# AN ENDEMIC RADIATION OF MALAGASY SONGBIRDS IS REVEALED BY MITOCHONDRIAL DNA SEQUENCE DATA

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*Abstract.*—The bird fauna of Madagascar includes a high proportion of endemic species, particularly among passerine birds (Aves: Passeriformes). The endemic genera of Malagasy songbirds are not allied obviously with any African or Asiatic taxa, and their affinities have been debated since the birds first were described. We used mitochondrial sequence data to estimate the relationships of 13 species of endemic Malagasy songbirds, 17 additional songbird species, and one species of suboscine passerine. In our optimal trees, nine of the 13 Malagasy species form a clade, although these birds currently are classified in three different families. In all optimal trees, the sister to this endemic clade is a group of Old World warblers including both African and Malagasy birds. The endemic Malagasy songbird clade rivals other island radiations, including the vangas of Madagascar and the finches of the Galapagos, in ecological diversity.

*Key words.*—Adaptive radiation, cytochrome *b,* Madagascar, molecular phylogeny, Pycnonotidae, Sylviidae, Timaliidae.

Adaptive radiation has been an important concept in evolutionary biology since the time of Darwin, because it is a manifestation of natural selection—often, a spectacular manifestation. Adaptive radiation can be defined as a rapid diversification of species from a common ancestor accompanied by phenotypic divergence and specialization to exploit newly available resources (Schluter 2000). Perhaps the best-known examples of adaptive radiation involve insular endemics, including the finches of the Galapagos and the honeycreepers of the Hawaiian islands. Molecular systematics has provided a boon to the study of adaptive radiation. Molecular data allow the inference of phylogenetic relationships without reliance on phenotypic characters, so the evolution of the phenotypic characters can be studied using an independently derived phylogeny (Givnish 1997).

The island of Madagascar is renowned as a hotspot for adaptive radiations. Madagascar has been separated from mainland Africa since the end of the Jurassic, about 150 million years ago (Hallam 1994), and this long isolation is reflected in the high percentage of endemics in its fauna. Among birds, 52% of the breeding birds are endemic; among passerines (Aves: Passeriformes), the fraction is an impressive 80% (Moreau 1966). The isolation of Madagascar predates the origin of passerines (Benton 1990), so the Malagasy endemics presumably are descended from colonists that reached the island by long-distance dispersal. Most endemic Malagasy birds are thought to be derived from African lineages, a reasonable assumption given the proximity of the

African continent (Moreau 1966). The Mozambique Channel separating Madagascar from Africa is wide ($> 400$ km), but dispersal might have been facilitated during periods of low sea level by exposed seamounts that could serve as stepping stones. Geological evidence suggests that three seamounts in the Mozambique Channel probably were exposed for some period during the Miocene (Leclaire et al. 1989).

In this study, we use mitochondrial sequence data to estimate the relationships of several species of endemic Malagasy songbirds, both to each other and to possibly closely related species from Africa and Asia. The term ''songbird'' refers to the oscine passerines, one of two distinct clades within the order Passeriformes: oscines and suboscines. The oscine passerines are characterized by possessing a complex vocal organ, the syrinx, whereas the suboscines have a less complex vocal organ. The monophyly of these two groups is supported by several morphological and molecular analyses (Sibley and Ahlquist 1990; Edwards et al. 1991; Raikow and Bledsoe 2000). Our study includes Malagasy songbirds in the genera *Phyllastrephus, Neomixis, Hartertula, Oxylabes, Mystacornis,* and *Thamnornis.* These species have been considered by some authors to constitute a single radiation (Salomonsen 1934; Irwin 1983; Olson 1989), but the birds are currently classified in three different families (bulbuls, Pycnonotidae; babblers, Timaliidae; warblers, Sylviidae; Morony et al. 1975). Our study also includes *Cryptosylvicola randrianasoloi,* a recently described species from the eastern rainforests of Madagascar, placed in the family Sylviidae (Goodman et al. 1996).

Two recent studies based on mitochondrial sequence data have focussed on the relationships of different subsets of the birds included in our dataset. Fjeldså et al. (1999) discovered

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TABLE 1.   List of taxa, sample and GenBank accession numbers, and collection locality. ANSP, Academy of Natural Sciences of Philadelphia; FMNH, Field Museum of Natural History, Chicago; MNHN, Muséum National d'Histoire Naturelle, Paris; ZMUC, Zoological Museum of Copenhagen. The classification follows Howard and Moore (1991).

