Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 41 (2006) 333–344
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

# Nuclear and mitochondrial sequence data reveal the major lineages of starlings, mynas and related taxa

Dario Zuccon [a,b,*], Alice Cibois [c], Eric Pasquet [d,e], Per G.P. Ericson [b]

[a] *Dipartimento di Biologia Animale e dell'Uomo, Università di Torino, Via Accademia Albertina 13, 10123 Torino, Italy*
[b] *Department of Vertebrate Zoology and Molecular Systematics Laboratory, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden*
[c] *Department of Mammalogy and Ornithology, Natural History Museum, CP 6434, CH-1211 Geneva 6, Switzerland*
[d] *UMR5202, Origine, Structure et Evolution de la Biodiversité, Département Systématique et Evolution, Muséum National d'Histoire Naturelle, 55 rue Buffon, 75005 Paris, France*
[e] *Service Commun de Systématique Moléculaire, IFR CNRS 101, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75005 Paris, France*

Received 31 December 2005; revised 22 April 2006; accepted 11 May 2006
Available online 19 May 2006

**Abstract**

We investigated the phylogenetic relationships among the major lineages of the avian family Sturnidae and their placement within the Muscicapoidea clade using two nuclear (RAG-1 and myoglobin) and one mitochondrial gene (ND2). Among Muscicapoidea, we recovered three clades corresponding to the families Cinclidae, Muscicapidae and Sturnidae (*sensu* [Sibley, C.G., Monroe Jr., B.L., 1990. Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven, CT]). Within the sturnoid lineage Mimini and Sturnini are sister groups, with *Buphagus* basal to them. We identified three major lineages of starlings: the Philippine endemic genus *Rhabdornis*, an Oriental-Australasian clade (genera *Scissirostrum*, *Gracula*, *Mino*, *Ampeliceps*, *Sarcops*, *Aplonis*), and an Afrotropical-Palaearctic clade (all African taxa, *Sturnus* and *Acridotheres*). We discuss the biogeographic implications of our findings and suggest an Asiatic origin for this family. The congruence between the age of major clades, estimated by NPRS, and palaeoclimatic data present evidence for the role of climatic changes in shaping present day distribution of the group.


*Keywords:* Sturnidae; Phylogeny; RAG-1; Myoglobin intron 2; ND2

## 1. Introduction

Most recent studies of passerine relationships suggest that this group began to diversify already during the Cretaceous (Barker et al., 2002, 2004; Ericson et al., 2002). Despite this long time for diversification the passerines show a remarkable anatomical uniformity, except for structures adapted for feeding and locomotion (e.g. Irestedt et al., 2004). Similar morphologies resulting from the occupation of the same ecological niches by representatives of different phylogenetic lineages of passerines have been notoriously difficult to separate from similarities due to a recent common ancestry. This has led avian taxonomists to group many taxa that are now known to be unrelated (cf. Sibley and Ahlquist, 1990).

The starlings (Sturnini *sensu* [Sibley and Monroe, 1990]) constitute one example of a group in which the internal affinities are misunderstood due to several cases of morphological convergence. The starlings are distributed in the Old World (one species has been introduced in the New World). They are small to medium-sized birds, robustly built, with quite long wings, strong legs and feet and usually a short tail. The sexes are alike and the young are usually rather different from the adults (Feare and Craig, 1998; Fry and Keith, 2000). It has been a long practice to associate starlings with crows and allies (Corvidae),

* Corresponding author. Fax: +46 8 51954212.
*E-mail address:* dario.zuccon@nrm.se (D. Zuccon).

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.05.007

icterids (Icteridae), or weaverbirds (Ploceidae), because of their bulky body shape, strong bill and often dark/black glossy plumage (Gray, 1840, 1870; Bonaparte, 1850–1857; Sharpe, 1890, 1891, 1909; Wetmore, 1930; Mayr and Greenway, 1956; Voous, 1977). A relationship with corvids was dismissed by Bock (1962) since starlings like other non-corvidian passerines have two fossae in the proximal end of the humerus, not a single fossa as in corvids.

Based on analyses of DNA–DNA hybridization data, Sibley and Ahlquist (1990) recognised three major lineages among oscine passerines. One of them, the superfamily Muscicapoidea, comprises four families; Bombycillidae (waxwings, silky flycatchers, palm chat), Cinclidae (dippers), Muscicapidae (thrushes in subfamily Turdinae, Old World flycatchers and chats in subfamily Muscicapinae), and Sturnidae. Sturnidae was further divided into the tribes Sturnini (starlings) and Mimini (mockingbirds). The latter result was especially surprising, although a starling–mockingbird relationship had been proposed earlier based on morphology (Beecher, 1953), serological complement fixation (Stallcup, 1961) and egg-white protein similarities (Sibley, 1970). Although all higher-level relationships of passerines suggested by Sibley and Ahlquist (1990) have not been supported by subsequent studies (Harshman, 1994; Sheldon and Gill, 1996; Ericson and Johansson, 2003; Barker et al., 2004), the monophyly of a "core Muscicapoidea" (i.e. Sibley's group waxwings and allies excepted) is now generally accepted.

However, many internal relationships in Muscicapoidea are less clear and phylogenetic hypotheses differ depending on which dataset is analysed. It has proven especially difficult to determine the sister group of Sturnidae and the position of Cinclidae. Nuclear datasets (Cibois and Cracraft, 2004; Barker et al., 2004; Beresford et al., 2005) agree with the results based on DNA–DNA hybridization data in placing Sturnidae as sister to a Cinclidae–Muscicapidae clade but the position of Bombycillidae, basal to all other Muscicapoidea, is supported only in a few analyses in Barker et al. (2004). Analysis of a combination of mitochondrial and nuclear data instead suggests that Cinclidae and Sturnidae are sisters and that they are most closely related to Turdinae (Voelker and Spellman, 2004). According to this dataset Muscicapinae belong to a separate clade, whereas the Bombycillidae are not part of Muscicapoidea at all (Voelker and Spellman, 2004).

The tribe Sturnini is a rather well-defined taxon, including 114 extant and 5 extinct species in 29 genera (Sibley and Monroe, 1990). Starlings occur in Africa, Eurasia, across the Wallacea to New Guinea and in most Pacific islands, but only marginally in Australia. There are two main biodiversity centers in Sturnini: in the sub-saharan Africa and in the Oriental-Australasian regions (Feare and Craig, 1998). Most recent studies used an insufficient taxon sampling to properly address intergeneric relationships and the starling diversification is poorly understood. Conflicting hypotheses in the literature concern for example the postulated relationship between *Scissirostrum* and *Buphagus* (Beecher, 1978), whether *Sturnus–Acridotheres* are the sister group of *Lamprotornis–Cinnyricinclus* or actually nested within it (Sibley and Ahlquist, 1990; Cibois and Cracraft, 2004), and the proposed monophyly of the Afrotropical taxa (Craig, 1997).

Several enigmatic taxa have been allied with the starlings in the past, like the bush-crow *Zavattariornis stresemanni* and the huia *Heterolocha acutirostris*. Both these have been shown to belong to the corvid assemblage (Barker et al., 2004; Ericson et al., 2005). Recently the Philippine genus *Rhabdornis*, a taxon never thought to be close to starlings, was identified as the sister group of Sturnidae (Barker et al., 2004; Cibois and Cracraft, 2004). These poorly known, small arboreal birds are found only in the Philippines where they are rather common in lowland and montane forests. *Rhabdornis* has a vague resemblance to *Certhia* creepers and has accordingly often been placed in Certhiidae (e.g. Sharpe, 1903).

In the present paper we use a combination of nuclear and mitochondrial genes to investigate the evolutionary and biogeographic history of the starlings. We also aim at determine which taxa should be included in the family and to calculate the age of the diversification of the major lineages in Sturnidae. The role of climate changes in driving the diversification of the family will also be explored.

