conducted using PAUP*b8 (Swofford 1999), except for maximum-likelihood reconstructions generated using Bayesian Markov chain Monte Carlo (MCMC) searches, which were conducted using the program MRBAYES 2.01 (Huelsenbeck and Ronquist 2001). As expected given the complete linkage of mitochondrial markers and the similar nucleotide frequencies across the mitochondrial genes we studied, partition-homogeneity tests (Farris et al. 1995) identified no significant differences among single-gene partitions in the 45-taxon, five-gene data set ($P = 0.40$), and we therefore combined the mtDNA sequences obtained from each individual for subsequent analyses of phylogenetic relationships.

We used both maximum-likelihood and maximum-parsimony techniques to reconstruct phylogenetic relationships. All analyses were rooted using the *Vireo latimeri* sequence, because that taxon is a representative of the corvid assemblage that is basal to the other oscine passerines (Sibley and Ahlquist 1990), as has been confirmed by many studies employing different molecular markers (e.g. Sheldon and Gill 1996, Lovette and Bermingham 1999).

Computational constraints precluded some types of phylogenetic reconstruction for the largest of our data sets (the 128 partial cytochrome-*b* sequences). For this mtDNA data set alone, maximum-parsimony analyses were conducted using heuristic searches with 100 stepwise addition replicates, with each replicate stopped after 1×10^8 rearrangements were assessed. A strict consensus was constructed from the shortest trees identified across the 100 replicates. We employed three character weighting schemes: equal weighting, downweighting of all transitions by 0.2 relative to transversions, and downweighting of third-position changes by 0.2 relative to all other substitutions (''Yoder weighting''; Yoder et al. 1996). Bootstrap values for the cytochrome-*b* data set were derived using the ''fast stepwise addition'' option in PAUP*, again because computation time constraints precluded more intensive methods of analysis.

For the remaining two mtDNA data sets, maximum-parsimony analysis were conducted via full heuristic searches with 100 stepwise addition replicates. Support for individual nodes was assessed via maximum-parsimony heuristic bootstrap for a single addition sequence replicate and 100 bootstrap replicates. We employed the same three character-weighting schemes described above. Major similarities and differences among those reconstructions are discussed in the text. Figures depict the results of the Yoder-weighted searches because that weighting scheme is the most biologically realistic and because that weighting was also used by Klicka et al. (2000) and thereby enhances the comparability of topologies across our respective studies.

Maximum-likelihood analyses were conducted for the two most sequence-intensive data sets via MCMC searches that were parameterized using the general time reversible model (*nst* = 6), with site-specific rate variation partitioned by codon. Those MCMC searches were run for 250,000 generations and sampled every 1,000 generations; graphical inspection of the resulting maximum-likelihood scores suggested that stationarity was reached before 25,000 generations in all searches, and we therefore conservatively discarded the 50 topologies sampled from the first 50,000 generations. Majority-rule consensus topologies were generated in PAUP* or MRBAYES from the remaining 201 sampled trees. In some cases, phylograms were generated in MRBAYES for those consensus topologies, with branch lengths determined by averaging across the 201 sampled trees. Support for particular branches was estimated and assessed by the proportion of sampled trees in which a given branch appeared, a value that corresponds to the posterior probability of that branch in the MCMC search (Huelsenbeck and Ronquist 2001).

c-mos Analyses

In a previous phylogenetic analysis (Lovette and Bermingham 2000) of passerine relationships based on sequences from the nuclear-encoded *c-mos* proto-oncogene, we found that the two wood-warbler genera sequenced (*Dendroica* and *Basileuterus*) had a one-codon deletion relative to the three nonparulid nine-primaried oscine genera we sequenced (*Coereba*, *Saltator*, and *Icterus*). That deletion involves the codon at positions 1263–1265 in the Gallus *c-mos* sequence (GenBank M19412; Schmidt et al. 1988). Although the same codon deletion is found in representatives of at least two other avian orders, it is not present in lineages representing a diverse group of suboscine and oscine passerines (Lovette and Bermingham 2000). Those preliminary results suggested that within passerines that indel might be a synapomorphic character restricted to a monophyletic group of parulid genera.

To further explore the utility of *c-mos* as a phylogenetic marker for parulid systematics, we obtained 579–582 nucleotides of *c-mos* sequence from almost all the taxa included in our analyses of long mtDNA sequences, including *c-mos* sequences from represen-

tatives of all but one genus (*Phaeothlypis*) currently placed within the Parulidae and a number of other nine-primaried oscine taxa (Appendix). Those *c-mos* sequences were generated using primers 944 and 1550 from Cooper and Penny (1997), following the laboratory protocols described in Lovette and Bermingham (2000). All *c-mos* sequences were derived from completely overlapping double-stranded sequencing reactions generated via PCR directly from genomic DNA.

In addition to surveying for presence or absence of the possibly diagnostic indel at sites 1263–1265, we also reconstructed relationships based on *c-mos* nucleotide substitutions. Those searches were rendered computationally difficult by the pattern and magnitude of *c-mos* sequence divergence, particularly the many parulid taxa with *c-mos* sequences that differed by only one or two substitutions. Preliminary analyses showed that this low magnitude of *c-mos* variation resulted in a very large number of alternative, apparently shortest topologies, and computation time constraints precluded techniques that examined each of those alternative trees in turn (e.g. branch swapping in full maximum-parsimony or maximum-likelihood heuristic searches). We therefore conducted two alternative types of phylogenetic analysis of the *c-mos* sequences. First, we conducted an unweighted maximum-parsimony search for 100 random-addition stepwise replicates; each replicate was stopped and the best trees saved when 1×10^6 alternative topologies had been examined. A strict consensus topology was then generated from the many (>70,000) equally shortest trees identified across those 100 replicates. A 100-replicate heuristic bootstrap was performed using 10 addition replicates per bootstrap replicate and a 1×10^5 rearrangement limit per bootstrap replicate. Second, we conducted a Bayesian MCMC search as described above for the mtDNA data sets. In both the maximum-parsimony and MCMC analyses, the three length-variable nucleotide sites were excluded and the reconstructions where rooted using the outgroup taxon *Vireo latimeri*.

RESULTS

*Cytochrome-*b*-based phylogenetic reconstructions.*—Our most taxonomically inclusive analyses included 128 partial cytochrome-*b* sequences of 894 nucleotides. All phylogenetic reconstructions based on those partial cytochrome-*b* sequences were characterized by poor topological resolution: consensus trees contained more polytomies than bifurcating nodes. Bootstrap support for most nodes was correspondingly negligible, except for some nodes that involved pairs of very closely allied taxa. Owing to the large physical size of those 128-taxa trees and their low information content, no cytochrome-*b*-based reconstructions are reproduced here.

