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Assessing the passerine "Tapestry": phylogenetic relationships of the Muscicapoidea inferred from nuclear DNA sequences

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Abstract

This study presents new comparative sequence data from the nuclear RAG-1 gene for an increased taxon sample in order to investigate phylogenetic relationships among a diverse songbird superfamily, the Muscicapoidea, which has variously included the waxwings, silky flycatchers, Palm Chat, dippers, starlings, mockingbirds, thrushes, chats, and Old World flycatchers. At the same time, our results provide a test of the often-cited relationships inferred from the phenetic studies of Sibley and Ahlquist [Phylogeny and Classification of Birds: A Study in Molecular Evolution. Yale University Press, New Haven, 1990] using DNA hybridization distances. Nuclear DNA sequences confirm the monophyly of the "core muscicapoid" group, as defined by Barker et al. [Proc. R. Soc. Lond. B 269 (2002) 295] and also support the sister-group relationship of the Sturnidae and Mimidae, on the one hand, and the large-bodied thrushes (Turdini) + the Old World flycatchers and robins, on the other. The results of the phylogenetic analysis allow preliminary inferences about muscicapoid biogeographic history.

Keywords: Passeriformes; Muscicapoidea; Nuclear DNA; RAG-1; Phylogeny

1. Introduction

The perching birds (Order Passeriformes) represent nearly 60% of extant avian species diversity. Relationships among this vast assemblage have traditionally been uncertain as can be seen in the differences among older, widely-used classification systems (e.g., Mayr and Amadon, 1951; Mayr and Greenway, 1956; Wetmore, 1960). This situation changed dramatically with the publication of the DNA–DNA hybridization tree, the so-called "Tapestry," of Sibley and Ahlquist (1990). Over the past decade the DNA hybridization tree has become widely used as a comparative framework to generate inferences about the evolution of ecological and behavioral traits across passerine birds, including songbirds (e.g., Owens and Bennett, 1995; Sheldon and Whittingham, 1997; Slikas, 1998).

Recently, significant advances in our understanding of passerine phylogeny have resulted from the accumulation of molecular studies employing increasingly better taxon samples (Barker et al., 2002, submitted; Cracraft et al., in press; Ericson et al., 2002; Irestedt et al., 2001). This work has resulted in the possibility of testing the hypotheses proposed by Sibley and Ahlquist (1990) using direct character evidence instead of phenetic distance similarity, and the aim of this paper is to re-examine the relationships within one of their primary passerine clades, the superfamily Muscicapoidea. The muscicapoids contain a large diversity of primarily Old World species within the passeridan songbirds (Barker et al., 2002; Sibley and Ahlquist, 1990); the "core muscicapoid" clade (Barker et al., 2002, submitted; Cracraft et al., in press) includes the waxwings, silky flycatchers, Palm Chat, dippers, starlings, mockingbirds, thrushes, chats, and Old World flycatchers. Although these birds have been generally divided into a number of families on the basis of morphological, behavioral, and ecological features, meaningful diagnostic characters are lacking. Recent molecular investigations using increased taxon

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samples (Barker et al., 2002, submitted; Cracraft et al., in press) have supported the monophyly of the "core muscicapoids."

Several morphological studies have proposed that the waxwings, silky flycatchers, and Palm Chat (the monotypic genus *Dulus*) are closely related and should be included in a single family Bombycillidae (Arvey, 1951; Beecher, 1953). On the basis of egg-white proteins Sibley (1970) suggested a relationship between waxwings and silky flycatchers but not between these and the Palm Chat. Later, Sibley (1973) suggested that the silky flycatchers might be related to the turdine genus *Myadestes*. The results of DNA hybridization agreed with the morphological studies, and Sibley and Ahlquist (1990) divided the waxwings, silky flycatchers, and Palm Chat into three "tribes" within the Bombycillidae, and placed the family in a basal position among the Muscicapoidea (Fig. 1). The dippers (Cinclidae) comprise five species included in one genus (*Cinclus*); three of the species are found in the Eurasia and Africa, and two are distributed in the New World. The wrens (Troglodytidae) and thrushes (Turdidae) are usually thought to be close relatives of the dippers (Mayr and Amadon, 1951; Wetmore, 1960). Sibley (1970) suggested that egg-white protein patterns support a relationship between dippers and thrushes, but rejected a dipper–wren alliance. Using DNA hybridization Sibley and Ahlquist (1990) concluded that the cinclids are the sister-group of the muscicapid-sturnid clade and that wrens belong to another passeridan superfamily, the Sylvioidea. The starlings, mynas, and oxpeckers have usually been grouped into the Old World family Sturnidae, but there has been no consensus about their relationships to other songbirds. Earlier classifications placed them close to the Old World orioles, family Oriolidae (Amadon, 1943, 1956; Howard and Moore, 1994) or to the weavers (Ploceidae; Berndt and Meise, 1962; Voous, 1977). Mayr and Amadon (1951) included the starlings in a group that included the Ploceidae, Oriolidae, and Dicruridae (drongos), followed by the crows and their allies (Corvidae). The mockingbirds and thrashers comprise the New World family Mimidae, and most classifications have placed them between wrens and thrushes (Mayr and Amadon, 1951; Voous, 1977; Wetmore, 1960). In contrast, some authors have argued that morphological (Beecher, 1953) and molecular data (Sibley and Ahlquist, 1984, 1990; Stallcup, 1961) support a relationship between the starlings and mockingbirds.

