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A molecular phylogenetic analysis of the "true thrushes" (Aves: Turdinae)

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Abstract

The true thrushes (Passeriformes: Muscicapidae, subfamily Turdinae) are a speciose and widespread avian lineage presumed to be of Old World origin. Phylogenetic relationships within this assemblage were investigated using mitochondrial DNA (mtDNA) sequence data that included the cytochrome *b* and ND2 genes. Our ingroup sampling included 54 species representing 17 of 20 putative turdine genera. Phylogenetic trees derived via maximum parsimony and maximum likelihood were largely congruent. Most of the Turdine taxa sampled can be placed into one of six well supported clades. Our data indicate a polyphyletic *Zoothera* which can be divided into at least two (Afro-Asian and Austral-Asian) main clades. The genus *Turdus*, as presently recognized, is paraphyletic but forms a well supported clade with the addition of three mostly monotypic genera (*Platycichla*, *Nesocichla*, and *Cichlherminia*). We identify an exclusively New World clade that includes a monophyletic *Catharus*, *Hylocichla*, *Cichlopsis*, *Entomodestes*, *Ridgwayia*, and *Ixoreus*. Members of the morphologically and behaviorally distinct genera *Sialia*, *Myadestes*, and *Neocossyphus* unexpectedly form a basal clade. Using multiple outgroup choices, we show that this group is distantly related, but unequivocally the sister group to the remaining Turdines sampled. The Turdinae appear to be a relatively old songbird lineage, originating in the mid to late Miocene. If the Turdinae are indeed Old World in origin, our data indicate a minimum of three separate invasions of the New World.

Keywords: Turdinae; True thrushes; Systematics; Biogeography; Cosmopolitan distribution; Bayesian methods

1. Introduction

Although the "true thrushes" (Turdinae, sensu Sibley and Monroe, 1990) are one of the most widespread and well-known "families" of birds in the world, their taxonomic affiliations with other lineages, and relationships among constituent genera have long confounded taxonomists. The root of these issues is a lack of definitive taxonomic characters. Often, representatives of presumably closely related groups "merge imperceptibly through intermediate species from one group to the next" (Hartert, 1910). Although most previous taxonomies have suggested a close relationship with chats (Saxicolini) and Old World flycatchers (Muscicapini), the true thrushes have also been linked historically with groups as diverse as babblers (Timaliinae), gnatcatchers (Polioptilinae), wrens (Troglodytidae), and dippers (Cinclidae) (see Sibley and Ahlquist, 1990 for a thorough taxonomic review, also see Clement, 2000 for color plates and an excellent review of relevant literature). With the advent of molecular systematics, relationships among these major lineages have begun to come into focus. The important DNA–DNA hybridization work of

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Sibley and Ahlquist (1990) resulted in the first classification in which the Turdinae (''true thrushes'') were identified as a distinct taxonomic entity, closely related to the Muscicapinae (including the tribes Saxicolini and Muscicapini). Recent molecular work (Pasquet et al., 1999; Voelker and Spellman, 2004) has improved on this work by more clearly defining generic membership in each of these subfamilies and tribes.

While the ''true thrush'' clade continues to be redefined with improved precision, relationships among its constituent genera remain largely unknown. Few previous studies providing testable phylogenetic hypotheses are available and the lack of defining morphological characters that plague higher taxonomy are also problematic within the subfamily. Voelker and Spellman's (2004) generic level revision of Sibley and Monroe's (1990) Turdinae suggests 147 species organized into 20 genera. Of these 147 species, 101 are lumped into one of two large genera, *Zoothera* (36 spp.) and *Turdus* (65 spp.). Many authors (e.g., Phillips, 1991) acknowledge that these large groups represent taxonomic ''catch-alls'' but due to a lack of distinguishing taxonomic characters, reasonable alternatives are lacking. That improved taxonomy within this group is needed is evidenced by the following statement: ''Our recognition of the genus *Zoothera* for the bar-winged forest thrushes of Africa-rather than Turdus... follows Irwin (1984), although it is to some extent an act of convenience, to reduce to manageable size the large genus *Turdus*'' (Dowsett and Dowsett-Lemaire, 1993, p. 353). Of the remaining 17 genera, 11 represent monotypic forms, most of which exhibit morphological peculiarities that exclude them from inclusion in larger genera. Relationships among these monotypic forms remain poorly understood as evidenced by the lack of consensus regarding their placement within the various available linear taxonomies (e.g., Ripley, 1964; Sibley and Monroe, 1990). In sum, much confusion exists at both higher and lower levels of turdine taxonomy.

In the work reported on here, we use mitochondrial (mtDNA) sequence data from 17 of 20 turdine genera to address a number of specific issues concerning turdine relationships. First, we examine whether genera historically placed within the subfamily Turdinae (= Turdidae of American Ornithologists' Union, 1998) are indeed members of this clade. Second, our sampling allows us to test hypotheses of monophyly for most turdine genera, in particular the speciose *Zoothera* and *Turdus*. Third, we assess the relationships and validity of several problematic (i.e., monotypic) thrush genera, most notably the New World forms *Ixoreus*, *Hylocichla*, and *Ridgwayia* and the Old World genus *Psophocichla*. Fourth, once genera are more clearly defined we reconstruct phylogenetic relationships among them and explore briefly these relationships from a biogeographic and temporal perspective.

2. Materials and methods

2.1. Sampling strategy

Our starting point in attempting to better define the ''true thrushes'' was the taxonomy of Sibley and Monroe (1990), the most recent, comprehensive treatment available. In their classification they include 21 thrush genera in their subfamily Turdinae. According to these authors, the monotypic New World forms *Ixoreus*, *Ridgwayia*, and *Hylocichla* (American Ornithologists' Union, 1998) are merged into either *Zoothera* (the former two) or *Catharus* (the latter). Because the taxonomic placement of these taxa is controversial, we prefer to recognize their potential generic status and refer to them using their monotypic names herein.

A recent higher-level taxonomic revision based on sequence data (Voelker and Spellman, 2004) demonstrated that five of Sibley and Monroe's (1990) putative turdine genera; *Brachypteryx*, *Alethe*, *Myiophonus*, *Pseudocossyphus*, and *Monticola*, are more correctly placed within a revised tribe Saxicolini (Chats). In this same work it was shown that the putative muscicapine (Muscicapini; Old World flycatchers) genus *Cochoa* falls instead within the turdine assemblage. Thus, we began this study with a revised Turdinae comprised of 20 genera (including *Ixoreus*, *Ridgwayia*, and *Hylocichla*) which include 147 species. In this work, 17 of these 20 genera were sampled; at the genus level we were lacking only three monotypic Sulawesi forms (*Heinrichia*, *Geomalia*, and *Cataponera*). Wherever possible, multiple representatives were used for each included genus. In all, 54 (of 147) species were sequenced as part of the Turdinae ingroup (see the Appendix). It should be noted that most of the specimens used in this study were obtained through the ''general collecting'' efforts of the institutions listed in Table 1. A study of this scope would be impossible to achieve by any single researcher or institution.

2.2. Outgroups

The proper selection of outgroup is a critical step in reconstructing phylogenetic trees (Swofford et al., 1996). In most cases, the best outgroup is composed of the taxa (or taxon) that are most closely related to, but not a part of, the ingroup (Smith, 1994; Wheeler, 1990). Unfortunately, the sister group to the ''true thrushes'' is not known with certainty. Sibley and Ahlquist's (1990) ''tapestry'' (based on DNA–DNA hybridization temperatures) indicates a turdine–muscicapine sister relationship; whereas, Voelker and Spellman (2004, in a study based on nuclear and mitochondrial DNA sequences) conclude that a clade comprised of Cinclidae (dippers) and Sturnidae (starlings, mockingbirds, and thrashers) is closest to Turdinae. Due to this ambiguity, we performed a full series of independent

Table 1
Overall and codon position-specific dynamics of the cyt-*b* and ND2 genes for all ingroup taxa

Position	Number of sites	Variable sites	Phylogen. informative	Relative rate	%A	%C	%G	%T	χ^2	Ts/Tv	α
Cyt-b											
All	998	418	359	5.2	28.1	34.0	13.8	24.0	$P = 0.992$	4.0	0.227
1st	333	82	56	3.0	24.0	29.5	24.8	21.8	$P = 0.999$	5.0	0.157
2nd	333	27	16	1.0	20.3	26.4	12.9	40.4	$P = 1.000$	2.4	0.008
3rd	332	307	287	11.4	40.0	46.3	3.5	10.2	$P = 0.977$	7.6	1.422
ND2											
All	1041	594	516	7.0	29.9	35.0	12.0	23.1	$P = 0.998$	7.7	0.342
1st	347	169	133	6.0	33.7	30.0	18.1	18.2	$P = 1.000$	7.3	0.295
2nd	347	84	58	3.0	16.6	34.0	10.6	38.8	$P = 1.000$	13.9	0.163
3rd	347	341	324	12.1	39.3	41.0	7.2	12.4	$P = 0.641$	8.7	2.630

Mean base composition is averaged over all sequences using PAUP*. Transition–transversion ratio (Ts/Tv) values are the average number of changes reconstructed on one of four topologies obtained with all sites having equal weight. Ts/Tv and α values were estimated simultaneously for each partition.

analyses (see below) using either muscicapines or Sturnidae + Cinclidae (in turn) to root our trees. To represent the Muscicapini, we used exemplars of the following genera: *Myiophonus*, *Pseudocossyphus*, *Monticola*, *Brachypteryx*, *Melaeornis*, and *Muscicapa*. The Cinclidae–Sturnidae outgroup included the genera *Cinclus* (dippers), *Toxostoma* (thrashers), *Lamprotornis*, and *Creatophora* (both starlings, see the Appendix for species identifications).