## 2. Materials and methods

### 2.1. Taxon sampling strategy

Taxon sampling in this study was designed in order to: (1) test the monophyly of the starlings (Sturnidae *sensu* [Sibley and Monroe, 1990]) and the general placement of this group within the Muscicapoidea clade, and (2) identify the major lineages within the starling clade. The dataset consists of 49 taxa (Table 1). The ingroup comprises 29 starling species, representing all major morphological and ecological types. Two species of *Rhabdornis* have been included, following the findings of Cibois and Cracraft (2004) and Barker et al. (2004). Other members of the Muscicapoidea included in the analysis are representatives of mockingbirds (Mimini, six species), thrushes, chats and flycatchers (Muscicapidae, five species), and dippers (Cinclidae, one species). As outgroup were chose *Bombycilla* (Bombycillidae) following the results of Sibley and Ahlquist (1990), as well as *Regulus* (Regulidae), *Troglodytes*, *Polioptila*, *Certhia* (Certhiidae), and *Sitta* (Sittidae) following Voelker and Spellman (2004) and Barker et al. (2004).

In seven cases the composite nucleotide sequences obtained from more than one individual were used in the analysis. In all cases the two species were congeneric. For example, the RAG-1 sequence for the nuthatch is from *Sitta pygmaea*, while the myoglobin and ND2 sequences are from an individual of *Sitta europaea*. We do not believe this will affect the results in any significant way since all the

Table 1
A summary of all samples and sequences included in this study, with museum accession numbers and collection localities

| Taxon | Accession number | Locality | RAG-1 | Myoglobin | ND2 |
|---|---|---|---|---|---|
| **Bombycillidae** | | | | | |
| *Bombycilla garrulus* | NRM 986044 | Sweden | AY056981 [1]* | AY228286 [4] | DQ466855 |
| **Cinclidae** | | | | | |
| *Cinclus cinclus* | NRM 20016138 | Sweden | AY056985 [1]* | AY228291 [4] | DQ146344 [10]* |
| **Muscicapidae** | | | | | |
| *Catharus guttatus* | NRM 20016341 | United States | AY307184 [2]* | DQ466820 | AY049524 [7]* |
| *Turdus philomelos* | NRM 20036737 | Sweden | AY307214 [2]* | DQ466848 | DQ466886 |
| *Ficedula hypoleuca* | NRM 976132 | Sweden | DQ466798 | AY228300 [4] | DQ146345 [10]* |
| *Erithacus rubecula* | NRM 976377 | Sweden | AY307191 [2]* | AY228296 [4] | DQ466861 |
| *Copsychus malabaricus* | NRM 20036774 | Vietnam | AY307188 [2]* | DQ466823 | DQ466859 |
| **Sturnidae Sturnini** | | | | | |
| *Aplonis tabuensis* | UWBM 42907 | Tufuwai, Tonga Islands | DQ466796 | DQ466818 | DQ466854 |
| *Aplonis grandis* | AMNH MKL-65 | Isabel Island, Solomons | DQ466795 | DQ466816 | DQ466852 |
| *Aplonis panayensis* | AMNH PRS692 | Singapore | AY307182 [2] | DQ466817 | DQ466853 |
| *Poeoptera lugubris* | AMNH PRS2126 | Central African Republic | AY307207 [2] | DQ466834 | DQ466872 |
| *Onychognathus morio* | UWBM 71314 | South Africa | DQ466802 | DQ466831 | DQ466869 |
| *Onychognathus tenuirostris* | FMNH 356555 | Uganda | DQ466803 | DQ466832 | DQ466870 |
| *Lamprotornis corruscus* | ZMUC 119501 | Kenya | DQ466801 | DQ466828 | DQ466865 |
| *Lamprotornis chalybaeus* | ZMUC 117680 | Kenya | DQ466800 | DQ466827 | DQ466864 |
| *Lamprotornis splendidus* | AMNH PRS2163 | Central African Republic | AY307194 [2] | DQ466829 | DQ466866 |
| *Lamprotornis caudatus* | FMNH 396797 | Ghana | DQ466799 | DQ466826 | DQ466863 |
| *Cinnyricinclus sharpii* | FMNH 356553 | Uganda | AY307187 [2] | DQ466833 | DQ466871 |
| *Cinnyricinclus leucogaster* | UWBM 72577 | Malawi | DQ466797 | DQ466822 | DQ466858 |
| *Spreo fischeri* | UMMZ T-1873 | Captivity, UK | DQ466807 | DQ466842 | DQ466880 |
| *Spreo bicolor* | UWBM 52795 | South Africa | DQ466806 | DQ466841 | DQ466879 |
| *Saroglossa aurata* | FMNH 384699 | Madagascar | DQ466804 | DQ466839 | DQ466876 |
| *Creatophora cinerea* | UWBM 70373 | South Africa | AY307189 [2]* | DQ466824 | DQ466860 |
| *Sturnus sinensis* | NRM 20036882 | Beijing Bird Market, China | DQ466810 | DQ466845 | DQ466883 |
| *Sturnus vulgaris* | NRM 966615 | Sweden | DQ466812 | AY228322 [4] | DQ146346 [10]* |
| *Sturnus unicolor* | ZMUC 119334 | Spain | DQ466811 | DQ466846 | DQ466884 |
| *Sturnus cineraceus* | UWBM 59925 | Mongolia | DQ466808 | DQ466843 | DQ466881 |
| *Sturnus nigricollis* | NRM VNM2002-067 | Vietnam | DQ466809 | DQ466844 | DQ466882 |
| *Acridotheres tristis* | AMNH PRS701 | Singapore | DQ466794 | DQ466814 | DQ466850 |
| *Acridotheres fuscus* | AMNH PRS693 | Malaysia | AY307180 [2] | DQ466813 | DQ466849 |
| *Ampeliceps coronatus* | AMNH PRS1243 | Captivity, US | AY307181 [2] | DQ466815 | DQ466851 |
| *Mino anais* | FMNH 363230 | Captivity | AY307200 [2] | DQ466830 | DQ466868 |
| *Sarcops calvus* | ZMUC 119595 | Philippines | AY307212 [2] | DQ466838 | DQ466875 |
| *Gracula religiosa* | AMNH PRSL344 | Captivity, US | AY307193 [2] | DQ466825 | DQ466862 |
| *Scissirostrum dubium* | ZMUC uncat. | Captivity | DQ466805 | DQ466840 | DQ466877 |
| *Buphagus erythrorhynchus* | A. Helbig uncat. | Unknown | AY307183 [2]* | DQ466819 | DQ466856 |
| **Sturnidae Mimini** | | | | | |
| *Dumetella carolinensis* | | | AY319981 [2]* | AF140878 [5]* | AF140890 [5]* |
| *Melanotis caerulescens* | | | AY307199 [2]* | AF140882 [5]* | AF140894 [5]* |
| *Mimus saturninus* | NRM 966912 | Paraguay | | AY228304 [4] | DQ466867 |
| *Mimus patagonicus* | | | AY057005 [1]* | | |
| *Toxostoma curvirostre* | | | AY307213 [2]* | | |
| *Toxostoma redivivum* | NRM 20016345 | United States | | DQ466847 | DQ466885 |
| *Ramphocinclus brachyurus* | | | AY307209 [2]* | AF140886 [5]* | AF140898 [5]* |
| *Margarops fuscatus* | | | AY307197 [2]* | AF140879 [5]* | AF140891 [5]* |
| **Sittidae** | | | | | |
| *Sitta europaea* | NRM 976163 | Sweden | | AY064257 [6] | DQ466878 |
| *Sitta pygmaea* | | | AY057030 [1]* | | |
| **Certhiidae** | | | | | |
| *Certhia familiaris* | NRM 976184 | Sweden | AY056983 [1]* | DQ466821 | DQ466857 |
| *Troglodytes troglodytes* | | | | AY228325 [4]* | |
| *Troglodytes aedon* | | | AY057038 [1]* | | AY460233 [8]* |
| *Polioptila caerulea* | | | AY443320 [3]* | | AY329446 [9]* |
| *Polioptila dumicola* | | | | AY228317 [4]* | |

(*continued on next page*)

Table 1 (*continued*)

| Taxon | Accession number | Locality | RAG-1 | Myoglobin | ND2 |
|---|---|---|---|---|---|
| **Regulidae** | | | | | |
| *Regulus regulus* | NRM 20016439 | Sweden | | DQ466835 | |
| *Regulus calendula* | | | AY057028 [1]* | | AY329435 [9]* |
| **Sylviidae** | | | | | |
| *Rhabdornis mystacalis* | ZMUC 119523 | Philippines | AY307210 [2] | DQ466837 | DQ466874 |
| *Rhabdornis inornatus* | ZMUC uncat. | Philippines | AY320000 [2]* | DQ466836 | DQ466873 |

