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# A comprehensive molecular phylogeny of the starlings (Aves: Sturnidae) and mockingbirds (Aves: Mimidae): Congruent mtDNA and nuclear trees for a cosmopolitan avian radiation

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**Abstract**

We generated a comprehensive phylogeny for the avian families Sturnidae (starlings, mynas, *Rhabdornis*, oxpeckers, and allies) and Mimidae (mockingbirds, thrashers, and allies) to explore patterns of morphological and behavioral diversification. Reconstructions were based on mitochondrial DNA sequences from five coding genes (4108 bp), and nuclear intron sequences from four loci (2974 bp), for most taxa, supplemented with NDII gene sequences (1041 bp) derived from museum skin specimens from additional taxa; together the 117 sampled taxa comprise 78% of the 151 species in these families and include representatives of all currently or recently recognized genera. Phylogenetic analyses consistently identified nine major clades. The basal lineage is comprised of the two *Buphagus* oxpeckers, which are presently confined to Africa where they are obligately associated with large mammals. Some species in nearly all of the other major clades also feed on or around large vertebrates, and this association may be an ancestral trait that fostered the world-wide dispersal of this group. The remaining taxa divide into sister clades representing the New-World Mimidae and Old-World Sturnidae. The Mimidae are divided into two subclades, a group of Central American and West Indian catbirds and thrashers, and a pan-American clade of mockingbirds and thrashers. The Sturnidae are subdivided into six clades. The Phillipine endemic *Rhabdornis* are the sister lineage to a larger and substantially more recent radiation of South Asian and Pacific island starlings and mynas. A clade of largely migratory or nomadic Eurasian starlings (within which the basal lineage is the model taxon *Sturnus vulgaris*) is allied to three groups of largely African species. These reconstructions confirm that *Buphagus* should not be included in the Sturnidae, and identify many genera that are not monophyletic. They also highlight the substantial diversity among the major Sturnidae subclades in rates of species accumulation, morphological differentiation, and behavioral variation.


*Keywords:* Sturnidae; Mimidae; Buphagidae; Phylogeny; Biogeography

## 1. Introduction

The Mimidae and Sturnidae are avian sister radiations that show striking parallels in a number of ecological and behavioral traits. The Sturnidae (Starlings and Mynas) are restricted to the Old World (except for human-mediated introductions elsewhere), with centers of diversity in Southeast Asia and Africa. The Mimidae have diversified in southwestern North America, the West Indies, and Central/South America and its satellite islands. Although taxa in both groups continue to serve as models in studies of behavioral (e.g., Derrickson, 1988; Kroodsma and Byers, 1991; Pinxten et al., 2002; Duffy and Ball, 2002; Gentner and Margoliash, 2003; Polo et al., 2004; Rubenstein, 2007a) and life history trait evolution (e.g., Ricklefs and Williams, 1984; Cordero et al., 2001; Christians et al., 2001; Komdeur et al., 2002. Rubenstein, 2007b), neither

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group has previously been the subject of phylogenetic analysis with robust taxonomic sampling. Here, we use a combination of mtDNA and nuclear DNA sequences to explore the phylogenetic relationships of all genera and most species within this cosmopolitan avian radiation.

Despite earlier evidence from studies of jaw musculature and cranial osteology (Beecher, 1953), and seriology (Stallcup, 1961), the sister relationship of the Sturnidae and Mimidae was not broadly recognized until it was featured in the DNA–DNA hybridization studies of Sibley and Ahlquist (1980, 1990). Although this finding initially met with controversy, subsequent phylogenetic studies based on DNA–DNA hybridization (Sheldon and Gill, 1996), physiological traits (Malcarney et al., 1994), and various mitochondrial and nuclear DNA sequence loci (Voelker and Spellman, 2004; Ericson and Johansson, 2003; Cibois and Cracraft, 2004; Barker et al., 2004; Zuccon et al., 2006) have been completely concordant in grouping the Mimidae and Sturnidae as sister clades, usually with very strong topological support.

The reliable characterization of the full set of taxa that fall within a monophyletic Sturnidae/Mimidae group has been strengthened by recent phylogenetic surveys of related passerine songbird groups, particularly an intensively sampled study (Cibois and Cracraft, 2004) of the deeper Muscicapoidea radiation within which the Sturnidae and Mimidae are nested. Cibois and Cracraft (2004) included many taxa that had not been sampled previously in any molecular phylogenetic analysis, and thereby helped confirm that all major lineages within the Sturnidae/Mimidae clade have been assigned correctly to this group. Their most surprising finding involving the Sturnidae/Mimidae was the recognition that *Rhabdornis*, a genus endemic to the Phillipines with previously uncertain family-level affinities, is a morphologically aberrant member of the Sturnidae. A phylogenetic enigma involving a second morphologically unusual genus, the *Buphagus* oxpeckers of Africa, remains somewhat less well resolved. *Buphagus* has been variously treated as its own family (Buphagidae), or more commonly included within the family Sturnidae. All molecular phylogenies that have included the *Buphagus* lineage have placed it as a long branch at the base of the Sturnidae/Mimidae clade (e.g., Cibois and Cracraft, 2004; Zuccon et al., 2006), but with low support for distinguishing whether it is the basal lineage in this entire group, or alternatively the sister lineage to either the Sturnidae or Mimidae.

Less is known about relationships within and among the major subclades of the Sturnidae/Mimidae radiation. Although most of the species, as well as all of the genera, of Mimidae have been included in previous DNA-based phylogeographic or phylogenetic studies (Sibley and Ahlquist, 1990; Zink et al., 1997, 1999, 2001; Zink and Blackwell-Rago, 2000; Hunt et al., 2001; Sgariglia and Burns, 2003; Barber et al., 2004; Cibois and Cracraft, 2004; Arbogast et al., 2006), these previous reconstructions have each primarily addressed relationships among sets of closely allied species and no single reconstruction has included a complete sample of Mimidae genera. The few previous DNA-based studies of relationships within the Sturnidae have similarly been taxonomically circumscribed. For example, the most inclusive survey of Sturnidae (Zuccon et al., 2006) sampled only 30 (of 117) Sturnidae species along with 6 (of 34) Mimidae species, and did not include many genus-level lineages with long-debated affinities.

Here, we use a combination of mitochondrial (mtDNA) and nuclear DNA sequences to reconstruct the phylogenetic relationships of all well-differentiated lineages within the Sturnidae/Mimidae. By using both modern, high-quality blood and tissue samples and skin-snips taken from dried museum specimens, we included 117 of 151 (78%) taxa representing all extant genera recognized by any recent taxonomic revision, and multiple species from most polytypic genera. From the high-quality samples, we obtained substantial mitochondrial DNA (4108 bp of protein-coding gene sequence) and nuclear intron (4 loci, 2974 aligned bp) sequences, to which we added shorter (NDII only, 1041 bp) sequences from samples derived from museum skin source materials. The majority of nodes in the resulting phylogenetic reconstructions have high topological support and provide strong evidence for the historical pattern of diversification in this world-wide avian radiation.

## 2. Materials and methods

### 2.1. Taxon sampling

We designed our taxonomic sampling strategy to include at least one representative of all morphologically or biogeographically distinctive lineages in the Sturnidae and Mimidae, including representatives of all genera recognized by any of the five most influential taxonomic treatments of the Sturnidae of the past half-century (Table 1). Here we employ the nomenclature of the most recent "Howard and Moore" checklist (Dickinson, 2003), which recognizes 26 genera and 114 extant species of Sturnidae inclusive of the two species in the "Rhabdornithidae," which Cibois and Cracraft (2004) showed to fall well within the Sturnidae, and not including the Mascarene starling *Necropsar leguati*—a monotypic genus first described by Forbes (1898)—that recent investigations have shown to be based on fraudulently labeled specimens correctly assignable to the Mimidae genus *Cinclocerthia* (Olson et al., 2005). Three additional known species in the large genus *Aplonis* and the monotypic *Fregilupus* are extinct. There has been substantial recent volatility in the genus- and species-level taxonomy of the Sturnidae. Table 1 compares the Sturnidae genera and species of Dickinson (2003) against those recognized by Amadon (1962), Wolters (1982), Sibley and Monroe (1990), and Feare and Craig (1999). In total these authors have recognized 43 genera of extant Sturnidae (Table 1). We sampled 91 species (80% of the Sturnidae)

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Table 1
Genera and species of extant Sturnidae recognized in five recent taxonomic treatments, and those included in this study

| Dickinson (2003) | | In this study | Amadon (1962) | Wolters (1982) | Sibley and Monroe (1990) | Feare and Craig (1999) |
|---|---|---|---|---|---|---|
| Genus | Species | | | | | |
| *Rhabdornis*[a] | *mysticalis* | × | — | — | — | NC |
| *Rhabdornis*[a] | *inornatus* | × | — | — | — | NC |
| *Aplonis* | *metallica* | × | — | *Lamprocorax* | — | — |
| *Aplonis* | *mystacea* | | — | *Rhinopsar* | — | — |
| *Aplonis* | *cantoroides* | × | — | — | — | — |
| *Aplonis* | *crassa* | | — | — | — | — |
| *Aplonis* | *feandensis* | | — | — | — | — |
| *Aplonis* | *insularis* | × | — | — | — | — |
| *Aplonis* | *magna* | | — | *Lamprocorax* | — | — |
| *Aplonis* | *brunneicapillus* | × | — | *Rhinopsar* | — | — |
| *Aplonis* | *grandis* | × | — | *Lamprocorax* | — | — |
| *Aplonis* | *dichroa* | | — | *Lamprocorax* | — | — |
| *Aplonis* | *zelandica* | | — | — | — | — |
| *Aplonis* | *striata* | | — | — | — | — |
| *Aplonis* | *atronitens*[b] | | — | ssp *striata* | ssp *striata* | ssp *striata* |
| *Aplonis* | *santovestris* | | — | — | — | — |
| *Aplonis* | *panayensis* | × | — | *Lamprocorax* | — | — |
| *Aplonis* | *mysolensis* | | — | *Lamprocorax* | — | — |
| *Aplonis* | *minor* | × | — | *Lamprocorax* | — | — |
| *Aplonis* | *opaca* | | — | — | — | — |
| *Aplonis* | *pelzelni* | × | — | — | — | — |
| *Aplonis* | *tabuensis* | × | — | — | — | — |
| *Aplonis* | *atrifusca* | | — | — | — | — |
| *Aplonis* | *cinerascens* | × | — | — | — | — |
| *Mino* | *dumontii* | | — | — | — | — |
| *Mino* | *kreffti* | × | ssp *dumontii* | ssp *dumontii* | ssp *dumontii* | — |
| *Mino* | *anais* | × | — | — | — | — |
| *Basilornis* | *celebensis* | × | — | — | — | — |
| *Basilornis* | *galeatus* | | — | — | — | — |
| *Basilornis* | *corythaix* | | — | — | — | — |
| *Basilornis* | *miranda* | × | — | *Goodfellowia* | — | — |
| *Sarcops* | *calvus* | × | — | — | — | — |
| *Streptocitta* | *albicollis* | × | — | — | — | — |
| *Streptocitta* | *albertinae* | | — | — | — | — |
| *Enodes* | *erythrophris* | × | — | — | — | — |
| *Scissirostrum* | *dubium* | × | — | — | — | — |
| *Saroglossa* | *spiloptera* | × | — | — | — | — |
| *Saroglossa* | *aurata* | × | — | *Hartlaubius* | — | — |
| *Ampeliceps* | *coronatus* | × | — | — | — | — |
| *Gracula* | *ptilogenys* | × | — | — | — | — |
| *Gracula* | *religiosa* | × | — | — | — | — |
| *Gracula* | *indica*[b] | × | ssp *religiosa* | — | ssp *religiosa* | — |
| *Gracula* | *robusta*[b] | | ssp *religiosa* | ssp *religiosa* | ssp *religiosa* | — |
| *Gracula* | *enganensis*[b] | | ssp *religiosa* | ssp *religiosa* | ssp *religiosa* | — |
| *Acridotheres* | *grandis* | × | — | *Aethiopsar* | — | — |
| *Acridotheres* | *cristatellus* | × | — | *Aethiopsar* | — | — |
| *Acridotheres* | *javanicus* | × | ssp *fuscus* | ssp *fuscus* | — | — |
| *Acridotheres* | *cinereus* | | *Sturnus* | *Aethiopsar* | ssp *fuscus* | — |
| *Acridotheres* | *fuscus* | × | — | *Aethiopsar* | — | — |
| *Acridotheres* | *albocinctus* | | — | *Aethiopsar* | — | — |
| *Acridotheres* | *ginginianus* | × | — | — | — | — |
| *Acridotheres* | *tristis* | × | — | — | — | — |
| *Acridotheres* | *melanopterus* | | *Sturnus* | *Leucopsar* | *Sturnus* | — |
| *Leucopsar* | *rothschildi* | × | — | — | — | — |
| *Sturnus* | *burmannicus* | | — | *Leucopsar* | — | *Acridotheres* |
| *Sturnus* | *nigricollis* | × | — | *Gracupica* | — | *Gracupica* |
| *Sturnus* | *contra* | × | — | *Sturnopastor* | — | *Gracupica* |
| *Sturnus* | *sturninus* | | — | *Agropsar* | — | *Sturnia* |
| *Sturnus* | *philippensis* | × | — | *Agropsar* | — | *Sturnia* |
| *Sturnus* | *sinensis* | × | — | *Sturnia* | — | *Sturnia* |
| *Sturnus* | *malabaricus* | × | — | *Temenuchus* | — | *Sturnia* |
| *Sturnus* | *erthropygius* | | — | *Temenuchus* | — | *Sturnia* |