2.3. Laboratory protocols

Total genomic DNA was extracted from tissue (or blood, see the Appendix) samples using a Qiaquick (Qiagen) tissue extraction kit. Overlapping sequence fragments were amplified via polymerase chain reaction (PCR) using various combinations of the following published primers: L14841 and H15299 (Kocher et al., 1989), B3, B4, B5 (Lanyon, 1994), H4A (Harshman, 1996), and L15114, L15609, and H15547 (Edwards et al., 1991) for the cytochrome *b* (cyt-*b*) gene; and, L5215 (Hackett, 1996), L5758, H5776, H5578, and H6313 (Johnson and Sorenson, 1998), and L5758.2 (Voelker, 2002) for ND2. All fragments were amplified in 50 μl reactions under the following conditions: denaturation at 94 °C followed by 40 cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 2 min. This was followed by a 10 min extension at 72 °C and a 4 °C soak. Products were purified using a Qiagen PCR purification kit following the manufacturer's protocols. Standard, 20 μl sequencing reactions were performed using 4 μl of BigDye (ABI) and 20–40 ng of purified and concentrated PCR product. Products of these reactions were purified using Centrisep columns following the manufacturer's protocol, dried in a centrivap concentrator, and run out on Long Ranger (BMA) acrylamide gels with an ABI 377 automated sequencer.

Full complementary strands of each gene were unambiguously aligned using Sequencher 4.1 (GeneCodes). The veracity of the sequence data was supported in several ways. Both light and heavy strands were sequenced for all PCR fragments and many of these fragments were overlapping. No gaps, insertions, or deletions were apparent in the aligned sequences and all data was translated (using MEGA2 version 2.1, Kumar et al., 2001) without problem into amino acid form. The resulting sequences include most of the cyt-*b* gene (998 bp) and all of ND2 gene (1041 bp) for a total of 2039 bp of concatentated sequence data. All of the sequences generated as a part of this study have been deposited in GenBank (Accession Nos. AY752319–AY752402).

2.4. Phylogenetic protocols

Phylogenetic analyses were preceded by data exploration. Using PAUP 4.0b4a (Swofford, 2000), we constructed genetic distance matrices using both inter- and intrageneric pairwise comparisons. The relatively high genetic distances uncovered suggested potential problems due to homoplasy. We addressed this possibility by plotting pairwise comparisons of corrected and uncorrected distances for each codon position for both genes. The evolutionary dynamics of each gene and gene partition (codon position) was investigated using all ingroup taxa. Parameters examined include: Ts/Tv (transition/transversion ratio), relative rates of evolution, percent nucleotide composition, and the gamma shape parameter (α). Because of the large genetic distances apparent within the ingroup we were concerned about the potential affect of nucleotide composition bias on phylogenetic reconstructions. A series of χ^2 tests of homogeneity were conducted on each gene and gene partition using only the informative data. For each of these partitions, we also plotted the relative proportions of each nucleotide for each taxon used (e.g., C vs T and A vs G). Outliers in such plots likely indicate taxa that are problematic with respect to nucleotide composition biases.

Phylogenetic analyses were performed using both likelihood (ML) and parsimony (MP) approaches. We first executed a partition homogeneity test (the incon-

gruence length difference test [ILD] of Farris et al., 1994), in PAUP* to ensure that the data sets for each gene contained congruent phylogenetic signal. This test consisted of 100 replicates and considered only informative characters (Cunningham, 1997). With no significant differences identified ($P = 0.63$), the data were combined for all subsequent analyses (but see Reed and Sperling, 1999). Both weighted and equal-weighted parsimony analyses were conducted. In the former, transitions were downweighted relative to transversions using empirically derived Ts/Tv ratios. Under this weighting scheme, transitions within cyt-*b* were downweighted by 1/3 and within ND2 by 1/5. Independent heuristic MP searches (20 replicate random stepwise additions) were conducted using either a Muscicapini or Cinclidae–Sturnidae outgroup (taxa listed in the Appendix). Support for individual nodes was assessed using MP heuristic bootstrap with 500 pseudoreplicates, each with 10 random addition sequence replicates.

Modeltest 3.04 (Posada and Crandall, 1998) was used to select the most appropriate model of sequence evolution for ML analyses. Hierarchical likelihood ratio tests (LRTs) and the Akaike Information Criterion (AIC) both identified GTR + I + Γ as the model that best fits the combined data, regardless of outgroup choice. When analyzed independently, Modeltest indicated that the GTR + I + Γ model was the most appropriate model for the ND2 data whereas the TVM + I + Γ was selected for cyt-*b*. These models differ only in the number of estimated transition rates (2 rates for GTR, 1 for TVM) used. Because we wanted to use a single model that could be used to generate a comparable ML tree (using PAUP*) and because the difference in these models would likely have a negligible affect on topology, we chose to use the GTR + I + Γ model across all ML analyses.

It is well understood that one of the shortcomings of MP is its inability to detect homoplasy on long branches, a potential source of bias in phylogeny estimation (Felsenstein, 1978; Swofford et al., 1996). Because the model chosen is more resistant to error caused by homoplasy (Kuhner and Felsenstein, 1994; Huelsenbeck, 1995), we decided a priori to consider our likelihood topology as our best estimate of a phylogenetic hypothesis for the true thrushes. Due to the size of our data set, we opted to use the successive approximations approach of Swofford et al. (1996) to obtain a ML estimate of phylogeny. The initial likelihood search was started using a LogDet NJ topology and the parameters indicated by Modeltest. After several days running, additional rearrangements were having a negligible affect on the likelihood score. A subsequent search was initiated using this "improved" topology on which parameters were reoptimized. This process was repeated until the analysis ran to completion. For verification, a second analysis using the ultimate parameters and a starting NJ tree was initiated and run to completion. This procedure was repeated using each of three different outgroups: the Muscicapini, the Cinclidae–Sturnidae, and an outgroup comprised of a *Sialia–Myadestes–Neocossyphus* clade. The rational for using this latter outgroup will be discussed below. Shimodaira and Hasegawa (1999) tests (with the RELL approximation) were used to compare this phylogenetic reconstruction with alternative (traditional) phylogenetic hypotheses.

For another approach using likelihood, we implemented the recently described PHYML program (Guindon and Gascuel, 2003). This program uses a hill-climbing algorithm that adjusts tree topology and branch lengths simultaneously. An advantage of this method is that relatively fewer iterations are required to reach an optimum, resulting in a drastic reduction in required computer time. The GTR + I + Γ model of nucleotide evolution was used along with an initial NJ tree. The program was allowed to estimate parameters, reoptimizing regularly as tree scores improved.

Bayesian inference (Rannala and Yang, 1996) was used primarily as a means of assessing support for nodes obtained via other (ML, MP) tree-building methods. The program Mr Bayes (Huelsenbeck and Ronquist, 2001, Ver. 3.0b4) was implemented and the GTR + I + Γ model of sequence evolution was once again assumed. Specific nucleotide substitution model parameters were left undefined and estimated as part of the analysis. All Bayesian analyses were initiated from random starting trees. Four Markov chain Monte Carlo chains were run for one million generations and sampled every 100 generations, yielding 10,000 trees. The first 100,000 generations (= 1000 trees) were discarded to ensure that chain stationarity had been reached. To ensure that the Markov chain was sampling from the posterior distribution, this procedure was repeated two more times. Because all three runs converged on the same distribution, all trees (excluding those sampled before "burn-in") were combined yielding a total of 27,000 topologies from which a 50% majority rule consensus tree was reconstructed. Nodes having posterior probability values of 95% or greater on this tree were deemed significantly supported (after Huelsenbeck and Ronquist, 2001). For heuristic purposes, we also ran independent, identical sets of Bayesian analyses on data sets that were partitioned either by gene (2 partitions) or by gene-specific codon position (6 partitions).