Published sequences used in this study are listed together with their GenBank accession numbers and references. Museum acronyms: AMNH, American Museum of Natural History; FMNH, Field Museum of Natural History, Chicago; NRM, Swedish Museum of Natural History; UMMZ, University of Michigan, Museum of Zoology; UWBM, Burke Museum, University of Seattle; ZMUC, Zoological Museum, University of Copenhagen. [1], Barker et al. (2002); [2], Cibois and Cracraft (2004); [3], Barker et al. (2004); [4], Ericson and Johansson (2003); [5], Hunt et al. (2001); [6], Ericson et al. (2002); [7], available in Genbank but unpublished; [8], Drovetski et al. (2004); [9], Voelker and Spellman (2004); [10], Fuchs et al. (2006); *, published sequences obtained from samples different from those included in this study.

species in the species-pairs are closely related and with a minimal genetic distance compared to those between other taxa in the dataset. Furthermore, composite sequences were only used for outgroup taxa.

### 2.2. DNA isolation and sequencing

Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen), according to the manufacturer's protocol. The mitochondrial NADH dehydrogenase II (ND2) gene and two nuclear genes, the protein coding RAG-1 and the intron 2 of the myoglobin, were analysed. RAG-1 and myoglobin intron 2 were amplified and sequenced using standard primers and amplification profiles as described in Barker et al. (2002), Irestedt et al. (2002), Cibois and Cracraft (2004). ND2 was amplified as a single fragment using primers L5219 and H6313 (Sorenson et al., 1999), and sequenced also with the newly designed internal primers ND2-intL (5′-TGR ATR GGV CTN AAY CAR AC-3′) and ND2-intH (5′-CCY ARR TGR RGA RAT RGA TGA-3′). PCR products were cleaned using QIAquick PCR Purification Kit (Qiagen). Sequencing reactions were electrophoresed on an ABI 377 or ABI 3100 automated sequencer (Applied Biosciences, Perkin–Elmer).

GenBank accession numbers are listed in Table 1. The DNA sequences were aligned with DNASTAR and, for myoglobin only, adjusted manually.

### 2.3. Gene characterization and phylogenetic analyses

Protein coding regions were translated into amino acids using the appropriate codes in order to detect the possible amplification of pseudogenes. No unexpected stop codons or unusual aminoacidic substitutions were observed, however.

Since heterogeneity in base composition is known to affect phylogenetic inference (Lockhart et al., 1994; Yang and Roberts, 1995), variation among sequences in base pair composition was examined. Base composition homogeneity was tested using a $\chi^2$ analysis of base frequencies across taxa, examining each codon position separately for the coding genes. This test does not correct for phylogenetic relationships among taxa, but should be conservative, since phylogenetic autocorrelation will reduce the probability of detecting the effect of shifts in base composition (Barker, 2004).

Genetic distances and the number of transitions and transversions were evaluated with MEGA version 2.1 (Kumar et al., 2001). For each gene, levels of sequences saturation were investigated empirically by plotting the number of transition substitutions against the number of transversions. In order to identify saturation levels in specific partitions of ND2 the same analysis were performed for each codon position separately.

The genes were analysed independently and combined under the parsimony, likelihood and Bayesian criteria. Maximum parsimony (MP) and maximum-likelihood (ML) analyses were conducted using PAUP* (v4.0b10; Swofford, 1998).

Parsimony analyses were performed with all characters weighted equal or, for ND2 sequences, applying a weight to third codon positions. Gaps in the alignment were treated as 5th base. All searches were heuristic, using the tree-bisection and reconnection (TBR) branch-swapping algorithm, with 1000 random additions of taxa. Branch supports were evaluated using 1000 bootstrap replicates.

Analyses under the maximum-likelihood criterion were conducted using a heuristic search, TBR branch-swapping and 100 random additions of taxa. Models for nucleotide substitutions were evaluated with ModelTest 3.06 (Posada and Crandall, 1998). Nodal supports were estimated using 100 bootstrap replicates with model parameters optimized for each replicate, and initial trees obtained by random addition of taxa. To reduce computer-time the bootstrap values were estimated with TreeFinder (ver. April 2004, Jobb et al., 2004), instead with PAUP*. In cases when a maximum-likelihood bootstrap analysis was performed with both PAUP* and TreeFinder the results were found to be identical.

The likelihood ratio test (Felsenstein, 1981) was used to test if the data adhere to a molecular clock. In case of hypothesis rejection a non-parametric procedure was

applied. Relative nodal ages were established by non-parametric rate smoothing (NPRS, Sanderson, 1997). Rate smoothing of the optimized tree was performed with TreeEdit (Rambaut and Charleston, 2002). Bootstrap estimates of the relative divergences were derived by rate smoothing of 100 pseudoreplicate datasets (Efron and Tibshirani, 1993), and were used to estimate 95% confidence intervals. The smoothed molecular clock tree was calibrated with the split between Sturnini and Mimini (Fig. 3). Barker et al. (2004) used two methods to estimate this split, resulting in two similar values: $20 \pm 2.8$ and $22 \pm 2.0$ mya (mean $\pm$ SE), for NPRS and penalised likelihood, respectively. From Barker et al.'s data we recalculated the two corresponding 95% confidence intervals, 20.4–19.6 and 22.5–21.5 mya, respectively. We used these two values to generate two calibrated trees. For each tree node we obtained two 95% confidence intervals, one derived from the 20 mya calibration, and one from the 22 mya calibration. For a more conservative temporal estimate, for each node we selected the more extreme values of the two intervals and combined them in an extended confidence interval.

Bayesian inference analyses were performed with MrBayes 3.01 (Ronquist and Huelsenbeck, 2003). The models of nucleotide substitutions used in the analyses were chosen using MrModelTest 2.0 (Nylander, 2004). The Bayesian analyses were conducted with uniform interval priors, except for base frequencies which were assigned a Dirichlet prior, Metropolis coupling with four incrementally heated Markov chains ($MC^3$, default heating parameter). Chains were run for $5 \times 10^6$ generations with trees sampled every 100 generation. The log-likelihood of each chain was evaluated as a function of generation number, in order to determine the minimum number of generations to discard from the beginning of the chain as 'burn-in'. Posterior estimates of model parameters and taxon bipartitions were derived from the complete sample of chains minus those discarded as burn-in. Two runs from random starting points were performed for each dataset, and the equilibrium log-likelihood, parameter values, and bipartition frequencies of each run were compared to evaluate the stability of posterior estimates.

All figures were drawn using MrEnt 1.2 (Zuccon and Zuccon, 2006).

Congruence of phylogenetic estimates from the individual and combined datasets was evaluated qualitatively and quantitatively. Significant node incongruence was inferred where conflicting nodes were recovered in separate analyses. Only nodes receiving a support higher than 70% for bootstrap (Hillis and Bull, 1993) and 95% for the Bayesian posterior probabilities (Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999) were taken into consideration in the comparisons between phylogenetic trees. Alternative a priori phylogenetic hypotheses were compared using the Shimodaira–Hasegawa (SH) test statistic (Shimodaira and Hasegawa, 1999), as implemented in PAUP*, with a fully optimized resampling-estimated log-likelihood (option Fullopt) and 1000 bootstrap replicates.

## 3. Results

### 3.1. Gene properties

The final alignment of the three concatenated genes is 4670 bp long. A summary of molecular properties of each gene is shown in Table 2. The proportions of potentially informative characters are 13%, 19% and 53% in RAG-1, myoglobin, and ND2, respectively, but they decrease to 10%, 14% and 50% when only ingroup taxa are considered. Base compositions are more A and C skewed in the mitochondrial ND2 gene than in the nuclear genes. A $\chi^2$ analysis of base frequencies across taxa could not reject a null hypothesis of homogeneity in all three genes ($P = 0.98$ or higher). But a $\chi^2$ analysis of base frequencies for each codon of ND2 gene recovered a significant non-homogeneity for the 3rd codon position (1st codon: $\chi^2 = 30.59$, $df = 144$, $P = 1.00$; 2nd codon: $\chi^2 = 9.76$, $df = 144$, $P = 1.00$; 3rd codon: $\chi^2 = 287.84$, $df = 144$, $P < 0.001$).

