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# PHYLOGENETIC RELATIONSHIPS OF THE WRENTIT BASED ON MITOCHONDRIAL CYTOCHROME *b* SEQUENCES

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*Abstract.* The phylogenetic relationship of the Wrentit (*Chamaea fasciata*) to other passerine birds is understood poorly. A variety of taxa have been proposed as closely related to the Wrentit, but in general this species is placed in a monotypic taxon to emphasize its morphological distinctiveness and lack of clear relationship to other groups of birds. We used the mitochondrial cytochrome *b* gene to infer the relationship of this monotypic genus to other avian groups including representatives of Sylvioidea, Muscicapoidea, and Passeroidea. Results of this study corroborate in part earlier proposals based on DNA-DNA hybridization. Maximum parsimony, maximum likelihood, and Bayesian phylogenetic analyses support the Wrentit, *Sylvia*, and babblers (*Garrulax*, *Illadopsis*, *Leiothrix*, *Pomatorhinus*, *Stachyris*, and *Yuhina*) as a clade, with the Wrentit sharing a more recent common ancestor with *Sylvia* than with babblers. A *Sylvia* and Wrentit association is further supported by similarities in morphology and ecology. In addition to findings on Wrentit relationships, our study in general agrees with the major groups of oscine passerines identified by earlier DNA hybridization studies.

*Key words: babbler,* Chamaea fasciata, *mitochondrial DNA, phylogeny,* Sylvia, *Wrentit.*

## Relaciones Filogenéticas de *Chamaea fasciata* Basadas en Secuencias del Citocromo *b* Mitocondrial

*Resumen.* La relación filogenética de *Chamaea fasciata* con otros paserinos es vagamente entendida. Una variedad de taxa ha sido propuesta como relacionada cercanamente a *C. fasciata*, pero en general esta especie es situada en un taxón monotípico para enfatizar su morfología distintiva y la falta de claridad en la relación con otros grupos de aves. Utilizamos el gen del citocromo *b* mitocondrial para inferir la relación de este género monotípico con otros grupos de aves incluyendo representantes de Sylviodea, Muscicapoidea y Passeroidea. Resultados de este estudio corroboran parcialmente propuestas anteriores basadas en hibridación de ADN-ADN. Análisis filogenéticos de máxima parsimonia, de máxima probabilidad y bayesianos respaldan a *C. fasciata*, *Sylvia*, *Garrulax*, *Illadopsis*, *Leiothrix*, *Pomatorhinus*, *Stachyris* y *Yuhina* como un clado, con *C. fasciata* compartiendo un ancestro común más reciente con *Sylvia*. Una asociación entre *Sylvia* y *C. fasciata* es respaldada por similitudes en morfología y ecología. En adición a los descubrimientos en las relaciones de *C. fasciata*, nuestro estudio coincide en general con los grupos mayores de paserinos oscinos identificados en estudios previos mediante hibridación de ADN.

## INTRODUCTION

The Wrentit (*Chamaea fasciata*) is a small songbird that frequents brushy habitats from northern Baja California to Oregon. This species is unusual among North American birds in that it apparently is not closely related to any other New World species. The evolutionary relationship of the Wrentit to other birds has been debated since Gambel (1845) first described the species. Titmice (Paridae), wrens (Troglodytidae), bushtits (*Psaltriparus*), mockingbirds (Mimidae), babblers (Timaliidae), and Old World warblers (Sylviidae) have all been considered the Wrentit's closest relatives (Grinnell 1913, Sibley 1970, Sibley and Ahlquist 1982, 1990). Reasons for this uncertainty stem from the lack of definable similarities in behavior, ecology, and morphology to other North American species. Because of its lack of clear relationship to other birds, the Wrentit has been assigned to a monotypic taxon at various taxonomic levels (genus, tribe, family).

Most recent classifications consider the Wrentit to be related to Old World warblers and babblers (traditionally defined as the families Sylviidae and Timaliidae, respectively; Wetmore 1960, Howard and Moore 1991). For example,

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Sibley (1970) compared electrophoretic mobility of egg-white proteins and was unable to unequivocally define the sister taxon, but narrowed the possibilities to either Timaliidae or Sylviidae. However, this result is not very enlightening because the limits and monophyly of these two families are uncertain (Sibley and Ahlquist 1982, 1990, Cibois et al. 1999). Babblers are a diverse group that primarily inhabit tropical areas of Asia and Africa. They do not have well-recognized synapomorphies, and genera without other obvious taxonomic affinities have been included among the babblers (Sibley and Monroe 1990, Howard and Moore 1991). Although babbler monophyly has been questioned, Cibois et al. (1999, 2001) demonstrated the monophyly of several babblers including species in the genera *Garrulax*, *Illadopsis*, *Leiothrix*, *Pomatorhinus*, *Stachyris*, and *Yuhina*. Old World warblers (Sylviidae) are another extensive radiation of birds that occupy a variety of niches and are abundant in Europe, Asia, and Africa. Although the group is not monophyletic (Sibley and Ahlquist 1990), recent systematic studies have identified several monophyletic assemblages within Old World warblers. These include the reed warblers (*Acrocephalus*, *Chloropeta*, and *Hippolais*) and some leaf warblers (*Phylloscopus*), based on studies by Helbig and Seibold (1999) and Price et al. (1997, 1998), respectively.