(*continued on next page*)

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Table 1 (*continued*)

| Dickinson (2003) | | In this study | Amadon (1962) | Wolters (1982) | Sibley and Monroe (1990) | Feare and Craig (1999) |
|---|---|---|---|---|---|---|
| Genus | Species | | | | | |
| *Sturnus* | *albofrontatus* | × | — | *Temenuchus* | — | *Sturnia* |
| *Sturnus* | *pagodarum* | × | — | *Temenuchus* | — | *Temenuchus* |
| *Sturnus* | *roseus* | × | — | *Pastor* | — | *Pastor* |
| *Sturnus* | *sericeus* | × | — | *Sturnopastor* | — | — |
| *Sturnus* | *cineraceus* | × | — | *Sturnopastor* | — | — |
| *Sturnus* | *vulgaris* | × | — | — | — | — |
| *Sturnus* | *unicolor* | × | — | — | — | — |
| *Creatophora* | *cinerea* | × | — | — | — | — |
| *Lamprotornis* | *nitens* | × | — | — | — | — |
| *Lamprotornis* | *chalybaeus* | × | — | — | — | — |
| *Lamprotornis* | *chloropterus* | × | — | — | — | — |
| *Lamprotornis* | *elizabeth*[b] | | ssp *chlorop.* | — | — | ssp *chlorop.* |
| *Lamprotornis* | *chalcurus* | × | — | — | — | — |
| *Lamprotornis* | *splendidus* | × | — | — | — | — |
| *Lamprotornis* | *ornatus* | × | — | — | — | — |
| *Lamprotornis* | *iris* | × | — | *Coccycolius* | *Coccycolius* | — |
| *Lamprotornis* | *purpureus* | × | — | — | — | — |
| *Lamprotornis* | *purpuroptera* | × | — | — | — | — |
| *Lamprotornis* | *caudatus* | × | — | — | — | — |
| *Lamprotornis* | *regius* | × | *Cosmopsarus* | *Cosmopsarus* | *Cosmopsarus* | — |
| *Lamprotornis* | *mavesii* | × | — | — | — | — |
| *Lamprotornis* | *australis* | × | — | — | — | — |
| *Lamprotornis* | *acuticaudus* | × | — | — | — | — |
| *Lamprotornis* | *corruscus* | × | — | — | — | — |
| *Lamprotornis* | *superbus* | × | *Spreo* | *Lamprospreo* | — | — |
| *Lamprotornis* | *hildebrandti* | × | *Spreo* | *Lamprospreo* | — | — |
| *Lamprotornis* | *shelleyi* | × | ssp *hildebrandti* | *Lamprospreo* | — | — |
| *Lamprotornis* | *pulcher* | × | *Spreo* | *Lamprospreo* | — | — |
| *Lamprotornis* | *purpureiceps*[c] | × | — | — | — | *Hylopsar* |
| *Lamprotornis* | *cupreocauda*[c] | × | — | — | — | *Hylopsar* |
| *Lamprotornis* | *unicolor* | × | *Cosmopsarus* | *Cosmopsarus* | *Cosmopsarus* | *Spreo* |
| *Lamprotornis* | *fischeri* | × | — | *Lamprospreo* | *Spreo* | *Spreo* |
| *Cinnyricinclus* | *femoralis*[d] | × | *Cinnyricinclus* | *Arizelopsar* | *Cinnyricinclus* | *Poeoptera* |
| *Cinnyricinclus* | *leucogaster* | × | — | — | — | — |
| *Spreo* | *bicolor* | × | — | — | — | — |
| *Spreo* | *albicapillus* | × | — | *Poneropsar* | — | — |
| *Onychognathus* | *morio* | × | — | — | — | — |
| *Onychognathus* | *tenuirostris* | × | — | — | — | — |
| *Onychognathus* | *fulgidus* | × | — | — | — | — |
| *Onychognathus* | *walleri* | × | — | — | — | — |
| *Onychognathus* | *blythii* | × | — | — | — | — |
| *Onychognathus* | *frater* | × | — | — | — | — |
| *Onychognathus* | *tristamii* | × | — | — | — | — |
| *Onychognathus* | *nabouroup* | × | — | — | — | — |
| *Onychognathus* | *salvadorii* | × | — | — | — | — |
| *Onychognathus* | *albirostris* | × | — | — | — | — |
| *Onychognathus* | *neumanni* | | ssp *morio* | ssp *morio* | ssp *morio* | — |
| *Poeoptera* | *stuhlmanni* | × | — | *Onychognathus* | — | — |
| *Poeoptera* | *kenricki* | × | — | *Onychognathus* | — | — |
| *Poeoptera* | *lugubris* | × | — | *Onychognathus* | — | *Pholia* |
| *Pholia* | *sharpii* | × | *Cinnyricinclus* | — | *Cinnyricinclus* | — |
| *Grafisia* | *torquata* | × | — | — | — | — |
| *Speculipastor* | *bicolor* | × | — | — | — | — |
| *Neocichla* | *gutturalis* | × | — | — | — | — |
| *Buphagus* | *erythrorhynchus*[e] | × | — | — | — | — |
| *Buphagus* | *africanus*[e] | × | — | — | — | — |

— indicates congruence with "Howard and Moore checklist" taxonomy of Dickinson (2003); ssp: treated as a subspecies of the species named thereafter; NC: not considered.

[a] *Rhabdornis* was only recently recognized as a member of the Sturnidae (Cibois and Cracraft, 2004).

[b] Taxa ranked as subspecies in Dickinson (2003) but as full species in one or more of the previous treatments.

[c] Also assigned to *Hylopsar* in some additional regional treatments (e.g., Craig, 1997; Fry et al., 2000).

[d] Also assigned to *Pholia* in some additional regional treatments (e.g., Craig, 1997; Fry et al., 2000).

[e] Sometimes treated as a separate family, Buphagidae.

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representing all 43 genera. All Sturnidae DNA sequences analyzed here were generated in our laboratory.

Dickinson (2003) recognizes 12 genera and 34 species of Mimidae, all of which are extant (with the possible exception of the highly endangered Cozumel Thrasher, *Toxostoma guttatum*). Although the genus-level taxonomy of the Mimidae has been fairly stable over the past half-century (Davis and Miller, 1960; Sibley and Monroe, 1990; AOU, 1998; Brewer, 2001; Cody, 2005), recent molecular evidence (Hunt et al., 2001; Banks et al., 2002) supported the re-separation of *Allenia* from *Margarops*, two Caribbean genera that had been merged in some earlier treatments. *Donacobius*, a monotypic genus of long-debated affinities, has sometimes been assigned to the Mimidae in the past, but molecular phylogenetic studies have now shown it to be a member of the Sylviidae and not closely related to the Mimidae or Sturnidae (Barker, 2004; Alström et al., 2006), and we did not include it here. We obtained sequences from 25 species (74% of the Mimidae) representing all recently recognized genera. Some or all DNA sequences from 16 Mimidae taxa (Appendix A) were derived from previous studies (Hunt et al., 2001; Barber et al., 2004; Arbogast et al., 2006).

We employed three outgroup taxa based on recent phylogenetic surveys that have addressed relationships between the Sturnidae/Mimidae clade and related passerine groups. These reconstructions show that the waxwing *Bombycilla* is the most basal outgroup lineage in our study, and that the thrushes *Catharus* and *Myadestes* represent the two early and well differentiated lineages (Klicka et al., 2005) within a large clade that is either sister to the Sturnidae/Mimidae (Barker et al., 2004; Cibois and Cracraft, 2004; Zuccon et al., 2006), or sister to a clade that contains the dippers (Cinclidae) and the Sturnidae/Mimidae (Ericson and Johansson, 2003; Voelker and Spellman, 2004).

The majority of samples that served as sources of DNA for our study were frozen tissues associated with traditionally vouchered specimens, either from existing museum collections or from our own collecting activities (Appendix A). We added additional taxa based on DNAs extracted from the toe-pads of traditionally prepared museum skin specimens. From some taxa, high-quality tissue samples were available only from non-vouchered tissue, blood, or feather samples provided by field researchers or taken from live captive birds in zoological or private avicultural collections. In these cases where otherwise high-quality DNA materials were not associated with voucher specimens, we sequenced the NDII gene (see below) from conspecific museum-skin specimens. This allowed us to generate much greater amounts of sequence from the robust but non-vouchered samples, while confirming their phylogenetic affinities by comparison to the NDII sequences from conspecific vouchered samples. We likewise replicated nearly all sequences derived from skin-snips, as well as a number of samples from vouchered frozen tissues, using samples from different conspecific individuals. In all but two cases, these replicated NDII sequences were identical or nearly identical matches to their conspecific counterparts; investigations of the two mismatches (one each from two different museum-based frozen tissue collections) showed them to be vouchered specimens misidentified by the field preparators, and consequently accessioned incorrectly. To reduce computation times we included only one representative per species in the reconstructions reported here; however, the replicated sequences used only in preliminary analyses are also archived in GenBank (Appendix A).

### 2.2. Laboratory methods

DNA was extracted from muscle tissue and feather samples using DNAeasy kits (Qiagen) and from blood samples using Perfect gDNA Blood Mini kits (Eppendorf). To amplify the mitochondrial NDII gene, we used primers METb and TRPc (Eberhard and Bermingham, 2004). To amplify the mitochondrial region spanning the COI and ATPase6 genes, we used various combinations of primers COIf and COIa (Kessing et al., 1989); GQL and HMH (Hunt et al., 2001); IL6591L (Lovette, 2004); and IL7513h (ATGGATAGCATGGCTCATACTATTCC), sturnCOIf2 (GACACCTACTACGTWGTAGCYCACT TCC), sturnCOIa2 (GGAAACCGARTTGTGAGTGGT TGG), IL8232l (ATGTTGGTTTCAAGCCAACCGC), and ILLYSh (CCTCTTTCTCCAGCTTAAAAGGCT AG). Amplification of β-fibrinogen introns 5 and 7 used primer pairs Fib-5 (CGCCATACAGAGTATACTGTGA CA) and Fib-6 (GCCATCCTGGCGATTCTGAA) provided by F.K. Barker (personal communication), and FIB-BI7U and FIB-BI7L (Prychitko and Moore, 1997), respectively. To amplify rhodopsin intron 1, we used Rho-I1F and Rho-I1R (Primmer et al., 2002). To amplify intron 5 of transforming growth factor β-2, we used primers TGFB2-I5F and TGFB2-I5R (Primmer et al., 2002).