Saturation seems to be absent in RAG-1 and myoglobin: the observed number of pairwise transitions and transversions are linearly correlated in both genes (data not shown). However, the corresponding graph for the ND2 sequences suggest this gene to be saturated, but an analysis by codon shows that saturation is restricted to the third codon. Consequently, third positions have been excluded in MP analyses.

ModelTest and MrModelTest both suggested the GTR+Γ+I evolutionary model for the combined dataset and RAG-1 and ND2 alone. For myoglobin TVM+Γ and GTR+Γ were selected by ModelTest and MrModelTest, respectively. The GTR+Γ+I model was applied in the ML analysis and in the BI of the combined dataset.

### 3.2. Phylogenetic results

The MP analysis for the combined dataset recovered four most parsimonious trees (3378 steps). The strict consensus tree calculated from these is shown in Fig. 1.

Table 2
Sequence characteristics of RAG-1, myoglobin, and ND2 genes

| Gene region | RAG-1 | Myoglobin | ND2 |
|---|---|---|---|
| Number of bases | 2875 | 754 | 1044 |
| Number of variable bases (%) | 782 (27%) | 302 (40%) | 629 (60%) |
| Number of parsimony informative bases (%) | 378 (13%) | 141 (19%) | 550 (53%) |
| % A nucleotides (range) | 31.3 (31.7–29.9) | 28.7 (27.1–29.1) | 30.1 (27.8–33.2) |
| % C (range) | 20.9 (22.6–20.1) | 21.7 (20.5–22.9) | 35.1 (29.9–37.1) |
| % G (range) | 24.2 (23.9–25.6) | 23.0 (21.8–24.2) | 11.9 (9.8–13.8) |
| % T (range) | 23.6 (24.3–21.8) | 26.6 (25.4–27.4) | 22.9 (20.5–26.3) |
| $\chi^2$ ($df = 144$) | 23.85 ($P = 1.00$) | 12.34 ($P = 1.00$) | 111.42 ($P = 0.98$) |

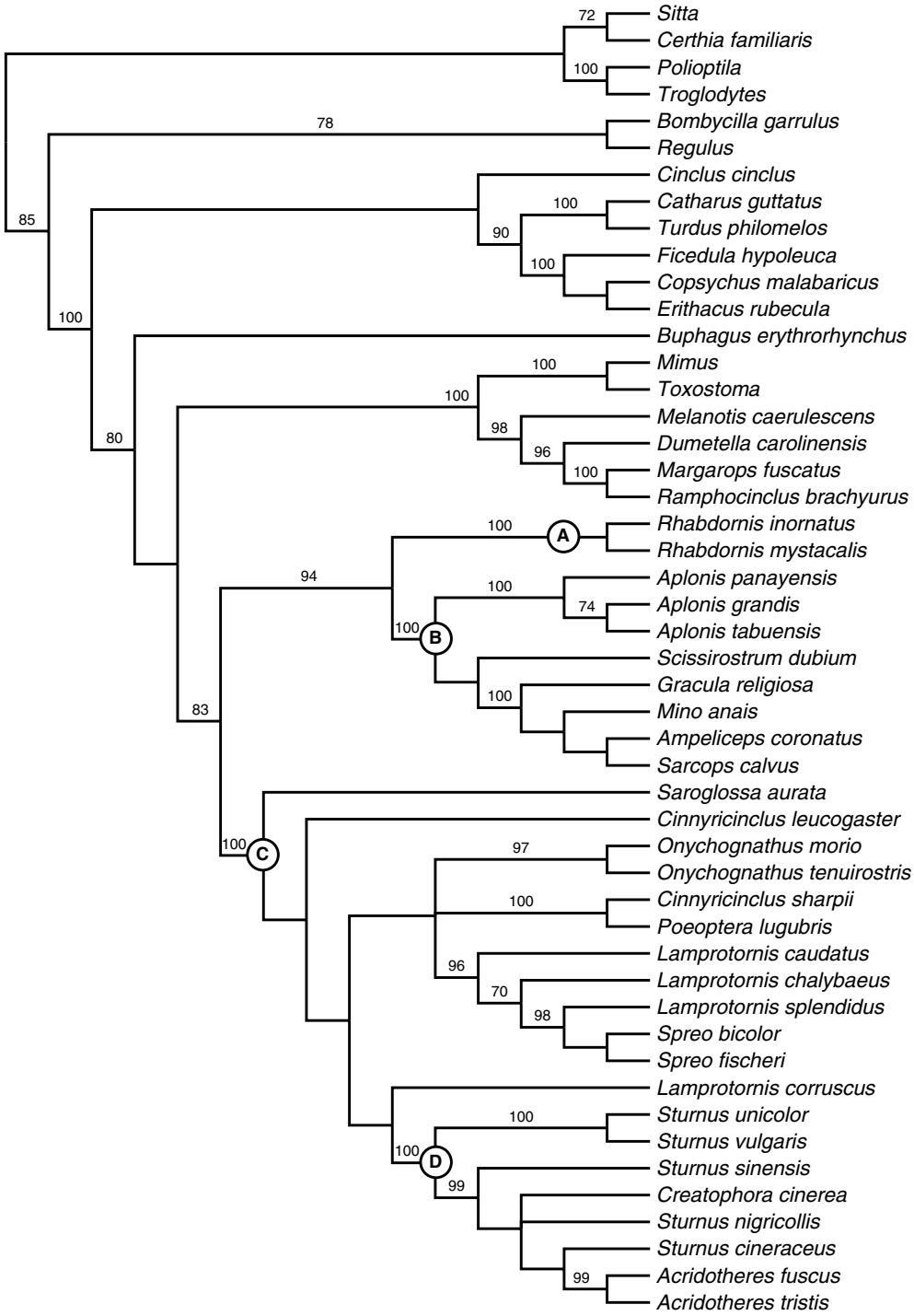


Fig. 1. MP analysis: the strict consensus tree based on 4 most parsimonious trees (tree length 3378 steps, CI = 0.567, RI = 0.641). Nodal support values are calculated from 1000 bootstrap replicates (only values at or above the 70% threshold level are shown).

The maximum-likelihood tree (−ln = 35879.67916, not shown) recovered in all 100 heuristic search replicates is almost identical to the BI tree (Fig. 2). The only difference is that two nodes are collapsed in the latter tree yielding two polytomies, while the ML tree these are fully resolved.

The tree topologies recovered from the MP and ML analyses are almost fully congruent with the BI topology. The trees were rooted using *Polioptila*, *Troglodytes*, *Sitta*, and *Certhia* as outgroup.

The clade including *Bombycilla* and *Regulus* (MP bootstrap support value: 87%, ML bootstrap support: 97%, BI posterior probability support value: 100%) is sister to all other taxa in Muscicapoidea (100% support in all analyses).

Among the Muscicapoidea two clades always receive strong support: a ''thrush–flycatcher'' lineage with Turdinae sister to Saxicolini–Muscicapini (MP: 90%, ML: 71%, BI: 100%), and a ''starling–mockingbird'' lineage with

*Buphagus* basal to all other taxa (MP: 80%, ML: 94%, BI: 100%). Although a Sturnini–Mimini relationship is recovered in all analyses, it is supported only in the ML (bootstrap 75%) and BI (posterior probability 97%) analyses. The affinities of *Cinclus* are not resolved: it is associated with the "thrush–flycatcher" lineage in ML and BI, but with no support, whereas it is recovered in a trichotomy at the base of Muscicapoidea in MP.

Within Sturnini a basal clade includes the two *Rhabdornis* taxa (clade A in Figs. 1–3) and the graculid genera (clade B), but despite being recovered in all analyses, it receives support only in MP (94%). Clade B is fully supported in all analyses, but relationships among its members are less clear. One lineage includes *Gracula*, *Mino*, *Ampeliceps*, and *Sarcops*, while the three *Aplonis* taxa form another lineage. In the MP tree *Scissirostrum* is the sister clade of *Gracula*, *Mino*, *Ampeliceps*, and *Sarcops*, whereas in the ML and BI trees it is sister of the *Aplonis* clade.