The only recent, comprehensive systematic study of both Timaliidae and Sylviidae that included the Wrentit was the DNA-DNA hybridization study of Sibley and Ahlquist (1982, 1990). The taxonomic classification based on Sibley and Ahlquist (1990) was outlined in Sibley and Monroe (1990). Sibley and Ahlquist (1990) found the families Sylviidae and Timaliidae as traditionally defined (Wetmore 1960, Howard and Moore 1991) to be polyphyletic. Thus, Sibley and Monroe (1990) altered the classification of these groups. Polyphyly of the traditionally recognized Sylviidae and Timaliidae was indicated by the inclusion of the genus *Pomatostomus*, traditionally a babbler, in the parvorder Corvida and close association of the remaining babblers (tribe Timaliini) to *Sylvia* (an Old World warbler genus). Both Old World warblers and babblers are included in Sibley and Monroe's (1990) superfamily Sylvioidea (199 genera, 1195 species). Sylvioidea consists of 11 families, one of which Sibley and Monroe (1990) designated as Sylviidae. However, Old World warblers are found in three families of the Sylvioidea, including Sylviidae. Sibley and Monroe (1990) also placed most traditional babblers into two of the four subfamilies of Sylviidae (Garrulacinae and Sylviinae). One of these subfamilies (Sylviinae) includes the majority of the babblers (tribe Timaliini), the Old World warbler genus *Sylvia* (tribe Sylviini) and the Wrentit (tribe Chamaeini). These results were consistent with Sibley and Ahlquist's (1982) earlier DNA hybridization study in which they concluded that the Wrentit is approximately equidistant from babblers and from *Sylvia*. Thus, DNA hybridization evidence indicates that the Wrentit is closely related to the genus *Sylvia* and to a clade containing most traditional babblers (Timaliini).

Although the classification of Sibley and Monroe (1990) is becoming more widely used, several authors (e.g., Harshman 1994) have criticized some of the methods of analysis used by Sibley and Ahlquist (1990) to derive this taxonomy. Several recent DNA sequencing studies (Groth 1998, Ericson et al. 2000, Lovette and Bermingham 2000, Barker et al. 2001) have directly tested predictions made from these DNA hybridization studies. However, none of these studies have addressed the phylogenetic position of the Wrentit. In this study, we use mitochondrial DNA sequence data to identify the closest living relative of the Wrentit. In addition, we compare our phylogenies of oscine passerine relationships based on DNA sequence data to the DNA-DNA hybridization phylogenies produced by Sibley and Ahlquist (1982, 1990) and more recent DNA sequencing studies.

## METHODS

### TAXON SAMPLING

Because of the taxonomic uncertainty surrounding higher-level oscine passerine relationships, a broad sample of taxa was necessary to investigate phylogenetic relationships of the Wrentit. Forty-four representatives of parvorder Passerida (sensu Sibley and Monroe 1990) were sampled, including representatives of all three Passerida superfamilies (Muscicapoidea, Passeroidea, and Sylvioidea). Fourteen genera of Old World warblers (*Acrocephalus*, *Bradypterus*, *Cettia*, *Chloropeta*, *Cisticola*, *Hippolais*, *Hypergerus*, *Locustella*, *Orthotomus*, *Phylloscopus*, *Regulus*, *Scotocerca*, *Sylvia*, and *Thamnornis*) and seven genera of babblers (*Illadopsis*, *Garrulax*, *Leiothrix*, *Neomixis*, *Pomatorhinus*, *Stachyris* and *Yuhina*) were chosen to represent the Sylvioidea. Seven genera of Muscicapoidea (*Catharus*, *Copsychus*, *Erithacus*, *Ficedula*, *Muscicapa*, *Toxostoma*, and *Turdus*) and six genera of Passeroidea (*Anthus*, *Melospiza*, *Motacilla*, *Passerella*, *Piranga*, and *Vidua*) were sampled as well. Two Wrentit exemplars from opposite ends of its distribution (southern California and Oregon) were included. Because of the lack of conclusive evidence for relationships among Muscicapoidea, Passeroidea, and Sylvioidea, representatives of the parvorder Corvida (*Aphelocoma*, *Corvus*, *Lophorina*, *Pomatostomus*, and *Terpsiphone*) were included to root relationships among these Passerida superfamilies. Multiple exemplars were taken from Corvida, Muscicapoidea, Passeroidea, and Sylvioidea to break up long branches. This exemplar approach is appropriate because previous studies have narrowed the relationships among the groups of interest to Sylvioidea, and the inclusion of other groups (Passeroidea, Muscicapoidea, and Corvida) facilitated the rooting of Sylvioidea and the arrangement of taxa within this group. Previous studies with more inclusive data sets were used as a guideline to choose a pertinent subset of taxa to use in this study (i.e., single exemplars for genera or other higher-level groups). For example, Cibois et al. (1999, 2001) evaluated relationships among some babblers (but did not include the Wrentit), Helbig and Seibold (1999) resolved relationships between reed warbler species (*Acrocephalus*, *Chloropeta* and *Hippolais*), and Price et al. (1997, 1998) studied relationships among leaf warbler species (*Phylloscopus*).