All 10 μL PCR amplifications included 1 μL of undiluted genomic DNA (concentration: 10–50 ng/μL), 10 μM Tris–HCl (pH 8), 50 μM KCl, variable $MgCl_2$ (range: 1.5–4 mM), 0.25 mM of each nucleotide, 0.25 mM of each primer, and 0.025 U Jumpstart Taq polymerase (Sigma). All sets of PCR reactions included negative controls with no DNA template. Thermal cycling and subsequent cycle sequencing was conducted in PTC-220 Dyad Thermal Cyclers (MJ Research). Thermal cycling profiles varied among primer sets, but most commonly employed an initial denaturing at 95 °C for 4 min 30 s; 30–35 cycles of denaturing at 95 °C for 45 s, annealing at a variable temperature for 45 s, and extension at 72 °C for 1–2 min 20 s; and a final extension at 72 °C for 5 min.

PCR products were electrophoresed in 1.5% agarose TAE gels to confirm amplification and fragment sizes. To digest unincorporated nucleotides and primers, 0.5 U of Exonuclease (USB) and 0.5 U of Shrimp Alkaline Phosphatase (USB) were added to each remaining 7 μL of PCR product and incubated for 30 min at 37 °C, then for 10 min at 90 °C. Cycle sequencing was conducted using the amplification primers and many additional internal

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primers, some of which were designed for particular subclades or individual species. Cycle sequencing reactions employed the BigDye 3.1 (Applied Biosystems) chemistry and the recommended cycling conditions, and sequences were read using Applied Biosystems model 3100 or 3730 automated DNA sequencers. Nearly all fragments were confirmed by sequencing both DNA strands. Sequences were checked and overlapping fragments concatenated using Sequencer 4.5 (Genecodes). In nuclear DNA sequences, heterozygous nucleotides were assigned the corresponding IUPAC ambiguity codes. All sequence alignments were resolved readily by eye, except for several intron sites with single-nucleotide repeats that had high levels of indel mutation homoplasy.

### 2.3. Processing of museum-skin samples

Because of the degradation of the template DNAs in dried museum skin samples and the concomitantly higher risk of PCR template contamination, we employed a number of specialized protocols for the DNA extraction and PCR assembly of NDII sequences derived from museum skin toe-pads. All toe-pad DNA extractions and PCR set-ups were conducted in a custom-designed and equipped laboratory dedicated solely to work with degraded DNA. Protocols included many precautions that are recommended for ancient DNA studies on older materials (Willerslev and Cooper, 2005), including: physical and air-handling isolation of the degraded-DNA laboratory; prohibition of travel of personnel, reagents, samples, or equipment from the standard molecular markers lab to the degraded-DNA lab; set-up of all reactions within a laminar-flow clean bench with ISO class V air filtering; frequent sterilization of surfaces and equipment with intense 254 nm UV irradiation and 10% sodium hypochlorite (chlorine bleach); and interspersion of negative control reactions at both the extraction (1 control:1 tissue-containing extraction) and PCR (also 1:1) stages. PCR controls differed from the adjacent PCR reactions only in the absence of extracted DNA template. Despite the several hundred sets of PCR reactions required to complete these sequences, we saw no evidence of PCR amplification in any of the negative control PCR reactions, nor in the reactions to which we added solutions from the extraction negative controls.

Conspecific replicates were extracted and amplified from different skin specimens with planned temporal breaks of at least several weeks (and usually 2–3 months), over which interval many skin-snip samples of other taxa were processed using the same laboratory facility, equipment, primers, and reagents. The later comparison of these independently processed conspecific sequences helped ensure that any contaminant fragments would be identified as such. We identified no situations where a single PCR fragment or a concatenated NDII sequence differed by an unexpected magnitude from its conspecific replicate.

All skin-snip DNA extractions were performed with DNAeasy kits (Qiagen). Degraded DNA PCR amplifications targeted short (100–500 bp), overlapping regions of the NDII gene. We employed the NDII-flanking primers METb and TRPc as well as several dozen additional primers within binding sites within the NDII coding region, many designed for particular subclades or individual taxa. Sealed PCR reaction tubes were transported to the standard laboratory for the thermal cycling through automated sequencing steps described above.

### 2.4. Phylogenetic analysis

To reconstruct phylogenies, we used Bayesian methods as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), and maximum parsimony as implemented in Paup* 4.0b10 (Swofford, 2002). Because of the heterogeneous composition of the sequence obtained from different taxa, we ran phylogenetic reconstructions on four datasets: (1) mtDNA sequences from all taxa surveyed, including 87 taxa from which we obtained 4108 nucleotides of mtDNA protein-coding sequence and 30 additional taxa from which we obtained only the 1041-nucleotide NDII gene; (2) mtDNA sequences from only the 87 more robustly sampled taxa; (3) four-locus combined intron sequences totaling 2974 aligned nucleotides from those same 87 taxa; and (4) all mtDNA and intron data combined for the complete set of 117 taxa.

Bayesian MCMC chains were sampled every 100 generations, with two independent sets of three heated and one unheated chains. All analyses employed the default flat Dirichlet priors. Stationarity was evaluated graphically for all parameters, and by monitoring the convergence of the standard deviation of split frequencies in the two independent sets of chains. Chains were run for $2 \times 10^6$ generations after the average standard deviation of split frequencies fell below 0.01 (0.02 for runs involving four or five data partitions); samples from earlier generations were discarded. Congruence between independent runs based on identical datasets was assessed by comparing parameter estimates, tree topologies, and posterior probability scores for individual branches. Parameters were estimated separately for 1–5 data partitions, depending on the loci included in a particular Bayesian search. All mitochondrial coding sequences were grouped in a single partition. In analyses that included nuclear loci, each intron was represented by a separate partition.

Parsimony analyses were conducted via full heuristic searches with 100 stepwise addition replicates. In analyses that included only mitochondrial coding sequences, transitional substitutions at third-position codon sites were given one-fifth the weight of third-position transversions and all changes at first- and second-position sites. In analyses that included only nuclear intron sequences, all sites were weighted equally. In analyses that included both mtDNA and intron sequences, mtDNA third-position transitions were downweighted as in the mtDNA-only searches, with all other sites equally weighted. Support for individual nodes in parsimony reconstructions was assessed via

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heuristic bootstrap searches with five addition sequence replicates and 100 bootstrap replicates. In MP analyses of the nuclear intron sequences alone, the high similarity of many congeneric sequences resulted in many thousands of equally parsimonious shortest trees, and consequently, extremely long search times; these analysis were therefore run for a maximum of $1 \times 10^7$ TBR rearrangements per addition sequence replicate.

In all reconstructions, deletions were treated as missing data, except that the intron alignments contained a small number of indel sites (see Section 3) that could not be reliably aligned, and these sites were excluded entirely. Indel mutations that could be reliably aligned were later mapped onto the reconstructed topologies using MacClade 4.08 (Maddison and Maddison, 2005). In MP searches weighted by codon position, the frame-shifted 10-bp overlap between the ATPase6 and ATPase8 mtDNA genes was excluded, as each of these bases occupies two codon positions. All reconstructions were rooted to the outgroup taxa *Bombycilla*, *Catharus*, and *Myadestes*, but these are not shown in the trees.

## 3. Results

### 3.1. Sequence characteristics

Sequence alignments were straightforward except for one 6–12 bp region in each of the Fib-5, Fib-7, and Rho-1 intron comparisons, each of which involved single-nucleotide repeats of variable length that had unusually high insertion/deletion mutation rates leading to substantial homoplasy. Excluding these short regions of questionable alignment, we found a total of 66 indels among the ingroup taxa, of which 43 were present in more than one taxon and therefore represent potential synapomorphies.

All mitochondrial coding sequences were of identical length, except that the two NDII replicates from different *Cinnyricinclus leucogaster* individuals shared an unusual 1-codon (alanine) insertion just before the stop codon, making the gene 1044 nucleotides in length in this one taxon. The NDII amino acid sequence of this species was otherwise typical. With this one-taxon insertion excluded, the total aligned length of mtDNA coding sequence was 4118 nucleotides. In comparisons among the 84 ingroup taxa for which we had all five mtDNA gene sequences, 1906 mtDNA nucleotide sites were variable, of which 1723 were potentially phylogenetically-informative; an additional 222 sites varied in comparisons that included the three outgroup taxa.

In comparisons among these 84 ingroup and 3 outgroup taxa, the total aligned length of combined intron sequence was 2974 nucleotides, of which 1298 were variable and 612 parsimony informative. Considered by locus, the β-fibrinogen intron 5 alignment was 542 nucleotides, including 242 variable and 105 informative sites; β-fibrinogen 7 was 880 nucleotides, including 395 variable and 198 informative sites; TGFB2-5 was 562 nucleotides, including 234 variable and 106 informative sites; and rhodopsin 1 was 990 nucleotides, including 427 variable and 203 informative sites. All intron alignment lengths reported here exclude insertion sites present only in single taxa, as well as sites with questionable alignment.

Table 2 summarizes the post-burn-in means of parameters estimated for each data partition in the combined analysis of the 87 taxa for which we had the complete set of mitochondrial and nuclear sequences. As expected, all loci showed a predominance of transitional substitutions, but this bias was substantially greater for the mtDNA partition. As the mtDNA sequences were all protein-coding with relatively high amino acid conservation, they had a greater proportion of sites estimated as invariant than did the four intron sequences. Likewise, the shape parameter ($\alpha$) of the gamma distribution of rate variation among sites was higher for all nuclear intron loci than for the mtDNA dataset, and much greater for the two β-fibrinogen introns than for the TGFB or rhodopsin introns; these high $\alpha$ values indicate that these loci had lower coefficients of variation in rate among sites.

To compare relative rates of nucleotide evolution among loci, we calculated pairwise ML distances in Paup* using the parameters summarized in Table 2, and plotted pairwise divergences for each intron locus against the corresponding mtDNA divergence calculated from the combined NDII, COI, COII, ATPase6, and ATPase8 genes (Fig. 1). Overall rates of ML divergence were approximately tenfold lower for all nuclear loci relative to the mitochondrial divergence. Relative rates were similar across all four intron loci; ML distances were nearly identical among the Fib-5, Fib-7, and Rho-1 introns, and about 25% greater at the TGFB2-5 locus (Fig. 1).

Table 2
Estimated model parameters for each of five loci under the HKY + G + I model of sequence evolution

| Locus | Relative substitution rates | | | | | | Base frequencies | | | | $\alpha$ | p(*I*) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A–C | A–G | A–T | C–G | C–T | G–T | A | C | G | T | | |
| mtDNA | 0.29 | 12.65 | 0.52 | 0.21 | 6.71 | 1.00 | 0.37 | 0.40 | 0.08 | 0.15 | 0.82 | 0.47 |
| Fib-5 | 1.71 | 6.80 | 0.92 | 1.81 | 4.26 | 1.00 | 0.31 | 0.16 | 0.19 | 0.34 | 12.14 | 0.10 |
| Fib-7 | 1.17 | 4.15 | 0.55 | 1.80 | 3.71 | 1.00 | 0.32 | 0.17 | 0.18 | 0.33 | 57.83 | 0.14 |
| TGFB2-4 | 0.92 | 4.12 | 0.91 | 1.62 | 2.58 | 1.00 | 0.24 | 0.23 | 0.21 | 0.32 | 2.23 | 0.16 |
| Rho-1 | 1.41 | 4.52 | 0.98 | 1.64 | 5.05 | 1.00 | 0.24 | 0.23 | 0.25 | 0.28 | 2.03 | 0.09 |