3. Results

3.1. Sequence characteristics

As expected, the ND2 gene was slightly more variable than cyt-*b* (Table 1). Over the 2039 bp of combined se-

quence, 1012 sites were variable and of these, 875 were phylogenetically informative. Overall, more than 95% of third position sites varied. Multiple substitutions (homoplasy) at these sites were reflected in plots of third position transition distances versus corrected sequence divergences. These results are consistent with the relatively high inter-generic distances exhibited within the turdine clade (Table 2). For cyt-*b*, uncorrected percent sequence divergence ranges from 6.5% between our single exemplar of *Platycichla* (*P. leucops*, Pale-Eyed Thrush) and the monotypic *Nesocichla* (*N. eremita*, Tristan Island Thrush); to 14% between *Sialia* (bluebirds) and the Lesser Antilles endemic *Cichlhermina* (*C. lherminia*, Forest Thrush). Intrageneric cyt-*b* comparisons range from 1.2% among *Entomodestes* species to 10.1% among *Neocossyphus*. Corresponding values from ND2 distances are substantially greater in all comparisons.

Nucleotide composition and bias varies only slightly between these two genes; both display a deficiency of guanine and an excess of cytosine nucleotides. The base composition biases described here are similar to those recovered in other avian studies (e.g., Kornegay et al., 1993; Lovette and Bermingham, 2000). Tests of homogeneity of base frequencies across ingroup taxa were not significant for either gene or any gene (codon) partition (Table 1) and a similar test for both genes combined was also insignificant ($\chi^2_{159} = 55.77$, $P = 1.00$). Although including outgroup taxa did not change this result ($\chi^2_{204} = 80.57$, $P = 1.00$), plots of third position purine and pyrimidine content (Fig. 1) identify a possible nucleotide bias with respect to the Cinclidae–Sturnidae outgroup. Cinclidae, in particular appears to have a fundamentally different base composition for the cyt-*b* gene suggesting that for the combined data set, the Muscicapini may provide a more robust outgroup choice.

Not surprisingly, codon position-specific gamma-shape parameter (α) estimates indicate that among-site rate heterogeneity is a likely problem in this data set. The problem is most acute at cyt-*b* second positions where estimates of α (0.008) are two orders of magnitude lower that at third position sites for either gene (1.422 and 2.630 for cyt-*b* and ND2 respectively). In contrast, the α estimate for ND2 second positions, although low (0.163) was within the range (0.1–0.5, Yang, 1996) typical of gamma-shape parameter estimates.

3.2. Phylogenetic analyses

To illustrate the results held in common using alternative phylogenetic methods, we present a fully resolved weighted parsimony tree and one derived from a successive approximations ML analysis (Fig. 2). Both of these were rooted using ingroup taxa, the *Sialia–Myadestes–Neocossyphus* clade. This was done because all analyses (regardless of method) using either the Muscicapini or Cinclidae–Sturnidae outgroups unequivocally identified

Table 2
Observed intergeneric pairwise genetic distances for both the cyt-*b* (below the diagonal) and ND2 (above the diagonal) genes

Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 *Hylocichla**		0.181	0.169	0.165	0.168	0.176	0.165	0.179	0.168	0.155	0.165	0.173	0.161	0.170	0.185	0.191	0.211	0.199
2 *Psophocichla**	0.111		0.160	0.177	0.162	0.174	0.166	0.181	0.169	0.165	0.168	0.162	0.180	0.166	0.177	0.183	0.203	0.198
3 *Zoothera*	0.109	0.117	***0.099***	0.174	0.159	0.163	0.161	0.178	0.168	0.151	0.162	0.164	0.165	0.162	0.187	0.183	0.201	0.190
4 *Catharus*	0.100	0.111	0.108	***0.074***	0.171	0.173	0.174	0.171	0.159	0.157	0.177	0.170	0.169	0.178	0.196	0.188	0.208	0.198
5 *Cochoa**	0.089	0.096	0.099	0.090		0.127	0.173	0.172	0.161	0.155	0.165	0.171	0.173	0.173	0.199	0.181	0.203	0.187
6 *Chlamydochaera**	0.107	0.110	0.109	0.100	0.074		0.173	0.172	0.164	0.164	0.161	0.163	0.174	0.168	0.209	0.184	0.202	0.201
7 *Cichlherminia**	0.124	0.114	0.121	0.118	0.103	0.112		0.167	0.163	0.151	0.094	0.092	0.159	0.104	0.183	0.187	0.203	0.196
8 *Cichlopsis**	0.114	0.109	0.117	0.111	0.105	0.110	0.132		0.120	0.161	0.178	0.171	0.170	0.175	0.197	0.186	0.207	0.192
9 *Entomodestes*	0.117	0.121	0.112	0.101	0.106	0.109	0.110	0.077	***0.012***	0.148	0.181	0.170	0.165	0.174	0.186	0.171	0.200	0.197
10 *Ixoreus**	0.103	0.110	0.105	0.101	0.093	0.100	0.119	0.111	0.119		0.149	0.153	0.150	0.152	0.169	0.180	0.187	0.182
11 *Nesocichla**	0.114	0.111	0.104	0.100	0.093	0.097	0.074	0.119	0.114	0.097		0.084	0.163	0.097	0.177	0.191	0.198	0.199
12 *Platycichla**	0.128	0.124	0.120	0.116	0.098	0.113	0.075	0.130	0.119	0.112	0.065		0.161	0.099	0.185	0.192	0.201	0.201
13 *Ridgwayia**	0.095	0.112	0.101	0.091	0.085	0.091	0.115	0.108	0.107	0.095	0.101	0.114		0.163	0.184	0.191	0.202	0.191
14 *Turdus*	0.112	0.112	0.113	0.108	0.103	0.109	0.084	0.123	0.113	0.105	0.068	0.073	0.106	***0.075***	0.186	0.189	0.195	0.197
15 *Myadestes**	0.136	0.126	0.119	0.114	0.113	0.106	0.126	0.124	0.117	0.112	0.108	0.127	0.110	0.120		0.184	0.185	0.194
16 *Neocossyphus*	0.129	0.117	0.125	0.123	0.120	0.116	0.127	0.127	0.130	0.119	0.117	0.133	0.117	0.125	0.119	***0.101***	0.190	0.198
17 *Sialia*	0.135	0.134	0.129	0.122	0.125	0.131	0.140	0.134	0.119	0.126	0.125	0.132	0.125	0.130	0.119	0.122	***0.053***	0.201
18 *Muscicapini*	0.134	0.132	0.137	0.136	0.127	0.128	0.143	0.141	0.139	0.128	0.129	0.142	0.131	0.137	0.127	0.137	0.140	***0.115***

All values shown depict uncorrected (*p*) sequence divergence values. Those shown on the diagonal (in bold) represent intrageneric comparisons using only cyt-*b* distances. Taxa with asterisks are represented by a single species.

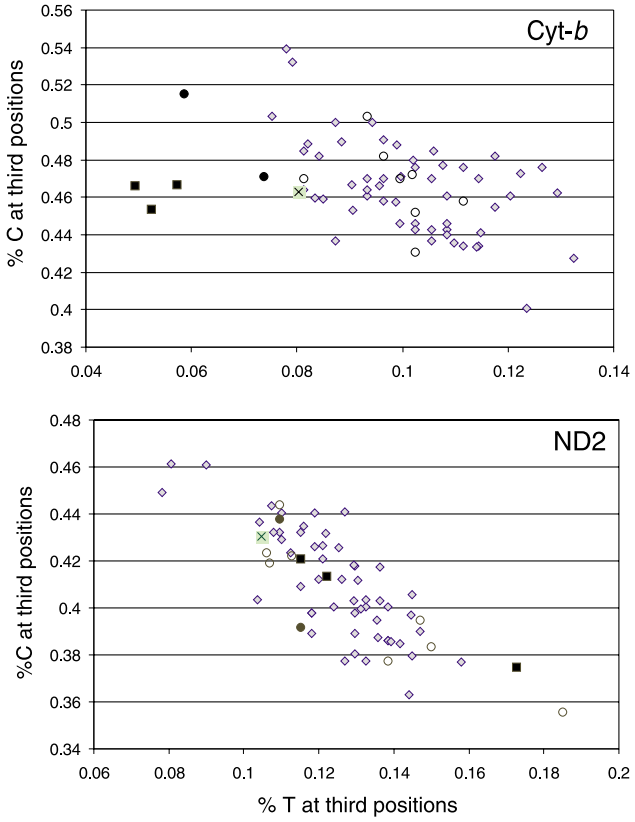

Fig. 1. Plots of relative measures of pyrimidine content at third positions for all taxa. The ingroup (Turdinae) is represented by gray diamonds. Muscicapini (outgroup one, Old World flycatchers) is represented by open circles. Cinclidae–Sturnidae (outgroup two) is depicted by darkened squares (*Cinclus*), darkened circles (*Lamprotornis*, *Creatophora*), and an X (*Toxostoma*). For the Cinclidae–Sturnidae, a qualitatively similar pattern was obtained when purine content was plotted in this way.

this clade as sister to the remaining turdine members. We reasoned that rooting with a distinct subset of the ingroup would contribute the least amount of homoplasy to the data set, thereby providing a more robust analysis. A comparison of the results using different outgroup choices will be discussed below.