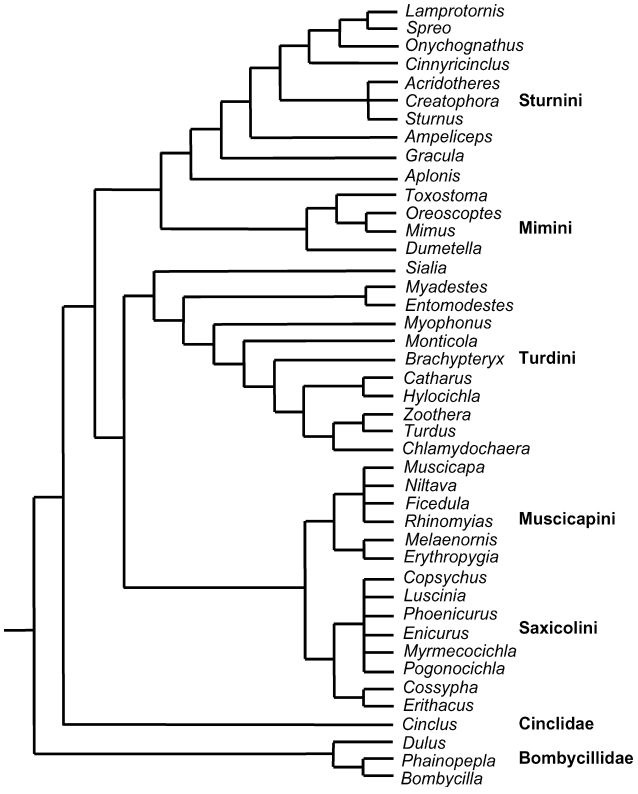

Fig. 1. Sibley and Ahlquist's (1990) hypothesis of phylogenetic relationships among the Muscicapoidea based on phenetic similarity among DNA hybridization distances.

The thrushes, chats, and Old World flycatchers form a remarkably diverse group within the Muscicapoidea that was originally defined by the presence of spots in the juvenile plumage. For a long time these birds were grouped together in the "primitive insect eaters," which also included a great number of Old World passerine families (Mayr and Greenway, 1956; Voous, 1977). A study of the passerine syrinx (Ames, 1975) provided an important new character for the definition of the turdine-musicapine group, namely a presumed derived pattern of the syringeal musculature (called "thumb-like"), whereas other non-muscicapid birds were found to have a generalized syrinx (Ames, 1975). Both thrushes and flycatchers are widely distributed in the Old World, but thrushes have also expanded substantially into the Americas.

This paper focuses on the large-scale phylogenetic relationships and biogeography of the Muscicapoidea superfamily, using nuclear sequences from the RAG-1 gene and an expanded taxon sample. At the same time, it provides an opportunity to assess the findings of DNA hybridization at several taxonomic scales.

2. Material and methods

2.1. Taxon sampling

Previous studies on passerine phylogeny included a relatively small number of muscicapoids taxa: five in Barker et al. (2002) (*Cinclus*, *Muscicapa*, *Turdus*, *Mimus*, and *Sturnus*), two in Ericson et al. (2002) (*Mimus* and

Sturnus). The data set analysed here has 44 musicapoid taxa as ingroup plus six other oscine birds as outgroups.

New sequences used in this study are listed in the Appendix A and have been deposited in GenBank. Previously published sequences were also used in this study and include: *Corvus corone* AY056989, *Sylvia nana* AY 057033, *Hirundo pyrrhonota* AY056997, *Certhia familiaris* AY056983, *Monarcha axillaris* AY057006, *Bombycilla garrulus* AY056981, *Cinclus cinlus* AY056985, *Sturnus vulgaris* AY057032, and *Mimus patagonicus* AY057005 (all from Barker et al., 2002), as well as *Tyrannus tyrannus* AF143739 (Groth and Barrowclough, 1999).

2.2. DNA isolation and sequencing

DNA was extracted from most tissues using QIAamp tissue kits (Qiagen). For some samples DNA was extracted by placing less than 20 mg of tissue in 250 μl of 5% Chelex (Bio-Rad) and heating at 100 °C for 15 min. Previously published primers (Barker et al., 2002; Groth and Barrowclough, 1999) and one new primer (R13D 5′-CAC CCT CTG ATG ACA GCC AG-3′) were used to amplify a large portion of the single exon of the RAG-1 locus. The thermocycling procedure (40 total cycles) was a touch-down hotstart PCR, with an initial 15 min at 95 °C, followed by 5 cycles of 95 °C for 15 s, 61 °C for 15 s, and 72 °C for 45 s. Two identical 5-cycle phases followed, with a 2 °C reduction in the annealing temperature in each phase (59 °C and 57 °C). The last phase also included 25 cycles at 55 °C. PCR products were purified using GeneClean (Bio101) kits. These products were resuspended in 12 μl of water and then sequenced in a ABI 9600 thermocycler in both directions in 7 μl total volume reactions containing 2.5 μl of PCR products, 3 μl of Terminator Mix (dRhodamine, Applied Biosystems), and 1.5 μl of primer (10 M). Sequenced reactions were cleaned of excess nucleotides by ethanol precipitation, using 74 μl of a solution containing 10 ml of Ethanol (70%), and 10 μl of Magnesium chlorate (0.5 M), dried, and resuspended in 1.8 μl formamide loading dye. Reactions were then electrophoresed on an Applied Biosystems 377 automated sequencer. Contig alignments were created using Sequencher (Genecodes, Ann Arbor, MI). Accuracy of the DNA sequencing was verified by sequencing both heavy and light strands of most PCR fragments, and by using overlapping fragments.