### DNA SEQUENCING

Mitochondrial cytochrome *b* gene sequences were used to infer Wrentit relationships. Cytochrome *b* has been used extensively in other avian phylogenetic studies and has been demonstrated to be a useful phylogenetic marker for resolving generic and family level questions (Avise et al. 1987, Edwards et al. 1991, Moore 1995, Moore and DeFilippis 1997). In total, 51 sequences were used. Some of these sequences are new to this study (Table 1, GenBank accession numbers AY124538–AY124547) and others were obtained from previous studies (see Appendix for these GenBank numbers). DNA was isolated using Chelex® (5% solution, incubated for 20 min at 95°C, Walsh et al. 1991) from fresh liver, heart, or breast muscle stored at −80°C. Cytochrome *b* was amplified using three primer pairs (H15298/L14830, H1570/L15184, and H16108/L15635; Groth 1998) for three overlapping fragments. Initial amplification was performed in 10-μL reactions, in capillary tubes for 40 cycles of 94°C for 3 sec, 43°C for 1 sec, 71°C for 30 sec in a Rapid Cycler (Idaho Technology, Inc., Salt Lake City, Utah). Desired PCR products were verified on 2.3% agarose gels and excised using disposable glass pipets. Reamplification of melted plugs (74°C for 20 min in 250 μL $H_2O$) was also performed in capillary tubes in a Rapid Cycler but in larger reaction volumes for longer denaturing times and at higher temperatures (40-μL reactions, 94°C for 12 sec, 52°C for 4 sec, 71°C for 30 sec). Double-stranded product was purified using a BIO 101 genecleaning kit and subjected to cycle sequencing (ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase; Perkin Elmer, Foster City, California). Cycle sequencing was done in 10-μL reaction volumes with a DNA Engine (MJ Research, Inc., Reno, Nevada) under the following conditions: 96°C for 1 min and then 28 cycles of 96°C for 30 sec, 50°C for 15 sec, and 60°C for 4 min. DNA sequencing was performed on an ABI 377 automated sequencer. Accuracy of DNA sequencing was ensured in two ways: sequencing both heavy and light strands of all PCR fragments and using overlapping fragments of the target sequence (approximately 15% of the total sequence). DNA sequences were edited and linked using Sequencher™ 3.0 (Gene Codes Corp., Ann Arbor, Michigan). Completed cytochrome *b* sequences were aligned and translated using Se-al v1.0a1 (Rambaut 1995).

### PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed under maximum parsimony and maximum likelihood as implemented in PAUP* (version 4.0b8; Swofford 2001). Maximum parsimony (MP) analysis was performed under multiple models of evolution through the use of the following weighting schemes: (1) equal or uniform weighting, (2) transition/transversion weighting, (3) codon position partitioning, and (4) six-parameter (nucleotide) step matrix. The four different weighting schemes were used to test sensitivity of parsi-

TABLE 1. Species names, voucher numbers, GenBank numbers, and locality information of sequences not previously reported. MVZ = Museum of Vertebrate Zoology at the University of California at Berkeley, FMNH = Field Museum of Natural History, SDSU = San Diego State University Vertebrate Collections.