The cytochrome-*b* reconstructions nonetheless provided two important types of information regarding the phylogenetic relationships of the Parulidae. First, the strict consensus trees generated under all three weighting schemes contained a clade of 19 parulid genera (*Vermivora*, *Parula*, *Dendroica*, *Catharopeza*, *Mniotilta*, *Setophaga*, *Protonotaria*, *Helmitheros*, *Limnothlypis*, *Seiurus*, *Oporornis*, *Geothlypis*, *Wilsonia*, *Cardellina*, *Ergaticus*, *Myioborus*, *Euthlypis*, *Basileuterus*, and *Phaeothlypis*; collectively referred to hereafter as the ''typical parulids'') that was recovered with much stronger support in analyses based on the larger mtDNA data sets described below. Six genera traditionally assigned to the Parulidae (*Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus*, and *Xenoligea*) always fell outside that typical parulid clade in the cytochrome-*b* consensus trees and in all subsequent analyses. Second, the cytochrome-*b* reconstructions contained many nonparulid taxa that were not represented in the more sequence-intensive data sets discussed below. Because none of those taxa fell within the clade of typical parulids in any cytochrome-*b*-based reconstruction, it is unlikely that any of them have previously unrecognized parulid affinities.

*Combined cytochrome-*b* and ND2-based reconstructions.*—Topological resolution was much better in the reconstructions based on the com-

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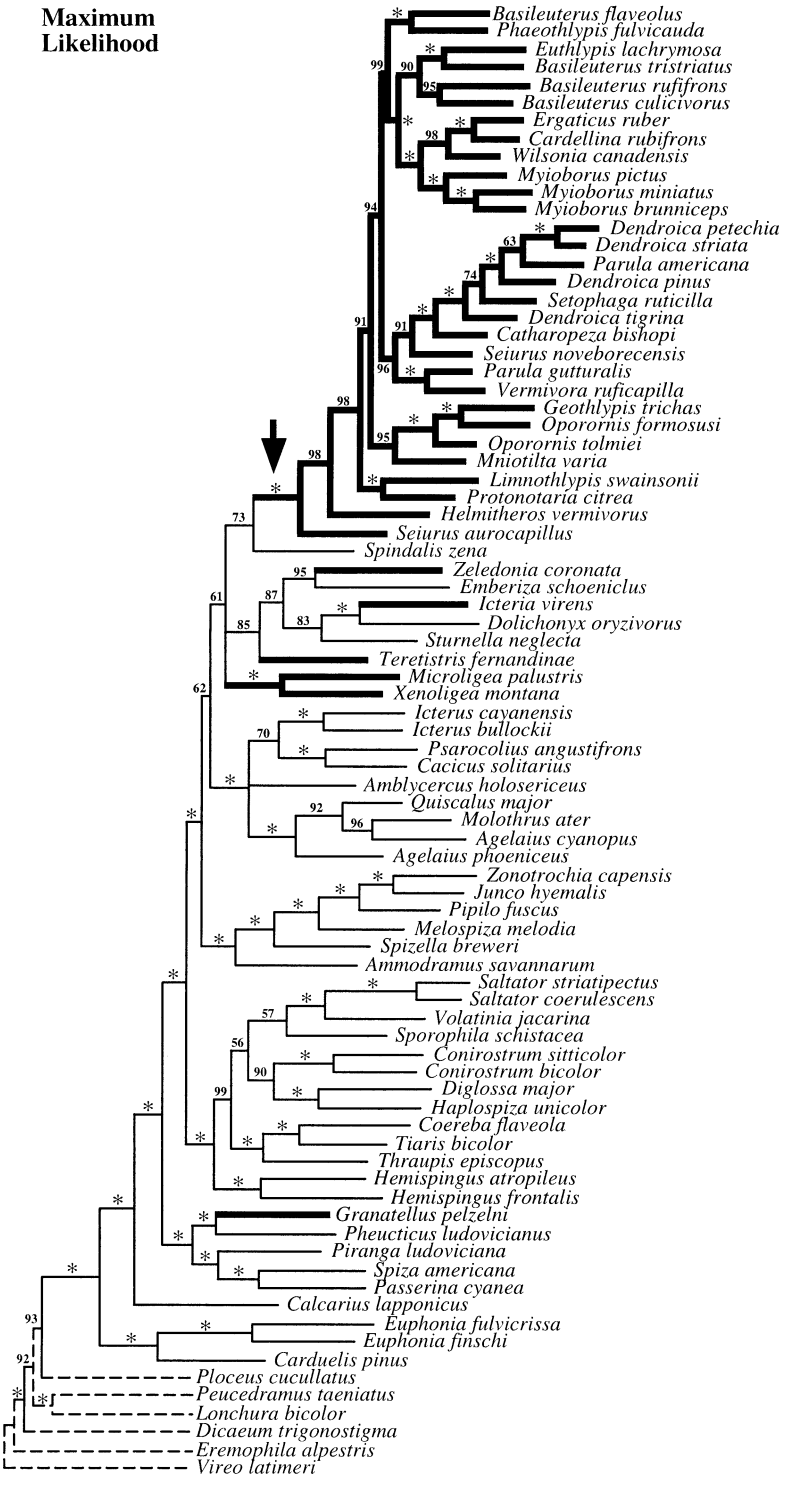
FIG. 1. Phylogenetic relationships among the Parulidae and other nine-primaried oscines based on maximum-likelihood analyses of 1935 nucleotides of mitochondrial cytochrome-*b* and ND2 nucleotide sequence from 82 taxa. Phylogram shown is a 50% majority-rule consensus of topologies sampled during a Bayesian MCMC search in MRBAYES (Huelsenbeck and Ronquist 2001). The lengths of dashed branches involving the non-nine-primaried oscine taxa at the root of the phylogram are not drawn to scale. Thick branches connect lineages currently placed in the Parulidae (AOU 1998), and the large arrow identifies the well-supported internode that defines a clade consisting of 19 genera of morphologically typical parulids. Numbers adjacent



to branches indicate the percentage of sampled trees in which that branch was found and correspond to their posterior probabilities (Huelsenbeck and Ronquist 2001); stars indicate branches found in 100% of sampled trees.