2.3. Phylogenetic analysis

Phylogenetic analyses were first undertaken using maximum parsimony, conducted with PAUP* 4.0b10 (Swofford, 2002). Tree topologies were evaluated with heuristic searches including 200 replicates of the random taxon sequence addition option with tree-bisection and reconnection (TBR) branch swapping. Searches were undertaken with gaps treated as missing data, and data were treated with equal weight. The robustness of the clades was assessed by bootstrap analysis with 500 iterative resamplings using TBR heuristic searches (Felsenstein, 1985). All trees were rooted using the suboscine passeriform *T. tyrannus*.

The data were also analyzed under the maximum-likelihood criterion. The fit of several nested models was evaluated using the program ModelTest 3.06 (Posada and Crandall, 1998), with the starting tree being, first, a neighbor-joining tree fitted to Jukes–Cantor distances (Jukes and Cantor, 1969), which is the default option implemented by ModelTest, and second, the MP topology. Both searches resulted in the same model of evolution, selected by comparison of nested models with increasing complexity using the likelihood ratio statistic (2L, where L is the difference in log-likelihood between the two models tested) compared to a mixed χ^2 (as proposed by Ota et al. (2000) and Goldman and Whelan (2000)). The ML search was conducted using the selected model and parameters, performed using TBR branch swapping and "as-is" sequence addition. Bootstrap replicates are computationally intensive under the ML criterion, and therefore were not performed with our large data set (50 taxa).

Finally, the data were submitted to Bayesian phylogenetic analysis, using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). Huelsenbeck et al. (2002) recently reviewed the pitfalls of Bayesian inferences in phylogenetic reconstruction and made several recommendations linked to the specificity of the method and its relevant problems: (1) run several long chains, (2) run simultaneous multiple chains ("heated" chains), (3) monitor the different parameters of the model for convergence, and (4) monitor the effect of adding prior information to the analysis. To address these recommendations several runs of 50,000, 100,000, 250,000, 500,000, and 10^6 generations were performed, with four chains running simultaneously from random trees ("heated" chains), with trees sampled every 10 generations, and with the "burnin" period estimated graphically. The convergence of the tree topology and posterior probabilities (as calculated from the 50% majority-rule consensus tree, with the "burnin" period excluded) was monitored between each run. We tested the effect of adding prior information to the analysis using the ML tree as the starting topology, and using the parameters of the model as defined by ModelTest as starting values for the likelihood searches. The results of the runs with prior information were compared to the runs performed without prior information. Because MrBayes only accepts strictly bifurcating trees, we modified slightly the ML tree, using Amadon's (1943) hypothesis of a close relationship between *Ampeliceps* and *Mino* to resolve the node among the four starlings for which the topology

was ambiguous: the topology used was then (*Sarcops* (*Ampeliceps* (*Gracula*, *Mino*))).

Different topologies as well as different a priori hypotheses about the position of particular taxa were compared using the Shimodaira–Hasegawa (SH) test statistic (Goldman et al., 2000; Shimodaira and Hasegawa, 1999) and the approximately unbiased test (AU; Shimodaira, 2002). Both of these tets are statistically appropriate in comparing both a priori and a posteriori hypotheses. The AU test uses a multiscale bootstrap procedure and is less conservative than the SH test (Strimmer and Rambaut, 2002). We used PAUP* to conduct SH tests, with resampling-estimated log-likelihood (RELL) optimization and 100,000 bootstrap replicates. The software package CONSEL (Shimodaira and Hasegawa, 2001) was used to calculate the AU test.

2.4. Biogeographic analysis

The biogeographic origin of major muscicapoid groups was evalued with ancestral area analysis (Bremer, 1992, 1995). The origin for each taxon was coded for Old World or New World origin, with a distinction between Africa and Asia for the Old World taxa. Calculations of area gains and losses with alternative ancestral states under Camin–Sokal (Camin and Sokal, 1965) parsimony were conducted using MAC CLADE 4.0 (Maddison and Maddison, 2000).

3. Results

3.1. Sequence variation

Alignment of RAG-1 was straightforward, with only four indels for the total data set: one-codon deletion for the clade (*Cercotrichas*, *Copsychus*, *Melaenornis*, and *Rhinomyias*), four-codon deletion for *Cinnyricinclus*, one-codon deletion for *Tyrannus*, and a one-codon insertion for the Bombycillidae (*Bombycilla* and *Phaenoptila*). A matrix of 2875 characters was then used for all analyses (matrix submitted to EMBL database). Given this alignment, 30.8% of the sites were variable, and 16.4% were parsimony-informative. Variation and phylogenetic information were codon-position dependant, with 20.2% of the variable characters in the first position (19% of the parsimony-informative characters), 13.6% in second (10.3% of the parsimony-informative characters), and 66.2% in third position (70.6% of the parsimony-informative characters). Pairwise sequence divergence varied from 0.348% between *Cinclus palasii* and *Cinclus cinlus* to 7.43% between the outgroup *T. tyrannus* and *Turdus philomelos* (the average for the complete data set is 3.81 ± 1.07).