The second major starling clade (clade C in Figs. 1–3), receives 100% support in all analyses and includes all Afrotropical taxa. One group consisting of *Sturnus*, *Acridotheres* and *Creatophora* (clade D in Figs. 1–3) is nested within clade C. The basal relationships in clade B are conflicting in the three analyses and no topology receives support. For example, the *Lamprotornis-Spreo-Poeoptera* lineage is associated with *Onychognathus* in the MP tree, but in the ML and BI trees this lineage takes a basal position in clade C. Three genera proved to be non-monophyletic by all analyses: the genus *Sturnus* is paraphyletic (including also *Acridotheres* and *Creatophora*), *Cinnyricinclus* and *Lamprotornis* are polyphyletic, and the genus *Spreo* is nested within a *Lamprotornis* clade.

The analyses of the single genes recovered much less resolved topologies and with fewer supported nodes. Some alternative relationships are suggested, but their support values are low (bootstrap values <70%, posterior probability values <95%). The only two exceptions are: a clade with *Cinclus* as sister to the Sturnidae clade receives a 80% support value in the ML myoglobin tree, and *Buphagus* is placed as sister to Sturnini with 75% bootstrap support in the ML RAG-1 tree (instead as sister to the Sturnini + Mimini clade).

Although the positions of *Buphagus* and *Rhabdornis* are consistently recovered in all analyses, these nodes received only a limited support. Three alternative topologies were compared with the SH test: (1) *Buphagus* sister to the other Sturnini, with the exclusion of Mimini (Amadon's hypothesis, 1943); (2) *Buphagus* sister to the Sturnini clade, but pruned of *Rhabdornis*; (3) *Rhabdornis* sister to the other Sturnini. The SH test was unable to dismiss the first alternative hypothesis ($P = 0.70$), whereas the other two hypotheses were rejected ($P < 0.01$ and $P < 0.05$, respectively).

A likelihood ratio test rejected the molecular clock hypothesis for the combined dataset ($-2\ln\Lambda = 112.650$, $df = 47$, $P < 0.0001$), requiring the use of rate smoothing procedures. The non-parametric rate smoothed tree (Fig. 3) was calibrated assuming an age of 22.5–19.6 mya for the split between Sturnini and Mimini (derived from Barker et al. (2004), see Section 2.3 for details). The estimated ages for some selected clades in Fig. 3 are indicated in Table 3. The origin of the "core Muscicapoidea" clade (i.e. excluding *Regulus* and *Bombycilla*, clade 1 in Fig. 3) is estimated at 30.9–27.2 mya (combined confidence interval), the lineage leading to *Buphagus* splits off at 23.4–20.5 mya, and the major division within Sturnini occurred between 21.3 and 17.8 mya (clades 4 and 5). The graculid clade B radiated at 10.4–8.5 mya and the genus *Aplonis* at 5.3–4.3 mya. The Afrotropical radiation occurred between 17.0 and 12.9 mya (clades 8 and 9), and the Palaearctic lineage (clade D) evolved immediately after. The basal split between western and eastern Palaearctic lineages (clade 10) took place at 10.5–9.1 mya and the eastern clade radiated between 7.2 and 4.6 mya (clades 11 and 12).

## 4. Discussion

### 4.1. Phylogenetics

This study confirms previous studies that starlings are part of the monophyletic clade Muscicapoidea, together with mockingbirds, thrushes, chats, and flycatchers (Sibley and Ahlquist, 1990; Ericson and Johansson, 2003; Voelker and Spellman, 2004; Cibois and Cracraft, 2004; Barker et al., 2004). Waxwings and goldcrests (families Bombycillidae and Regulidae) are recovered with high support as sister branch to the "core Muscicapoidea", an arrangement that has never been identified before. *Regulus* has proved to be exceedingly difficult to place taxonomically (e.g. Ericson and Johansson, 2003; Barker et al., 2004; Alström et al., 2006). We regard this arrangement as tentative since our sampling is too incomplete. Among Muscicapoidea, three major clades corresponding to the families Cinclidae, Muscicapidae, and Sturnidae (*sensu* [Sibley and Monroe, 1990]) are recovered, but their relationships are not fully resolved. The results suggest that Cinclidae are more closely related to Muscicapidae than to Sturnidae, but the evidences are ambiguous.

The unusual arrangement in Voelker and Spellman (2004), with Turdinae sister of a clade Cinclidae–Sturnidae, is probably the result of a suboptimal choice of genes for the study. These authors concluded that "the nuclear c-*mos* gene contributed little if anything to the overall analysis of relationships" and the phylogeny is thus based mainly on the two mitochondrial genes ND2 and cytochrome *b*. It is possible that the relationships among "core Muscicapoidea" (i.e. Cinclidae, Muscicapidae, and Sturnidae) are too old to be reliably resolved by mtDNA data alone. More specifically it seems to be the rooting of the tree that is problematic. When placing the root of their Muscicapoidea clade between Cinclidae and Sturnidae, the tree topology becomes identical to the results herein,

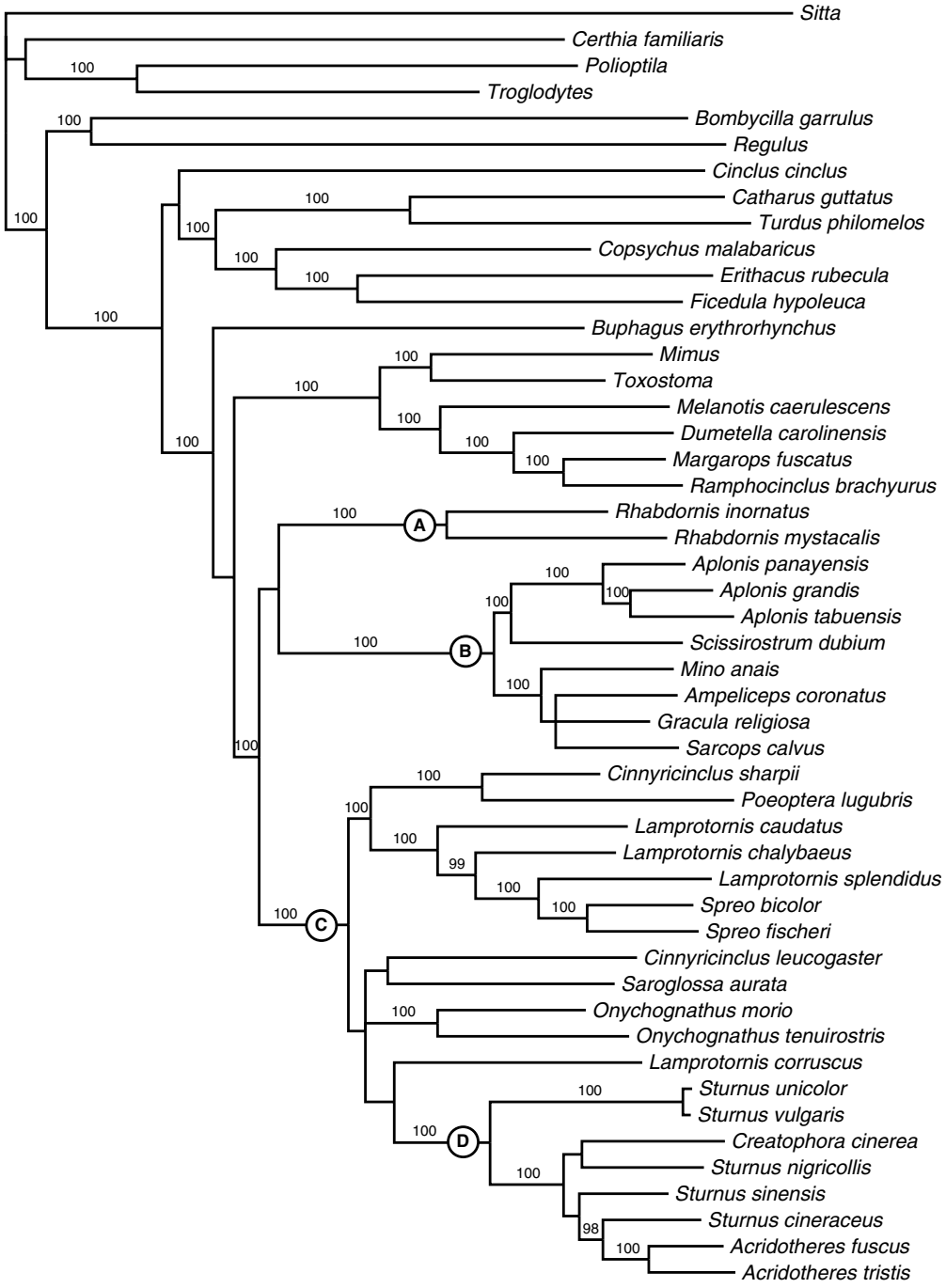


Fig. 2. BI analysis: 50% majority rule consensus tree estimated with GTR + Γ + I model of nucleotide substitution, with posterior probability values (only values at or above the 95% threshold level are shown).