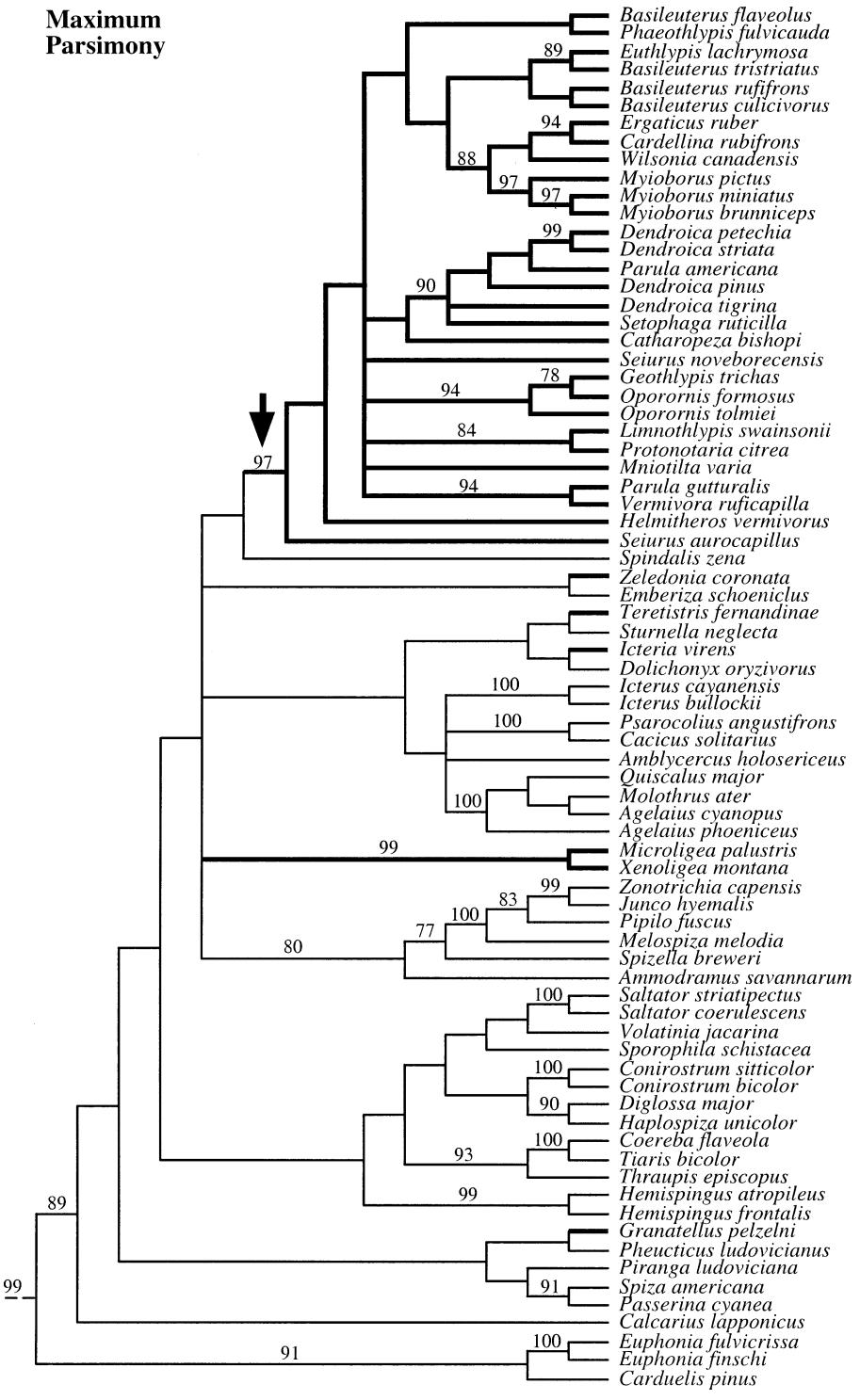


FIG. 2. Phylogenetic relationships among the Parulidae and other nine-primaried oscines based on maximum-parsimony analyses of the sequences used to generate the likelihood phylogram shown in Figure 1. The topology shown above is a strict consensus of the 14 equally shortest maximum-parsimony trees (length

bined cytochrome-*b* and ND2 sequences from 82 taxa (Figs. 1 and 2). Those reconstructions all contained the clade of 18 typical parulids identified in the cytochrome-*b* reconstructions, and the internode at the base of that clade received high support in both the maximum-likelihood (Fig. 1) and maximum-parsimony (Fig. 2) reconstructions; support for that clade was also >90% in maximum-parsimony reconstructions based on the other weighting schemes.

Long mtDNA-based reconstructions.—Figure 3 depicts the maximum-likelihood and maximum-parsimony consensus trees for the most nucleotide-intensive sample of sequences. As in the previous reconstructions, the typical parulid clade received very high support in all reconstructions, and we found a high degree of congruence across trees generated using different reconstruction techniques.

c-mos*-based reconstructions.*—Figure 4 depicts maximum-likelihood and maximum-parsimony consensus trees based on nucleotide substitutions in the *c-mos* proto-oncogene with the three length-variable nucleotide sites discussed below excluded. The magnitude of *c-mos* nucleotide variation among the 26 typical parulid taxa was very low (mean = 2.8 substitutions, range 0–10 substitutions in pairwise comparisons). As in the mtDNA-based reconstructions, a long basal internode separated the typical parulid clade from the other nine-primaried oscines analyzed, including the six traditionally parulid genera (*Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus*, and *Xenoligea*) that fell outside of the typical parulid clade in the mtDNA reconstructions.

The *c-mos* indel at positions 1263–1265 further supports the monophyly of that clade of typical wood-warblers. All 26 taxa sampled from the typical parulid clade identified in the mtDNA trees had a one-codon deletion at those sites, whereas all other passerines sequenced to date (this study, Lovette and Bermingham 1999, I. J. Lovette unpubl. data) have an aspartic acid or glutamic acid codon. Passerine taxa that lack that deletion include the six traditionally parulid genera that fell outside of the typical parulid clade in the mtDNA- and *c-mos* nucleotide-based trees (Figs. 1–4): *Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus*, and *Xenoligea*.

Discussion

Relationships among major nine-primaried oscine lineages.—Of the three data sets we employed, the combined cytochrome-*b* and ND2 analyses (Figs. 1 and 2) provide perhaps the best compromise between nucleotide and taxon sampling for addressing general relationships among nine-primaried oscine lineages. Those intermediate-level analyses add a large number of putatively parulid taxa and a smaller number of other nine-primaried oscine taxa to those studied by Klicka et al. (2000). Except for the placement of some parulid and thraupid taxa that we added, our reconstructions are broadly consistent with those of Klicka et al. (2000), who discussed the systematic implications of their reconstructions in detail.

One of Klicka et al.'s (2000) primary findings was that the six traditionally recognized nine-primaried oscine families (sensu AOU 1998, but variously ranked as tribes or subfamilies in other classifications) are all either para- or polyphyletic. Our enhanced taxonomic sample reinforces that general conclusion about the poor correspondence between mtDNA-based phylogenetic reconstructions and traditional nine-primaried oscine taxonomy. In Figure 2, relationships involving taxa new to our study that do not correspond to traditional family-level classification include the placement of (1) *Zeledonia coronata* as the sister lineage of the *Emberiza schoeniclus*; (2) *Icteria virens* and *Teretistris fernandinae* within a clade of icterids; (3) the *Microligea–Xenoligea* clade as a lineage that roots deeply in the nine-primaried oscine tree; (4) *Granatellus pelzelni* in a clade that contains taxa traditionally placed in the Cardinalidae

←

5558.6 steps) found using a heuristic search with third position transitions downweighted by 0.2 relative to all other substitutions. The topology of non-nine-primaried oscine taxa (not shown) at the dashed root of the tree was identical to the topology shown in Figure 1. Numbers adjacent to branches indicate bootstrap proportions ≥75%. As in Figure 1, thick branches connect lineages currently placed in the Parulidae and the large arrow identifies the well-supported internode that defines a clade consisting of 19 genera of morphologically typical parulids.