3.2. Phylogenetic analyses

Parsimony analysis yielded 24 equally parsimonious trees (1756 steps), with the strict consensus exhibiting unresolved nodes only among starlings (Fig. 2). Bootstrap percentages are relatively high for most of the basal nodes of the Muscicapoidea, except for the positions of *Cinclus*, *Buphagus*, and *Rhabdornis*, as well as a few nodes among starlings and thrushes. Although *Buphagus* is resolved as the sister-group of the sturnid + mimid clade, there is no strong support for this. Likewise, *Rhabornis* is depicted as the basalmost starling, but again without strong support. The dippers, Cinclidae, cluster with the muscicapines and saxicolines, but that nodes is also not well supported. The core muscicapoids (Barker et al., 2002) are strongly supported and the bombycillids lie outside that clade (Fig. 2).

Under the maximum-likelihood criterion, the model fitting the data best was the TrN + I + G model (Tamura

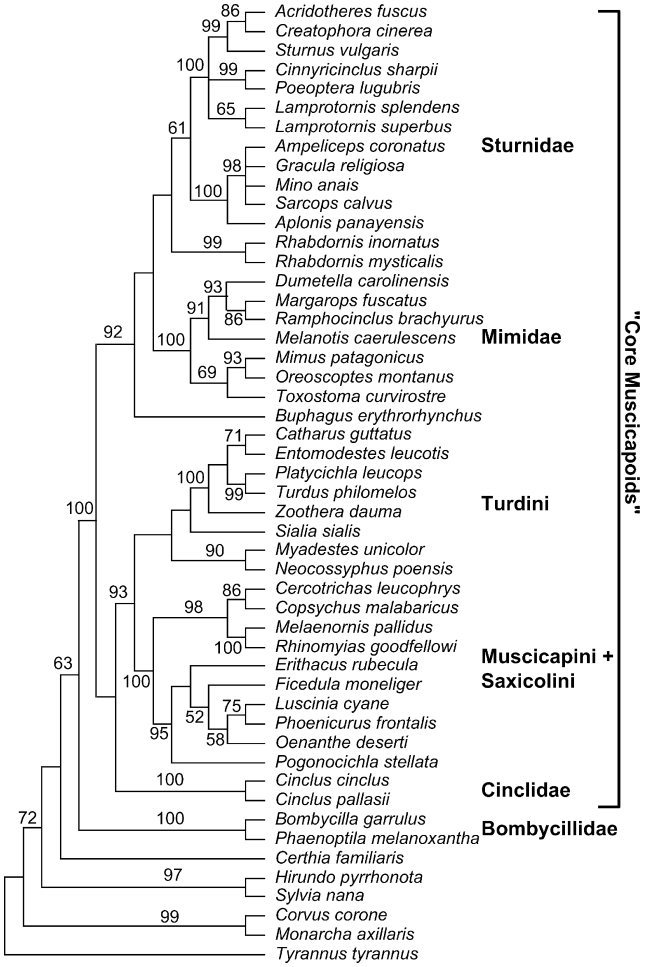

Fig. 2. A maximum parsimony tree (strict consensus, 24 most parsimonious trees, $L = 1756$ steps) for the Muscicapoidea using RAG-1 nuclear sequences. Numbers along branches are the proportion of 500 bootstrap replicates.

and Nei, 1993), which assumes equal substitution rates for transversions but different rates for transitions. The parameters defined were the following: the probabilities for the six substitution types Rmat = 1.0000 4.5826 1.0000 1.0000 10.4149 1, proportion of invariant sites I = 0.4258, and shape parameter = 0.9506. The MP consensus tree differs from the ML topology only in four nodes, involving *Zoothera*, *Sialia*, *Ficedula-Erithacus-Pogonocichla*, and *Certhia* (noted with an asterisk in Fig. 3); none of these nodes, however, was supported by bootstrap values greater than 52% in the MP tree. We compared the likelihood of the ML tree with and without a molecular clock, using a likelihood ratio test LRT (Swofford et al., 1996), which assumes that the test statistic (2L, where L is the difference in log-likelihood between the clock and non-clock trees) follows a χ^2