| Species | Voucher number | GenBank number | Locality |
|---|---|---|---|
| *Bradypterus cinnamomeus* | FMNH 355750 | AY124541 | Uganda: Kasese, Nyabitaba, 10 km NW Ibanda, Mubuku Valley, Rwenzori Mts. |
| *Chamaea fasciata* | SDSU 2189 | AY124547 | USA: California, San Diego Co., 4 miles west Corte Madera Mt., 32°45.2′N, 116°39.7′W |
| *Chamaea fasciata* | SDSU 2225 | AY123546 | USA: Oregon, Lincoln Co., 6 miles southeast of Tidewater 44°21.5′N, 123°48.2′W |
| *Illadopsis pyrrhopterum* | FMNH 355676 | AY124539 | Uganda: Kasese, Nyabitaba, 10 km NW Ibanda, Mubuku Valley, Rwenzori Mts. |
| *Passerella iliaca* | MVZ 170456 | AY124544 | USA: California, Lake Co., Hull Mt. 4 miles S and 1 mile E |
| *Piranga ludoviciana* | SDSU 2212 | AY124545 | USA: California, Fresno Co., 4 miles west of Hume Lake, 36°47.8′N, 118°59.2′W |
| *Stachyris whiteheadi* | FMNH 433007 | AY124542 | Philippine Islands: Luzon, Camarines Sur, Mt Isarog |
| *Sylvia borin* | FMNH 385182 | AY124540 | Uganda: Kabale, Byumba |
| *Terpsiphone cinnamomea* | FMNH 358456 | AY124538 | Philippine Islands: Sibuyan, Mt. Guitinguitin, NW slope, 4.5 km S, 4 km E Magdiwang |
| *Toxostoma redivivum* | SDSU 2281 | AY124543 | USA: California, San Diego Co., 4 miles west Corte Madera Mt., 32°45.2′N, 116°39.7′W |

mony results to model and model parameters. Equal weighting and codon position partitioning involved weighting characters. In equal weighting, all characters were assigned a weight of one. Codon position partitioning involved assigning first and second positions a weight of five and third positions a weight of one. Transition/transversion weighting and six-parameter weighting involved weighting character-state transformations through use of a step matrix. For transition/transversion weighting, we assigned transversions five times the weight of transitions. Character transformation weights for the six-parameter step-matrix substitution model were estimated on a parsimony tree in MacClade 3.07 (Maddison and Maddison 1997) based on equal weights. Thus, for the six-parameter analysis, the following weights were used: transformations between A and C = 3.3, between A and G = 1.0, between A and T = 4.3, between C and G = 4.3, between C and T = 1.0, and between G and T = 5.3. All MP analyses were implemented under multiple, independent heuristic searches (100 random taxa-addition replicates with tree-bisection-reconnection (TBR) branch swapping). All parsimony analyses were bootstrapped for 100 replicates, with 10 random-addition replicates for each bootstrap replicate.

The second optimality criterion implemented for phylogenetic analysis was maximum likelihood (ML). Simulation studies have shown ML to be a consistent estimator of phylogeny that is robust with respect to small changes of model and model parameter estimates (Huelsenbeck 1995, Yang 1996). Maximum likelihood takes into account important attributes of molecular evolution (i.e., unequal base frequency and among-site rate variation) difficult to incorporate into parsimony analysis. Selection of a model of sequence evolution for maximum-likelihood analysis was explored in a hierarchical likelihood framework using ModelTest v3.0 (Posada and Crandall 1998). Fifty-six nested models of DNA sequence evolution were evaluated, using the likelihood-ratio test statistic with Bonferroni correction, to find the simplest model that yields a significantly higher likelihood. Initial topologies for estimating rate parameters and evaluating substitution models for ModelTest v3.0 were generated from three trees. The equal-weight parsimony tree, the six-parameter parsimony tree (with codon partitioning) and a neighbor-joining tree (the default setting for ModelTest) were used to test sensitivity of model selection to starting tree conditions. Using a successive approach to obtain the optimal ML phylogeny (Wilgenbusch and de Queiroz 2000), subsequent trees generated from the three initial ML searches were used to re-estimate models and parameters (in ModelTest) and then used in further ML searches until identical topologies were generated in successive searches.

In addition to parsimony and ML, Bayesian inference of phylogeny (as implemented in MrBayes 2.01, Huelsenbeck and Ronquist 2001) was also used to evaluate relationships. The Bayesian approach generates a pool of trees or a posterior probability distribution. The distribution of sample points after which trees converge on a likelihood score was used to produce a majority-rule consensus tree. Nodal support was inferred using a clade's posterior probability (the percentage of samples that contain that particular clade). Multiple independent Bayesian analyses were conducted and compared to ensure analyses were not entrapped on local optima. ModelTest identified TVM + I + $\Gamma$ as the preferred model for ML analysis, but MrBayes cannot implement this model. Instead the GTR + I + $\Gamma$ (nst = 6 rates = invargamma) was incorporated into the Bayesian analysis. GTR + I + $\Gamma$ varies slightly from TVM + I + $\Gamma$, in that all nucleotide substitutions are unequal and independent of each other in the GTR + I + $\Gamma$ model (transition substitutions are uniform with TVM + I + $\Gamma$). All Bayesian analyses were run for 250 000 generations and sampled every 100 generations. Thus, each analysis resulted in 2500 samples. Four Markov Chain Monte Carlo chains (being incrementally heated) were run for each analysis and parameters were not specified and thus were estimated as part of the analysis.