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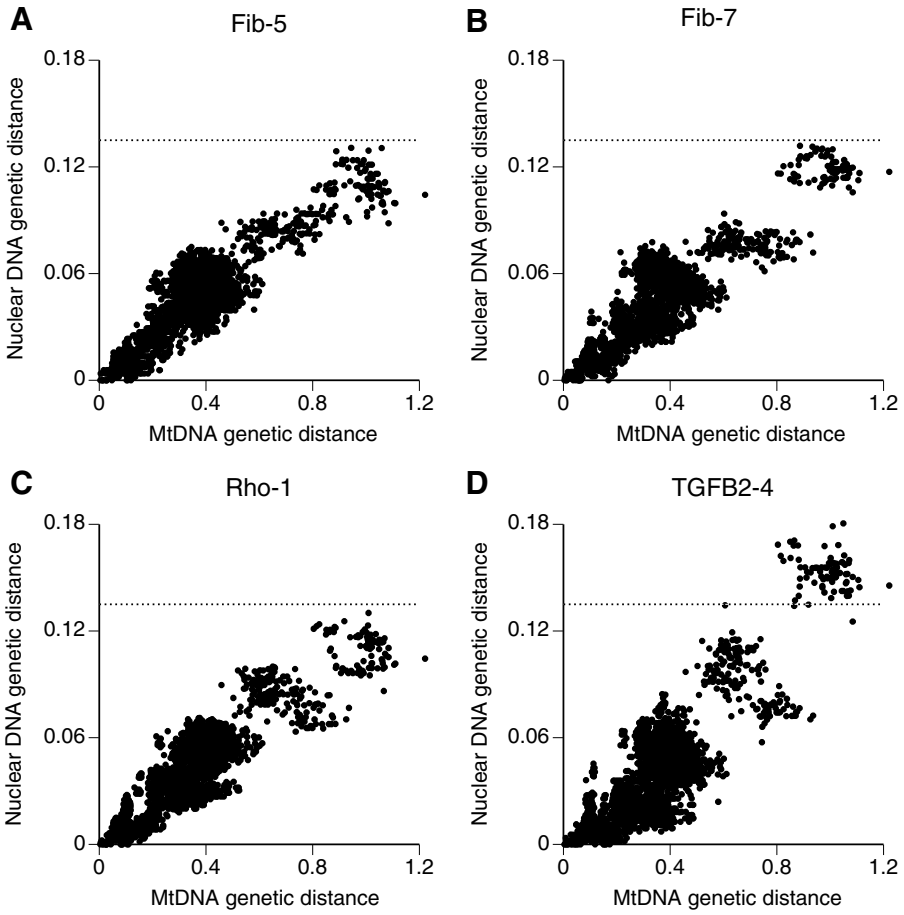


Fig. 1. Relative sequence divergence at protein-coding mtDNA genes versus four nuclear intron loci based on pairwise distances among 84 Sturnidae and Mimidae species and three outgroup taxa. (A) β-Fibrinogen intron 5, (B) β-fibrinogen intron 7, (C) rhodopsin intron 1, and (D) transforming growth factor β-2 intron 5. All comparisons were based on maximum-likelihood distances calculated using the locus-specific parameters given in Table 2. The dashed horizontal line is a visual reference to facilitate comparisons among nuclear loci, which indicate a ~25% faster rate of divergence at the TGFB2 intron 5 locus (D) relative to the other three intron loci (A–C). Mitochondrial distances were based on 4116 nucleotides of aligned sequence from five genes.

### 3.2. Phylogenetic reconstructions

We found high congruence among phylogenetic reconstructions based on different subsets of the combined nuclear and mitochondrial datasets, and among topologies based on Bayesian versus parsimony reconstruction methods (Figs. 2–4). In all reconstructions, the majority of nodes received substantial posterior probability (>90%) and bootstrap (>70%) support, and in no case did one of these moderately to highly supported nodes in a given topology conflict with a similarly well-supported node in an alternative reconstruction. The highest level of resolution was found in the combined-data reconstructions that included both mitochondrial and nuclear loci (Fig. 4). All reconstructions were consistent in the identification of nine major clades within this combined radiation; the composition of these clades and the relationships among them are reviewed in the Discussion below.

A gene-tree based only on mitochondrial sequences is depicted in Fig. 2. This analysis, which involved a mixed dataset of 4108 nucleotides for 87 taxa and 1041 nucleotides for the 30 remaining taxa, provided high resolution among most groups of allied species and genera, with somewhat more modest support for relationships among some of the more basal Sturnidae clades. In separate analyses (not shown) we investigated potential biases of including mitochondrial sequences of heterogeneous lengths in a single analysis by analyses of only the NDII region from taxa for which we had the full set of sequences; in no case did the position of these taxa differ in the reconstructions based on truncated data. We likewise confirmed the identification of many taxa, most importantly those based on degraded DNA samples derived from museum skin-snips, using the replicate NDII sequences listed in Appendix A. In all cases these replicate samples from different conspecific individuals had very low divergence and grouped together in tree-based reconstructions.

Gene-trees generated from single nuclear introns generally had low resolution, and we compare here the Bayesian tree based on the four intron loci combined with the

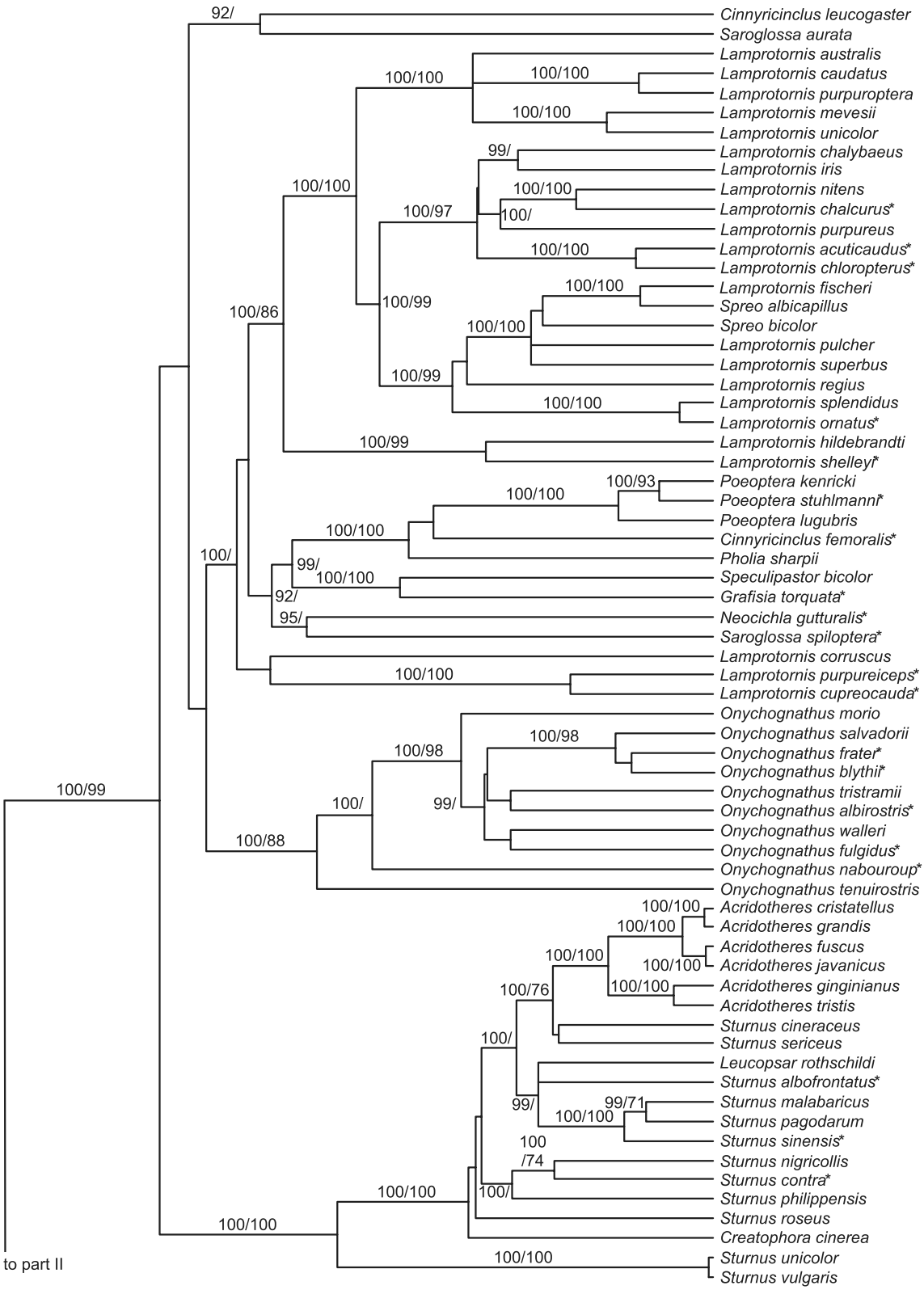


Fig. 2. Ultrametric Bayesian likelihood phylogeny for the Sturnidae and Mimidae based on mitochondrial protein-coding sequences (4116 nucleotides for most taxa; only 1041 bp NDII gene for taxa indicated with asterisks). Numbers above or adjacent to nodes indicate posterior probability values $\geqslant 90$ followed by maximum parsimony bootstrap scores $\geqslant 70$; missing values indicate scores below these thresholds. Owing to the dataset heterogeneity, branch lengths were calculated based on the NDII sequence region only. Tree was rooted to outgroups *Bombycilla*, *Myadestes*, and *Catharus* (not shown).

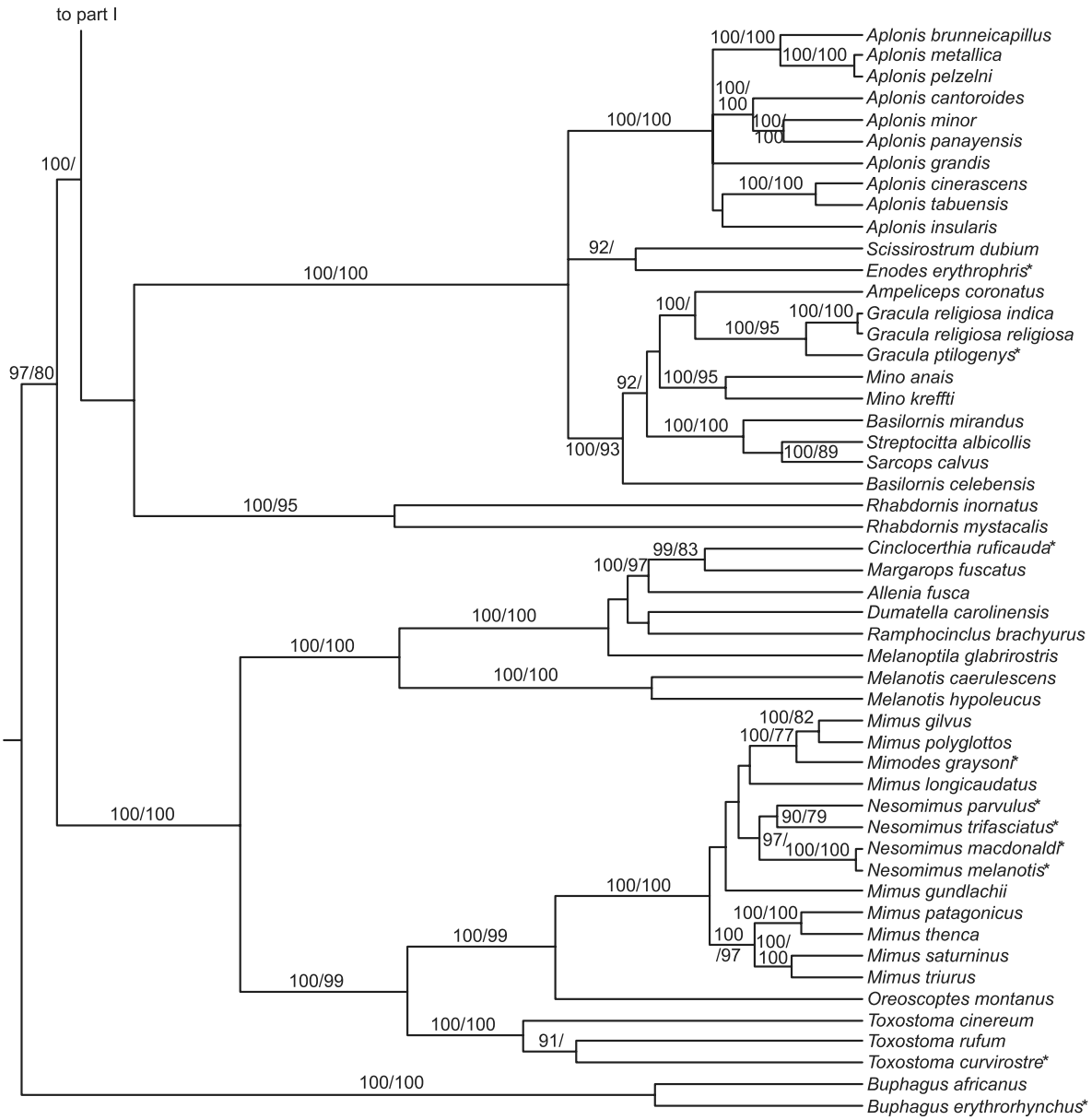


Fig. 2 (*continued*)

corresponding single gene-tree based on five mitochondrial loci. Because the taxa for which we had only NDII sequence are not included, this comparison involves 87 ingroup species, including representatives of all major clades within the combined radiation. The resolved nodes in these nuclear- and mtDNA-based topologies were topologically nearly identical, with the largest conflict involving the placement of the Bali Myna *Leucopsar* within the clade of Eurasian starlings (Fig. 3). Otherwise, these topologies differed only in the relationships of a few taxa within several very recently derived clusters of allied species (Fig. 3).