| Species | Sample number | GenBank accession | Collection locality | Taxonomic family |
|---|---|---|---|---|
| *Tyrannus melancholicus* | MNHN 9-46 | AF135051 | United States | Tyrannidae |
| *Corvus corone* | MNHN 21-16 | AF094613 | France | Corvidae |
| *Muscicapa striata* | MNHN 23-1A | AF096458 | France | Muscicapidae |
| *Andropadus latirostris* | MNHN 2-52 | AF096457 | Cameroon | Pycnonotidae |
| *Phyllastrephus scandens* | FMNH 389335 | AF199381 | Gabon | Pycnonotidae |
| *Phyllastrephus debilis* | FMNH 356727 | AF199383 | Tanzania | Pycnonotidae |
| *Phyllastrephus icterinus* | FMNH 357201 | AF199382 | Congo | Pycnonotidae |
| *Phyllastrephus madagascariensis* | FMNH 356644 | AF199387 | Madagascar | Pycnonotidae |
| *Phyllastrephus zosterops* | FMNH 356645 | AF199391 | Madagascar | Pycnonotidae |
| *Phyllastrephus apperti* | FMNH 393162 | AF199388 | Madagascar | Pycnonotidae |
| *Phyllastrephus cinereiceps* | FMNH 363827 | AF199389 | Madagascar | Pycnonotidae |
| *Alophoixus bres* | ANSP 1138 | AF199384 | Borneo | Pycnonotidae |
| *Tricholestes criniger* | ANSP 1179 | AF199385 | Borneo | Pycnonotidae |
| *Cisticola cherina* | FMNH 352959 | AF09425 | Madagascar | Sylviidae |
| *Prinia bairdii* | MNHN 2-45 | AF094617 | Cameroon | Sylviidae |
| *Thamnornis chloropetoides* | FMNH 356699 | AF094642 | Madagascar | Sylviidae |
| *Cryptosylvicola randrianasoloi* | FMNH 360058 | AF094641 | Madagascar | Sylviidae |
| *Acrocephalus aedon* | MNHN 4-8d | AF094623 | Burundi | Sylviidae |
| *Phylloscopus laetus* | FMNH 346427 | AF094624 | Burundi | Sylviidae |
| *Garrulax leucolophus* | MNHN 4-6E | AF094627 | Thailand | Timaliidae |
| *Illadopsis albipectus* | ZMUC 03192 | AF199392 | Uganda | Timaliidae |
| *Pomatorhinus ochraceiceps* | MNHN 5-38 | AF094630 | Laos | Timaliidae |
| *Neomixis tenella* | FMNH 352906 | AF094637 | Madagascar | Timaliidae |
| *Neomixis viridis* | FMNH uncatalogued | AF094638 | Madagascar | Timaliidae |
| *Neomixis striatigula* | FMNH 352909 | AF094640 | Madagascar | Timaliidae |
| *Neomixis flavoviridis* | FMNH 345858 | AF094628 | Madagascar | Timaliidae |
| *Turdoides rubiginosus* | ZMUC 42 | AF199386 | Kenya | Timaliidae |
| *Yuhina gularis* | MNHN 7-24 | AF094631 | Vietnam | Timaliidae |
| *Oxylabes madagascariensis* | FMNH 352918 | AF094629 | Madagascar | Timaliidae |
| *Crossleyia xanthophrys* | FMNH 363848 | AF199390 | Madagascar | Timaliidae |
| *Mystacornis crossleyi* | FMNH 352920 | AF094635 | Madagascar | Timaliidae |

that the Malagasy species in *Phyllastrephus,* a genus otherwise restricted to the African mainland, form a clade that is not the sister to the African *Phyllastrephus.* Cibois et al. (1999) investigated the relationships of Malagasy babblers (Timaliidae). Based on their trees, they concluded that *Oxylabes madagascariensis, Hartertula (Neomixis) flavoviridis, Thamnornis chloropetoides,* and *Cryptosylvicola randrianasoloi* constitute an endemic warbler clade, and that the other three species of *Neomixis* form a clade that is most closely related to cisticoline warblers from Africa and Madagascar. Here we extend the results of these preliminary studies on the genetic affinities of endemic Malagasy passerines.

METHODS

Our data matrix includes 13 species of endemic Malagasy birds, 17 species of Old World oscine passerines, and one suboscine passerine (*Tyrannus melancholicus*) (Table 1). We extracted whole genomic DNA from frozen or alcohol-preserved tissues using standard protocols, including digestion with proteinase K, phenol-chloroform purification, and ethanol precipitation. The sequences in our data matrix were obtained in three different laboratories (Field Museum of Natural History, Chicago; Muséum National d'Histoire Naturelle, Paris; National Zoological Park, Washington, D.C.), and amplification and sequencing protocols differed among laboratories (Cibois et al. 1999; Fjeldså et al. 1999). We obtained 888 base pairs of sequence, including most of the

mitochondrial cytochrome *b* gene. Sequences were aligned either manually or using the program Sequencher (Gene Codes Corporation, Ann Arbor, MI). No insertions/deletions were required for alignment.

We analyzed the sequence data matrix using both maximum-parsimony (MP) and maximum-likelihood (ML) criteria. Searches were implemented in PAUP* (Swofford 1999). We ran five different MP searches, varying the relative weighting of transitions and transversions. Searches were run with all changes equally weighted, with a step matrix imposed weighting transversions (tv) over transitions (ti) by different factors (2:1, 5:1, and 10:1), and with transitions at third codon positions excluded ($-3Ti$). The unequal weighting schemes were intended to discount the more saturated transitional changes, based on our saturation curves (Fig. 1). We also ran MP searches excluding the most distant outgroup, *T. melancholicus.*