Support for phylogenetic relationships was assessed using nonparametric bootstrapping of MP analyses and using Bayesian measures of nodal support (posterior probability values). Based on simulation studies and studies of known phylogenies, a bootstrap value of 70% or greater is generally regarded as strongly supported given phylogenetic model assumptions (Hillis and Bull 1993). In Bayesian analyses, posterior probabilities are true probabilities of clades with values of 95% or greater deemed significantly supported.

## RESULTS

### SEQUENCE VARIATION

All sequences aligned without gaps or insertions, as expected for a protein-coding gene. Of the 1143 total aligned nucleotide positions, 555 (49%) characters were constant, 100 (9%) were variable but uninformative, and 488 (43%) were parsimony informative. As expected, most variation occurred at the third codon position. Of the 588 variable characters, 369 occurred at third positions, 64 at second positions, and 155 at first codon positions. Uncorrected sequence divergence (*p*-distance) between genus-level exemplars ranged from 12% (*Chloropeta–Hippolais*) to 20% (*Anthus–Aphelocoma*). Intrageneric variation ranged from 0.4% (*Chamaea*) to 14% (*Sylvia*).

### PHYLOGENETIC ANALYSIS

In the ModelTest analyses, all three starting topologies (uniformly weighted parsimony tree, six-parameter step matrix parsimony tree, and a neighbor-joining tree) yielded TVM + I + $\Gamma$ as the preferred model. Each of these three trees (and their parameters estimated from the ModelTest analyses) were used as starting trees in separate heuristic ML searches using a successive approach. The results of the three separate ML analyses were the same. Regardless of starting tree used, all converged on the same optimal ML tree (Fig. 1, $-\ln$ likelihood = 17 276.8; $r_{AC} = 0.07$, $r_{AG} = 2.6$, $r_{AT} = 0.32$, $r_{CG} = 0.20$, $r_{CT} = 2.6$, $r_{GT} = 1.00$, %A = 38, %C = 43, %G = 5, %T = 14, proportion invariable sites = 0.37). In this tree, the Wrentit was the sister taxon of a clade including three species of *Sylvia* warblers, and the Wrentit and *Sylvia* clade was sister to a clade containing six babblers (*Yuhina*, *Stachyris*, *Garrulax*, *Leiothrix*, *Pomatorhinus*, and *Illadopsis*). The ML tree showed broad agreement with Sibley and Monroe's (1990) proposed classification of passerine birds. The parvorder Corvida was monophyletic as were the superfamilies Muscicapoidea and Passeroidea (but see Sheldon and Gill 1996, Groth 1998, and Barker et al. 2001). With the exception of *Regulus*, the superfamily Sylvioidea was also monophyletic. Within Sylvioidea, the reed warbler group (*Acrocephalus*, *Chloropeta*, and