The topologies shown are nearly identical with both identifying several well supported larger clades. These include a New World thrush clade comprised of *Catharus*, *Hylocichla*, *Ridgwayia*, *Ixoreus*, *Cichlopsis*, and *Entomodestes* (Fig. 2B, node 7). The genus *Zoothera*, as presently recognized, is polyphyletic with a basal Austral-Asian clade (B3) and a more derived assemblage comprised of African and Asian forms (B12). This latter clade is sister to a paraphyletic genus *Turdus* that includes several monotypic, "robin-like" genera including *Cichlherminia*, *Nesolichla*, and *Platycichla*. At the base of our trees is the root clade comprised of *Sialia*, *Myadestes*, and *Neocossyphus*. Although clearly belonging among the true thrushes, members of this "basal" assemblage are only distantly related to other members of this group.

The MP and ML trees shown differ only in the placement of the taxon pair *Cochoa* and *Chlamydochaera* (whose placement is not well supported by any analytical method), and the shifting placement of terminal taxa in the genera *Catharus* (*C. dryas*, *C. mexicanus*) and *Turdus* (*T. merula*). Other analyses were largely congruent with those shown. A ML tree generated using PHYML differed from the ML tree depicted only in the placement of *T. merula* and *Cichlherminia* of the poorly sampled *Turdus* clade (Fig. 2B, node 6). The various Bayesian analyses yielded a single topology and varied only slightly (a single node) with respect to significant posterior probabilities. This topology was identical to the ML tree depicted except that *Cochoa* and *Chlamydochaera* were placed as sister to the *Turdus* and Afro-Asian *Zoothera* clades as they are in the MP tree.

The results of analyses using alternative outgroup arrangements are summarized in Table 3. Nearly identical trees were obtained regardless of outgroup used, with one important exception. A well supported (via MP, ML, and Bayesian analyses) basal Austral-Asian *Zoothera* clade (Fig. 2B, node 3) was identified when either a *Neocossyphus* (and allies) or Muscicapini outgroup (taxa listed in Table 3) was used. When rooting with a Cinclidae–Sturnidae outgroup, however, Bayesian and ML (but not MP) analyses instead suggest (albeit weakly, posterior probability = 62) a sister relationship between the Austral-Asian *Zoothera* and the New World thrush assemblage (Fig. 2B, node 7). Whether this contradictory result is due to nucleotide composition bias (Fig. 1) or because Cinclidae and Sturnidae are relatively more distantly related (contra Voelker and Spellman, 2004) to the ingroup, is unclear. It does emphasize the importance of exploring various outgroup choices when contemplating phylogenetic reconstructions.

The use of Bayesian posterior probabilities as a means of evaluating node strength has recently come under criticism (e.g., Douady et al., 2003; Erixon et al., 2003; Suzuki et al., 2002). It is well understood that posterior probabilities are usually higher than corresponding non-parametric bootstrap frequencies and this is true for our data (e.g., see Table 3). The former are typically considered too "liberal" while the latter are viewed as conservative. In our data set, we note little discrepancy among these methods if we assume that a bootstrap value of 70% or greater indicates strong support as does a posterior probability of greater than 95%. Under these criteria, 33 well supported nodes are identified on each tree shown in Fig. 2. These trees have 29 supported nodes in common and 36 of 37 nodes identified as "supported" in either analysis occur in both topologies. The single conflicting node occurs in the *Catharus* clade (Fig. 2A) MP tree where a *C. aurantiirostris* and *C. dryas* relationship receives 73% bootstrap support.

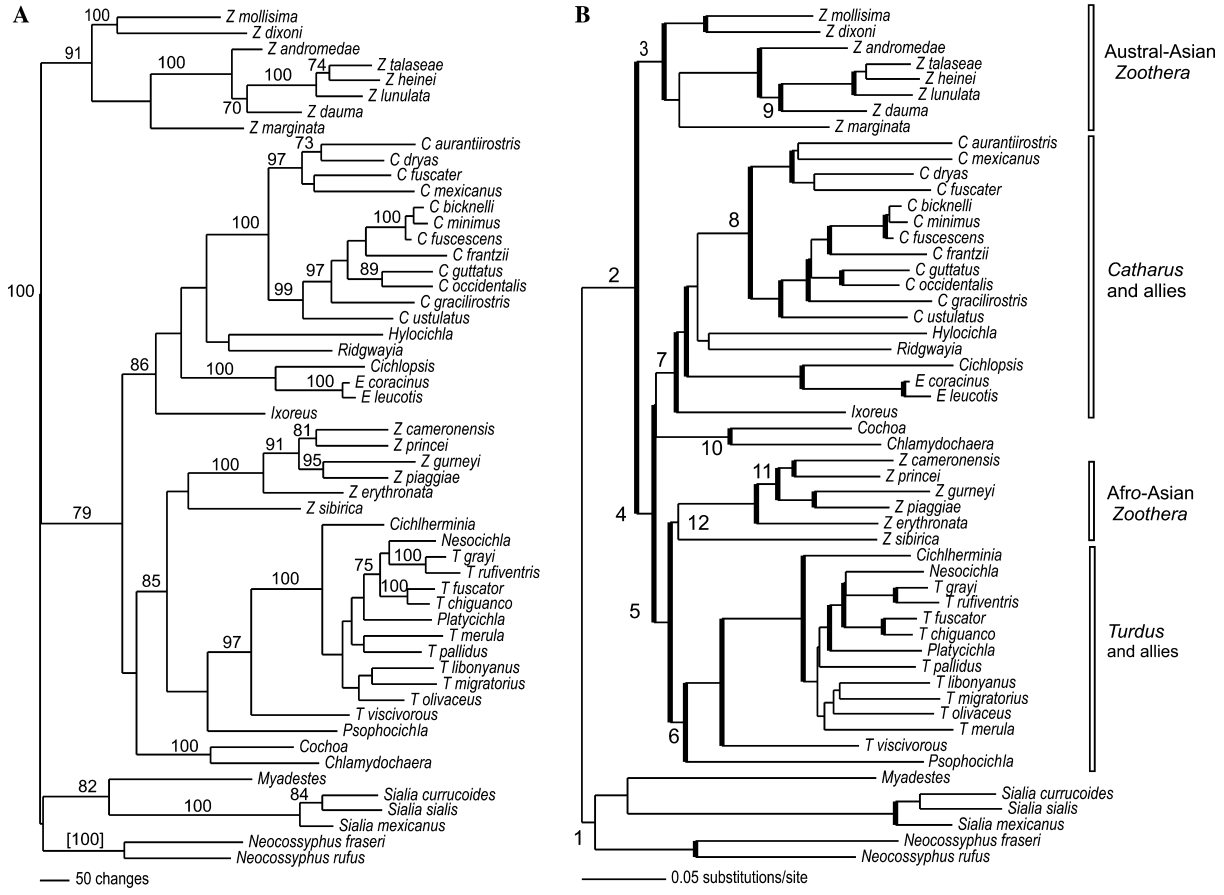

Fig. 2. A maximum parsimony tree (A) for which transversions in cyt-*b* were assigned three times the weight of transitions and transversions in ND2 were given five times the weight of transitions. These Ts/Tv ratios (1:3, 1:5) represent empirical estimates obtained independently from each of these genes. Bootstrap values shown are the result of 500 full heuristic MP search replicates under this weighting scheme. The maximum likelihood tree (B, −Ln length = 27,225.3188) depicted was obtained using a successive approximations approach (see Methods). The GTR + I + Γ model of sequence evolution was used with parameter settings of I = 0.46685 and $\alpha = 1.16759$ (R-matrix available upon request). Branch lengths are scaled to depict the relative numbers of reconstructed changes. Bold highlights indicate those nodes having significant (>95%) Bayesian support. Clades are numbered for reference in the text.