Indel characters were treated as missing data in our phylogenetic reconstructions, and mapping these characters on the mtDNA and nuclear topologies provides further support for many internodes (Fig. 3). Of the 65 indels that could be aligned with high confidence, 21 were single-taxon apomorphies, 38 mapped on the mtDNA and nuclear trees as singe-mutation synapomorphies, and 6 mapped with two or three inferred changes. Several of these latter indels (labeled A–F in Fig. 3) are shared by taxa that are separated, with strong support, in the independent topologies based on nuclear and mitochondrial nucleotide substitutions; these conflicts may derive from indel mutation homoplasy, or possibly from within-locus recombination followed by lineage sorting.

Given the high congruency among reconstructions derived from different data partitions, the reconstructions based on all combined data (Fig. 4) mirror the features seen in trees based on subsets of the total available data, but with increased support for many internodes. We consider these combined mtDNA and nuclear DNA reconstructions to be the most informative.

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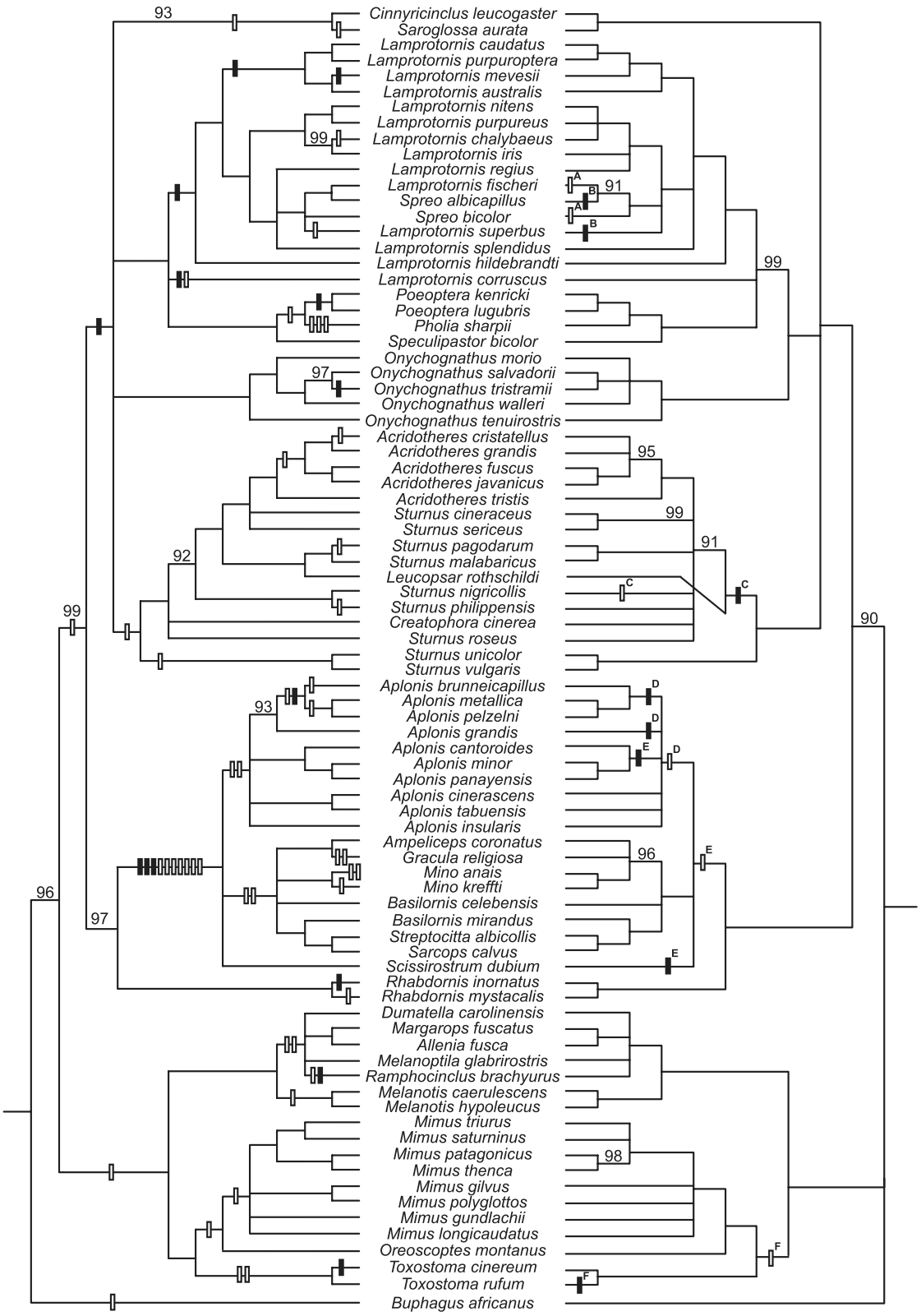


Fig. 3. Comparison of Bayesian 90% consensus trees for 82 Sturnidae and Mimidae taxa based on nucleotide substitutions in five mtDNA coding genes (left; 4116 bp) and four nuclear intron loci (right; 2974 bp). All resolved internodes lacking posterior probability scores were supported at 100%. Box symbols along branches indicate insertion (open boxes) and deletion (filled boxes) mutations in the four intron loci mapped onto these topologies via maximum parsimony. The 58 indel mutations indicated on the left-hand topology each mapped with only a single step; the 5 indels (A–F) indicated on the right-hand tree each required 2–3 steps. Trees were rooted to outgroups *Bombycilla*, *Myadestes*, and *Catharus* (not shown).

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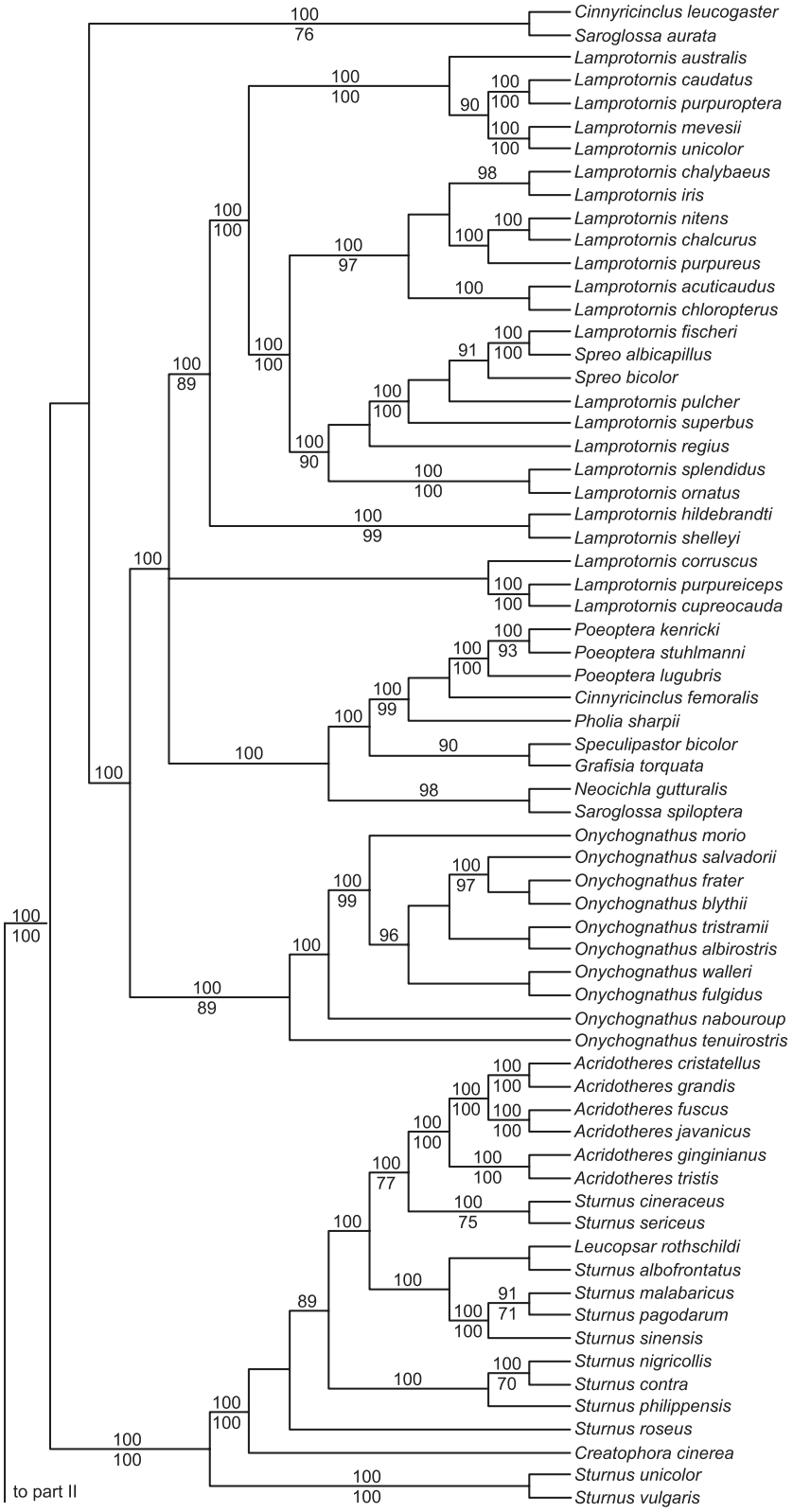


Fig. 4. Total evidence Bayesian consensus tree from analysis of combined mtDNA and nuclear intron sequences for 120 taxa. Numbers above branches indicate posterior probability values $\geqslant 90$; numbers below branches indicate maximum parsimony bootstrap scores $\geqslant 70$; missing values indicate scores below these thresholds. Tree was rooted to outgroups *Bombycilla*, *Myadestes*, and *Catharus* (not shown).