as well as those in the studies of Cibois and Cracraft (2004), Barker et al. (2004), and Beresford et al. (2005).

By feeding on ectoparasites of large African mammals the oxpeckers (*Buphagus*) exploit an ecological niche that is unique for birds. Although recognised as starlings, or starlings-like, since a long time, it has been questioned whether they represented an ancestral or a highly derived form. The oxpeckers are often placed in their own family or subfamily but this study unequivocally shows that they constitute the first branch within the sturnoid lineage (Figs. 1–3). Beecher (1978) and Raikow (1985) noted several similarities in the skull and leg musculature between *Buphagus* and *Scissirostrum dubium*, a monotypic Sulawesi endemic, but our results clearly show these similarities are due to convergence. *Scissirostrum* are colonial breeders that dig a nesting cavity in soft and decaying wood. Both genera use a similar "scissoring" movement of the beak, which presumably requires similar musculature, and they often perch on vertical substrates, which requires shorter legs and strong claws to increase the grasping power of the feet.

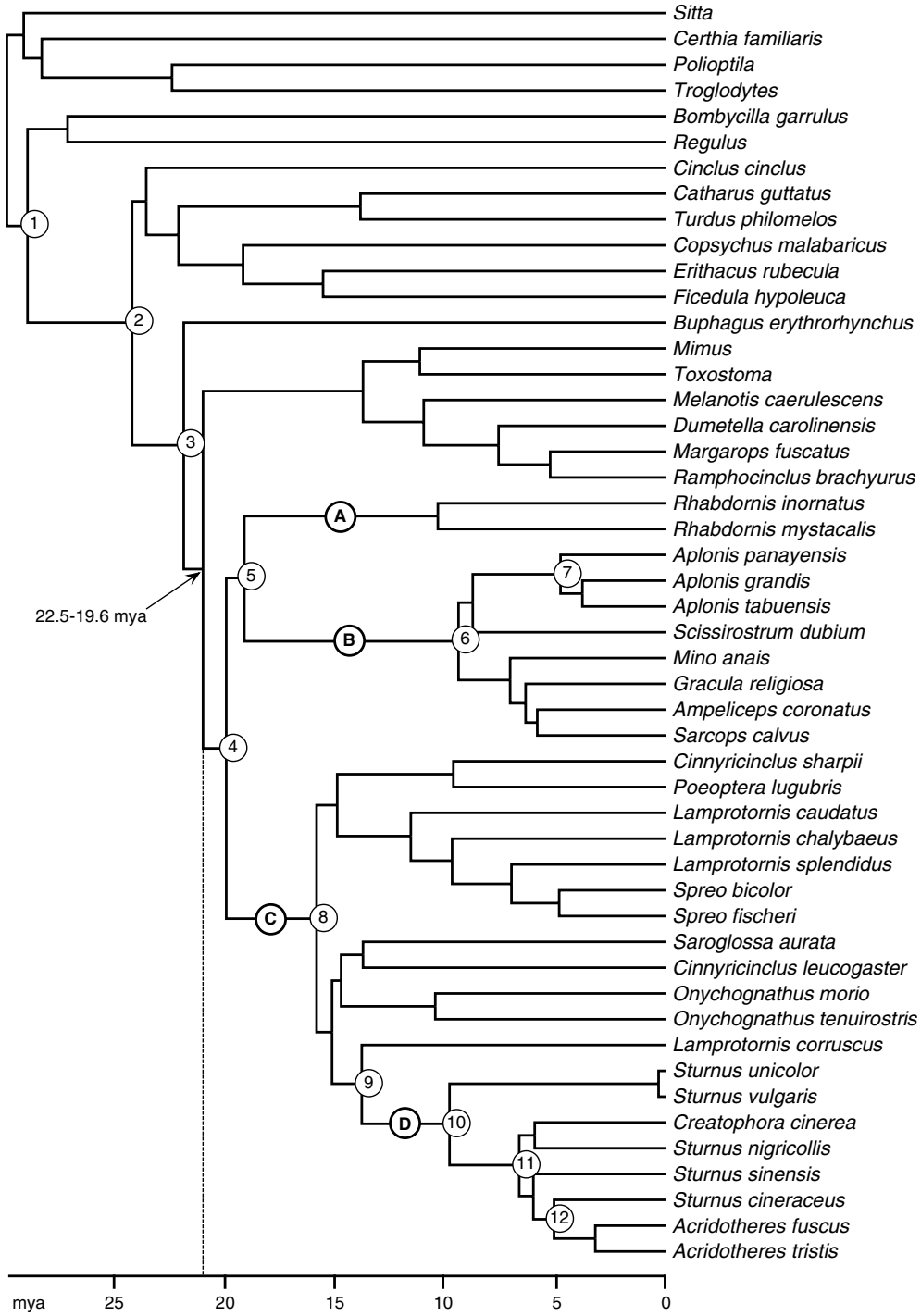


Fig. 3. Molecular clock tree obtained by NPRS of ML tree. Tree calibrated assuming an age of 22.5–19.6 mya for the split between starlings and mockingbirds (recalculated from Barker et al., 2004). Numbers in front of selected clades refer to Table 3.

The sister group relationship of mockingbirds (Mimini) and starlings (Sturnini) is now generally accepted and is also supported in the present study.

The relationships within Sturnini are more complex than previously supposed, and three major lineages can be identified. The placement of the rhabdornises (*Rhabdornis*) among the Muscicapoidea by Cibois and Cracraft (2004) was unexpected. This Philippines endemic genus includes three species with brown streaked plumage and long decurved bill. In general appearance they remind *Certhia* creepers, but they do not climb trunks creeper-like, instead hop along branches gleaning insects form under barks or leaves (Kennedy et al., 2000). The suggested sister group relationship between the rhabdornises and the starlings in that study received no statistical support. Herein we show that they are not only close to the starlings, but nested

Table 3
Age of selected clades by NPRS of ML tree: the estimated ages are combined 95% confidence intervals obtained by rate smoothing of 100 pseudoreplicate datasets; numbers in brackets refer to clades in Fig. 3

| Clade | Estimated age |
|---|---|
| Origin of "core Muscicapoidea" clade (1) | 30.9–27.2 |
| Origin of "sturnoids" clade (2) | 25.9–22.7 |
| Origin of *Buphagus* (3) | 23.4–20.4 |
| First division among Sturnini (4) | 21.3–18.6 |
| Separation of *Rhabdornis*/"graculids" clade (5) | 20.4–17.8 |
| Radiation of "graculids" (6) | 10.1–8.7 |
| Radiation of *Aplonis* (7) | 5.2–4.4 |
| Radiation of main African clades (8 and 9) | 17.0–12.9 |
| Origin of Palaearctic clade (9) | 14.8–12.9 |
| Separation of Western and Eastern Palaearctic clades (10) | 10.6–9.1 |
| Radiation of Eastern Palaearctic clade (11 and 12) | 7.2–4.7 |

within this group (clade A in Figs. 1–3). The rhabdornises thus represent an early and morphologically and ecologically peculiar adaptation among starlings that has not previously been recognised.