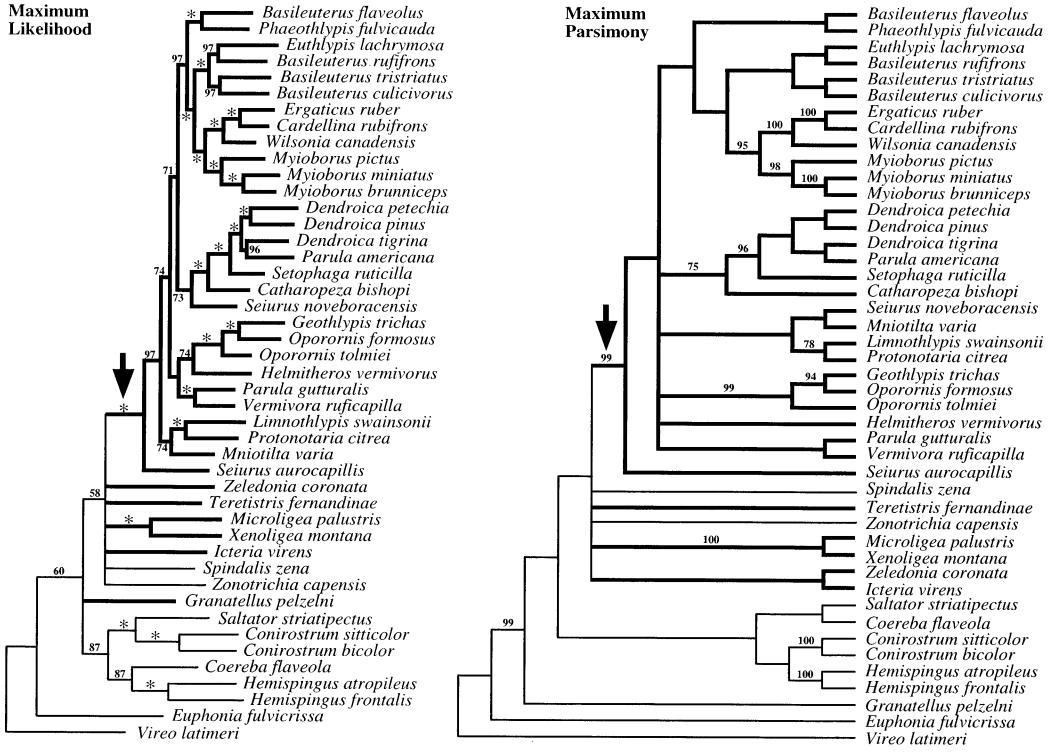


FIG. 3. Phylogenetic relationships among the Parulidae and some other nine-primaried oscines based on analyses of 3,638 nucleotides of mitochondrial protein-coding sequence from 45 taxa. Topology at left is a 50% majority-rule phylogram of topologies sampled during a Bayesian MCMC search. Numbers adjacent to branches indicate the percent of sampled trees in which that branch was found and correspond to their posterior probabilities (Huelsenbeck and Ronquist 2001); stars indicate branches found in 100% of sampled trees. Topology at right is a strict consensus of the two equally shortest maximum-parsimony trees (length 4738.6 steps) found using a heuristic search with third position transitions downweighted by 0.2 relative to all other substitutions. Numbers above branches indicate bootstrap proportions ≥75%. As in previous figures, thick branches in both trees connect lineages currently placed in the Parulidae and the large arrows identify the clade of 19 typical parulid genera.

and Thraupidae; and (5) *Spindalis zena* as the sister to the large clade of typical parulids. Those relationships are present but more weakly supported in the maximum-parsimony reconstruction (Fig. 2), but they nonetheless suggest that those taxa have affinities that are not reflected in traditional classifications.

Nine-primaried oscine taxonomy should ultimately reflect phylogenetic relationships, but these results demonstrate that our understanding of those relationships is at present highly incomplete. A rigorous examination of nine-primaried oscine relationships will probably require very extensive taxonomic sampling, particularly of lineages that are morphologically or behaviorally atypical of the families in which they are placed in traditional classifications.

Characterizing a monophyletic Parulidae.—All our mtDNA- and *c-mos*-based reconstructions contained a clade consisting of 19 wood-warbler genera (Figs. 1–4). Those genera have all long been placed in the Parulidae on the basis of nonphylogenetic assessments of morphological similarity, and we therefore termed that clade the ''typical parulids''. Three independent lines of evidence support the monophyly of those 19 genera with respect to the other nine-primaried oscines. First, the clade appeared in all mtDNA-based analyses, where it received strong support across a broad spectrum of both taxonomic and nucle-