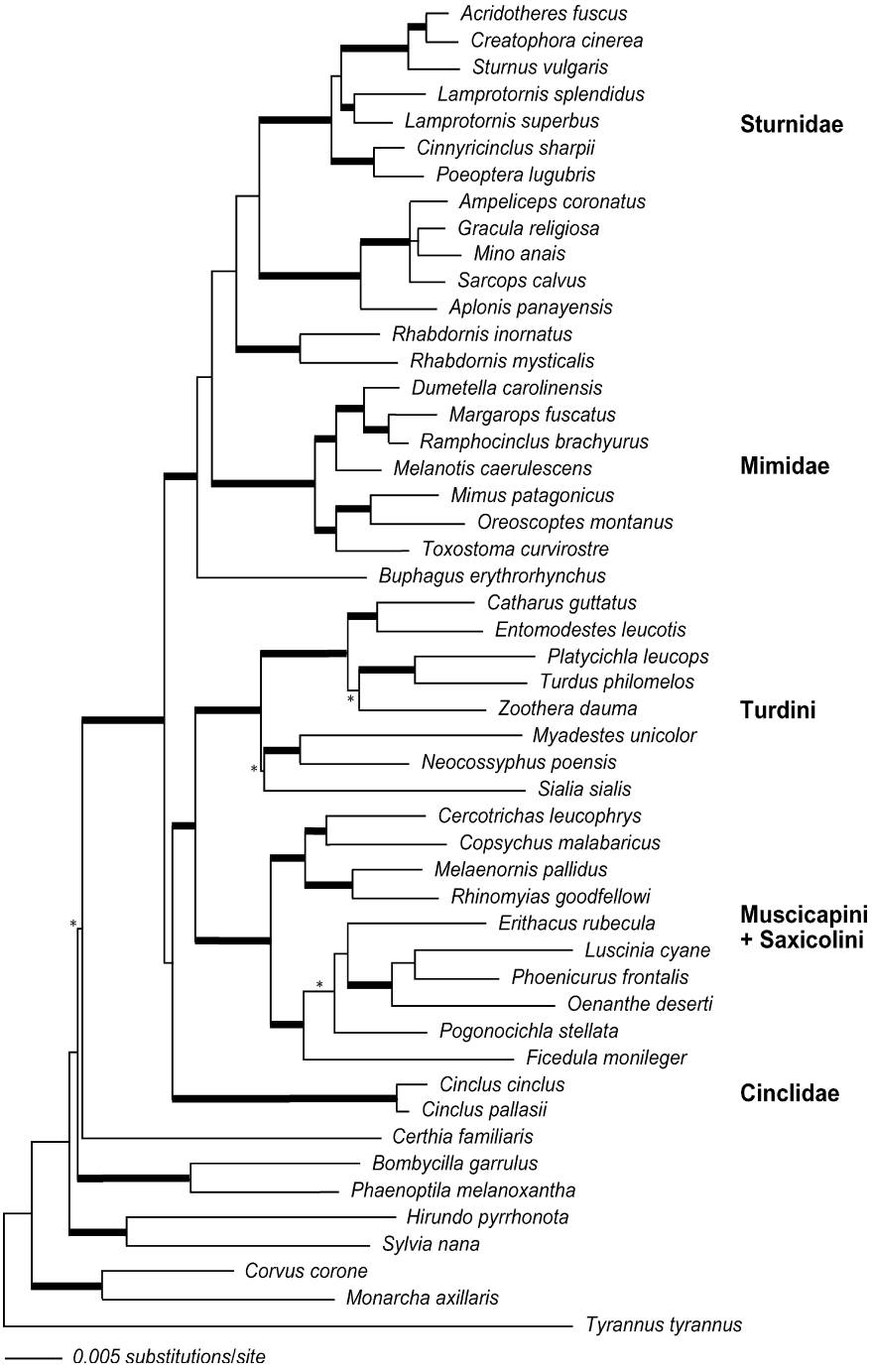

Fig. 3. A maximum-likelihood tree for the Muscicapoidea based on RAG-1 sequences (see text for details). Branch lengths are proportional to the number of substitutions per site. Thick branches indicate clades that were recovered in all Bayesian searches with a posterior probability ⩾95%. The four nodes that differ between the ML tree and MP consensus tree are marked with an asterisk.

Table 1
Ancestral area analyses (Bremer, 1992, 1995) of the Muscicapoidea

Clade	Ancestral area hypothesis	Inferred gains	Inferred losses	G/L
Sturnidae *s.l.*	Africa	4	4	1
	Asia	3	4	0.75
Turdini	Old World	3	4	0.75
	New World	3	2	1.5
Muscicapoidea	Old World	4	2	2
	New World	2	4	0.5

distribution with N-2 degrees of freedom when N is the number of taxa (Felsenstein, 1988). The likelihood of the tree with a molecular clock imposed was significantly worse than the tree without a molecular clock ($P < 0.001$), suggesting rate variation among taxa. This result was seemingly influenced by the very distant outgroup, *T. tyrannus*, for when that taxon was excluded from the analysis, the hypothesis of a molecular clock among the remaining taxa could not be rejected ($P = 0.99$).

The result of Bayesian searches indicated that the likelihood of the trees reached stabilization after approximately 100,000 generations and did not increase afterward. All runs gave the same topology for the ingroup, the only difference being the position of *Certhia* in the first run (50,000 generations) which was not long enough to evaluate the parameters of the trees properly. There was no significant variation in the posterior probabilities among the different runs, and adding the ML topology as a starting tree and/or the ML parameters as priors to the search did not affect the results. Thus, all searches converged to a topology identical to the tree obtained with ML search (Fig. 3), and we consider this to be the best hypothesis for the phylogenetic relationships among the Muscicapoidea given the current data set.

In this phylogenetic tree, the monophyly of the Muscicapoidea, as defined by Barker et al. (2002) and also found by Ericson and Johansson (2003), that is, with the Bombycillidae excluded, is recovered with strong support. Most of the basal nodes are well supported among the Muscicapoidea, except for the position of *Cinclus*, and the relative positions among *Buphagus*, *Rhabdornis*, Sturnidae, and the Mimidae. Within starlings, the relationships among *Ampeliceps*, *Sarcops*, and the clade *Mino* + *Gracula* are unresolved. Among the muscicapids, only a few nodes lack strong support. Although all methods and analyses resulted in the same position for *Buphagus* and *Cinclus*, the AU and SH tests were unable to reject alternative topologies, including, for example, Sibley and Ahlquist's (1990) hypothesis of *Cinclus* being sister-taxon to all other Muscicapoidea (Fig. 1), and Amadon's (1943) hypothesis for the basal position of *Buphagus* among starlings (AU test, $P = 0.43$ and 0.36; SH test $P = 0.41$ and 0.34, for the *Cinclus* and the *Buphagus* hypotheses, respectively).