Shimodaira and Hasegawa (1999) tests of competing topologies provide additional support for some of our conclusions (Table 4). The monotypic New World taxa *Ridgwayia* and *Ixoreus* are placed by some authors (e.g., Sibley and Monroe, 1990) in the genus *Zoothera* but making *Ridgwayia* sister to either *Zoothera* clade results in significantly worse topologies ($P = 0.048$, $P = 0.010$). According to our data, constraining *Turdus* to be monophyletic (by removing *Nesocichla*, *Cichlherminia*, and *Platycichla*) also yields a significantly worse tree ($P = 0.001$). Even though many nodes on our tree are well supported we were unable to reject several competing (i.e., traditional) taxonomic arrangements although this is not unexpected given the conservative nature of this test (Goldman et al., 2000). Forcing *Ixoreus* into a basal position of either *Zoothera* clade results in worse topologies, but not significantly so ($P = 0.350$, $P = 0.129$). Similarly, trees in which *Zoothera* was constrained to be monophyletic had worse $-\ln L$ scores but the difference was not significant ($P = 0.120$, $P = 0.109$). The taxonomic position of the monotypic form *Hylocichla* has been the frequent subject of debate (summarized in Winker and Rappole, 1988) with numerous authors placing it within *Catharus*. Our data suggest a *Hylocichla–Ridgwayia* relationship although a *Hylocichla–Catharus* pairing can not be rejected ($P = 0.941$).

4. Discussion

4.1. Systematics overview

In this study, we sampled 37% (54 of 147) of the species that comprise a revised (Voelker and Spellman, 2004) subfamily Turdinae. Nevertheless, we obtained relatively well supported trees that were nearly identical across a variety of analyses. The trees shown (Fig. 2) are well resolved at internal nodes with only the placement of *Z. sibiricus* and the *Cochoa–Chlamydochaera* pairing in question. Less resolution is apparent nearer the tips of branches. This may be an artifact of taxon sampling with

Table 3
A comparison of parsimony bootstrap and Bayesian support values for critical clades using different outgroups

Clades of interest		Outgroups					
		Cinclidae–Sturnidae		Muscicapini		*Necossyphus* and allies	
Clade							
—	Turdinae, all	98	[100]	100	[100]	—	—
2	Turdinae "ingroup"	98	[100]	100	[100]	98	[100]
3	Austral-Asian *Zoothera*	95	[100]	94	[100]	91	[100]
7	*Catharus* and allies	<50	[100]	79	[100]	86	[100]
12	Afro-Asian *Zoothera* (including *Z. sibirica*)	68	[75]	65	[79]	68	[83]
6	*Turdus* and allies	75	[95]	87	[94]	69	[97]
1	Root clade, *Sialia-Myadestes-Neocossyphus*	<50	[99]	<50	[94]	—	—
5	"*Turdus*" (6) plus Afro-Asian *Zoothera* (12)	82	[100]	87	[100]	85	[100]
4	"Ingroup", exclusive of Austral-Asian *Zoothera* (3)	<50	[*]	77	[97]	79	[100]
9	Scaly thrushes (*dauma* group)	72	[81]	64	[89]	70	[94]
8	*Catharus* thrushes	98	[100]	99	[100]	99	[100]
11	African "spot-winged" thrushes	94	[100]	90	[99]	91	[100]
10	*Cochoa-Chlamydochaera*	100	[100]	100	[100]	100	[100]

MP values, depicted on the left side of columns, were obtained from weighted (Tv × 3 for cyt-*b*, Tv × 5 for ND2, see text) analyses. Corresponding Bayesian support values are indicated on right, in brackets. The asterisk indicates that a particular clade was lacking in that analysis. Outgroup memberships as follows: Cinclidae–Sturnidae = *Cinclus mexicanus*, *C. pallasi*, *C. schultzi*, *Toxostoma lecontei*, *Lamprotornis nitens*, and *Creatophora cinerea*; Muscicapini = *Myiophonus caeruleus*, *Pseudocossyphus bensoni*, *Monticola saxatilis*, *Brachypteryx montana*, *Melaenornis ardesiacus*, and *Muscicapa adusta*; *Neocossyphus* and allies = *Neocossyphus fraseri*, *Neocossyphus rufus*, *Sialia currucoides*, *S. mexicanus*, *S. sialis*, and *Myadestes townsendi*.

Table 4
Shimodaira–Hasegawa tests of alternative phylogenetic hypotheses

Constraint	−ln *L*	Δ−ln *L*	*P*
(1) "Best" tree (Fig. 2B)	27225.32		
(2) *Hylocichla* and *Catharus* (8) as sisters	27225.50	0.18	0.992
(3) *Ridgwayia* and Austral-Asian *Zoothera* (3) are sisters	27272.24	46.92	0.048*
(4) *Ridgwayia* and Afro-Asian *Zoothera* (12) are sisters	27285.28	59.96	0.010*
(5) *Ixoreus* and Austral-Asian *Zoothera* (3) are sisters	27246.75	21.43	0.350
(6) *Ixoreus* and Afro-Asian *Zoothera* (12) are sisters	27257.71	32.3 9	0.129
(7) A monophyletic *Zoothera* (move clade 12 to 3), sister to rest of ingroup (4)	27261.05	35.73	0.120
(8) A monophyletic *Zoothera* (move clade 3 to 12), sister to *Turdus*	27262.00	36.68	0.109
(9) *Cochoa–Chlamydochaera* (10) and Afro-Asian *Zoothera* (12) are sisters	27248.59	23.27	0.276
(10) A monophyletic *Turdus*	27328.49	103.17	0.001*

Clade designations (in parentheses) refer to Fig. 2. Likelihoods were obtained using the GTR + I + Γ model of sequence evolution with parameters optimized on the ML tree shown in Fig. 2. Values of $P \leqslant 0.05$ indicate a significantly worse estimate of phylogeny and are marked with an asterisk.

only 10 of 65 putative *Turdus* species and 16 of 36 putative *Zoothera* species having been sampled. Increased resolution in terminal nodes will require more extensive sampling of these two groups in particular. Lanyon (1993) advises that systematists identify both a "best estimate" and a "reliable estimate" of phylogenetic relationships. In that spirit, we consider Fig. 2B to be our best estimate of relationships within the true thrushes and we consider the consensus tree (Fig. 3) as our most reliable (i.e., conservative) estimate of relationships.

4.1.1. Zoothera

Members of the large (36 extant species *fide* Sibley and Monroe, 1990) genus *Zoothera* are thought to represent an older and more primitive thrush radiation, relative to the genus *Turdus* (Urban et al., 1997). All *Zoothera* (or "ground thrush") species share a single morphological character, a striking ("geocichline") under-wing pattern in which the bases of secondary and inner primary feathers are white, contrasting sharply with an otherwise dark under-wing surface (Ripley, 1952). Despite this "unifying" morphological character, there has long been a lack of consensus as to taxonomic boundaries of *Zoothera*. For example, the African "spot-winged" thrushes (represented by *cameronensis*, *princei*, *gurneyi*, and *piaggiae* in this study) have been assigned either to *Zoothera* (Irwin, 1984; Urban et al., 1997) or *Turdus* (Hall and Moreau, 1970). *Ridgwayia*, *Ixoreus*, and *Psophocichla* have also been placed variously within *Turdus* or *Zoothera* (e.g., Ripley, 1952, 1964; Sibley and Monroe, 1990; Urban et al., 1997).

Notable among our findings is the confirmation of polyphyly within this genus, with all trees depicted (Figs. 2 and 3) supporting the existence of multiple *Zoothera* lineages. The African spot-winged thrushes are a well-