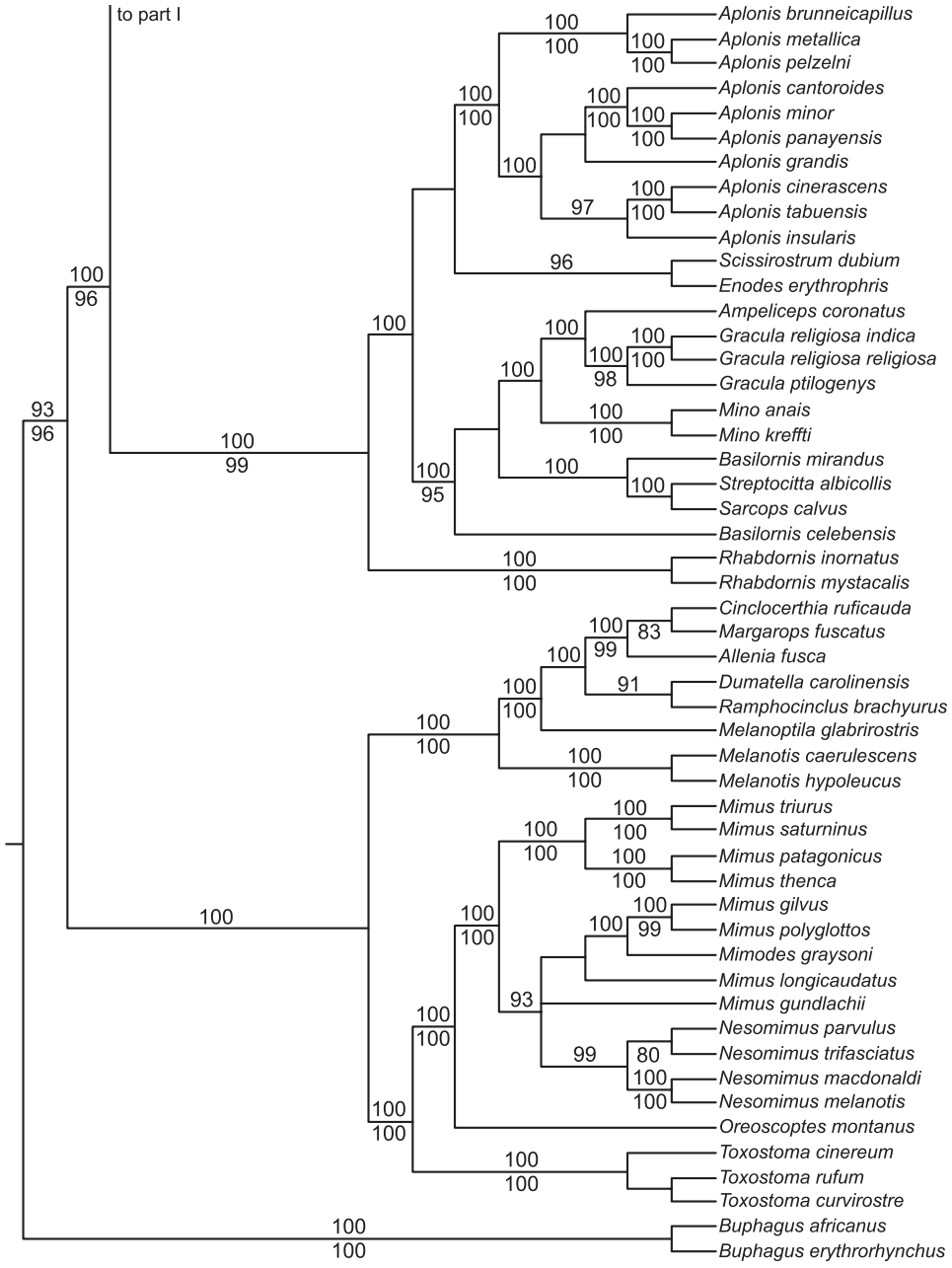


Fig. 4 (*continued*)

### 3.3. Nodes of topological uncertainty

Although the relationships among most lineages within the combined Sturnidae/Mimidae radiation are well supported in all or most reconstructions, several regions of the trees remain imperfectly resolved. With the exception of the four subclades that together link the African and Eurasian Sturnidae, relationships among the major basal clades are generally very highly supported, and those clades themselves are each well defined by long basal internodes (Fig. 5). The relationship among the Eurasian starling group (*Sturnus*, *Acridotheres*, and allies) and the three groups of African starlings is less certain, as these four clades are involved in various alternative (but always negligibly supported) topologies in the mtDNA- and nuclear-only trees (Figs. 2 and 3). In the combined data reconstructions, there is strong (100%) support in the Bayesian tree for a sister relationship between the large African Starling clade and the Red-winged (and also African) starling clade, but this sister relationship does not receive high parsimony bootstrap support.

Two apparent sister species, the Madagascar Starling *Saroglossa aurata* and the Amethyst Starling *C. leucogaster*, form one of the clades involved in this polytomy. These two species are highly divergent from one another, and

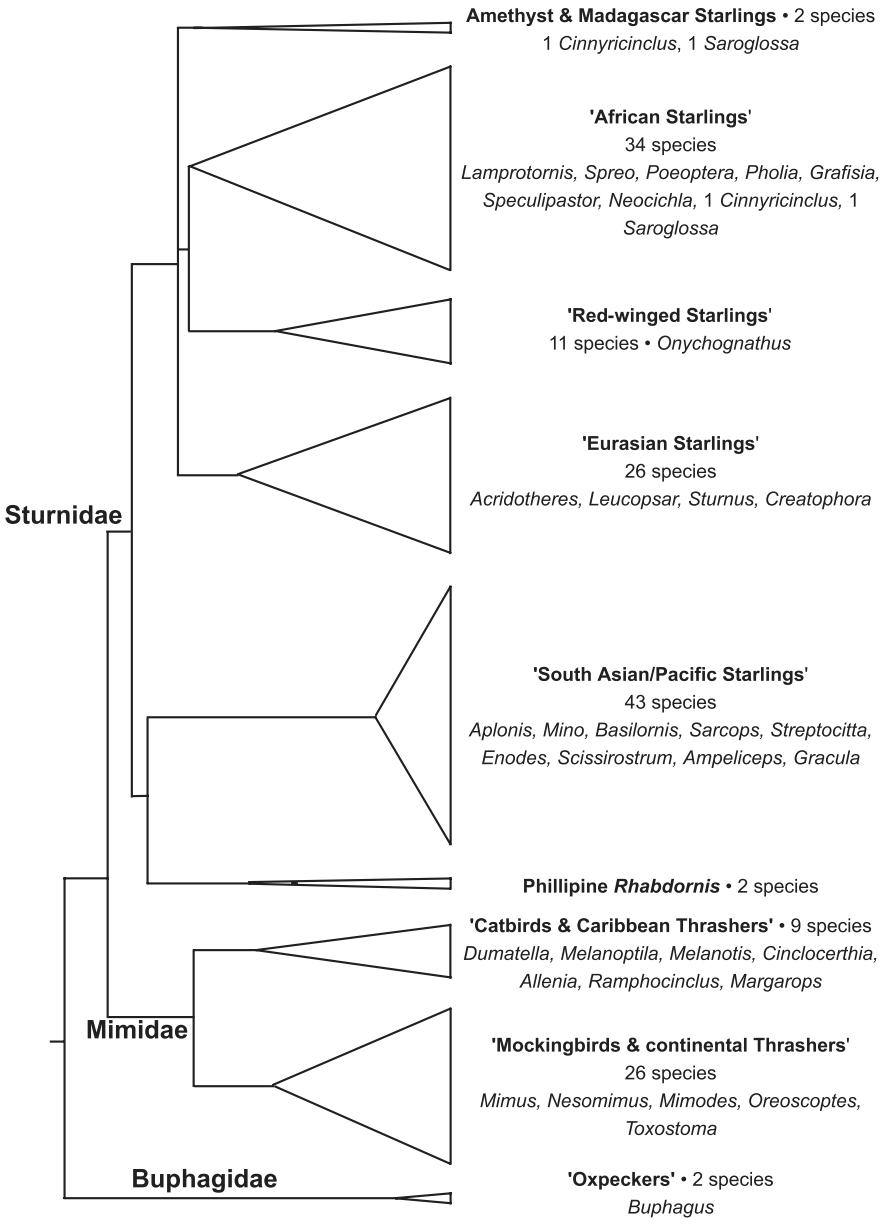


Fig. 5. Schematic representation of the relationships among, and species diversities within, the nine well-supported major clades of starlings, mockingbirds, and oxpeckers. The height of the triangle denoting each group is proportional to its species diversity, whereas the width approximates the relative divergence of the earliest bifurcation within the clade.

both likely represent old lineages with no close extant allies. Their sister relationship could therefore be driven by long-branch attraction (Felsenstein, 1978; Bergsten, 2005), although we note that these species group together in all reconstructions, and that they share a synapomorphic intron deletion.

Several relatively old and well differentiated lineages are also present within the large African clade, and some of these are included here only on the basis of NDII sequences; more extensive sequence data from *Grafisia*, *Neocichla*, *Saroglossa spiloptera*, *Lamprotornis purpureiceps*, and *Lamprotornis cupreocauda* would likely help resolve their placements in these reconstructions. Finally, the six clades with moderate to high species diversities each include some subclades of species that radiated recently and rapidly, but within which resolution is weak.

## 4. Discussion

### 4.1. Major clades

All reconstructions were consistent in defining three major clades within the combined Sturnidae/Mimidae radiation (summarized in Fig. 5). These three clades correspond taxonomically to the families Buphagidae, Mimidae, and Sturnidae. They can be further subdivided into two major subclades within the Mimidae and six within the Sturnidae.

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#### 4.1.1. Buphagidae (oxpeckers)

All reconstructions were consistent in placing the two *Buphagus* oxpeckers together as the basal lineage within this entire radiation. With only three outgroup taxa, our present study was not designed to test the monophyly of *Buphagus* + Mimidae + Sturnidae, as previous studies with robust sampling of allied avian groups have provided universally strong support for this clade that forms the ingroup here (Cibois and Cracraft, 2004; Voelker and Spellman, 2004; Ericson and Johansson, 2003; Barker et al., 2004; Zuccon et al., 2006). The placement of *Buphagus* in our reconstructions confirms relationships seen in several recent studies, in which *Buphagus* has appeared in the same position relative to the Mimidae/Sturnidae (Cibois and Cracraft, 2004; Zuccon et al., 2006). In those previous studies the nodes defining the basal position of *Buphagus* were poorly supported, but with our more substantial taxon and nucleotide sampling, support for the basal position of the *Buphagus* lineage was high in both our mitochondrial-only and nuclear-only reconstructions (Figs. 2 and 3), and in the analyses of combined data where the defining internode had a 93% Bayesian posterior probability and a 96% MP bootstrap score (Fig. 4).

Over the past century, *Buphagus* has either been placed in the monotypic family Buphagidae (Fry et al., 2000), or lumped into the Sturnidae, with most recent taxonomic treatments following this latter classification (e.g., Sibley and Monroe, 1990; Feare and Craig, 1999), often with the Buphaginae ranked as a subfamily (Amadon, 1943, 1956, 1962; Dickinson, 2003). Our strong and independent nuclear and mitochondrial evidence for the basal placement of *Buphagus*, in conjunction with the identical, but more tenuous, results from other recent phylogenetic studies, argues for the recognition of the family Buphagidae in any classification that treats the Mimidae and Sturnidae as separate families, as the inclusion of *Buphagus* renders the family Sturnidae paraphyletic.

#### 4.1.2. Mimidae (mockingbirds and thrashers)

In all reconstructions (Figs. 2–5), the traditional Mimidae form a well supported clade with two subclades, a division that has also been found in previous studies that included subsets of these Mimidae taxa (Arbogast et al., 2006; Cibois and Cracraft, 2004; Hunt et al., 2001; Barber et al., 2004; Zuccon et al., 2006). The most diverse Mimidae subclade includes the *Toxostoma* and *Oreoscoptes* thrashers, and all mockingbirds in the genera *Mimus*, *Nesomimus*, and *Mimodes*. Our sampling included only three of the ten *Toxostoma* species, but previous molecular phylogenies of this genus indicate that it is monophyletic (Zink et al., 1999). The monotypic Sage Thrasher *Oreoscoptes* is the sister taxon to the *Mimus* mockingbird group. Our sampling of *Mimus* and its close allies is congruent with recent studies showing that *Mimodes* and *Nesomimus* fall within *Mimus* (Barber et al., 2004; Arbogast et al., 2006), but some species' relationships within this combined mockingbird clade remain unclear, in part probably because several mockingbird taxa (including all *Nesomimus* and *Mimodes*) were included in our trees only on the basis of NDII sequences. The low resolution at some of the corresponding internodes leaves open the identity of the continental sister lineage of the Galápagos mockingbirds (*Nesomimus*), an important question given the historical prominence of the *Nesomimus* radiation in Charles Darwin's conceptualization of evolutionary modification following from island colonization (Arbogast et al., 2006).

The second Mimidae subclade includes three genera of North and Central American catbirds (*Melanotis*, *Melanoptila*, and *Dumatella*), and four genera of thrashers endemic to the islands of the West Indies (*Ramphocinclus*, *Margarops*, *Allenia*, and *Cinclocerthia*). The two species of *Melanotis* are basal sister species within this subclade. *Melanoptila*, a monotypic genus endemic to the Yucatan Peninsula, is sister to a clade comprised of the four endemic Caribbean genera plus the Grey Catbird *Dumatella*, a long-distance migrant that breeds in continental North America and that over-winters throughout the Greater Antilles and broadly in North and Central America around the Caribbean basin. These relationships are largely congruent with those found previously in molecular phylogenies of this group (Hunt et al., 2001; Barber et al., 2004), except that our reconstructions resolve the placement of *Melanoptila* as basal to the Caribbean endemics + *Dumatella* group. This finding that a Yucatan endemic is basal to a largely Caribbean clade is suggestive of a pathway of colonization into the Antilles from Central America via Cuba as proposed by Hunt et al. (2001), but the placement of *Dumatella* within the Caribbean clade adds a complication to this simple colonization scenario, because it suggests alternatively that either (1) the West Indian taxa are derived from one or more colonization events by a previously migratory ancestor, or that (2) the *Dumatella* lineage has a West Indian origin and has re-evolved the trait of long-distance migration.