3.3. Biogeography

Results of the ancestral area analysis for the main clades are presented in Table 1. This analysis indicates that the most probable ancestral component for the Muscicapoidea (Waxwings and allied excluded) is the Old World region. The Sturnidae sensu lato (starlings and mimids, including *Buphagus* and *Rhabdornis*) are suggested to be of African origin, an hypothesis that is strongly dependant of the position of *Buphagus*. The thrushes (Turdini) are suggested to have originated in the New World, with several subsequent returns to the Old World (for *Neocossyphus*, *Zoothera*, and *Turdus*).

4. Discussion

4.1. Phylogenetics

All analyses suggest that the Muscicapoidea are composed of three main groups: the Cinclidae (dippers), the Muscicapidae sensu lato (thrushes and Old World flycatchers), and the Sturnidae sensu lato (starlings and mimids, including *Buphagus* and *Rhabdornis*). Our results agree with those of Barker et al. (2002) in finding that the Bombycillidae and allies lie outside the core Muscicapoidea and that *Cinclus* is the apparent sister-taxon of the thrushes and flycatchers, in contrast to the DNA hybridization findings of Sibley and Ahlquist (1990, Fig. 1). Nevertheless, an alternative topology for the position of *Cinclus* (Sibley and Ahlquist, 1990; Fig. 1) could not be rejected, and resolution of the basal position of the Cinclidae will have to await the accumulation of more data. Thrushes and Old World flycatchers form two main clades, the first one comprising all flycatchers and several small thrushes (the Muscicapini and Saxicolini, respectively, of Sibley and Monroe (1990)), and the second being restricted to the large thrushes (Turdini). The phylogeny of this diverse group will be considered elsewhere with a larger taxon sampling. The genus *Buphagus* (the oxpeckers, two African species) was traditionally placed among starlings (Sturnidae) because it shares with starlings the harsh call and the habit of nesting in holes of trees. Amadon (1943), however, called attention to its strikingly different bill morphology and habits (eating ectoparasites of large

mammals) and placed the genus in a different subfamily, the Buphaginae, as the sister-taxon to all other starlings in his phylogenetic tree of the group. Beecher (1978) noted that oxpeckers, in addition to their divergent jaw musculature, also show a foot morphology that is different from all other starlings. Although the best RAG-1 trees using both MP and ML place *Buphagus* as the sister-taxon to mimids and starlings (including *Rhabdornis*), the alternative hypothesis, in which *Buphagus* is at the base of the starlings, could not be rejected. Nevertheless, this study, comprising the largest data set to date for starlings and muscicapines, suggests that *Rhabdornis* shares a closer relationship with starlings than does *Buphagus*.

The phylogenetic hypothesis for the starlings presented here shows two main clades (Fig. 3), the first for Asian starlings only, and the second for African as well as Asian taxa. The Asian clade includes the closely related mynahs (*Mino*, *Gracula*, *Ampeliceps*) and the Bald Starling (*Sarcops*), which together comprise the sister-group of the genus *Aplonis*. The Jungle Myna (*Acridotheres*) is not closely related to the other mynas but belongs instead with *Sturnus* and *Creatophora*, inside the Afro-Asian clade (Fig. 3). *Acridotheres*, *Sturnus*, and *Creatophora* possess a derived jaw musculature and skull adaptation linked to their prying feeding habit, a particular behavior that is found only in these three starling genera (Beecher, 1978). Their monophyly suggests this behavior arose only once in the family, although it has also evolved in the very distantly related New World orioles and blackbirds, the Icteridae (Beecher, 1951).

Most of the species of the African glossy starlings exhibit spectacularly iridescent plumage, produced by the structure of melanin granules and not by pigmentation (Craig and Hartley, 1985). This group is represented here by four taxa, *Cinnyricinclus*, *Poeptera*, *Lamprotornis splendidus*, and *L. superbus*, the latter being sometimes placed in the genus *Spreo*. Craig (1997) studied the structure of feather melanin granules and other morphological characters for all African starlings (including *Buphagus*), assuming the monophyly of the group. Without more comprehensive taxon sampling it is not possible to compare our results to Craig's, but we found no convincing support for the monophyly of the African glossy starlings given these data (Fig. 3). This portion of the topology has very short branches and low support in every analysis.

The monophyly of the Mimidae is strongly supported. This group is endemic to the New World, and its close relationship with starlings has been suggested on the basis of DNA hybridization (Sibley and Ahlquist, 1990) as well as nuclear sequence data (Barker et al., 2002). Among mimids, the West Indies endemics *Ramphocinclus* and *Margarops* are sister-taxa, closely related to the catbird *Dumetella*, which ranges from North to Central America. This topology agrees with previous phylogenetic relationships of Antillean mimids based on mitochondrial sequence data (Hunt et al., 2001). The Blue Mockingbird *Melanotis* is sister-taxon to the *Dumetella* + West Indies mimid group. Sibley and Ahlquist (1990) suggested that the monotypic Sage Thrasher (*Oreoscoptes*) is more closely related to the mockingbirds (*Mimus*) than to the thrashers (*Toxostoma*), as opposed to traditional classifications that placed the Sage Thrasher near the other thrashers (e.g., Davis and Miller, 1960; Morony et al., 1975). Our analysis based on nuclear sequence data (Fig. 3) agrees with DNA hybridization in finding *Oreoscoptes* closely related to *Mimus*, with *Toxostoma* being the sister-taxon to these two genera.