We also ran a ML search. To find the optimal model and parameter values for the ML search, we followed a protocol suggested by Swofford (1999). Using a MP tree (the single MP tree obtained in a search with a 5:1 tv:ti weighting), we calculated the likelihoods and optimal parameter values for six different evolutionary models, varying the number of substitution types (two or six) and the type of rate variation across sites (no variation; site specific, according to codon position; gamma distributed). Our goal was to find the simplest model and associated parameter values yielding a sig-
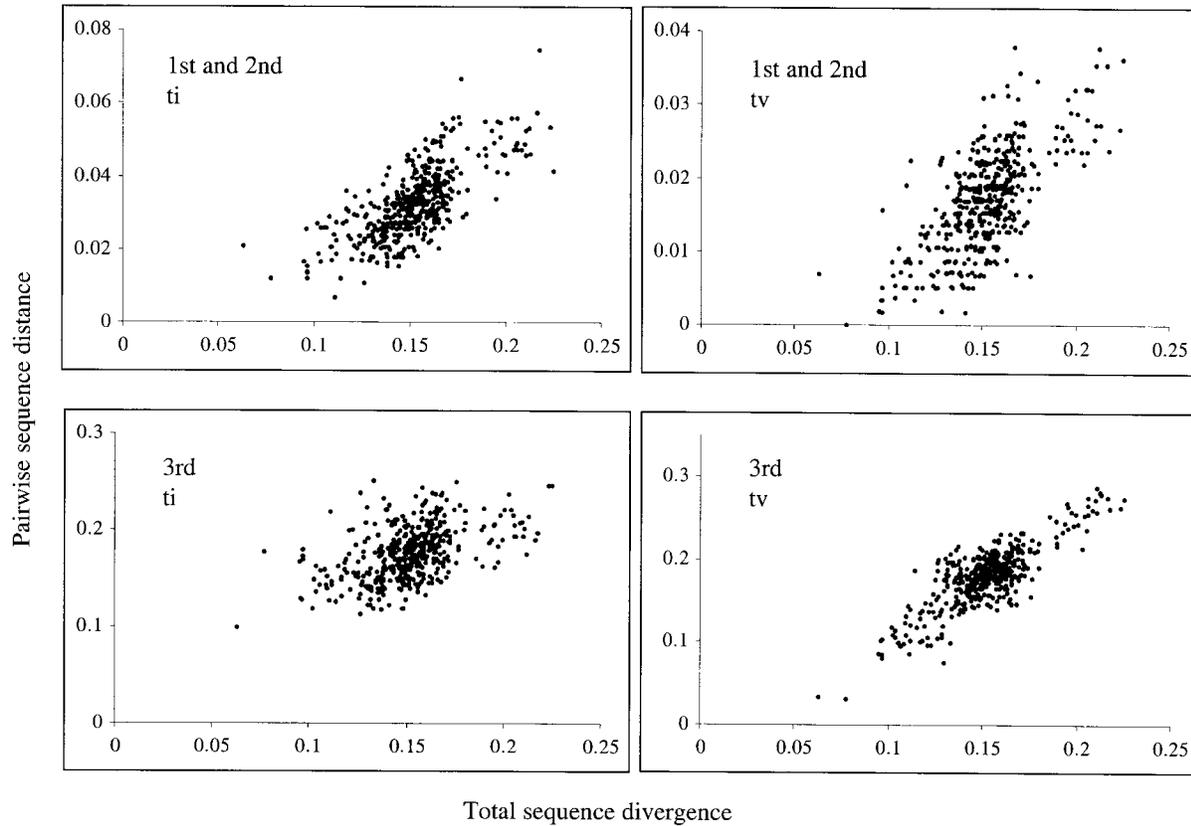
FIG. 1. Pairwise transition (ti) and transversion (tv) sequence distance (uncorrected distance) plotted against total sequence divergence.

nificantly higher likelihood than all others, based on the likelihood-ratio test (Swofford et al. 1996). We selected a model including six substitution types and gamma-distributed rate variation across sites (Table 2). This a priori selection of parameter values reduces computational time considerably, compared to a search in which the parameters are estimated during the search.

All searches were heuristic, with TBR branch swapping and the MULPARS option in effect. For MP searches, taxa were added randomly, with 10 addition-sequence replicates. For ML searches, taxa were added ''as is.'' We used bootstrapping (100 replicates, TBR branch swapping, simple sequence addition) to assess support for nodes in the MP trees

TABLE 2. Likelihood values for the maximum-parsimony tree (Fig. 2) under six different evolutionary models. The model yielding the highest likelihood (model 6, **) was significantly better than the model yielding the next highest likelihood (mode 3, *), based on the likelihood-ratio test ($2\Delta L = 149.7$, df = 4; $P \ll 0.001$).

| Model | No. of substitution types | Rate variation across sites | -ln (likelihood) |
|---|---|---|---|
| 1 | 2 | no variation | 10794.8302 |
| 2 | 2 | according to codon position | 9495.0269 |
| 3 | 2 | gamma distributed | 9382.6674* |
| 4 | 6 | no variation | 10585.0235 |
| 5 | 6 | according to codon position | 9427.1339 |
| 6 | 6 | gamma distributed | 9307.8077** |

(Felsenstein 1985). The bootstrapping for the ML tree (100 replicates) was performed using SPR branch swapping, with the starting tree obtained by neighbor joining: these options were chosen to reduce computational time. We also calculated Bremer's decay indices for the MP trees, using Autodecay (Eriksson 1997).

The Kishino-Hasegawa test (Kishino and Hasegawa 1989) commonly is used to compare the optimality scores of different tree topologies. However, Goldman et al. (2000) demonstrate that this test cannot be applied validly to compare an optimal tree to an alternative phylogeny, due to assumptions about the distribution of the test statistic. Therefore, we employed the SOWH test, described by Goldman et al. (2000), to compare our trees to topologies suggested by previous taxonomic classifications. We followed the protocol suggested by N. Goldman, J. P. Anderson and A. G. Rodrigo on Goldman's website (http://www.zoo.cam.ac.uk/zoostaff/goldman/tests/). First, we used the program Seq-Gen (Rambaut and Grassly 1997) to generate 100 simulated datasets via parametric bootstrapping. For each simulated data set, PAUP* (Swofford 1999) was used to calculate the likelihood scores of the null and ML topologies. The difference between these likelihood scores was used as the null distribution for the test statistic in the SOWH test (Goldman et al. 2000). We performed the SOWH tests using partial optimization, because our data matrix is large (31 taxa). The ML searches on the simulated datasets were performed using SPR branch swapping, with the starting tree obtained by neighbor joining.