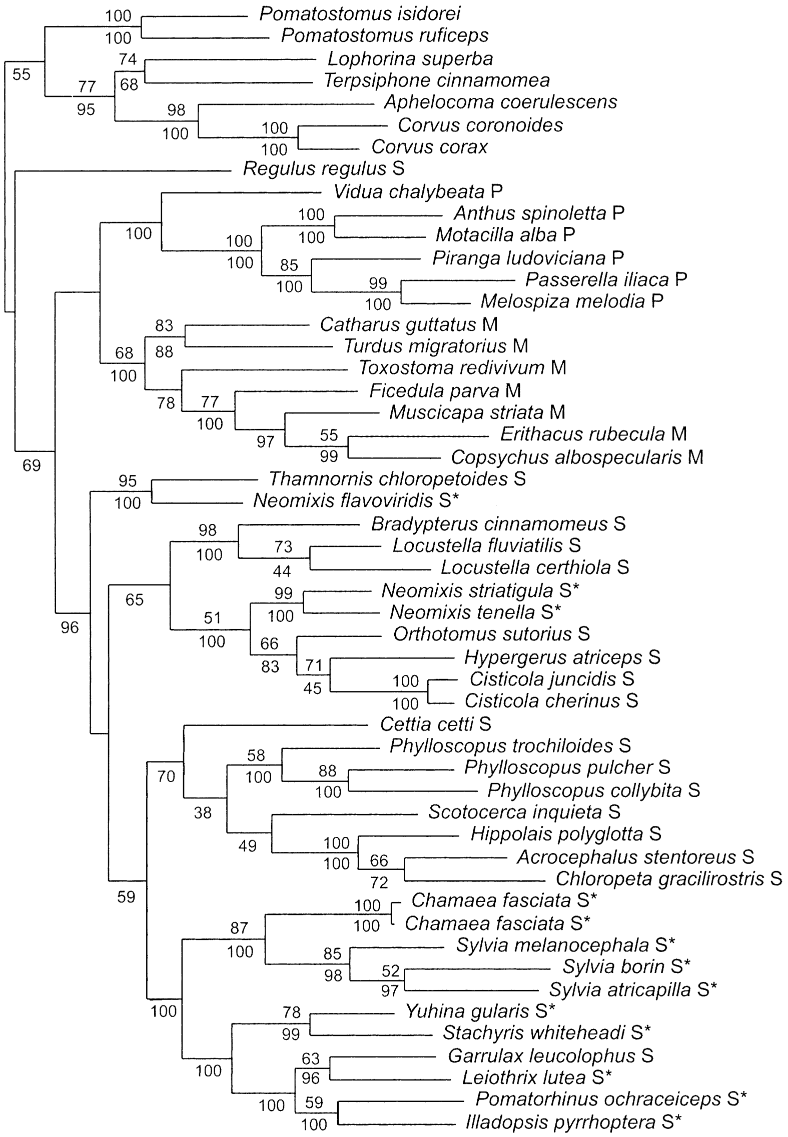

FIGURE 1. Phylogenetic relationships of the Wrentit (*Chamaea fasciata*) as identified by maximum likelihood. Branch lengths are proportional. Numbers above nodes indicate bootstrap support for the transition/transversion analysis. Nodes without values above the node were not supported in greater than 50% of bootstrap replicates. Numbers below nodes indicate Bayesian posterior probability values. Nodes without values below the node represent parts of the tree that conflict between the Bayesian and maximum-likelihood trees. For members of the parvorder Passerida, the letter following taxon name indicates the superfamily according to Sibley and Monroe's (1990) taxonomy (S = Sylvioidea, M = Muscicapoidea, P = Passeroidea). Members of Sibley and Monroe's subfamily Sylviinae are indicated with an asterisk.

*Hippolais*) and three species of leaf warblers (*Phylloscopus*) each formed a clade. The ML analysis supports the placement of *Regulus* outside of all Passerida superfamilies (Muscicapoidea, Passeroidea and Sylvioidea) in agreement with Sheldon and Gill (1996) and Barker et al. (2001). ML analysis of the mtDNA also showed Muscicapoidea and Passeroidea as being sister taxa.

Five separate Bayesian analyses were conducted and all five converged onto similar, stable log-likelihood scores well before 25 000 generations. Thus, we eliminated the first 250 samples for all analyses and constructed majority-rule consensus trees using the remaining 2250 samples. All five independent Bayesian analyses generated identical trees. This tree was broadly congruent with the ML tree. Posterior probabilities for the five Bayesian analyses varied only slightly, and values of the first analysis were mapped onto the ML tree (Fig. 1). All clades with posterior probabilities of 95 or greater are considered to be significantly supported. Support for the sister relationship of the Wrentit to a clade including three species of *Sylvia* warblers was high (100%) as was support for the sister relationship of these two taxa to a clade including six species of babblers (100%). In addition, support was high for the monophyly of Passeroidea and Muscicapoidea, as represented in this study. Two nodes differed between the ML tree and the Bayesian tree. The Bayesian tree differed from the ML tree in the relationships among the three Passerida superfamilies. The Bayesian tree identified the Sylvioidea as the sister taxon of the Passeroidea (posterior probability of 48%), while the ML analyses identified the Muscicapoidea as the sister taxon of the Passeroidea. In the ML tree, *Thamnornis chloropetoides* and *Neomixis flavoviridis* are sister to the rest of the Sylvioidea (Fig. 1), while in the Bayesian tree, these two taxa are the sister group to a subset of the Sylvioidea (posterior probability = 47%).

Like the Bayesian analyses, the parsimony analyses agreed with the ML tree in the placement of several taxa (bootstrap values of transition/transversion weighted tree shown, Fig. 1). Depending on the weighting scheme used, the parsimony analyses varied in degree of resolution and number of bootstrap-supported nodes. Number of trees found, consistency indices excluding uninformative characters, and number of bootstrap nodes above 50% for each of the four analyses were as follows: equal weighting (6 trees, 0.23, 22 nodes), transition/transversion weighting (2 trees, 0.20, 32 nodes), codon position weighting (1 tree, 0.25, 23 nodes), six-parameter weighting (3 trees, 0.21, 33 nodes). In general, the weighting schemes that attempted to account for saturation showed more resolution and higher bootstrap values than the equally weighted tree. This is not surprising, given the distant relationships of many of the taxa included in this study.