4.2. Biogeography

The three main groups among the Muscicapoidea, the Cinclidae, Muscicapidae sensu lato, and Sturnidae sensu lato have diversified in both Old and New Worlds (Fig. 4). Voelker (2002) proposed a phylogenetic hypothesis for the five species of *Cinclus* using mitochondrial sequence data that showed North and South American species being more closely related to each other than to those in Eurasia. He proposed an Old World origin for the family followed by dispersion to North and South America (short branches at the base of the *Cinclus* mitochondrial tree, however, led to a failure to reject alternative topologies). Among thrushes and flycatchers (Muscicapidae sensu lato) the Muscicapini–Saxicolini clade is restricted to Eurasia and Africa, whereas the Turdini is comprised of a mix of taxa having Old and New World distributions. Ancestral area analysis suggests a New World origin for the Turdini (Table 1), although this solution may merely reflect the taxon sample of the present study. The position of *Cinclus*, basal to the Muscicapidae sensu lato, supports an Old World origin for the group (Fig. 4). These hypotheses, however, require further investigation using a larger taxon sampling among thrushes and flycatchers. The Mimidae are the New World sister-group to the Old World Sturnidae. The basal position of the African *Buphagus* to the mimid + sturnid clade indicates an African origin for the group as a whole (Table 1), but additional work is needed on starling systematics to assess carefully the phylogenetic relationship of *Buphagus* relative to the starlings.

Groth and Barrowclough (1999) used the divergence between oscine and suboscine taxa (*Passer* and *Tyrannus*) to infer a rate calibration for passerines of 0.13% divergence per My based on RAG-1 sequence data. This rate was used here to estimate roughly the different Old World and New World divergences experienced during the muscicapoid radiation under the hypothesis of a molecular clock (see Section 3). The oldest event is the

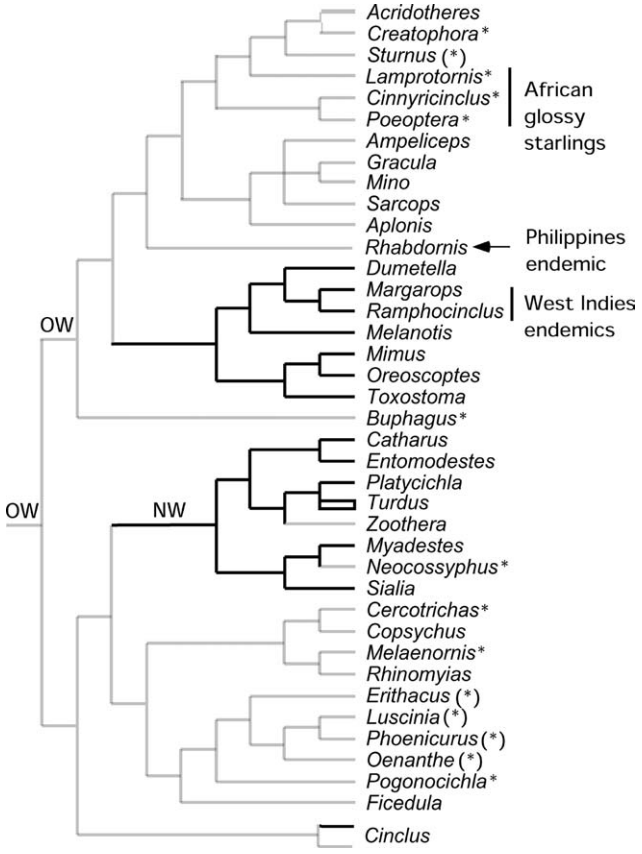

Fig. 4. Distribution and biogeographic reconstruction for the Muscicapoidea. The topology corresponds to the tree presented in Fig. 3. Grey branches indicate an Old World distribution (OW), black branches a New World distribution (NW). The white branch of *Turdus* indicates that this taxon is present in both continents. African endemics are shown with an asterisk, and taxa whose distributions cover both Africa and Asia have an asterisk in parenthesis. The hypothesis of origin for several basal nodes, as suggested by ancestral area analysis (Bremer, 1992, 1995), are mapped on the tree. We followed Voelker's (2002) hypothesis for the biogeography of *Cinclus*.

separation between the Turdini and Muscicapini–Saxicolini, estimated at 30.73 My $\pm$ 3.4, whereas the divergence between the mimids and the starlings dates to 22.54 My $\pm$ 0.22. Barker et al. (submitted) estimated a similar timing for the invasion of mimids to the New World at 24 My $\pm$ 2, based on a larger passerine data set, including the Rag1 and Rag 2 nuclear genes, and using non-parametric rate-smoothing methods (Sanderson, 1997). The divergence between the American and Eurasian dippers is a much more recent event, estimated by Voelker (2002) at 4.13 My on the basis of mitochondrial sequence data. We compared the date estimated for the divergence between *Cinclus pallasii* and *Cinclus cinclus* using nuclear sequence (2.68 My) to the estimation given by Voelker (2002) with mitochondrial genes (2.54 My), and found that the dates were similar. Applying the same rate calibration for the node uniting *Myadestes*, a South American taxon, and *Neocossyphus*, an African endemic, gives an early Miocene estimation (19.83 My).