A second lineage within Sturnini includes arboreal and frugivorous species, as some mynas (*Scissirostrum*, *Gracula*, *Ampeliceps*, *Mino*), the bald starling *Sarcops*, and the shining starlings *Aplonis* (clade B in Figs. 1–3). While most of these genera are monotypic or include only two species, the genus *Aplonis* is remarkably speciose and is represented all the way from the Malay Peninsula to the Samoa Islands in the east. This lineage seems to include surviving species of an old radiation which occurred in and near the Wallacea region. This hypothesis is supported by the great morphological diversity exhibited in this clade when compared to the rest of the family.

The third Sturnini lineage is the largest and includes all Afrotropical and Palaearctic taxa, as well as many Oriental species (clade C in Figs. 1–3). Although the basal relationships are poorly resolved and lack support, two clades are well-defined. One of them includes the genera *Spreo* and *Poeoptera*, most *Lamprotornis* species and *Cinnyricinclus sharpii*. The genera *Spreo* and *Lamprotornis* were considered closely related (e.g. Amadon, 1956). Indeed morphologically they are rather similar and almost all differences lay on plumage colours. The blue iridescence was assumed to be an apomorphic state and *Lamprotornis* derived from *Spreo* (Craig, 1997). Our findings indicate the opposite, with the genus *Spreo* nested within *Lamprotornis*. The iridescence seems to be the ancestral state in afrotropical starlings and it has been lost several times.

The second well-defined clade includes the Palearctic and Oriental genera *Sturnus* and *Acridotheres* and the Afrotropical genus *Creatophora* (clade D in Figs. 1–3). These taxa exhibit a peculiar feeding behaviour, defined as prying, associated with adaptations in the skull structure and musculature (Beecher, 1978). The morphological adaptations are more marked in *Sturnus* than in *Acridotheres*, and the more advanced complexity is observed in *Sturnus vulgaris* and *S. unicolor*. Our molecular data are conflicting with the morphology, suggesting that morphological structures associated with feeding may be strongly adaptive. Indeed, in our trees the genus *Sturnus* is paraphyletic. *S. vulgaris* and *S. unicolor* represent the most basal branch in clade D, whereas *Acridotheres* is of much more recent origin.

The results of our study suggest that some starling genera, as traditionally defined, are not monophyletic but a taxonomic reassessment must await a more inclusive dataset.

### 4.2. Biogeography

It is difficult to draw biogeographic conclusions about the origin of the starlings. Most major Sturnidae clades have reciprocally separate distributions: *Buphagus* is an Afrotropical genus, Mimini are Nearctic–Neotropical, clades A and B (Fig. 3) are mainly Oriental. Only clade C (Fig. 3) is distributed in both Africa and Eurasia. According to an ancestral area reconstruction Asia and Africa are equally parsimonious ancestral areas for the Sturnidae.

Voelker (2002) suggested a Palaearctic origin of Cinclidae and Cibois and Cracraft (2004) supported an Old World origin for the Muscicapoidea clade. The sister clade of Muscicapoidea includes Bombycillidae and Regulidae, both mainly Holoarctic, marginally extending in range into the Neotropical (Bombycillidae only) and Oriental region (Regulidae only). Thus the Northern hemisphere seems to have played a role in the early diversification of the whole Muscicapoidea clade. In such case an Asiatic origin of the Sturnidae would be more likely but an alternative scenario can be also considered.

Fortelius et al. (2002) presented an hypothesis for the evolution of Eurasian palaeoclimate since the Miocene. During the early Miocene (24–15 mya) the Eurasian climate was wet, favoring the spread of forests. The Sturnidae lineage split from the other Muscicapoidea at about 24 mya, with *Buphagus* evolving at 22 mya (Fig. 3). The New World dispersal of the Mimini lineage is estimated to have occurred at about 21 mya (Barker et al., 2004). These data suggest two possible scenarios for the biogeographic evolution of the starlings, with an African or Asian origin. In the first hypothesis, *Buphagus*, a sturnid with very peculiar morphology and food habit, would be the only extanct relictual taxon from early african lineages. Moreover, in both scenarios the remaining African diversity (clade C) would be the result of a recent split from Asian lineages (see below). Because an African origin would require the extinction of all but one early African lineages, we then favor the second scenario in which the ancestor of all Sturnidae would have been a forest species inhabiting Eurasia. A first lineage colonised Africa, leading to *Buphagus*, while a second lineage split into two clades, one of them colonising North America and leading to the Mimini. The ancestor of Sturnini presumably followed two dispersal directions:

one lineage diversified in the South East Asia and split early (18 mya) into the Philippine endemic *Rhabdornis* (clade A), and the more successful clade including *Gracula*, *Scissirostrum* and related genera (clade B). Like the ancestor of all Sturnidae, also the species belonging to clades A and B are mainly forestal species. The Wallacean area has the highest biodiversity of graculid starlings, including endemic taxa like *Scissirostrum* and *Sarcops*, and it is possible that their diversification took place here. The radiation of the extant graculid species occurred at about 10 mya (node 6 Fig. 3).

A second major dispersal route leads to Africa. Beginning in the early Miocene, African climate became dryer, with a reduction of forests (Axelrod and Raven, 1978). The radiation of the major Afrotropical lineages occurred at 16–14 mya (early Middle Miocene). Only a minority of the extant Afrotropical taxa are strictly forest birds and this radiation may have been favored by the general climate drying. The radiation includes two lineages that dispersed out of Africa, one colonising Madagascar (*Saroglossa aurata*) and the other reinvading Palaearctic (*Sturnus* and *Acridotheres*). The latter lineage includes taxa adapted to open landscapes and it is remarkable that the separation of a Western and an Eastern Palaearctic group (node 10 Fig. 3) occurred simultaneously with a major climatic transition to more arid conditions in the Eastern Palaearctic at 11 mya (Fortelius et al., 2002). The diversification of Eastern Palaearctic taxa took place between the late Miocene and early Pliocene (6–5 mya), i.e. at the same time of a further increase in Eastern Palaearctic aridity. Another intercontinental dispersal events occurred later, with *Creatophora cinerea* colonising the Afrotropics at about 6 mya.

## Acknowledgments

We are grateful to the institutions and curators that provided some of the samples used in this study: Joel Cracraft and Paul Sweet (American Museum of Natural History, New York), Jon Fjeldså and Jan Bolding Kristensen (Zoological Museum, University of Copenhagen), Scott Edwards and Sharon Birks (Burke Museum, University of Seattle), David Willards (Field Museum of Natural History, Chicago), Janet Hinshaw (University of Michigan, Museum of Zoology), and Andreas Helbig (Vogelwarte Hiddensee, University of Greifswald). Pia Eldenäs and Mari Källersjö provided help and advice in the laboratory. Financial support was provided by the Swedish Research Council (Grant No. 621-2004-2913 to P.E.), by the High Lat program (Swedish Museum of Natural History, Stockholm, to D.Z.), and by the ParSystCol program (Muséum National d'Histoire Naturelle, Paris, to D.Z.).

## References

Alström, P., Ericson, P.G.P., Olsson, U., Sundberg, P., 2006. Phylogeny and classification of the avian superfamily Sylvioidea. Mol. Phylogenet. Evol. 38, 381–397.

Amadon, D., 1943. The genera of starlings and their relationships. Amer. Mus. Novit. 1247, 1–16.

Amadon, D., 1956. Remarks on the starlings, family Sturnidae. Amer. Mus. Novit. 1803, 1–41.

Axelrod, D.I., Raven, P.H., 1978. Late Cretaceous and Tertiary vegetation history in Africa. In: Werger, M.J.A. (Ed.), Biogeography and Ecology of Southern Africa. Junk Publ., The Hague, pp. 77–130.

Barker, K.F., Barrowclough, G.F., Groth, J.G., 2002. A phylogenetic analysis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proc. R. Soc. Lond. B 269, 295–305.

Barker, F.K., Cibois, A., Schikler, P., Feinstein, J., Cracraft, J., 2004. Phylogeny and diversification of the largest avian radiation. Proc. Natl. Acad. Sci. USA 101, 11040–11045.