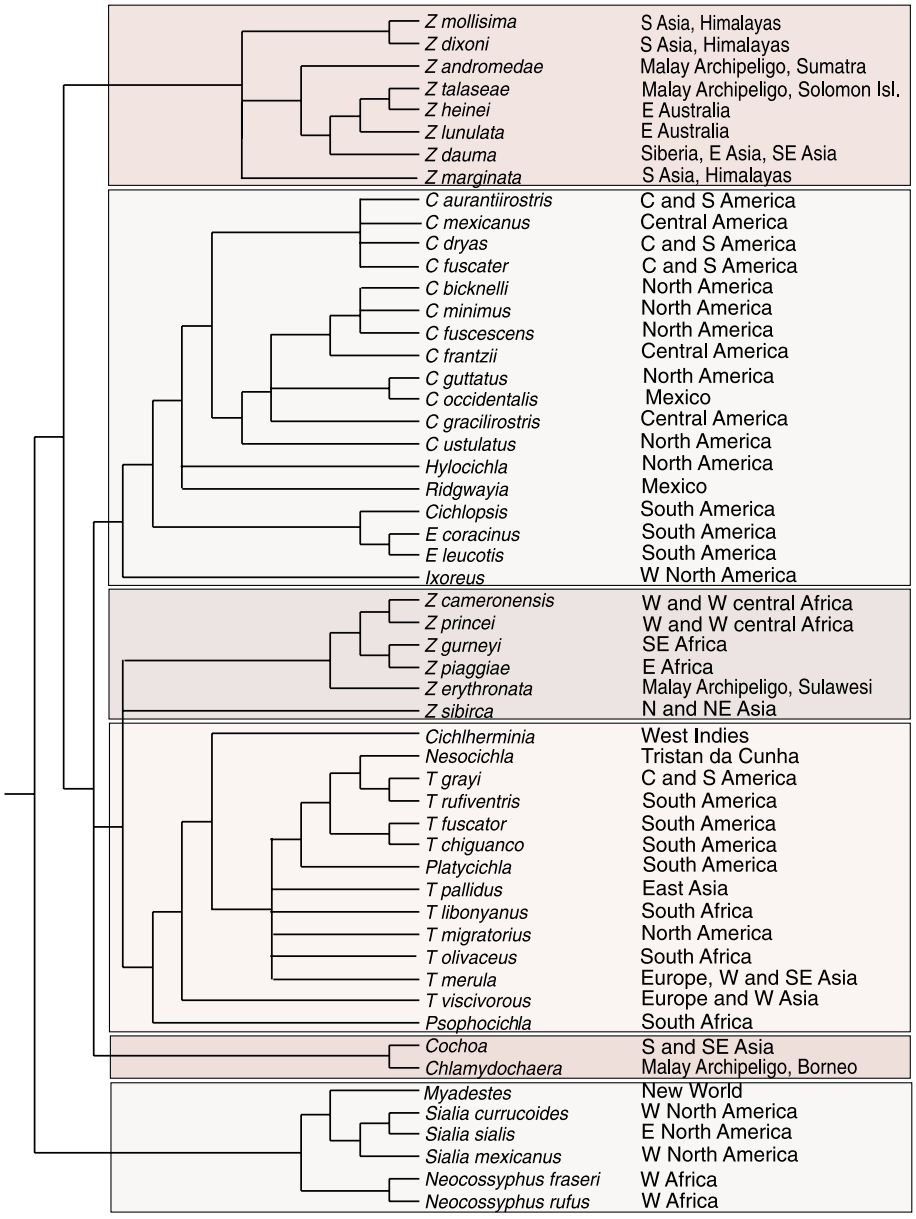

Fig. 3. Consensus tree based on MP, ML, and Bayesian analyses. All weakly supported and conflicting nodes have been collapsed. This tree represents our most "reliable estimate" (Lanyon, 1993) of phylogenetic relationships among the true thrushes. Variable shading identifies the major clades that are discussed in the text.

supported component of the Afro-Asian *Zoothera* clade that is indeed sister to the *Turdus* assemblage. Morphology would suggest that the unsampled African representatives of this group, *crossleyi* and *oberlaenderi* almost certainly belong here. This clade also includes the Indonesian form *Z. erythronotata*, suggesting that the additional Indonesian *Zoothera* having similar pigmentation and wing patterns (e.g., *dumasi*, *interpres*, *dohertyi*, *peronii*, and *citrina*) likely belong in this clade. Our results consistently place *Z. sibirica* in a basal position, distant from other members of the Afro-Asian clade, although support is lacking. Morphologically, *sibirica* seems an unlikely fit with this group and additional sampling of taxa may reveal that its true affinities lie elsewhere. Both *Ixoreus* and *Ridgwayia* are members of a strongly supported North American clade, (discussed below) having no clear affinity with either *Turdus* or *Zoothera.*

The Austral-Asian clade of *Zoothera* thrushes are mostly brown and characterized by having a "scaly" feather appearance on either the ventral or ventral and dorsal surface. The two Australian *Zoothera* (*heinei* and *lunulata*) cluster with the narrowly distributed Pacific Island form *talaseae* and these are embedded within the clade, indicating a relatively recent colonization of Australia. These are part of a morphologically well-defined group including *Z. dauma*, *major*, *horsfieldi*, and *machiki*, all of which likely belong together in this clade.

Indeed, all but *talaseae* have previously been recognized as subspecies of *dauma* (Ripley, 1964). Nearer the base of the Austral-Asian clade are the phenotypically similar Himalayan endemics *molissima*, *dixoni*, and *marginata*. Based on overall morphological, geographical, and ecological similarities, *Z. monticola* (unsampled) probably clusters with these other Himalayan forms.

There remain a number of unsampled Asian *Zoothera* thrushes that are phenotypically distinct from any of the groups sampled or described thus far (e.g., *shistacea*, *guttata*, *spiloptera*, and *wardi*). Sampling of these taxa may identify the presence of additional independent "*Zoothera*" lineages. Regardless, it is clear that the genus *Zoothera*, as it is presently defined, is little more than a taxonomic catch-all. This is due in no small part to a lack of clear, defining characters. The one character that they do all possess, the "geocichline" wing pattern is apparently sympleisiomorphic. We note that the same pattern (without the stark contrast) also occurs within representatives of the thrush genera *Myadestes*, *Sialia*, and *Catharus*. Taxonomy has also been confounded by a lack of geographic structuring within the group. The African spot-winged thrushes have long been considered a clade but their affinity with Asian and Indonesian forms was not suspected. Even with our limited sampling, it is clear that the true thrushes as a group have been highly vagile, thus complicating an interpretation of relationships.

4.1.2. Catharus and allies

Excepting those taxa in the *Turdus* and "basal" (i.e., *Sialia*, *Myadestes*) assemblages, all other New World thrushes are combined in this strongly supported clade. This result was not predicted in any previous linear taxonomy of the group. Ripley (1952) indicated that *Catharus* was nearest to *Turdus*, that *Ridgwayia* and *Ixoreus* belonged among the *Zoothera*, and that the South American solitaires *Cichlopsis* and *Entomodestes* were lumped in with the *Myadestes* solitaires which he excluded entirely from the true thrushes. Sibley and Monroe (1990) did suggest a relationship between *Cichlopsis–Entomodestes* and *Catharus*, placing them between *Myadestes* and *Turdus*.

Our results indicate that *Turdus* is only distantly related to members of this New World clade. On morphological grounds, the linking of *Cichlopsis–Entomodestes* and *Myadestes* would seem appropriate but they clearly represent independent lineages that converged upon the "solitaire" phenotype. Despite having the morphological appearance of *Zoothera*, both *Ixoreus* and *Ridgwayia* are placed in this New World clade. Surprisingly, they are not sister taxa, with *Ixoreus* more distantly related to other clade members while *Ridgwayia* is a distant (13.0% uncorrected distance overall) but sister taxon to *Hylocichla*. *Ixoreus* and *Ridgwayi* have evidently retained elements of the ancestral morphology (e.g., "geocichline" wing pattern, overall shape, and plumage patterns) that others in this clade have not. The monotypic form *Hylocichla* has a checkered taxonomic history, being shuttled back and forth between *Turdus* and *Catharus* (see thorough review in Winker and Rappole, 1988). In an allozyme study, Avise et al. (1980) noted that "phenetically and cladistically" *Hylocichla* was aligned with *Catharus* and in subsequent classifications it has either been lumped with *Catharus* (Sibley and Monroe, 1990) or it retains monotypic status (American Ornithologists' Union, 1998). The data that we present could be used to support either placement. The genus *Catharus* was the focus of a recent systematic study (Outlaw et al., 2003). We note that our results and theirs are nearly the same, differing only at nodes that are unsupported in either work.

4.1.3. Turdus and allies

Having sampled only 10 of 65 putative *Turdus* taxa, we can draw few conclusions concerning relationships within this group. This clade is well supported and is sister to the Afro-Asian *Zoothera* clade. Where *Turdus* begins taxonomically awaits a more comprehensive sampling of taxa (Voelker, in prep.). In our trees, *Psophocichla* and *T. viscivorus* lie far outside the remaining *Turdus* assemblage. Neither of these taxa are especially turdine in appearance, both looking rather like plump *Catharus*. *Psophocichla*, with plumage traits characteristic of some *Zoothera* (*spiloptera*, *guttata*) and *Turdus* (*viscivorus*, *philomelos*, and *mupinensis*) species, has historically been placed in either of these genera (e.g., Hall and Moreau, 1970; Irwin, 1984; Ripley, 1964). The morphological intermediacy of *Psophocichla* is consistent with its placement in our toplogy, being a basal lineage within "*Turdus*" and near the Afro-Asian *Zoothera* clade. It has been suggested that the resemblance of *Psophocichla* to *Turdus* taxa (i.e., *viscivorus*, *philomelos*, and *mupinensis*) is superficial and due to convergence (Urban et al., 1997). Our data instead indicate a phylogenetic basis for this similarity. On these grounds, we predict that these similarly plumaged *Turdus* thrushes are likely to also fall out near the base of the *Turdus* clade.