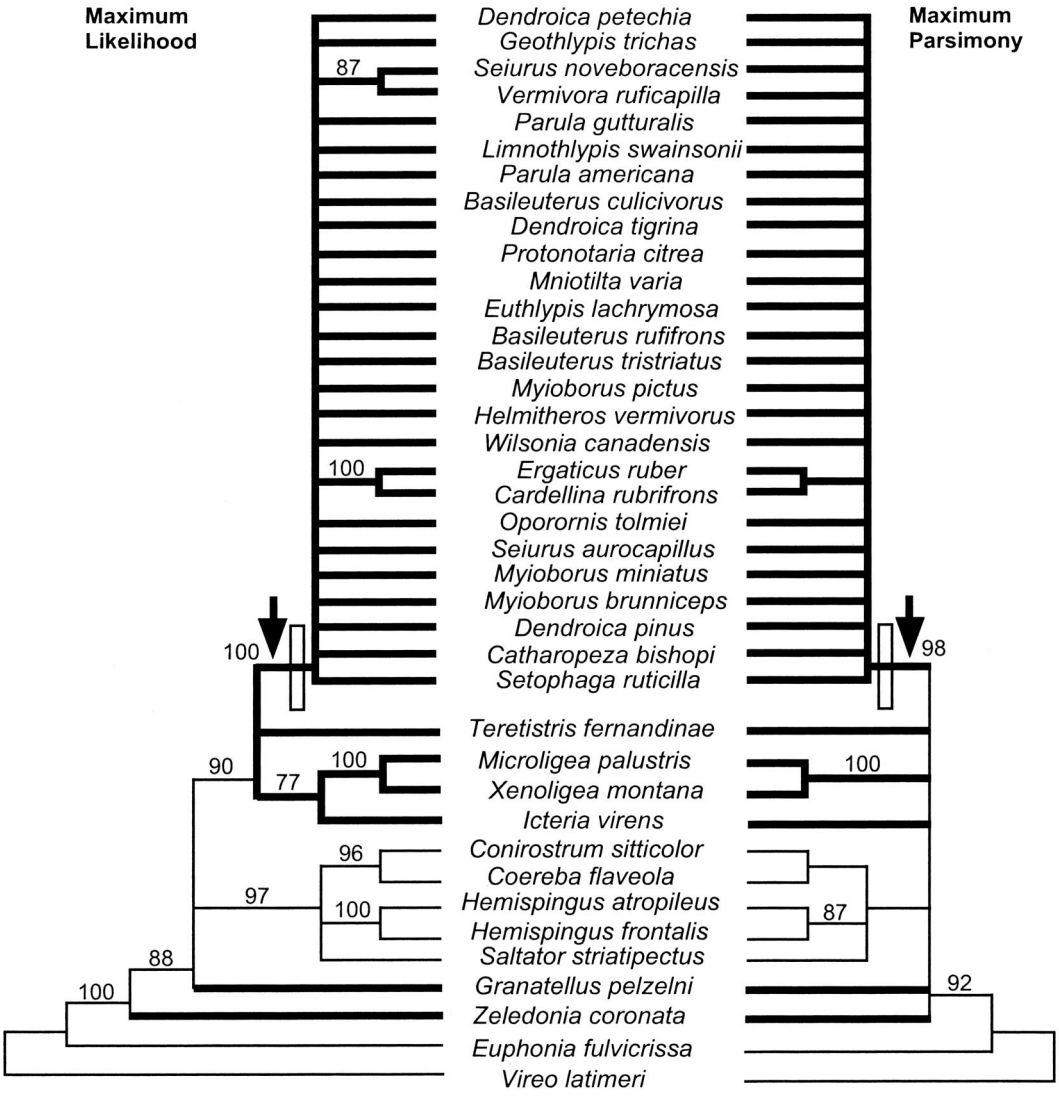


FIG. 4. Phylogenetic relationships among the Parulidae and some other nine-primaried oscines based on analyses of 579 nucleotides of nuclear-encoded *c-mos* proto-oncogene sequence. Topology at left is a 50% majority-rule consensus of trees sampled during a Bayesian MCMC search. Numbers adjacent to branches indicate the percentage of sampled trees in which that branch was found. Topology shown at right is a strict consensus of 70,321 equally shortest trees (tree length = 140 steps) found in an unweighted partial maximum-parsimony analysis. Numbers above maximum-parsimony branches indicate all bootstrap proportions ≥75%. As in previous figures, thick branches indicate lineages currently placed in the Parulidae and the large arrows indicate the base of the clade of 19 typical parulid genera. The vertical bars depict the inferred origin of a one-codon deletion found in all lineages of typical parulids and in no other passerine taxa surveyed, including *Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus*, and *Xenoligea*.

otide sampling. Second, it appeared in reconstructions based on nucleotide substitutions in the independent, nuclear-encoded, and much more slowly evolving *c-mos* gene (Fig. 4). Finally, a one-codon *c-mos* deletion is shared by all 19 typical parulids genera but is not present in other passerine lineages. Deletions in the avian *c-mos* gene are rare (Lovette and Bermingham 1999), and that deletion therefore probably represents a synapo-

morphy with low potential for homoplasy within the passerines.

Although this typical parulid clade was present and strongly supported in all analyses, the identity of its sister lineage remains uncertain. Although the Stripe-headed Tanager (*Spindalis zena*) is the sister lineage of the typical parulids in both reconstructions based on ND2–cyt-*b* sequences (Figs. 1 and 2), *Spindalis* was not resolved as the sister taxon in the more information rich reconstructions shown in Figure 3. The root of the typical parulid clade apparently falls in the deep region of the nine-primaried oscine radiation where support for a particular bifurcating topology is low.

Considered in concert, the mtDNA and nuclear DNA reconstructions provide strong support for the monophyly of the clade composed of *Vermivora*, *Parula*, *Dendroica*, *Catharopeza*, *Mniotilta*, *Setophaga*, *Protonotaria*, *Helmitheros*, *Limnothlypis*, *Seiurus*, *Oporornis*, *Geothlypis*, *Wilsonia*, *Cardellina*, *Ergaticus*, *Myioborus*, *Euthlypis*, *Basileuterus*, and *Phaeothlypis*. Six genera traditionally classified as parulids (*Microligea*, *Teretistris*, *Zeledonia*, *Icteria*, *Granatellus*, and *Xenoligea*) are not members of that assemblage, nor are the most morphologically wood-warbler-like lineages traditionally placed in other families (e.g. *Conirostrum*, *Hemispingus*, and *Coereba*), as discussed below.

Relationships within the typical parulids.—The same set of 29 typical parulid taxa were included in all mtDNA-based analyses. Figure 3 depicts maximum-likelihood and maximum-parsimony reconstructions based on the largest amount of sequence per taxon. Although the maximum-parsimony tree contained many nodes with weak support, the maximum-likelihood topology was highly resolved and many branches were highly supported. The grouping of a number of sets of taxa in that tree is in accordance with the observations of previous workers, including the placement of *Catharopeza*, *Setophaga*, and *Parula americana* close to *Dendroica* (e.g. Kepler and Parkes 1972, Parkes 1961, Ficken and Ficken 1965, Mayr and Short 1970, Lowery and Monroe 1968, Eisenmann 1955, Lovette and Bermingham 2001); the placement of *Phaeothlypis* with *Basileuterus* (Ridgely and Tudor 1989); the affinity of *Oporornis* and *Geothlypis* (Lowery and Monroe 1968, Mayr and Short 1970); and the grouping of *Parula gutturalis* with *Vermivora* (Lovette and Bermingham 2001). The reconstructions further suggest that at least five parulid genera (*Parula*, *Dendroica*, *Oporornis*, *Basileuterus*, and *Seiurus*) are not monophyletic.

Although our analyses included all extant parulid genera, many polytypic genera were sparsely sampled at the species level: the 29 taxa included represent only 28% of the 105 species in the 19 typical parulid genera. Given the high likelihood that some of these additional 76 species have unanticipated generic affinities, we remain cautious about discussing intraparulid relationships in detail until reconstructions that include a much more robust sample of taxa are available.

Systematic implications for aberrant parulid genera.—The molecular phylogenetic reconstructions provide information on the general affinities of a number of morphologically atypical wood-warbler genera that represent long-standing taxonomic enigmas. We first summarize the systematic histories of those genera to provide insight into why they have been considered parulid warblers. We then discuss the implications of their placement in our phylogenetic reconstructions.

Microligea *and* Xenoligea.—These genera have had volatile systematic histories, with uncertainties surrounding both their relationship to one another and their affinities to other nine-primaried oscines. Both monotypic genera are endemic to the island of Hispaniola in the Greater Antilles, where the Green-tailed Ground Warbler (*Microligea palustris*) is broadly distributed from coastal lowlands to montane forests (Wetmore and Swales 1931, McDonald 1987), and the endangered White-winged Warbler (*Xenoligea montana*) is restricted to high elevation forests. *Microligea palustris* was described by Cory (1884), who placed it along with *Geothlypis* in the ''Geothlypeae group'' of the Sylvicolidae. In the initial description of *Xenoligea*, Chapman (1917) commented on its similarities to *M. palustris* and considered the two taxa congeneric. Ridgway (1902) noted similarities between *M. palustris* and the *Geothlypis* yellowthroats, and he inserted *Microligea* between *Geothlypis* and the endemic Caribbean genera *Leucopeza* and *Teretistris* in his taxonomic sequence, a placement followed by most later authorities (e.g. Hellmayr 1935; Bond 1956; Lowery and Monroe 1968; AOU 1983, 1998; Sibley and Monroe 1990). Bond (1956) initially dis-

puted this affinity but later stated that *Geothlypis* and *M. palustris* share similarities in behavior, habitat selection, and morphology (Bond 1971). Bond also observed that *M. palustris* and ''*Microligea*'' *montana* differed greatly in bill shape and plumage, leading him to erect *Xenoligea* for the latter species (Bond 1968). Although Paynter and Storer (1970) and Bond (1971) both noted similarities between *Xenoligea* and the Hispaniolan *Phaenicophilus* palm-tanagers, Bond (1974) later stated that this resemblance was ''superficial and of no taxonomic import'' and ultimately lent his support to a *Xenoligea–Dendroica* relationship (Bond 1974, 1978). Lowery and Monroe (1968) agreed with Bond that *M. palustris* and *X. montana* were highly distinct, possibly to the degree that they belong to different avian families. They noted that whereas *M. palustris* resembles the yellowthroat *Geothlypis*, *X. montana* seemed to them to be allied to the tanagers, and they placed *Xenoligea* as a genus *incertae sedis* near the end of their wood-warbler taxonomic sequence.