Importantly, regardless of weighting scheme used, all parsimony trees showed the Wrentit to be the sister taxon of the clade containing three species of *Sylvia*. Bootstrap support for this relationship ranged from 54% (codon position weighting) to 87% (both six-parameter and transition/transversion weighting). The reed warbler (*Acrocephalus*, *Hippolais*, *Chloropeta*) and leaf warbler (*Phylloscopus*) groups also were monophyletic in all the most-parsimonious trees. However, in general, parsimony trees showed less agreement with previous hypotheses of relationships than did the ML and Bayesian trees. In addition, fewer than half of the nodes in the most-parsimonious trees had greater than 70% bootstrap support, and most well-supported nodes joined pairs or trios of terminal taxa. The species representing the Corvida and Muscicapoidea were not monophyletic in the equally weighted tree because the genus *Pomatostomus* fell within the Muscicapoidea. In all parsimony analyses, the Passeroidea was not monophyletic, because *Vidua* always fell outside this otherwise monophyletic group. The Sylvioidea also was not monophyletic in the parsimony analyses, because passeroid taxa were nested within this group.

As found in other studies (e.g., Leaché and Reeder 2002), posterior probability values of the Bayesian analyses were generally higher than the bootstrap parsimony values. In addition, posterior probabilities and bootstrap values were only somewhat correlated. Using the bootstrap values of the transition/transversion tree (Fig. 1), 19 nodes had a posterior probability greater than 95% and a bootstrap value greater than 70%. An additional 13 nodes had greater than 95% posterior probability, but weaker than 70% bootstrap support. Only four nodes showed strong bootstrap support (greater than 70%), but a posterior probability less than 95%.

## DISCUSSION

### COMPARISON TO PREVIOUS SYSTEMATIC STUDIES

Previous phylogenetic studies supported monophyly of the following groups: (1) *Aphelocoma*, *Corvus*, *Lophorina*, *Pomatostomus*, and *Terpsiphone* (Corvida; Sibley and Monroe 1990, but see Barker et al. 2001); (2) *Phylloscopus* (Price et al. 1997, 1998); (3) *Acrocephalus*, *Chloropeta*, and *Hippolais* (Sibley and Monroe 1990, Helbig and Seibold 1999); (4) *Anthus*, *Vidua*, *Melospi-*

*za*, *Motacilla*, *Passerella*, and *Piranga* (Passeroidea; Sibley and Monroe 1990); (5) *Catharus*, *Copsychus, Erithacus, Ficedula, Muscicapa, Toxostoma*, and *Turdus* (Muscicapoidea; Sibley and Monroe 1990); and (6) *Thamnornis*, *Regulus*, *Neomixis*, *Bradypterus*, *Locustella*, *Orthotomus*, *Hypergerus*, *Cisticola*, *Cettia*, *Phylloscopus*, *Scotocerca*, *Hippolais*, *Acrocephalus*, *Chloropeta*, *Chamaea*, *Sylvia*, *Yuhina*, *Stachyris*, *Garrulax*, *Leiothrix*, *Pomatorhinus*, and *Illadopsis* (Sylvioidea; Sibley and Monroe 1990). Our parsimony analysis generated slightly varying topologies and all deviated from these groupings to some extent (all demonstrated Corvida, Muscicapoidea, Passeroidea, or Sylvioidea paraphyly). Both the ML tree and the Bayesian trees showed much broader agreement with the clades described above. The first five clades were found in the ML and Bayesian trees, and Sylvioidea was monophyletic with the exception of *Regulus* (see below). More extensive sampling is necessary (see Barker et al. 2001) to fully address the monophyly of most of these groups.

In addition to the similarities with Sibley and Monroe (1990) outlined above, our study also identified differences. In agreement with Sheldon and Gill (1996) and Barker et al. (2001), the ML tree and the Bayesian tree placed *Regulus* outside of Sibley and Monroe's (1990) Sylvioidea. ML phylogenetic hypotheses show that *Garrulax* is closely related to babblers assigned to Timaliini with strong Bayesian support (100% posterior probability). This conclusion is concordant with results from Cibois et al. (1999, 2001), but contrasts with Sibley and Monroe's (1990) classification. Also in contrast to Sibley and Monroe (1990), in none of the phylogenetic analyses was *Neomixis* monophyletic nor did it form a close association to other Timaliini. Cibois et al. (1999, 2001) also demonstrated *Neomixis* paraphyly with regards to intrageneric babbler relationships. Inferred relationships between and within families of Sylvioidea (except for the distinction of the subfamily Sylviinae, i.e., Wrentit, *Sylvia*, and Timaliini) were supported weakly. For example, the families Cisticolidae (*Cisticola*, *Hypergerus*, and *Scotocerca*) and Sylviidae (*Thamnornis*, *Neomixis*, *Bradypterus*, *Locustella*, *Orthotomus*, *Cettia*, *Phylloscopus*, *Hippolais*, *Acrocephalus*, *Chloropeta*, *Chamaea*, *Sylvia*, *Yuhina*, *Stachyris*, *Garrulax*, *Leiothrix*, *Pomatorhinus*, *Illadopsis*) were paraphyletic. More genera, including representatives from all Sylvioidea families, are needed to provide conclusive evidence for the paraphyly of these families as well as other relationships within Sylvioidea.