Due to inadequate sampling, nodes at the interior of the Turdus clade, not unexpectedly, lack support. Our results do however, allow us to offer comment on the relative placement of a few obscure and problematic thrush genera. On the basis of minor morphological differences, the South American *Platycichla* forms (2 species) have been placed in their own genus (Goodwin, 1957) although they are similar overall in appearance to other *Turdus* members. This is not the case for the monotypic forms *Nesocichla* and *Cichlherminia*. Like *Psophocichla*, the morphological peculiarities exhibited have left taxonomists little room to assign them to existing genera,

and they were subsequently given monotypic status for lack of a better solution. Interpretation of morphological characters is further confounded by the fact that both of these forms occur only on islands. In his "evolutionary tree," Ripley (1952) represents *Nesocichla* and *Cichlherminia* as nubs on the terminal branch leading to a *Catharus–Turdus* dichotomy. In the linear taxonomy of Sibley and Monroe (1990) they are placed between *Zoothera* and *Myadestes*. Clearly, a consensus is lacking. While we can not define precisely the relationships of *Cichlherminia*, *Nesocichla*, and *Platycichla* with these data, we do show that all are best considered members of the genus *Turdus*.

4.1.4. Cochoa and Chlamydochaera

Chlamydochaera (monotypic) was, for most of its history, placed within the Old World tribe Oriolini. Syringeal morphology (Ames, 1975) and DNA–DNA hybridization (Ahlquist et al., 1984) evidence supported its inclusion among the true thrushes although establishing affinities within that group was problematic. Only recently (Voelker and Spellman, 2004) was it discovered that *Cochoa* (four species), traditionally placed among the Muscicapini (Sibley and Monroe, 1990) belongs instead among the true thrushes. All *Chlamydochaera* and *Cochoa* species are frugivorous with striking and boldly patterned plumages. This study clearly indicates that these are long-separated (10.2% uncorrected overall genetic distance) sister genera although their placement within the true thrushes remains equivocal. Evidently, this clade represents the remaining members of a group that diverged from other thrush lineages relatively early in turdine history.

4.1.5. The "basal" assemblage

The genera *Neocossyphus*, *Sialia*, and *Myadestes* have long been recognized as being unique among taxa grouped within the true thrushes. *Neocossyphus*, for example, is lacking the turdine syringeal morphology (Ames, 1975) and juveniles lack the spotted plumage that is characteristic of most young thrushes. Myadestes species also possess a distinctive non-turdine syrinx (Ripley, 1962) leading some authors to propose erecting a separate subfamily Myadestinae (Olson, 1989; Pasquet et al., 1999) for these distinctive forms. Given their behavior and morphology, Ripley (1952) concluded that *Sialia* most resembled a "redstart" (genus *Erithacus*, Saxicolini) and he omitted all three of these basal genera from his classification of the "true thrushes." Sibley and Monroe (1990) include them in their subfamily Turdinae although their placement is equivocal. In their linear taxonomy, a relationship between *Sialia* and *Myadestes* is inferred but *Neocossyphus* is placed at the beginning among non-turdine taxa (see Voelker and Spellman, 2004) such as *Pseudocossyphus*, *Monticola*, and *Myiophonus*. Recent molecular work (Pasquet et al., 1999) suggests a *Myadestes–Neocossyphus* relationship although *Sialia* was not included.

In this study, *Neocossyphus*, *Myadestes*, and *Sialia* form a clade in all analyses that included more distant outgroups (trees not shown). A Bayesian analysis rooted with a Muscicapini clade yielded a posterior probability of 94% for the ancestral node whereas a 99% Bayesian probability was recovered using a Cinclidae–Sturnidae root. Despite this apparent relationship, we note that none of these three genera appear to be close relatives. With an average corrected sequence divergence of around 18% among groups (Table 5), these taxa have had very long histories independent of one another. To our knowledge, the trees shown are the first to depict relationships among all members of the genus *Sialia*. *S. sialis* (Eastern Bluebird) and *S. mexicanus* (Western Bluebird) have historically been considered a closely related sister taxon pair (e.g., Mengel, 1970). Because they are morphologically similar, known to hybridize, and have broad, parapatric east-west distributions, this pair has figured prominently in the development of models of songbird evolution (Mengel, 1970; see Klicka and Zink, 1997). Our data indicate that *S. currucoides* and *S. sialis* are sister taxa, exclusive of *S. mexicanus*. The long history and small number of species in the *Sialia* clade suggest that this is a relict group. Despite the ancient

Table 5
A matrix of K2-P (Kimura, 1980), α-corrected cyt-*b* distances estimates, derived following the protocol of Fleischer et al. (1998)

Clade	1	2	3	4	5	6	7
1 Austral-Asian *Zoothera* [1] (0.111)							
2 *Catharus* and allies [2] (0.123)	0.154						
3 Afro-Asian *Zoothera* [3] (0.131)	0.162	0.164					
4 *Turdus* and allies [4] (0.110)	0.167	0.163	0.173				
5 *Cochoa* and *Chlamydochaera* (0.096)	0.147	0.135	0.151	0.152			
6 *Neocossyphus* (0.148)	0.183	0.190	0.209	0.191	0.175		
7 *Sialia* (0.065)	0.187	0.185	0.211	0.202	0.194	0.182	
8 *Myadestes*	0.175	0.173	0.185	0.182	0.158	0.181	0.178

The α value (0.227) used was taken from Table 1. Within clade distances are shown below clade designations; among clade distances are below the diagonal. Numbers in brackets refer to clades identified in Fig. 2B.

history we note that the extant members of this group are all of relatively recent origin with a corrected within-clade sequence divergence of 6.5% (Table 5), the lowest value reported for any of the clades recovered.

4.2. Biogeography and the timing of divergences

The greatest number and complexity of turdine forms occur in the Old World (Ripley, 1952) and most workers today suggest a likely Asian origin for the group (e.g., Clement, 2000; Ripley, 1952). Present day thrush distributions, taken together with our topology (Fig. 3) are consistent with this interpretation although we consider our taxon sampling too incomplete to allow a formal analysis. We note that southern and southeastern Asian distributions occur in four of the five interior (i.e., excluding the "basal assemblage") clades, figuring prominently in three of these, suggesting a critical role for this region in the evolution of modern day thrush distributions. Basal nodes in our topology suggest an early occupation of at least three continents by ancestral thrush forms; Africa (proto-*Neocossyphus*), North America (proto-*Myadestes*/*Sialia*), and the ancestor to all remaining thrush taxa (node 2, Fig. 2B). The latter, and most speciose thrush clade, has the Austral-Asian *Zoothera* a clade in a basal position with subsequent divergence in the New World (*Catharus* and allies) and in Africa (Afro-Asian *Zoothera*) indicated.

With respect to New World thrushes (*Catharus* and allies), Ripley (1952) postulated three or four independent invasions from Old World thrush stock. According to Ripley, a colonization by the island forms *Cichlherminia* and *Nesocichla* was followed by a radiation within *Catharus*. A third invasion brought the North American "*Zoothera*" forms (*Ridgwayia* and *Ixoreus*) followed by "a multitude of true thrushes of the genus *Turdus*." At least three separate New World colonizations are apparent in our study but not in the way that Ripley envisioned. According to our topology, early in thrush-history, a *Myadestes*–*Sialia* ancestor gave rise to these two New World genera. Later, the New World clade (*Catharus* and allies, Fig. 2B) arose, followed by at least one colonization of the New World by *Turdus*. Whether multiple independent *Turdus* invasions might have occurred is currently under investigation (Voelker, in prep.).

Incomplete taxon sampling, extraordinarily large genetic distances, and a rejection of the molecular clock assumption for this data set precludes a thorough discussion of the timing of divergence events in the history of the true thrushes. Accordingly, we will only discuss divergence times briefly and in most general terms. The genetic distances shown (Tables 2 and 5) are consistent with those of a relatively old (and perhaps taxonomically undersplit) assemblage. Using the clock methodology of Fleischer et al. (1998; Kimura 2-parameter, gamma corrected cyt-*b* data only [Table 5] and a rate of 1.6% corrected sequence divergence per MY) we estimate that the ancestor of *Necossyphus*, *Sialia*, and *Myadestes* split from proto-thrush stock in the mid-to-late Miocene some 11 MYA. The remaining major clades of thrushes (Fig. 3) arose within a relatively short span of time around one MY later. *Turdus* (with *Cichlherminia* as the basal taxon) and *Catharus* radiations occurred nearer the end of the Miocene, 7–8 MYA. According to the distance data, the three extant *Sialia* species have the most recent origin of those taxa sampled, diverging from a proto-*Sialia* ancestor during the mid-Pliocene.

4.3. Taxonomic implications

Among the Old World clades examined, *Zoothera* and *Turdus* are polyphyletic and paraphyletic respectively. Clearly taxonomic changes are required for these taxa but we think it most appropriate that additional sampling be done before formal recommendations are made. With respect to the distinctive basal assemblage (*Neocossyphus*, *Myadestes*, and *Sialia*), our data are consistent with the recognition of these as members of a proposed subfamily Myadestinae (Olson, 1989), a more "primitive" group that is sister taxon to the true thrushes, Turdinae (Pasquet et al., 1999). However, the subfamily as proposed does not include *Sialia* and the correct taxonomic placement of several additional putative members (*Modulatrix*, *Pinarornis*) is unresolved. Our results unequivocally indicate that *Neocossyphus*, *Myadestes*, and *Sialia* do fall out within the Turdinae and until more data become available we suggest that they be recognized as basal members of this assemblage.