More recently, biochemical and morphological studies by McDonald (1987, 1988) have suggested that *Microligea* might also have thraupid affinities. In searching for the sister taxon of the *Phaenicophilus* palm-tanagers, she compared allozyme and morphological characters between *Microligea* and a variety of parulid and thraupid genera. Although McDonald's skeletal morphology-based reconstructions were equivocal, her allozyme trees consistently placed *Microligea* within a thraupid clade as a sister taxon to *Spindalis zena*. *Xenoligea* was not included in her analyses.

Our mtDNA-based reconstructions (Figs. 1–3) all grouped *Microligea* and *Xenoligea* as sister taxa, a pattern mirrored in the *c-mos*-based reconstruction (Fig. 4). Bootstrap support for that relationship was invariably high. The sister relationship of those taxa suggests that Bond's (1968) erection of *Xenoligea* was based at least in part on an erroneous hypothesis of relationship, but the mitochondrial divergence between those taxa (e.g. 7.8–8.2% uncorrected cytochrome-*b* divergence) is similar to that between many genera in the typical parulid clade. The broader affinities of the *Microligea–Xenoligea* lineage are unclear. In the cytochrome-*b* tree (not shown), the *Microligea–Xenoligea* lineage formed a clade (albeit with negligible bootstrap support) with the *Phaenicophilus* palm tanagers, an affinity predicted by Paynter and Storer (1970) on the basis of similarities in plumage coloration. Those three genera may hence represent an autochthonous Hispaniolan radiation. *Phaenicophilus* was not included in the more nucleotide-intensive analyses, in which the *Microligea–Xenoligea* lineage was rooted deep within the nine-primaried oscine radiation (Figs. 1–3).

Teretistris.—The genus *Teretistris* is composed of two species endemic to Cuba, the Yellow-headed Warbler (*T. fernandinae*) and Oriente Warbler (*T. fornsi*). *Teretistris* has been considered a parulid in all modern taxonomic treatments and has usually been placed near *Geothlypis*, *Microligea*, and *Leucopeza* in linear classifications (e.g. Hellmayr 1935, Lowery and Monroe 1968, Sibley and Monroe 1990, AOU 1998). The mtDNA and *c-mos* reconstructions, however, indicate that *T. fernandinae* falls outside the clade of typical wood-warblers and suggest instead that it may be allied to taxa traditionally placed in the Icteridae. That latter relationship was not strongly supported, however, and may be a spurious result of long-branch attraction. *Teretistris fornsi* was not included in our study.

Leucopeza.—In light of the placement of both *Teretistris* and the *Microligea–Xenoligea* lineages outside of the typical parulids, it seems that Caribbean taxa have evolved confusingly convergent wood-warbler-like morphologies at least twice. The nonparulid affinities of *Teretistris*, *Microligea*, and *Xenoligea* raise doubts about the affinities of Semper's Warbler (*Leucopeza semperi*), an additional monotypic West Indian endemic genus. *Leucopeza* is known only from the Lesser Antillean island of St. Lucia; it is probably extinct (AOU 1998, Raffaele 1998) and was not included in our study owing to a lack of modern DNA samples. Like *Teretistris*, *Leucopeza* has been consistently classified as a parulid, but it is placed adjacent to *Teretistris* and *Microligea* in linear classifications, and inspection of museum specimens suggests similarities in gross morphology between it and *Teretistris* (I. J. Lovette pers. obs.; see Curson et al. 1994 and Raffaele 1998 for illustrations of these taxa). Testing thatypothesis is likely to be possible using DNA extracts obtained from museum specimens, most easily by surveying for the presence or absence in *Leucopeza* of the

c-mos deletion that is diagnostic of the typical parulid clade.

Granatellus.—The three species of *Granatellus* chats are distributed among southwestern Mexico, northeastern Central America, and the Amazonian lowlands of South America. *Granatellus* is a morphologically atypical genus that nonetheless has traditionally been considered a parulid (e.g. Ridgway 1902, Hellmayr 1935, Sibley and Monroe 1990). Some recent linear classifications have retained the genus within the parulids while noting that it may not have paruline affinities (Lowery and Monroe 1968, AOU 1998); Ridgely and Tudor (1989) commented that it has a tanager-like bill morphology. Our reconstructions suggest that *Granatellus* is not closely allied to the typical parulid clade nor to any other taxa traditionally placed within the Parulidae (Figs. 1–4) and suggest that it is allied instead to taxa traditionally placed in the Cardinalidae.

Zeledonia.—The Wrenthrush (*Zeledonia coronata*) is endemic to the highlands of western Panama and Costa Rica. Its taxonomic history has been detailed by Sibley (1968) and Sibley and Ahlquist (1990). In brief, *Zeledonia* was placed in its own monotypic family and considered allied to the thrushes (Turdidae; e.g. Ridgway 1907) until comparisons of egg-white proteins and a reconsideration of morphological and behavioral characters suggested that it is a member of the nine-primaried oscine assemblage (Sibley 1968, Hunt 1971). A cladistic analysis of myological characters (Raikow 1978) further suggested that *Zeledonia* is a parulid allied to the speciose Neotropical genus *Basileuterus*. Although *Zeledonia* was not included in their DNA–DNA hybridization study, Sibley and Ahlquist (1990:696) stated that it ''may be regarded as a terrestrial member of [*Basileuterus*].''

The mtDNA and *c-mos* reconstructions indicate that *Zeledonia* is a nine-primaried oscine that is not closely allied to *Basileuterus* or to any other genus within the typical parulid clade. *Zeledonia* surprisingly grouped with Emberiza in the reconstructions in which that taxon appeared (Figs. 1 and 2).

Icteria.—The monotypic Yellow-breasted Chat (*Icteria virens*) is the best known example of a parulid taxon of long-standing systematic debate, as summarized by Sibley and Ahlquist (1982). Although the parulid affinities of *Icteria* have long been considered dubious (Eisenmann 1962, Ficken and Ficken 1962, Mayr and Short 1970, Lowery and Monroe 1968, AOU 1998), it has been retained as a parulid in all recent classifications, and it appeared as the basal lineage in the clade comprised of nine parulids in Sibley and Ahlquist's (1990) DNA–DNA hybridization phylogeny.