TABLE 3. Summary of results from five maximum-parsimony analyses, run with different step matrices weighting transversions (tv) over transitions (ti). Bootstrap percentages (above) and Bremer indices (below) are reported for 15 nodes common to all optimal trees (Fig. 2). Node K (bold type) supports the clade of Malagasy endemics. TV*j* designates a *j*:1 weighting of tv:ti; -3Ti designates that third position transitions were excluded.

| | A | B | C | D | E | F | G | H | I | J | **K** | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unweighted (3 trees) | <50 | 71 | <50 | 70 | 54 | 79 | 100 | 76 | <50 | <50 | **66** | 80 | 95 | <50 | <50 |
| | — | 9 | 4 | 6 | 7 | 7 | 18 | 8 | 3 | — | **3** | 4 | 9 | 6 | — |
| TV2 (1 tree) | 53 | 75 | <50 | 78 | 70 | 88 | 100 | 65 | 53 | 66 | **90** | 79 | 99 | 58 | 56 |
| | 2 | 5 | 5 | 8 | 9 | 11 | 23 | 7 | 5 | 2 | **9** | 8 | 17 | 6 | 4 |
| TV5 (1 tree) | 78 | 78 | <50 | 76 | 59 | 69 | 99 | 64 | 50 | 65 | **94** | 78 | 98 | <50 | 71 |
| | 13 | 13 | 5 | 14 | 17 | 16 | 35 | 8 | 8 | 12 | **26** | 14 | 37 | 18 | 23 |
| TV10 (2 trees) | 72 | 77 | <50 | 83 | 63 | 62 | 95 | <50 | <50 | 75 | **92** | 71 | 100 | <50 | 64 |
| | 21 | 29 | 3 | 27 | 25 | 17 | 48 | 6 | 6 | 25 | **45** | 21 | 74 | 31 | 49 |
| -3Ti (2 trees) | 56 | 75 | <50 | 78 | 81 | 87 | 95 | 62 | <50 | 65 | **93** | 78 | 94 | 65 | 57 |
| | 1 | 3 | 1 | 1 | 4 | 5 | 8 | 2 | 3 | 3 | **5** | 5 | 9 | 6 | 4 |

## RESULTS

Across all taxa, 419 (47%) of the sites were variable and 348 (39%) were parsimony informative. Of the variable sites, 23% were at first, 9% at second, and 68% at third codon positions. Of the parsimony-informative sites, 20% were at first, 5% at second, and 75% at third codon positions. The average base composition of the sequences is skewed. First codon positions show little bias. Second codon positions show a deficiency of adenine and guanine and an overabun-

dance of thymine. Third codon positions show a strong deficiency in guanine (3.3%) and thymine (10.6%) and an overabundance of adenine (40.2%) and cytosine (45.9%). This bias in base composition does not differ significantly across taxa, based on a chi-squared test (implemented in PAUP*, $P = 0.99$). These patterns of variability and base composition bias are typical for avian cytochrome *b* (e.g., Bloomer and Crowe 1998; Voelker 1999), suggesting that our sequences are actual mitochondrial sequences, not nuclear homologues of mitochondrial sequences (Arctander 1995). Additionally, we converted our DNA sequences to amino acids using MacClade (Maddison and Maddison 1997), and we found no stop codons.

To assess saturation in our sequences, we plotted uncorrected total sequence divergence versus divergence based on transition and tranversion substitutions (Fig. 1). The curve for third position transitions clearly plateaus, implying saturation of transitions for distances greater than about 8% total uncorrected sequence divergence. Similar values for the onset of saturation have been reported for cytochrome *b* in other groups of birds (Hackett 1996; Bloomer and Crowe 1998).

The translated amino acid sequences gave a matrix of 296 characters, and 17.2% of the characters were parsimony informative. MP analysis of this matrix yielded 76 trees. The strict consensus of these trees includes several polytomies, and only two clades were supported by bootstrap percentages greater than 50%: one clade including all taxa except *Tyrannus* and *Corvus,* and another including the pair *Neomixis tenella* and *N. striatigula*.