Barker, F.K., 2004. Monophyly and relationships of wrens (Aves: Troglodytidae): a congruence analysis of heterogeneous mitochondrial and nuclear DNA sequence data. Mol. Phylogenet. Evol. 32 (2), 486–504.

Beecher, W.J., 1953. A phylogeny of oscines. Auk 70, 270–333.

Beecher, W.J., 1978. Feeding adaptations and evolution in the starlings. Bull. Chicago Acad. Sci. 11, 269–298.

Beresford, P., Barker, F.K., Ryan, P.G., Crowe, T.M., 2005. African endemics span the tree of songbirds (Passeri): molecular systematics of several evolutionary enigmas. Proc. R. Soc. B 272, 849–858.

Bock, W.J., 1962. The pneumatic fossa of the humerus in the Passeres. Auk 79, 425–443.

Bonaparte, C.L., 1850–1857. Conspectum generum avium. Ludguni Batavorum, Brill.

Cibois, A., Cracraft, J., 2004. Assessing the passerine "Tapestry": phylogenetic relationships of the Muscicapoidea inferred from nuclear DNA sequences. Mol. Phylogenet. Evol. 32, 264–273.

Craig, A.J.F.K., 1997. A phylogeny for the African starlings. Ostrich 68, 114–116.

Drovetski, S.V., Zink, R.M., Fadeev, I.V., Nesterov, E.V., Koblik, E.A., Red'kin, Y.A., Rohwer, S., 2004. Mitochondrial phylogeny of *Locustella* and related genera. J. Avian Biol. 35, 105–110.

Efron, B., Tibshirani, R.J., 1993. An Introduction to the Bootstrap. Chapman & Hall, London.

Ericson, P.G.P., Johansson, U.S., 2003. Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. Mol. Phylogenet. Evol. 29, 126–138.

Ericson, P.G.P., Christidis, L., Cooper, A., Irestedt, M., Jackson, J., Johansson, U.S., Norman, J.A., 2002. A Gondwanan origin of passerine birds supported by DNA sequences of the endemic New Zealand wrens. Proc. R. Soc. Lond. B 269, 235–241.

Ericson, P.G.P., Jansén, A.L., Johansson, U.S., Ekman, J., 2005. Intergeneric relationships of the crows, jays, magpies and allied groups (Aves: Corvidae) based on nucleotide sequence data. J. Avian Biol. 36, 222–234.

Feare, C., Craig, A., 1998. Starlings and Mynas. Helm, London.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Fortelius, M., Eronen, J., Jernvall, J., Liu, L., Pushkina, D., Rinne, J., Tesakov, A., Vislobokova, I., Zhang, Z., Zhou, L., 2002. Fossil mammals resolve regional patterns of Eurasian climate change over 20 million years. Evol. Ecol. Res. 4, 1005–1016.

Fry, C.H., Keith, S., 2000The Birds of Africa. vol. 6, Picathartes to Oxpeckers. Academic Press, London.

Fuchs, J., Fjeldså, J., Bowie, R.C.K., Voelker, G., Pasquet, E., 2006. The African warbler genus *Hyliota* as a lost lineage in the Oscine songbird tree: molecular support for an African origin of the Passerida. Mol. Phylogenet. Evol. 39, 186–197.

Gray, G.R., 1840. A List of the Genera of Birds. Taylor, London.

Gray, G.R., 1870. Hand-list of Genera and Species of Birds, Part II. British Museum, London.

Harshman, J., 1994. Reweaving the tapestry: what can we learn from Sibley and Ahlquist (1990)? Auk 111, 377–388.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES. Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Hunt, J.S., Bermingham, E., Ricklefs, R.E., 2001. Molecular Systematics and Biogeography of Antillean Thrashers, Tremblers, and Mockingbirds (Aves: Mimidae). Auk 118, 35–55.

Irestedt, M., Fjeldså, J., Johansson, U.S., Ericson, P.G.P., 2002. Systematic relationships and biogeography of the tracheophone suboscines (Aves: Passeriformes). Mol. Phylogenet. Evol. 23, 499–512.

Irestedt, M., Fjeldså, J., Ericson, P.G.P., 2004. Phylogenetic relationships of woodcreepers (Aves: Dendrocolaptinae)—incongruence between molecular and morphological data. J. Avian Biol. 35, 280–288.

Jobb, G., von Haeseler, A., Strimmer, K., 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol. Biol. 4 (1), 18.

Kennedy, R.S., Gonzales, P.C., Dickinson, E.C., Miranda Jr., H.C., Fisher, T.H., 2000. A Guide to the Birds of Philippines. Oxford University Press, Oxford.

Kumar, S., Tamura, K., Jakobsen, J.B., Nei, M., 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17 (12), 1244–1245.

Larget, B., Simon, D., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Lockhart, P.J., Steel, M.A., Hendy, M.D., Penny, D., 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. Mol. Biol. Evol. 11, 605–612.

Mayr, E., Greenway Jr., J.C., 1956. Sequence of passerine families (Aves). Breviora 58, 1–11.

Nylander, J.A.A., 2004. MrModeltest v. 2.0. Program distributed by the author. Department of Systematic Zoology, Uppsala University, Uppsala 2004 <http://www.ebc.uu.se/systzoo/staff/nylander.html/>.

Posada, D., Crandall, K.A., 1998. ModelTest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Raikow, R.J., 1985. The locomotor system. In: King, A.S., McLelland, J. (Eds.), Form and Function in Birds, vol. 3. Academic Press, London, pp. 57–147.

Rambaut, A., Charleston, M., 2002. TreeEdit 1.0a10. Program distributed by the authors <http://evolve.zoo.ox.ac.uk/software.html?id=treeedit/>.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14, 1218–1231.

Sharpe, R.B., 1890Catalogue of Birds of the British Museum, vol. 13. British Museum, London.

Sharpe, R.B., 1891. A review of recent attempts to classify birds. In: Proc. Second Intl. Orn. Congr., Budapest.

Sharpe, R.B., 1903A Hand-list of the Genera and Species of Birds, vol. 4. British Museum, London.

Sharpe, R.B., 1909A Hand-list of the Genera and Species of Birds, vol. 5. British Museum, London.

Sheldon, F.H., Gill, F.B., 1996. A reconsideration of songbird phylogeny, with emphasis on titmice and their sylvioid relatives. Syst. Biol. 45, 473–495.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Sibley, C.G., Ahlquist, J.E., 1990. Phylogeny and Classification of Birds. Yale University Press, New Haven, CT.

Sibley, C.G., Monroe Jr., B.L., 1990. Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven, CT.

Sibley, C.G., 1970. A comparative study of egg-white proteins of passerine birds. Bull. Peabody Mus. Nat. Hist. 32, 1–131.

Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogenet. Evol. 12, 105–114.

Stallcup, W.B., 1961. Relationships of some families of the suborder Passeres (songbirds) as indicated by comparisons of tissue proteins. J. Grad. Res. Center, Southern Methodist Univ. 29, 43–65.

Swofford, D.L., 1998. PAUP*: Phylogenetic Analysis using Parsimony (* and other methods), version 4.0. Sinauer Associates, Sunderland, MA.

Voelker, G., 2002. Molecular phylogenetics and the historical biogeography of dippers (*Cinclus*). Ibis 144, 577–584.

Voelker, G., Spellman, G.M., 2004. Nuclear and mitochondrial DNA evidence of polyphyly in the avian superfamily Muscicapoidea. Mol. Phylogenet. Evol. 30, 386–394.

Voous, K.H., 1977. List of Holoarctic bird species. Passerines. Ibis 119, 223–250, 376–406.

Wetmore, A., 1930. A systematic classification for the birds of the world. Proc. U. S. Natl. Mus. 76, 1–8.

Yang, Z., Roberts, D.Mc.L., 1995. On the use of nucleic acid sequences to infer early branchings in the tree of life. Mol. Biol. Evol. 12, 451–458.

Zuccon, A., Zuccon, D., 2006. MrEnt v1.2. Program distributed by the authors. Department of Vertebrate Zoology and Molecular Systematics Laboratory, Swedish Museum of Natural History, Stockholm <http://www.nrm.se/inenglish/researchandcollections/zoology/vertebratezoology/>.