The New World clade of *Catharus* and allies is the only one for which sampling is complete at the species level; therefore, we restrict our formal taxonomic recommendations to these taxa. In some current taxonomies (e.g., Sibley and Monroe, 1990), *Ixoreus* and *Ridgwayia* (*fide* American Ornithologists' Union, 1998, this manuscript) are placed within the *Zoothera*. We favor the retention of monotypic status for both of these forms as well as for the genus *Hylocichla*. According to this study, all are members of a New World radiation and all have long branches with no close extant relatives. The fact that these represent relatively distinct taxonomic entities on long, independent evolutionary trajectories is best reflected by them having unique names. The genera *Cichlopsis* and *Entomodestes*, when viewed from within the context of the overall phylogeny, are relatively similar both morphologically and genetically. Collectively, they form a well supported "solitaire" clade. Although we recognize that the merging of monophyletic higher taxon groups is subjective, we advocate the merging of *Cichlopsis* into *Entomodestes* (which has priority; Ripley, 1964).

5. Conclusion

Higher level molecular studies (Pasquet et al., 1999; Voelker and Spellman, 2004) support the earlier contention of Sibley and Ahlquist (1990) that the Muscicapidae is composed of two distinctive subfamilies, the Muscicapinae (muscicapine flycatchers and chats) and the Turdinae (true thrushes). Membership within the latter is becoming clear, as are relationships among the constituent genera. There *is* a well-supported true thrush clade and within it, a New World clade comprised of five genera (our taxonomy) has emerged. *Zoothera* as presently recognized is polyphyletic and *Turdus*, although paraphyletic, appears to be a well supported clade. Among the taxa sampled, only the generic-level placement of *Z. sibirica* and a *Cochoa–Chlamydochaera* clade are unresolved. Although relationships among the true thrushes are coming into focus, additional changes are likely as obscure and problematic taxa are added to the tree. The three monotypic, true thrush taxa (*fide* Sibley and Monroe, 1990), *Catponera*, *Geomalia*, and *Heinrichia* remain unsampled. Also worthy of examination are a suite of "non-thrush" taxa that have previously been considered true thrushes including *Modulatrix*, *Arcanator*, *Grandala*, and *Pinarornis*.

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Appendix

Species used, sample source, and locality information

Taxon	Sample source[a,b]	Collecting locality
Neocossyphus fraseri	FMNH 391727	Uganda: Masindi, Budongo Forest
Neocossyphus rufus	FMNH 389378	Gabon: Minvoul, 31 km ESE
Zoothera cameronensis	FMNH 391736	Uganda: Masindi, Budongo Forest
Zoothera dauma	FMNH 358376	Philippines: Sibuyan
Zoothera gurneyi	FMNH 356762	Tanzania: Tanga, Korogwe District
Zoothera piaggiae	FMNH 355649	Uganda: Nyabitaba, 10 km NW Ibanda
Zoothera talasea	MSP 064	Papua New Guinea: West New Britain Province
Zoothera heinei	CSIRO 46857	Australia: New South Wales
Zoothera lunulata	CSIRO 29221	Australia: New South Wales
Zoothera princei	AMNH 832142	Central African Republic: Sangha-Mbare Prefecture
Zoothera erythronata	AMNH 833602	Sulawesi: Bangai
Zoothera marginata	AMNH 25557 (skel.)	Vietnam: Quang Nam Province
Zoothera mollisima	MSUZM	Vietnam: Lao Cai Province
Zoothera dixoni	MSUZM	Vietnam: Lao Cai Province
Zoothera andromedae	CMNH 37010	Philippines: Negros Island
Zoothera sibirica	MSUZM	Vietnam: Lao Cai Province
Ridgwayia pinicola	BMNH 25591 (skin)	Mexico: Oaxaca
Ixoreus naevia	BMNH 42283	USA: Minnesota, Mille Lacs County
Nesocichla eremita	PF 464706*	Tristan da Cunha Islands
Cichlherminia lherminieri	STRI DO-CLH1	Dominican Republic
Sialia currucoides	MBM 5654	USA: Nevada, Clark County
Sialia mexicanus	BMNH JK95092	USA: Oregon, Wasco County
Sialia sialis	BMNH JK97041	USA: Minnesota, Clearwater County
Myadestes townsendi	MBM 5645	USA: Utah, Garfield County
Cichlopsis leucogenys	STRI EC-CLE11769	Ecuador: Esmeraldas Province
Entomodestes leucotis	STRI PU-ELE599	Peru: Puno Department
Entomodestes coracinus	ANSP 766	Ecuador: Carchi Province
Catharus dryas	MVZ 169692	Peru: Departamento Cajamarca
Catharus frantzii	LSUMNS B-28222	Panama: Chiriqui Province
Catharus fuscater	LSUMNS B-10003	Bolivia: Departamento LaPaz
Catharus mexicanus	MBM 7224	Honduras: Departamento Copan

Appendix (*continued*)

Taxon	Sample source[a,b]	Collecting locality
Catharus gracilirostris	LSUMNS B-2830	Panama
Catharus minimus	SUNY G769*	USA: New York
Catharus occidentalis	SFSU 97N4116*	Mexico: Jalisco
Catharus bicknelli	SUNY1531 48268*	USA: Vermont
Catharus fuscescens	SUNY V209*	USA: New York
Catharus guttatus	MVZ 177246	USA: California
Catharus aurantiirostris	MBM 6639	Honduras: Departamento Copan
Catharus ustulatus	CAS 596	USA: California
Hylocichla mustelina	MBM 6227	USA: Louisiana, Cameron Parish
Platycichla leucops	COP NK4-110291*	Ecuador: Loja Province
Psophocichla litsipsirupa	MBM 5853	South Africa: Northwest Province
Turdus chiguanco	MBM 5431	Argentina: Jujuy Province
Turdus fuscator	LSUMNS B-7678	Peru: Huanuco Department
Turdus grayi	MBM 6620	Honduras: Departamento Copan
Turdus libonyanus	UWBM 52923	South Africa: KwaZulu/Natal Province
Turdus merula	LSUMNS B-1335	Denmark: Vestjaelland County
Turdus migratorius	MBM 5137	Colorado: Las Animas County
Turdus olivaceous	MBM 5877	South Africa: Orange Free State
Turdus pallidus	UWBM 51130	Russia: Primorskiy Kray
Turdus rufiventris	LSUMNS B-25910	Paraguay: Caaguazu Department
Turdus viscivorus	UWBM 57249	Russia: Moscovskaya Oblast'
Chlamydochaera jefferyi	LSUMNS B-36481	Malaysia: Sabah
Cochoa viridis	AMNH 25555 (skel.)	Vietnam: Quang Nam Province
Myiophonus caeruleus	AMNH23244	Nepal: Betrabati
Pseudocossyphus bensoni	FMNH 396194	Madagascar: Fianarantsoa
Monticola saxatilis	UWBM 46533	Kazakhstan: Almaty Oblysy
Brachypteryx montana	FMNH 396295	Philippines: Luzon
Melaenornis ardesiacus	FMNH 385192	Uganda: Kisoro
Muscicapa adusta	MBM 7455	South Africa: W. Cape Province
Cinclus mexicanus	MBM 5778	USA: Nevada, White Pine County
Cinclus pallasi	UWBM 51144	Russia: Primorskiy Kray
Cinclus schultzi	MBM 6912	Argentina: Tucuman Province
Toxostoma lecontei	MBM 6000	USA: Nevada, Clark County
Lamprotornis nitens	MBM 8225	South Africa: Northwest Province
Creatophora cinerea	MBM 5948	South Africa: Orange Free State

[a] Museum tissue sources, abbreviations as follows: FMNH, Field Museum of Natural History; MSP, University of Wisconsin Museum of Zoology; CSIRO, Australian National Wildlife Collection; AMNH, American Museum of Natural History; MSUZM, Moscow State University Zoological Museum; BMNH, Bell Museum of Natural History; PF, Percy Fitzpatrick Institute, Cape Town; STRI, Smithsonian Tropical Research Institute; MBM, Marjorie Barrick Museum of Natural History; ANSP, Academy of Natural Sciences, Philadelphia; MVZ, Museum of Vertebrate Zoology; LSUMNS, Louisiana State University Museum of Natural Science; UWBM, University of Washington Burke Museum; SUNY, State Universities of New York, Syracuse and Albany; COP, Copenhagen Museum; CAS, California Academy of Science.

[b] Asterisks indicate those samples for which blood was used as mtDNA source.

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