The results of the MP searches on the sequence data are summarized in Table 3, where we list the bootstrap percentages and the Bremer's decay indices for 15 nodes common to all optimal trees. These nodes are mapped on the MP tree shown in Figure 2. The optimal MP trees all differ slightly in topology, and the ML tree (Fig. 3) also differs from the MP trees. However, differences between optimal trees are confined to nodes with less than 50% bootstrap support. With the distant outgroup *T. melancholicus* excluded, MP searches yielded five most-parsimonious trees with a 2:1 step matrix imposed and one most-parsimonious tree with either a 5:1 or 10:1 step matrix. These trees differ slightly in topology from the optimal trees obtained with *T. melancholicus* included in the analysis, but the differences again are restricted to nodes with less than 50% bootstrap support.
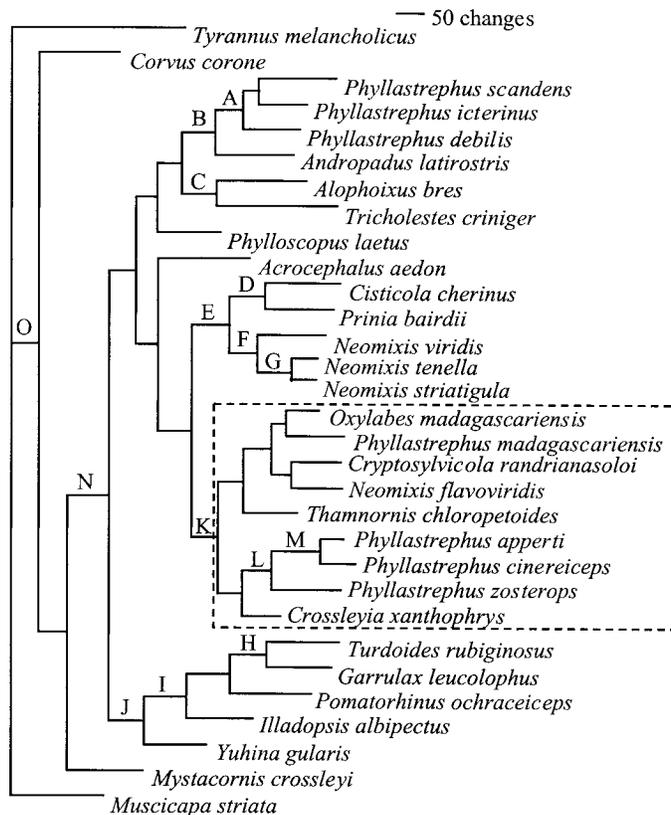


FIG. 2. Single most parsimonious tree obtained from a heuristic search with a 5:1 step matrix imposed weighting transversions over transitions. The box (dotted lines) surrounds a clade of endemic Malagasy songbirds. See Table 3 for a summary of support for labeled nodes.
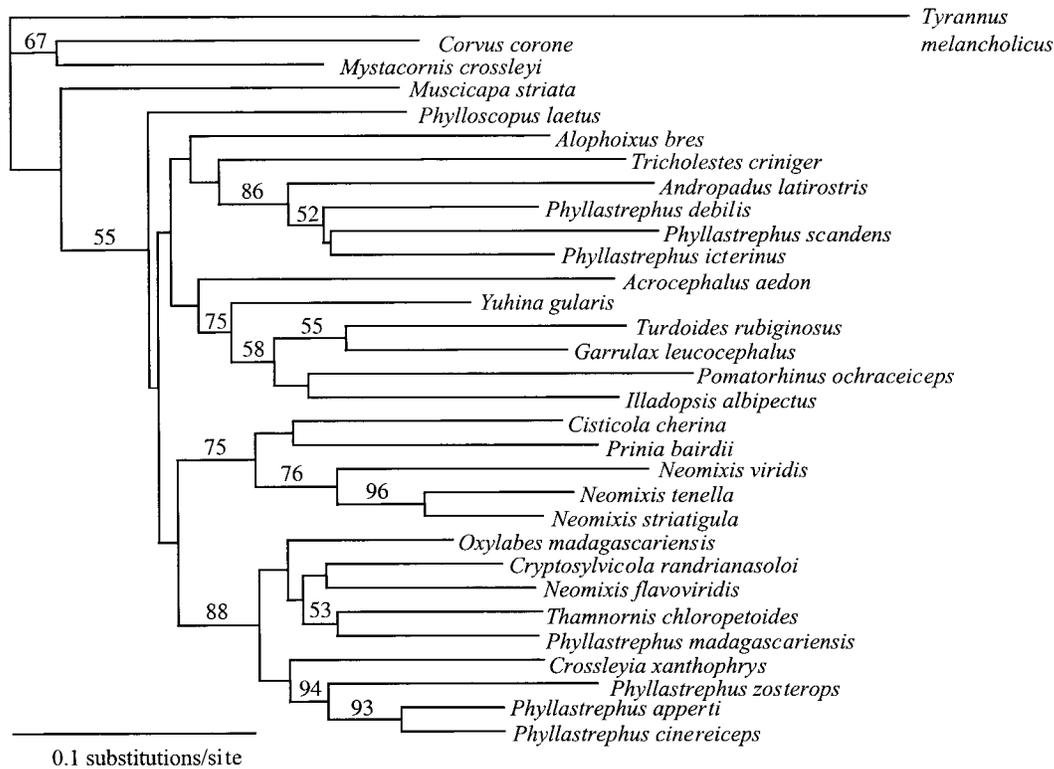
FIG. 3. Maximum-likelihood tree from a heuristic search implemented in PAUP* (Swofford 1999) with six substitution types (Rmatrix = 3.38, 7.49, 1.48, 0.47, 11.02, 1.00) and gamma-distributed rate variation across sites (alpha = 0.909). The starting trees were obtained via stepwise addition, and TBR branch swapping was used. Bootstrap percentages are based on 100 replicates.

We compared the likelihood of one MP tree (5:1 step matrix, Fig. 2) with and without a molecular clock imposed, using a likelihood-ratio test (Swofford et al. 1996). The likelihood-ratio test assumes that the test statistic ($2\Delta L$, where $\Delta L$ is the difference in log-likelihood between the clock and nonclock trees) follows a chi-squared distribution, with the degrees of freedom equal to ($N - 2$), where N is the number of taxa in the data matrix (Felsenstein 1988). The likelihood was significantly worse with a clock imposed ($P < 0.001$), suggesting rate variation among taxa. We repeated the test on a more limited set of taxa: the Malagasy endemic clade, its sister clade, and an outgroup (*Corvus corone*). The clock and nonclock models yielded likelihoods that were not significantly different ($P > 0.10$), suggesting no significant rate variation among this smaller set of taxa.