#### 4.1.3. Eurasian, South Asian, and Pacific Island Sturnidae

The traditional Sturnidae form a monophyletic group when *Buphagus* is excluded and *Rhabdornis* is included (Fig. 5), with support for this Sturnidae clade high in both mtDNA- and intron-only reconstructions, as well as in the analyses of combined dataset. This group is further defined by an indel synapomorphy, a one-nucleotide deletion in the β-fibrinogen intron 7 locus (Fig. 3). The basal division within the Sturnidae separates a clade of largely South Asian and Pacific Island starlings from a group of largely Eurasian and African starlings (Fig. 5). The South Asian/Pacific Island group is further divided into two subclades, the smaller of which includes only the Phillipine endemic *Rhabdornis*. The larger Eurasian/African group is subdivided into four subclades (Fig. 5).

The affinities of the *Rhabdornis* "Phillipine creepers" were uncertain until molecular phylogenetic reconstructions based on the RAG-1 gene placed *Rhabdornis* clearly within the Sturnidae (Cibois and Cracraft, 2004).

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Subsequent analyses of these RAG-1 sequences in conjunction with mtDNA and intron sequences clarified the position of *Rhabdornis* as the sister lineage to the South Asian/Pacific Island starling group (Zuccon et al., 2006), a finding mirrored in our reconstructions (Figs. 2–5).

The *Rhabdornis* lineage is sister to a large clade of Australasian mynas and starlings that includes a few lineages found on the Indian subcontinent and in continental southeast Asia, as well as several groups that have diversified more extensively on various archipelagos within the Indopacific region. This clade comprises species in the genera *Aplonis*, *Gracula*, *Mino*, *Basilornis*, and *Streptocitta*, as well as in the monotypic genera *Scissirostrum*, *Enodes*, *Ampeliceps*, and *Sarcops*. The phylogenetic affinities of these genera have been previously suspected based on shared morphological traits and geographical proximity (Feare and Craig, 1999). As viewed best in the ultrametric tree based on mtDNA sequences (Fig. 2), this entire group shares a recent common ancestor, and its remarkable species and morphological diversities result from a correspondingly recent period of rapid diversification. The *Aplonis* clade, which has explosively radiated across much of the Indopacific, is especially notable in this regard, as even within this recent broader group, *Aplonis* is defined by a long basal internode leading to a cluster of species with very low mitochondrial divergence (Fig. 2). Although our sample includes only 10 of the 22 *Aplonis* species, it includes representatives of most of the morphologically and geographically distinctive *Aplonis* subgroups, and thus suggests that this genus is monophyletic. Other island radiations within this broader clade include: (1) the monotypic and morphologically unusual genera *Enodes* and *Scissirostrum*, which are both endemic to Sulawesi and which are likely sister taxa; and (2) the *Basilornis*/*Sarcops*/*Streptocitta* mynas, which are distributed from the Phillipines south to Sulawesi.

The remaining Sturnidae include one clade of 'Eurasian starlings' and three clades of African taxa. A deep division within the Eurasian clade separates the well-known European Starling *Sturnus vulgaris* (and its close relative *S. unicolor*, which is often treated as a subspecies of *vulgaris;* Feare, 1984; de la Cruz-Cardiel et al., 1997) from the remaining taxa, which comprise lineages that radiated relatively recently (Fig. 2). This group includes species placed in the genera *Acridotheres*, *Leucopsar*, *Creatophora*, and *Sturnus* by Dickinson (2003), but divided among as many as 7 (Feare and Craig, 1999) to 11 (Wolters, 1982) genera in other recent treatments. One monotypic genus within this clade, *Creatophora*, is notable for its entirely African distribution, but its affinities to this otherwise Eurasian group have long been suspected (Amadon, 1956; Feare and Craig, 1999). Although we are still lacking molecular phylogenetic information for 6 of the 26 species in this group, the available evidence suggests that *Acridotheres* (sensu Dickinson, 2003) is monophyletic, but that the lineages assigned to the three remaining genera have a more complicated history than reflected in any previous classification (Table 1). The apparently rapid diversification of these lineages helps explain why the relationships within this group have been difficult to discern from morphological evidence.

#### 4.1.4. African Sturnidae

The three African clades include one that is comprised of a pair that are likely sister taxa, the Amethyst Starling *C. leucogaster* and the Madagascar Starling *S. aurata*. These species are highly divergent from one another at both mtDNA and nuclear intron loci (Figs. 2–5), suggesting that they both represent relatively old relictual lineages. They are both found largely in forested ecotones, and both move nomadically in flocks of conspecifics, a trait that may have facilitated the colonization of Madagascar by *S. aurata* and of most of sub-Saharan Africa by *C. leucogastor*.

A second well-supported clade is comprised of all species of 'red-winged' starlings in the genus *Onychognathus*. Nine of the 11 species in this genus are found in sub-Saharan continental Africa, with one species (*frater*) endemic to Socotra Island off the Horn of Africa, and one species (*tristramii*) distributed along the Arabian coast of the Red Sea and north into the Sinai Penninsula and Israel. Our species sampling of this group is complete, save for one species from central western Africa (*neumanni*) that was only recently split from the more widespread *O. morio* (Craig, 1998). Our results confirm the monophyly of *Onychognathus*, which has long been suspected on the basis of the morphological similarities of its constituent species (Fry et al., 2000). At the species level, our trees are in substantial conflict with some previous hypotheses of relationship within *Onychognathus*, including the suggestion that the longer tails and more pronounced sexual dimorphism seen in *morio*, *fulgidus*, *blythii*, and *tristrami* indicate their superspecies-level affinity (Hall and Moreau, 1970), and a phylogenetic analysis of morphological and ecological characters (Craig and Hulley, 1992) that suggested that *alirostris*/*blythii*/*salvadorii*/*neumanii*, *morio*/*tenuirostris*, and *fulgidus*/*walleri* each form clades. Our results show little support for these hypotheses and suggest that further work on this clade is warranted.

The largest African clade contains 34 species with a volatile genus-level history of classification (Table 1; also Fry et al., 2000). Our species sampling of this group is complete. Our trees support the monophyly and close genetic affinities of the three canopy forest *Poeoptera* species, which group into a deeper clade along with *Cinnyrinclus femoralis* and *Pholia sharpii*; these latter two species have frequently been considered congeneric in alternative classifications (Table 1). This well-supported group of five species is subsequently nested within a less strongly supported clade that also contains four deeply rooted, single-species lineages (*Speculipastor*, *Grafisia*, *Neocichla*, and *S. spiloptera*). The placement of *S. spiloptera*, the Spot-winged Starling, within the African starling group is unanticipated, as this species breeds in the Himalayan foothills and migrates nomadically east to the Indochinese

Peninsula, and it is the only member of this entire larger African group to occur outside of Africa. *S. spiloptera* is not closely allied to the one other *Saroglossa* species, the Madagascar Starling *S. aurata*.

We found two separate clades of species usually assigned to *Lamprotornis*. The smaller of these clades comprises two closely related species (*cupreocauda* and *purpureiceps*) previously recognized as a superspecies (Hall and Moreau, 1970; Fry et al., 2000) and placed together in *Hylopsar* by Feare and Craig (1999) on the basis of their unusual feather pigment structures. In all reconstructions that include these taxa, their sister lineage is the single species *Lamprotornis corruscus*, but this sister relationship between *cupreocauda/purpureicieps* and *corruscus* is not highly supported. Accordingly, the *corruscus* lineage appears to have originated early in the African radiation, much like *Neocichla*, *Speculipastor*, and *Grafisia*.

The larger *Lamprotornis* group comprises 22 species from sub-Saharan Africa. Support for the monophyly of this group is high in the sequence-based reconstructions (Figs. 2–4), and further supported by one indel synapomorphy (Fig. 3). Within this clade are four well-supported subclades, the most basal of which unites *hildebrandti* and *shellyi*, taxa that were formerly often considered conspecific (e.g., Amadon, 1962). Five species form a second clade notable for the extreme elongation of their tails, with the basal species (*australis*) showing an intermediate tail length. The long-tailed Ashy Starling *L. unicolor* is a member of this clade, rather than a member of the "*Spreo*" subgroup of *Lamprotornis* as often proposed (e.g., Feare and Craig, 1999). However, the long tailed Golden-breasted Starling *Lamprotornis regius* is not a member of this "long-tailed" sub-group. Seven shorter-tailed and ground-foraging 'glossy' starling form a third clade. Finally, eight species form a group that various authors have separated, in many combinations of species and little consistency among recent classifications, into the genus *Spreo* (Table 1; also Fry et al., 2000). Our results provide strong evidence that all previous treatments of *Spreo* render *Lamprotornis* paraphyletic. Aside from this issue of classification, the general grouping of these eight species is consistent with many previous suggestions that subsets of these taxa are closely allied to one another (Hall and Moreau, 1970; Feare and Craig, 1999; Fry et al., 2000). These eight species have notably high variation in plumage coloration, tail length, habitat affinities, and mating systems (Feare and Craig, 1999).

Phylogenetic relationships among most genera of African starlings have been assessed previously based on cladistic analysis of external color, feather ultrastructure (Craig and Hartley, 1985), body shape, skeletal, and behavioral characters (Craig, 1997), although this latter analysis was characterized by the author as preliminary owing to the lack of data for many species. We note that few nodes are shared between the DNA-based trees reported here and the previous non-molecular phylogenies, possibly because the radiation of the African Sturnidae has involved high rates of morphological change and concomitant morphological homoplasy.

### 4.2. Global biogeography and comparison with previous studies

Previous phylogenies for the Sturnidae/Mimidae clade provided the basis for biogeographic scenarios advanced by Sibley and Ahlquist (1990) and Zuccon et al. (2006). Our taxonomically more comprehensive and better resolved reconstructions allow us to evaluate some aspects of these prior historical hypotheses and suggest several modifications and alternatives.

Sibley and Ahlquist (1984, 1990) proposed that the common ancestor of the Sturnidae/Mimidae had a widespread distribution across the Northern Hemisphere during the Miocene, and that the subsequent long period of global cooling severed this range and fostered the diversification of the two families at more southern latitudes. This scenario was logical given their simple, but sparsely sampled, DNA–DNA hybridization-based trees, which did not include *Buphagus* and which divided the Mimidae cleanly from the Sturnidae with little phylogenetic structuring within either family. The temporal dating of this basal division was based on their assumption that the separation of these families was caused by early Pliocene climate change; because the relevant DNA–DNA hybridization distances were not congruent with dates derived from their previous (and chronologically much older) calibration points, Sibley and Ahlquist (1990) used their speculative late Miocene split between the Sturnidae/Mimidae to justify a twofold faster rate of genetic divergence for all birds with short generation times.

With better information on the pattern of diversification within these groups and a more robust calibration point for the Mimidae/Sturnidae split derived from Barker et al. (2004), Zuccon et al. (2006) examined several possible biogeographic histories for the Mimidae and Sturnidae, but favored a scenario much like that of Sibley and Ahlquist (1990) in which a forest-inhabiting Eurasian ancestral taxon gave rise first to the *Buphagus* lineage after colonizing Africa, and then to the respective Mimidae and Sturnidae clades after colonizing North America. The Old World Sturnidae then split into two clades, one that gave rise to *Rhabdornis* and then diversified in Wallacea, and the other which dispersed into Africa. They point out that the notable morphological diversity of this first "Wallacean" clade (which corresponds to our "South Asian/Pacific clade"; we prefer this alternate geographic descriptor because the majority of species in this clade occur outside of Wallacea proper) results from this group retaining the remnant lineages of an old radiation. In contrast, we found that both their trees and ours suggest instead that this largely island-inhabiting group has undergone a very recent period of explosive diversification and correspondingly shares a more recent common ancestor than any other speciose clade within the entire radiation. The remarkable diversity of this

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group is therefore more likely a result of ecological release than an example of the relictual retention of ancient differentiation.