If an approximate molecular clock holds across all muscicapoid taxa, the results suggest that the different muscicapoid groups did not disperse simultaneously between the Old and New Worlds. Three dispersal events can be postulated from an Old World ancestor to the New World: (1) an early Oligocene dispersal by a thrush (Turdini) ancestor, (2) a late Oligocene-early Miocene dispersal by a mimid ancestor, and (3) a Pliocene dispersal by a dipper ancestor. The analysis of Fig. 4 also suggests that several dispersal events back to the Old World have occurred among the Muscicapoidea, all of which are restricted to Turdini lineages: (1) within the *Turdus-Platycichla* and *Zoothera* clade, and (2) between *Myadestes* and *Neocossyphus*, the later being estimated as an early Miocene event. These results, although preliminary for the large thrush-flycatcher group, indicate that dispersal between the Old World and New World has at least occurred once in every major muscicapoid lineage but at different times. It appears that several reverse dispersals occurred subsequently within Turdini, a result that will have to be tested with a larger sampling of thrushes and flycatchers. Although this study focuses on a single passerine clade, the Musicapoidea, the results pertaining to Old and New World dispersal events are similar to inferences when all passerine clades are examined (Barker et al., submitted). Thus, different lineages did not respond simultaneously to climatic changes that facilitated connections between Afro-Eurasian and American faunas.

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Appendix A

List of species, tissue sample numbers, GenBank Accession numbers, and collection locality for the sequences new to this study

Species	Sample number	GenBank Accession	Collection locality
Acridotheres fuscus	AMNH PRS 693	AY307180	Malaysia
Ampeliceps coronatus	AMNH PRS 1243	AY307181	Captive
Aplonis panayensis	AMNH PRS 692	AY307182	Singapore
Buphagus erythrorhynchus	PFI, NC	AY307183	South Africa
Catharus guttatus	AMNH PRS 1638	AY307184	USA, New York
Cercotrichas leucophrys	AMNH ALP 78	AY307185	Central African Republic
Cinclus pallasii	AMNH PRS 2481	AY307186	Vietnam
Cinnyricinclus sharpii	FMNH 356553	AY307187	Uganda
Copsychus malabaricus	AMNH PRS 2210	AY307188	Vietnam
Creatophora cinerea	LSU B-34297	AY307189	South Africa
Dumetella carolinensis	AMNH PRS 1601	AY319981	USA, New York
Entomodestes leucotis	LSU B-1775	AY307190	Peru
Erithacus rubecula	AMNH PRS 2340	AY307191	United Kingdom
Ficedula monileger	AMNH PRS 2211	AY307192	Vietnam
Gracula religiosa	AMNH PRS 1344	AY307193	Captive
Lamprotornis splendidus	AMNH PRS 2163	AY307194	Central African Republic
Lamprotornis superbus	LSU B22546	AY307195	Captive
Luscinia cyane	AMNH RTC 612	AY307196	Vietnam
Margarops fuscatus	LSU B-11443	AY307197	Puerto Rico
Melaenornis pallidus	AMNH ALP 81	AY307198	Central African Republic
Melanotis caerulescens	FMNH 343277	AY307199	Mexico, Jalisco
Mino anais	FMNH 363230	AY307200	Captive
Myadestes unicolor	AMNH PRS 302	AY319990	Captive
Neocossyphus poensis	AMNH PRS 1988	AY307201	Central African Republic
Oenanthe deserti	AMNH JGG 1140	AY307202	Nepal
Oreoscoptes montanus	LSU B-21368	AY307203	USA, California
Phaenoptila melanoxantha	AMNH PEP 2205	AY307204	Costa Rica
Phoenicurus frontalis	AMNH JGG 1022	AY307205	Nepal
Platycichla leucops	AMNH PRS 845	AY307206	Venezuela
Poeoptera lugubris	AMNH PRS 2126	AY307207	Central African Republic
Pogonocichla stellata	FMNH 355539	AY307208	Uganda
Ramphocinclus brachyurus	LSU B-75	AY307209	Captive
Rhabdornis inornatus	FMNH 357586	AY320000	Philippines, Mindanao
Rhabdornis mysticalis	MZC O 3734	AY307210	Philippines, Luzon
Rhinomyias goodfellowi	FMNH 357498	AY307211	Philippines, Mindanao
Sarcops calvus	MZC O 3806	AY307212	Philippines, Luzon
Sialia sialis	AMNH PRS 991	AY320001	USA, New Jersey
Toxostoma curvirostre	AMNH GFB 1165	AY307213	USA, New Mexico
Turdus philomelos	AMNH PRS 2351	AY307214	United Kingdom
Zoothera dauma	AMNH PRS 2234	AY307215	Vietnam

AMNH: American Museum of Natural History, New York; FMNH: Field Museum of Natural History, Chicago; LSU: Louisiana State University Museum of Natural Science, Baton Rouge; MZC: Museum of Zoology Copenhagen, Denmark; PFI, Percy Fitzpatrick Institute, University of Cape Town, Republic of South Africa; NC, not catalogued.

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