All optimal sequence-based trees include a clade of Malagasy endemics comprised of four bulbuls in the genus *Phyllastrephus*; the babblers *Oxylabes madagascariensis, Crossleyia xanthophrys,* and *Neomixis flavoviridis;* and the warblers *Thamnornis chloropetoides* and *Cryptosylvicola randrianasoloi.* This clade has strong bootstrap and Bremer support (Table 3, node K). In most optimal trees, this clade of Malagasy endemics pairs with a clade including several species of African and Malagasy warblers (Sylviidae), but bootstrap and Bremer support for this sister relationship is weak (bootstrap < 50%, Bremer's indice ≤ 3). Within the endemic Malagasy clade, *P. cinereiceps* and *P. apperti* pair as sister species in all optimal trees, and bootstrap support for the pairing is strong (Table 3, node M). The *cinereiceps + apperti*

clade pairs with *zosterops* in all optimal trees, also with strong bootstrap support (Table 3, node L). Other relationships within the endemic clade, as well as the sister relationship of this clade, are inconsistent across the optimal trees and are supported by low bootstrap percentages.

In our optimal trees, nine endemic Malagasy species group as a clade (Fig. 2, clade K), contradicting previous hypotheses of relationship for these birds (Table 4). We used the SOWH test (Goldman et al. 2000) to determine if tree topologies based on four previously suggested classifications yielded likelihood values significantly worse than the value for our ML tree, using our sequence data matrix. We tested four topologies (T1, T2, T3, and T4) as null hypotheses against our ML tree ($T_{ML}$, Fig. 3). Because the four null hypothesis topologies are based on classifications, the trees include several polytomies. The program that we used to generate replicate datasets for the SOWH test (Seq-Gen; Rambaut and Grassly 1997) does not accept polytomies in the null hypothesis topology. So, we obtained four fully bifurcating trees by using each of the null-hypothesis topologies (T1, T2, T3, and T4) as a constraint tree in a ML search in PAUP*. Based on the SOWH test, the four trees based on previous classifications have a significantly lower likelihood than our ML tree ($P < 0.001$).

Among the nine taxa comprising the Malagasy radiation (Fig. 2, clade K), uncorrected sequence divergence ranged from 6.3% (between *apperti* and *cinereiceps*) to 14.0% (between *flavoviridis* and *zosterops*), with a mean of $11.2 \pm 3.1\%$ (error = 2 SD). Among the three African species in the genus

TABLE 4. The different classifications for the Malagasy endemics studied.

| Species | Delacour[1] T1 | Morony et al.[2] T2 | Howard and Moore[3] T3 | Olson[4] T4 | Cibois et al.[5] $T_{ML}$ |
|---|---|---|---|---|---|
| *Phyllastrephus madagascariensis* | Pycnonotidae | Pycnonotidae | Pycnonotidae | endemic radiation | endemic radiation |
| *Phyllastrephus zosterops* | Pycnonotidae | Pycnonotidae | Pycnonotidae | endemic radiation | endemic radiation |
| *Phyllastrephus apperti* | Pycnonotidae | Pycnonotidae | Pycnonotidae | endemic radiation | endemic radiation |
| *Phyllastrephus cinereiceps* | Pycnonotidae | Pycnonotidae | Pycnonotidae | endemic radiation | endemic radiation |
| *Thamnornis chloropetoides* | Sylviidae[6] | Sylviidae | Sylviidae | endemic radiation | endemic radiation |
| *Cryptosylvicola randrianasoloi* | (taxa described in 1996) | (taxa described in 1996) | Sylviidae | (taxa described in 1996) | endemic radiation |
| *Neomixis tenella* | Timaliidae | Timaliidae | Timaliidae | endemic radiation | Sylviidae |
| *Neomixis viridis* | Timaliidae | Timaliidae | Timaliidae | endemic radiation | Sylviidae |
| *Neomixis striatigula* | Timaliidae | Timaliidae | Timaliidae | endemic radiation | Sylviidae |
| *Neomixis flavoviridis* | Timaliidae | Timaliidae | Timaliidae | endemic radiation | endemic radiation |
| *Oxylabes madagascariensis* | Sylviidae | Timaliidae | Timaliidae | endemic radiation | endemic radiation |
| *Crossleyia xanthophrys* | Pycnonotidae | Pycnonotidae | Timaliidae | endemic radiation | endemic radiation |
| *Mystacornis crossleyi* | Sylviidae | Timaliidae | Timaliidae | endemic radiation | Corvoidea[7] |

[1] Delacour (1943) for the Pycnonotidae and Delacour (1946) for the Timaliidae and Sylviidae.
[2] Morony et al. (1975).
[3] Howard and Moore (1991).
[4] Olson (1989), who agreed with Salomonsen (1934) and Irwin (1983) for including all these Malagasy endemic taxa in a single radiation.
[5] The results founded in this study.
[6] According to Peters's classification (Watson et al. 1986), because Delacour (1943, 1946) gives not any information about this species.
[7] See Cibois et al. (1999).

*Phyllastrephus,* uncorrected divergence ranged from 11.1% to 13.0%, with a mean of 12.1 ± 1.8%. Thus, the Malagasy endemics and African *Phyllastrephus* show similar levels of intraclade divergence, based on the taxa included in this study. Between the Malagasy endemics and the African species in *Phyllastrephus,* uncorrected sequence divergence ranged from 12.7% to 16.4%, with a mean of 14.9 ± 2.2%.