The mtDNA and *c-mos* reconstructions demonstrate that *Icteria* is not a member of the typical parulid clade and suggest instead that it may be allied to a group of icterids, particularly *Dolichonyx* (Figs. 1 and 2; see also Klicka et al. 2000).

Other Morphologically Parulid-like Taxa

Given growing evidence that the existing family-level divisions in nine-primaried oscine taxonomy are partially incorrect, we included three taxa—*Coereba*, *Conirostrum*, and *Hemispingus*—in our survey because previous workers have suggested that those taxa might be allied to the Parulidae. As summarized below, none of those taxa fell within the typical parulid clade in our reconstructions.

Coereba.—*Coereba flaveola* has been variously placed in the Coerebidae, Thraupidae, and Parulidae (Ridgway 1902, Lowery and Monroe 1968, Sibley and Monroe 1990), and is placed in the Thraupini (Sibley and Monroe 1990) or retained as the sole taxon in the Coerebidae (AOU 1998) in the most recent taxonomic treatments. Other ''honeycreeper'' taxa previously placed in the Coerebidae along with *Coereba* (e.g. Sclater 1886, Ridgway 1902, Hellmayr 1936) include a number of small-bodied, nectivorous genera that are now placed within the Thraupidae (AOU 1998, Sibley and Monroe 1990). Burns' cytochrome-*b* phylogeny (1997) did not include *Coereba*, but it confirmed previous suspicions (Beecher 1951) that morphological similarities among these other putatively coerebid taxa arose convergently from several independent origins of nectivory. In our reconstructions, *Coereba* fell within a clade of tanagers and tanager-finches, and was grouped as the highly supported sister taxon of the grassquit *Tiaris* in the reconstructions that included both taxa (Figs. 1–2; cytochrome-*b* tree not shown; see also Klicka et al. 2000). In the partial cytochrome-*b* reconstruction (not shown), *Coereba*

was not close to any other nectivorous taxon previously placed in the Coerebidae, and *Coereba* is therefore likely to represent yet another independent evolution of nectivory. Because *Coereba* similarly fell within the Thraupidae in the hybridization-based phylogenies of Bledsoe (1988) and Sibley and Ahlquist (1990), the available phylogenetic evidence is consistent in placing *Coereba* as a derived lineage within a radiation of tanagers and tanager-finches.

Hemispingus.—Although *Hemispingus* is placed within the Thraupidae by all modern authorities, several authors have commented on the general resemblance of *Hemispingus* to the *Basileuterus* wood-warblers (Paynter and Storer 1970, Ridgely and Tudor 1989), and *Hemispingus* was formerly placed within the Parulidae (Ridgway 1902). Despite its wood-warbler-like morphology and behavior, *Hemispingus* is not closely allied to *Basileuterus* or to the other taxa in the typical parulid clade. Instead, the phylogenetic evidence is congruent with current taxonomic practice in placing *Hemispingus* within a clade that includes a number of typical thraupids and tanager-finches (Figs. 1–4).

Conirostrum.—The conebill genus *Conirostrum* is composed of 10 species found in Central and South America that resemble wood-warblers in body size and bill morphology. *Conirostrum* fell within the thraupid clade in the DNA–DNA hybridization study of Sibley and Ahlquist (1990) and recent classifications have placed *Conirostrum* in the Thraupidae (Sibley and Monroe 1990, AOU 1998). On morphological grounds, however, *Conirostrum* has long been considered intermediate between the wood-warblers and tanagers, and earlier authorities tentatively placed it in the Coerebidae (Hellmayr 1935) or the Parulidae (Ridgway 1902, Beecher 1951, Lowery and Monroe 1968). Ridgely and Tudor (1989) revisited earlier taxonomic practice in suggesting that *Conirostrum* is divisible into two distinct and possibly divergent groups, six Andean highland species in the ''true *Conirostrum*'' clade and four lowland species that were formerly separated as the genus *Ateleodacnis*. We sequenced one representative of each of those groups.

In the partial cytochrome-*b* reconstruction (not shown) those *Conirostrum* species grouped in a shallow, well-supported (100% bootstrap) three-taxon clade that also contained the monotypic genus *Oreomanes*, the Giant Conebill (*Oreomanes fraseri*), a taxon that has hybridized with *Conirostrum* in nature (Schulenberg 1985). The pairwise cytochrome-*b* distances among those three taxa were among the smallest we observed, suggesting that those taxa are particularly closely related and that the merger of *Ateleodacnis* and perhaps *Oreomanes* into *Conirostrum* is warranted, a possibility raised by Schulenberg (1985) on the basis of morphological similarities. In a broader systematic context, our reconstructions are consistent with those of Sibley and Ahlquist (1990) in placing the *Conirostrum–Oreomanes* clade within the thraupids rather than within the typical parulids (Figs. 1–4).

Summary.—The phylogenetic evidence presented here provides a somewhat contradictory perspective on the near-term future of nine-primaried oscine systematics. On one hand, the mtDNA and nuclear evidence allowed the identification of a well supported parulid clade that was robust to differences in phylogenetic reconstruction techniques and to variation in nucleotide and taxonomic sampling, and the results of both the mtDNA and nuclear DNA analyses provide the opportunity to define a monophyletic Parulidae based on complementary and congruent molecular phylogenetic criteria. The characterization of that clade is the first step in a more intensive study of systematic relationships within the Parulidae. Future studies that include comprehensive samples of other nine-primaried oscine families may provide equivalent resolution for those groups. On a more pessimistic note, this and other recent molecular studies have emphasized the difficulty of resolving deeper relationships within the nine-primaried oscines. Many nine-primaried oscine lineages seem to have arisen during a temporal window in which mtDNA loci suffer from high levels of homoplasy and nuclear-DNA loci have limited sequence variation. Resolving the deep nine-primaried oscine tree with therefore almost certainly require the generation of extensive molecular information from a very large sample of taxa, as well as advances in the phylogenetic analysis of such large data sets.

Acknowledgments

We thank the contributors to museum-based genetic resource collections who obtained many of the

tissues and associated voucher specimens through their general collecting activities. We express our gratitude to the Louisiana State University Museum of Natural Science, Burke Museum of Natural History, Field Museum of Natural History, and Academy of Natural Sciences, Philadelphia, for the loan of genetic samples, and we thank F. Sheldon, D. Dittmann, F. Gill, R. Ridgely, D. Agro, J. Bates, C. Wood, and S. Rohwer for facilitating those loans. We also thank the national and local authorities of the Bahamas, Barbuda and Antigua, the Dominican Republic, Honduras, Jamaica, Panama, Puerto Rico, and Trinidad and Tobago for granting the research, collecting, and export permits that have facilitated our fieldwork. We benefited greatly from the expert laboratory assistance of J. Hunt, M. Gonzalez, and G. Seutin.

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Associate Editor: F. Sheldon

APPENDIX. Taxa sequenced, sample sources, and GenBank accession numbers.