## WRENTIT RELATIONSHIPS AND BIOGEOGRAPHIC HISTORY

*Chamaea* (Wrentits) and a clade of three species of *Sylvia* share a more recent common ancestor with each other than with any other taxa in the data set. All optimal trees from ML, MP, and Bayesian analyses included this pairing, and Bayesian analyses provided significant levels of support (100% posterior probability) for this pairing. In addition, Wrentits, *Sylvia*, and babblers (*Pomatorhinus*, *Garrulax*, *Leiothrix*, *Stachyris*, *Illadopsis*, and *Yuhina*) formed a clade in all optimal trees. Thus the results of our study, in part, corroborate the earlier hypotheses of Sibley and Ahlquist (1982, 1990). Sibley and Ahlquist (1990) considered the Wrentit, the genus *Sylvia*, and a large group of traditional babblers to form a monophyletic group. However, Sibley and Ahlquist (1982, 1990) did not explicitly recognize a close relationship between *Sylvia* and the Wrentit to the exclusion of babblers. UPGMA trees and $\Delta T$ values indicated that the Wrentit was actually closer to *Sylvia* than to Timaliini (Sibley and Ahlquist 1990). However, Sibley and Monroe's (1990) classification did not indicate the close relationship between *Sylvia* and the Wrentit in relation to babblers, probably because the branch connecting *Sylvia* and the Wrentit was short and within the realm of error. Sibley and Monroe (1990) ranked all three groups at an equivalent taxonomic level, and together they compose the three tribes within Sylviinae. Our results indicate that recognizing a monophyletic group that consists of *Sylvia* and the Wrentit to the exclusion of other babblers would be appropriate, and that the branching diagrams illustrated in Sibley and Ahlquist (1990) do reflect the relationships of these taxa.

Overall similarity based on morphology and ecology lends additional support to an association between the Wrentit and *Sylvia* (as first noted by Sibley 1970). The Wrentit and some members of *Sylvia* are similar in plumage, iris coloration, and overall body size and shape. *Sylvia undata* (Dartford Warbler) has dark brown to brownish-gray upperparts with lighter streaked underparts (similar to Wrentits in the northernmost end of their distribution), while *Sylvia sarda* (Marmora's Warbler) has bluish-gray upperparts with a lighter, mottled underbelly (similar to Wrentits found in the southern end of their distribution). All three species have small rounded wings with long slim tails, which they hold cocked. Juveniles all have lighter, creamy orange or brown, speckled irises (only outer iris for the Wrentit) that become brighter with age. All *Sylvia* species are primarily insectivorous and partially frugivorous, build open cup-shaped nests in dense vegetation, and reach their highest density in the Mediterranean region. These characteristics are similar for the Wrentit except it reaches highest species density in California (with a similar, Mediterranean climate). Although these characteristics are morphological and ecological generalities, they do agree with the hypotheses generated in this study.

The phylogeny presented in this study provides a framework for addressing the biogeographic history of the Wrentit. Current distributions are allopatric (Wrentit: western North America, *Sylvia*: primarily Europe, the Middle East, and Africa). Both groups are more closely related to babblers of Timaliini than to any other Sylvioidea member. Babblers are widespread, but reach their greatest diversity and density in Asia and have been proposed to have Asian ancestry. One possible biogeographic scenario is that the Wrentit and *Sylvia* ancestor also originated in Asia. As each group dispersed, they colonized new yet similar climates and habitats. Wrentit ancestors dispersed east to western North American and the chaparral habitat of California, and *Sylvia* ancestors dispersed west to Europe and the maquis habitat of the Mediterranean.

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## APPENDIX

GenBank accession numbers for sequences that come from other studies. GenBank numbers for sequences unique to this study are reported in Table 1.

AF094618, AF094622, AF094625, AF094627, AF094628, AF094630, AF094631, AF094636, AF094637, AF094640, AF094642, AF135052, AF139826, AF197835, AF197837, AF276776, AF290159, AJ004318, AJ004762, AJ004764, AJ004765, AJ004789, AJ004797, AJ004798, AJ299689, AJ299690, NC_000880, U25733, U46773, U46777, U77335, U86031, X60937, X60938, X74261, Y08058, Y10732, Y10739, Z73474, Z73487, Z73494.