## DISCUSSION

### Adaptive Radiation

The history of the classification of the Malagasy birds included in this study is complex, reflecting the difficulty of assessing their relationships based on perceived similarities in plumage and external morphology. As summarized in Table 4, the 13 species have been distributed among as many as nine genera in three different families (Delacour 1943, 1946; Benson and Irwin 1975) or considered as a single radiation (Salomonsen 1934; Irwin 1983; Olson 1989). These previous classifications were based mostly on external characters, such as the shape of the bill or legs, although Olson (1989) based his assessments of relationship on cranial osteological characters. Contrary to the previous classifications, the sequence data strongly support a clade of endemic Malagasy songbirds, including the species in *Phyllastrephus, Oxylabes madagascariensis, Crossleyia xanthophrys, Hartertula* (*Neomixis*) *flavoviridis, Thamnornis chloropetoides,* and the recently discovered *Cryptosylvicola randrianasoloi* (Goodman et al. 1996), but excluding the other species in *Neomixis* and *Mystacornis crossleyia.*

Most previous classifications of these endemic Malagasy songbirds suggested colonization of Madagascar by at least three different lineages of forest-dwelling birds (babblers, bulbuls, warblers), but our phylogeny suggests a single colonization event. A single colonization seems more likely, based on the observation that successful colonization of islands by forest-restricted birds is rare. The avifauna of islands typically is comprised of habitat generalists (Whittaker 1998), while all nine species in the endemic Malagasy clade are forest dwelling. Most are confined to the humid eastern forests, although at least one species (*Thamnornis*) occupies the xerophytic scrub of southwestern Madagascar. In some localities, as many as seven species in this radiation occur sympatrically (e.g., Goodman and Putnam 1996; Hawkins et al. 1998; Hawkins and Goodman 1999). All species glean insect prey, but differ in body size, feeding stratum, and, to some extent, elevational distribution (Goodman and Putnam 1996; Hawkins et al. 1998; Hawkins and Goodman 1999). At least four of the species belonging to the endemic radiation of Malagasy songbirds are classified as threatened or nearly threatened (*P. cinereiceps, C. xanthophrys, H. flavoviridis,* and *P. tenebrosus*; Collar et al. 1994). Given the relatively ancient origin of this unique radiation, increased conservation efforts to protect these birds and their habitats are warranted.

The nine species comprising the endemic Malagasy clade display a heterogeneous array of bill shapes, wing and tail proportions, and tarsus lengths (Morris and Hawkins 1998). This diversity in morphology caused many ornithologists to overlook the monophyly of this endemic group. In classifications, the Malagasy birds were dispersed among as many

as three different families (Table 4), based on convergent similarities to continental groups. Similarly, the monophyly of endemic Australo-Papuan songbirds was overlooked for many decades due to convergent similarities to continental families, until Sibley and Ahlquist (1985) provided convincing evidence of an Australo-Papuan radiation based on DNA-DNA hybridization data.

Several other examples of endemic groups on Madagascar that were overlooked due to convergent similarities to continental lineages have been reported in the recent literature. Bossuyt and Milinkovitch (2000) used mitochondrial and nuclear gene sequence data to demonstrate that Malagasy ranid frogs constitute a monophyletic group including about 120 species, although the Malagasy frogs have evolved convergent similarities in morphology, development, and physiology to different clades of ranid frogs found on the Indian subcontinent. Caccone et al. (1999) demonstrated the monophyly of endemic Malagasy tortoises using mitochondrial sequence data. Previously, two of the four endemic tortoises had been place in a widespread genus (*Geochelone*) based on morphological and ecological similarities to other species in this genus. Yoder et al. (1996), using a combined dataset of morphological characters and cytochrome *b* sequences, demonstrated that Malagasy primates are monophyletic. Previously, one group of Malagasy primates, the cheirogaleids (mouse and dwarf lemur group) had been placed with the Afro-Asian loris group based on similarities in basicranial anatomy, and the phylogenetic position of the aye-aye was debated due to its unique morphological attributes. Olson (1999) used a combination of morphological and DNA sequence data to demonstrate the monophyly of the Malagasy tenrecs (family Tenrecidae), and Jansa et al. (1999) and Yoder and Flynn (2002) used DNA sequence data to demonstrate the monophyly of endemic Malagasy rodents and carnivores, respectively.

The bird fauna of Madagascar is depauperate and includes numerous endemic species (Dorst 1972), characteristics common to island bird communities (Whittaker 1998). Among nonpasserine birds, the endemic avifauna includes the ground rollers (Brachypteracidae, five species), mesites (three species), and couas (nine species; Johnson et al. 2000). Among passerine birds, about two-thirds of the breeding species are endemic, including two sizeable radiations: the vangas, including about 17 species (A. C. Driskell, S. J. Hackett, and T. S. Schulenberg, unpubl. data), and the radiation described in this paper (Fig. 2), including at least nine species. Thus, on Madagascar, the endemic passerine assemblage is not dominated by a single large radiation, in contrast to the situation on the isolated archipelagos of Hawaii and the Galapagos. The drepanidines in Hawaii (33 species known historically; James and Olson 1991) and Darwin's finches in the Galapagos (13 species; Sato et al. 1999) account for about 70% of the endemic songbirds in these archipelagos, whereas the vangas and the radiation described here comprise about 38% and 20%, respectively, of the endemic Malagasy songbirds. The difference in the size of these radiations undoubtedly is driven by the large size of Madagascar and its relative proximity to the African continent, both factors that increase the probability, and thus the frequency, of colonization. Presumably, on Madagascar, the size of songbird radiations was limited by the presence or arrival of competitors. This hypothesis implies that although adaptive radiations are believed to occur rapidly (McCune 1997), some period of isolation is necessary for a radiation to fill all available niches.