Species	Museum[a]	Catalog number	Locality	GenBank accession number				
				ATPases	COI	ND2	Cyt-*b*	*c-mos*
Vireo latimeri	STRI	PRVLA10	Puerto Rico: Maricao National Forest	AF279692	AF281025	AF281020	AF383108	AF254886
Vermivora ruficapilla	UWBM	CDS3525	USA: Washingon	AF256489	AF256519	AF256501	AF256510	AF383065
Parula gutturalis	LSUMZ	B26458	Panama: Chiriqui Province	AF256486	AF256516	AF256498	AF256507	AF383053
Parula americana	STRI	JAPAM2	Jamaica: St. Elizabeth Parish	AF256480	AF256514	AF256496	AF256503	AF383043
Dendroica petechia	STRI	BUDPE2	Antigua and Barbuda: Barbuda	AF382957	AF383068	AF383112	AF382996	AF383033
Dendroica tigrina	STRI	JADTI2	Jamaica: Clarendon Parish	AF382963	AF383069	AF383118	AF383002	AF383038
Dendroica pinus	ANSP	2933	USA: New Jersey	AF382988	AF383075	AF383143	AF383027	AF383061
Catharopeza bishopi	STRI	SVCBI1	St. Vincent: Cumberland Valley	AF382985	AF383080	AF383140	AF383024	AF383059
Mniotilta varia	STRI	JAMVA1	Jamaica: St. Andrew Parish	AF382967	AF383082	AF383122	AF383006	AF383042
Setophaga ruticilla	STRI	JASRU2	Jamaica: Westmoreland Parish	AF382969	AF383072	AF383124	AF383008	AF383045
Protonotaria citrea	LSUMZ	B23575	USA: Louisiana	AF382991	AF383104	AF383146	AF383030	AF383064
Helmitheros vermivorus	STRI	JAHVE1	Jamaica: St. Andrew Parish	AF382965	AF383088	AF383120	AF383004	AF383040
Limnothlypis swainsonii	STRI	JALSW2	Jamaica: St. Andrew Parish	AF382966	AF383089	AF383121	AF383005	AF383041
Seiurus aurocapillus	STRI	JASAU1	Jamaica: Westmoreland Parish	AF382968	AF383071	AF383123	AF383007	AF383044
Seiurus noveboracensis	STRI	HASNO150	Honduras: Cochino Pequeno	AF382262	AF383087	AF383117	AF383001	AF383037
Oporornis formosus	STRI	PROFO1	Puerto Rico: Carite National Forest	AF382978	AF383095	AF383133	AF383017	
Oporornis tolmiei	UWBM	CDS4192	USA: Washingon	AF382990	AF383103	AF383145	AF383029	AF383063
Geothlypis trichas	STRI	JAGTR1	Jamaica: St. Andrew Parish	AF382964	AF383070	AF383119	AF383003	AF383039
Microligea palustris	STRI	RDMPA1	Dominican Republic: La Altagracia Province	AF382982	AF383073	AF383137	AF383021	AF383057
Teretistris fernandinae	ANSP	5548	Cuba	AF382960	AF383085	AF383115	AF382999	AF383035
Wilsonia canadensis	ANSP	184471	Panama: Panama Province	AF382977	AF383094	AF383132	AF383016	AF383054
Cardellina rubrifrons	LSUMZ	B10178	USA: Arizona	AF382987	AF383101	AF383142	AF383026	AF383060
Ergaticus ruber	FMNH	BMM169	Mexico: Michoacan	AF382971	AF383090	AF383126	AF383010	AF383047
Myioborus pictus	FMNH	BMM239	Mexico: Oaxaca	AF382972	AF383091	AF383127	AF383011	AF383048
Myioborus miniatus	LSUMZ	B26421	Panama: Chiriqui Province	AF382976	AF383093	AF383131	AF383015	AF383052
Myioborus brunniceps	FMNH	339730	Venezuela: Bolivar	AF382992	AF383105	AF383147	AF383031	AF383066
Euthlypis lachrymosa	FMNH	BMM252	Mexico: Oaxaca	AF382970	AF383089	AF383125	AF383009	AF383046
Basileuterus flaveolus	LSUMZ	B14692	Bolivia: Santa Cruz Department	AF382955	AF383076	AF383110	AF382994	
Basileuterus culicivorus	STRI	TRBCU2	Trinidad and Tobago: St. George County	AF279694	AF281027	AF281022	AF383106	AF254891
Basileuterus rufifrons	ANSP	184482	Panama: Colon Province	AF382973	AF383078	AF383128	AF383012	AF383049
Basileuterus tristriatus	LSUMZ	B28336	Panama: Panama Province	AF382974	AF383079	AF383129	AF383013	AF383050
Phaeothlypis fulvicauda	LSUMZ	B5341	Costa Rica: Puntarenas Province	AF382958	AF383077	AF383113	AF382997	
Zeledonia coronata	LSUMZ	B19939	Costa Rica: San Jose Province	AF382959	AF383084	AF383114	AF382998	AF383034
Icteria virens	UWBM	CDS4131	USA: Washingon	AF382989	AF383102	AF383144	AF383028	AF383062
Granatellus pelzelni	LSUMZ	B18554	Bolivia: Santa Cruz Department	AF382956	AF383083	AF383111	AF382995	AF383032
Xenoligea montana	STRI	RDXMO1	Dominican Republic: Pedernales Province	AF382983	AF383074	AF383138	AF383022	AF383058
Coereba flaveola	STRI	ABCFA2	Bahamas: Abaco Island	AF382954	AF383067	AF383109	AF382993	AF254893
Conirostrum bicolor	STRI	TRCBC1	Trinidad and Tobago: St. George County	AF382986	AF383100	AF383141	AF383025	

APPENDIX. Taxa sequenced, sample sources, and GenBank accession numbers.

Species	Museum[a]	Catalog number	Locality	GenBank accession number				
				ATPases	COI	ND2	Cyt-*b*	*c-mos*
Conirostrum sitticolor	ANSP	185901	Ecuador: Carchi Province	AF382961	AF383086	AF383116	AF383000	AF383036
Hemispingus atropileus	LSUMZ	B1889	Peru: Pasco Department	AF382980	AF383097	AF383135	AF383019	AF383055
Hemispingus frontalis	LSUMZ	B1766	Peru: Pasco Department	AF382981	AF383098	AF383136	AF383020	AF383056
Spindalis zena	STRI	PRSZE3	Puerto Rico: Carite National Forest	AF382979	AF383096	AF383134	AF383018	
Zonotrichia capensis	STRI	RDZCA1	Dominican Republic: La Vega Province	AF382984	AF383099	AF383139	AF383023	
Euphonia fulvicrissa	STRI	PAEFU102	Panama: Panama Province	AF382975	AF383092	AF383130	AF383014	AF383051
Saltator striatipectus	STRI	CCSAL1	Trinidad and Tobago: Chacachacare Island	AF279695	AF281028	AF281023	AF383107	AF254892

[a] STRI = Smithsonian Tropical Research Institute; UWBM = University of Washington Burke Museum; LSUMZ = Louisiana State University Museum of Zoology; ANSP = Academy of Natural Sciences, Philadelphia.