*Issues of Resolution*

In general, our sequence data yield consistent resolution of certain major clades, with moderate to strong bootstrap support, but yield inconsistent and weakly supported resolution of relationships among clades. The well-supported clades include the Malagasy endemics, the African greenbuls (*Phyllastrephus scandens, P. icterinus, P. debilis, Andropadus latirostris*), the African and Malagasy warblers (*Neomixis viridis, N. tenella, N. striatigula, Prinia bairdii, Cisticola cherinus*), and the babblers (*Turdoides, Garrulax, Pomatorhinus, Yuhina, Illadopsis*). In part, the poor resolution of relationships among clades (i.e., more basal relationships in the trees) is attributable to saturation of the mitochondrial sequence data. However, a rapid diversification of these passerine clades would also contribute to difficulty in resolving interclade relationships. Studies of relationships among songbird families based on DNA-DNA hybridization distances have suggested such a rapid radiation (Sibley and Ahlquist 1990; Sheldon and Gill 1996).

Our sequence data lend strong support to the existence of an endemic Malagasy clade, but we have not determined with certainty the extent of this radiation. One member of the Malagasy *Phyllastrephus* is missing from our dataset, *P. tenebrosus.* This species is almost certainly a member of the endemic radiation, probably closely related to the *apperti + cinereiceps + zosterops* group (Benson and Irwin 1975). In addition, several other Malagasy endemics might fall within this radiation, including *Randia pseudozosterops, Tylas eduardi,* and species in the genera *Nesillas* (two species), *Newtonia* (four species), and *Dromaeocercus* (two species).

*Origin and Timing of Radiation*

Our data are insufficient to resolve with certainty the biogeographic origin of the Malagasy clade. In most optimal trees, the sister group to the Malagasy endemics is a clade of African and Malagasy warblers, suggesting an African origin; however, bootstrap support for this pairing is weak (Figs. 2, 3). The average uncorrected sequence divergence between the Malagasy clade and its putative sister group (*Neomixis, Cisticola, Prinia*) is 14.9 ± 1.6 % (error = 2 SD). The distance between the Malagasy endemics and other clades in our optimal trees is comparable: 14.9 ± 2.6% to the babblers (*Turdoides, Garrulax, Pomatorhinus, Illadopsis, Yuhina*) and 15.2 ± 2.1% to the bulbuls (*Phyllastrephus scandens, P. icterinus, P. debilis, Andropadus, Alophoixus, Tricholestes*). These uncorrected distances are compressed by saturation and thus give a minimum value for the sequence divergence among these clades. Corrected distances (Kimura two-parameters; Kimura 1980) give similar results. The average corrected sequence divergence is 16.7 ± 2.1% between the Malagasy clade and the African warbler group (*Neomixis, Cisticola, Prinia*), 16.5 ± 3.6% between the Malagasy clade and babblers, and 17.1 ± 2.7% between the Malagasy clade and bulbuls. These results show that there is no support in

the genetic distance for a clade grouping the Malagasy endemics and the African warblers. Assuming a rate of substitution of 1.6% per million years for cytochrome *b* (Fleischer et al. 1998), the age of the endemic Malagasy clade is at least 9 million years. If ML distances are used to calculate a divergence time, the estimate is about 17 million years. Thus, our data suggest that the ancestor of this endemic Malagasy clade colonized the island between 9 million and 17 million years ago, roughly. The fossil record of passerines on Madagascar is poor, so fossil data are not available to check this estimate (Unwin 1993). Caccone et al. (1999) gave a similar estimate (14–22 million years ago) for the colonization of Madagascar by the ancestor of an endemic clade of tortoises, also based on mitochondrial sequence data. Yoder et al. (1996) gave a much earlier estimate for the colonization of Madagascar by primates: about 54 million years ago, the earliest Eocene.

### Provisional Classification

The current classification of the Malagasy songbirds (Table 4) reflects a biased attempt to fit these endemics into familiar continental families. Our optimal trees imply that the classification is inconsistent with phylogeny, so we propose the following provisional changes. The nine species in the Malagasy songbird clade (Fig. 2, clade K) are a monophyletic group. All these species should be included in the same family, tentatively within the Sylviidae. The species *flavoviridis,* currently placed in *Neomixis,* should be removed to the monotypic genus *Hartertula* (Stresemann 1925), as suggested by Cibois et al. (1999).

Our optimal trees indicate that the genus *Phyllastrephus* is polyphyletic. In all optimal trees, the African species in this genus form a monophyletic group with strong support. The genus name *Phyllastrephus* is linked to the African species *terrestris,* so the Malagasy species must be removed to other genera. The Malagasy species in *Phyllastrephus* are not monophyletic. We suggest that *madagascariensis* be restored to the genus *Bernieria* (Bonaparte 1854) and the remaining species (*apperti, cinereiceps, zosterops*) be restored to *Xanthomixis* (Sharpe 1881) because our phylogeny (Fig. 2) suggests that the latter three species constitute a monophyletic group. Further alterations to the classification should await improved resolution of relationships both within the endemic Malagasy clade and between the Malagasy clade and other sylvioid songbirds.

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