In the Zuccon et al. (2006) scenario, the clade corresponding to our "Eurasian Starlings" represents a secondary colonization out of Africa, with the Madagascar Starling *S. aurata* also representing a colonization from Africa. Because the relationships among the Eurasian and various African clades are not well resolved, this out-of-Africa hypothesis for the Eurasian group is equally parsimonious with a number of alternative scenarios. The small number of early lineages within the Buphagidae/Mimidae/Sturnidae radiation and their simple pattern of geographical separation make it difficult to polarize biogeographic hypotheses with confidence, but we offer several further observations about the traits likely associated with the diversification of the group and suggest that the history of this group is more complicated than previously recognized. First, the present-day restriction of the basal lineage, the Buphagidae, to Africa could readily result from recent environmental changes elsewhere: the oxpeckers are obligately associated with large herbivores, and the presence of diverse megafauna communities across Eurasia and the Americas prior to the very late Pleistocene could have supported a geographically widespread oxpecker relative. The rapid extinction of the Pleistocene megafauna could have led to a parallel extinction of ancestral Buphagidae throughout North America and Eurasia. Unfortunately, it is not possible to reconstruct the geographic distribution of this common ancestor by reference to the distributions of outgroup taxa, as the two candidate sister clades both have very broad distributions across all (in the Turdidae + allies) or most (Cinclidae) continents. Moreover, as far as we know, there are no known fossils of the Buphagidae or its ancestral relatives.

Second, we suggest that the tendency to associate with large mammals is likely ancestral to the entire radiation, not a derived condition restricted to the Buphagidae: species in all of the major Sturnidae clades (except the forest-dwelling *Rhabdornis* creepers) regularly forage around the feet of large ungulates, and many frequently perch on these animals while foraging (Feare and Craig, 1999). Large mammal associations are more unusual in the Mimidae, but several populations of Galápagos mockingbirds are well known for their oxpecker-like behavior of eating ectoparasites, drinking blood, and picking at wounds on marine iguanas, seabirds, and sea lions (Curry and Anderson, 1987).

Third, we note that several clades contain lineages that have dispersed substantially beyond their current biogeographic centers of diversity. This is most notable in the Mimidae, in which the *Mimus* group appears to have colonized South America relatively recently; in the Asian taxon *S. spiloptera*, which is nested within the large African group; and in the African *Creatophora cinerea*, which is nested with the Eurasian group. Many additional species of Sturnidae move nomadically in large flocks during at least part of the year, often tracking fruit resources (Feare and Craig, 1999). This tendency for flocks of birds to disperse together likely facilitated their colonizations of new regions (Clegg et al., 2002), including the many Sturnidae and Mimidae populations now present on remote islands.

Finally, we found that several of the most diverse clades within the radiation are defined by long basal internodes (shown schematically in Fig. 5), such that the extant diversity of these groups results from a lineage that persisted for substantial periods before diversifying into the presently extant lineages. It is likely that at least some now-extinct lineages were contemporaneous with these now-basal lineages, but we know nothing about their diversity or geographic distributions. Factoring these patterns into even the most simple biogeographic scenarios adds substantial complexity. For example, it is possible that the early split between the Mimidae and Sturnidae resulted from the colonization of the New World by the ancestral mimid as suggested by both Sibley and Ahlquist (1990) and Zuccon et al. (2006), but given the much more recent basal split *within* the Mimidae clade (Fig. 5), it is equally possible that the ancestral mimid lineage persisted in the Old World for a substantial time and that the radiation of extant Mimidae occurred when one member of this group colonized the New World at a much later point.

### 4.3. Taxonomic recommendations

Our taxonomic recommendations are based on several conservative criteria: (A) the assignment of names at and above the genus level to monophyletic groups; (B) applying taxonomic revisions only when newly understood relationships are well supported by independent lines of evidence, such as robust and congruent mtDNA and nuclear gene trees; and (C) holding off on revisions in situations where information on some relevant taxa is missing, and where the inclusion of those missing lineages might alter the preferred classification.

At the family level, we recommend the recognition of the Buphagidae, Mimidae, and Sturnidae, as summarized in Fig. 5.

In classifications that include major divisions within families (e.g., subfamilies or tribes), we recommend the further subdivision of the Mimidae into two groups, and the Sturnidae into six groups, also as summarized in Fig. 5.

At the genus level, our results identify a number of genera that are not monophyletic, including *Mimus*, *Basilornis*, *Sturnus*, *Saroglossa*, *Cinnyricinclus*, *Lamprotornis*, and *Spreo*. We currently lack phylogenetic information on only a single species of *Mimus*, and we concur with recent suggestions (e.g., Barber et al., 2004) to merge *Nesomimus* and *Mimodes* into *Mimus*. We lack potentially important species of both *Basilornis* and *Sturnus*, and we therefore do not yet recommend an alternative classification of these genera, although we anticipate generic changes when the requisite data become available. As the two *Saroglossa* species are not sister-taxa (nor apparently otherwise closely

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related), we recommend referring *aurata* to *Hartlaubius* Bonaparte 1853. Similarly we recommend moving *Cinnyricinclus femoralis* (but not *C. leucogaster*) and *P. sharpii* to *Poeoptera* Bonaparte 1854.

We follow Feare and Craig (1999) in recognizing *Hylopsar* von Boetticher 1940 for the species more often treated as *L. cupreocauda* and *L. purpurieceips*. We further recommend making *L. corruscus* the sole member of *Notopholia* Roberts 1922, a genus for which *corruscus* is the type. Finally, we recommend subsuming all *Spreo* into *Lamprotornis*.

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## Appendix A

Taxa included in this study, tissue types, collecting localities, institutional sources, and GenBank Accession Numbers

| Taxon | Museum source[a] and sample no. | Type[b] | Locality [c] | Mitochondrial coding genes | | | | Nuclear intron loci | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NDII[d] | COI | COII | ATPases | Fib-5 | Fib-7 | Rho-1 | TGFB2-4 |
| *Rhabdornis mysticalis* | ZMUC-119523 | T | Philippines, Cayapa, Baliuag | EF468190 | EF486342 | EF484316 | EF486775 | — | EF471842 | EF472854 | EF484113 |
| *Rhabdornis inornatus* | FMNH-357586 | T | Philippines, Mindanao, Mt. Kitanglad, Baungon | EF468189 | EF484215 | EF484315 | EF486774 | EF468321 | EF471841 | EF472853 | EF484112 |
| *Aplonis metallica* | UWBM-63222 | T | Solomon Islands, Choiseul Island, Choiseul Prov. | EF468151 | EF484181 | EF484282 | EF486740 | EF468289 | EF471808 | EF472823 | EF484079 |
| *Aplonis cantoroides* | UWBM-63221 | T | Solomon Islands, Choiseul Island, Choiseul Prov. | EF468146 | EF484176 | EF484277 | EF486735 | EF468284 | EF471803 | EF472818 | EF484074 |
| *Aplonis insularis* | AMNH-6574 | T | Solomon Islands, Rennell Island, Tahatmatangi | EF468150 | EF484180 | EF484281 | EF486739 | EF468288 | EF471807 | EF472822 | EF484078 |
| *Aplonis brunneicapillus* | UWBM-60250 | T | Solomon Islands, Guadalcanal Island, Gold River | EF468145 | EF484175 | EF484276 | EF486734 | EF468283 | EF471802 | EF472817 | EF484073 |
| *Aplonis grandis* | UWBM-67899 | T | Solomon Islands, New Georgia, Arara | EF468149 | EF484179 | EF484280 | EF486738 | EF468287 | EF471806 | EF472821 | EF484077 |
| *Aplonis panayensis* | UWBM-64932 | T | Captive bird (JBP) | EF468153 | EF484183 | EF484284 | EF486742 | EF468291 | EF471810 | EF472825 | EF484081 |
| *Aplonis minor* | FMNH-357661 | T | Philippines, Mindanao, Mt. Kitanglad, Baungon | EF468152 | EF484182 | EF484283 | EF486741 | EF468290 | EF471809 | EF472824 | EF484080 |
| *Aplonis pelzelni* | AM-17550 | T | Captive bird (TZP) | EF468154 | EF484184 | EF484285 | EF486743 | EF468292 | EF471811 | EF472826 | EF484082 |
| *Aplonis tabuensis* | UWBM-42839 | T | Tonga, Eua, Houma | EF468155 | EF484185 | EF484286 | EF486744 | EF468293 | EF471812 | EF472827 | EF484083 |
| *Aplonis cinerascens* | UWBM-42817 | T | Cook Islands, Rarotonga, Avarua | EF468147 | EF484177 | EF484278 | EF486736 | EF468285 | EF471804 | EF472819 | EF484075 |
| *Mino kreffti* | UWBM-76294 | T | Solomon Islands, New Georgia, Lambet | EF468161 | EF484191 | EF484291 | EF486750 | EF468299 | EF471818 | EF472832 | EF484089 |
| *Mino anais* | LSUMNS-B20541 | T | Captive bird (PAC) | EF468160 | EF484190 | EF484290 | EF486749 | EF468298 | EF471817 | EF472831 | EF484088 |
| *Basilornis celebensis* | CUMV-51469 | T | Captive bird (HZG) | EF468156 | EF484186 | EF486341 | EF486745 | EF468294 | EF471813 | EF472828 | EF484084 |
| *Basilornis miranda* | FMNH-357664 | T | Philippines, Mindanao, Mt. Kitanglad, Baungon | EF468157 | EF484187 | EF484287 | EF486746 | EF468295 | EF471814 | EF472829 | EF484085 |
| *Sarcops calvus* | FMNH-358605 | T | Philippines, Sibuyan, Goangan | EF468163 | EF484193 | EF484293 | EF486752 | EF468301 | EF471820 | EF472834 | EF484091 |
| *Streptocitta albicollis* | CLOFBP-393013 | F | Captive bird (SDZ) | EF468162 | EF484192 | EF484292 | EF486751 | EF468300 | EF471819 | EF472833 | EF484090 |
| *Enodes erythrophris* | AMNH-299958 | S | Indonesia, Sulawesi | EF468227[d] | | | | | | | |
| *Enodes erythrophris* | AMNH-299946 | S | Indonesia, Sulawesi, Rujukan | EF468228 | | | | | | | |
| *Scissirostrum dubium* | LSUMNS-B20447 | T | Captive bird (PAC) | EF468164 | EF484194 | EF484294 | EF486753 | EF468302 | EF471821 | EF472835 | EF484092 |
| *Saroglossa spiloptera* | AMNH-203507 | S | Thailand, Um Parig | EF468220 | | | | | | | |
| *Saroglossa aurata* | FMNH-384699 | T | Madagascar, Toliara, Sakaraha | EF468142 | EF484172 | EF484273 | EF486731 | EF468280 | EF471799 | EF472814 | EF484070 |
| *Ampeliceps coronatus* | BG-020148 | F | Captive bird (BG) | EF468148 | EF484178 | EF484279 | EF486737 | EF468286 | EF471805 | EF472820 | EF484076 |
| *Gracula ptilogenys* | CUMV-15269 | S | Sri Lanka, PundaLaya | EF468237 | | | | | | | |
| *Gracula r. religiosa* | LSUMNS-B27008 | T | Captive bird (HZG) | EF468159 | EF484189 | EF484289 | EF486748 | EF468297 | EF471816 | EF472830 | EF484087 |
| *Gracula religiosa indica* | CLOFBP-AKD06 | F | Captive bird (MNHNPC) | EF468158 | EF484188 | EF484288 | EF486747 | EF468296 | EF471815 | EF484086 | — |
| *Acridotheres grandis* | AMNH-9614 | T | Malaysia, Kuala Lumpur | EF468168 | EF484198 | EF484298 | EF486757 | EF468305 | EF471824 | EF472838 | EF484095 |
| *Acridotheres cristatellus* | NMNH-B3778 | T | Phillippines, Luzon Island, Cagayan Prov. | EF468165 | EF484195 | EF484295 | EF486754 | EF468303 | EF471822 | EF472836 | EF484093 |
| *Acridotheres javanicus* | UWBM-67528 | T | Captive bird (JBP) | EF468169 | EF484199 | EF484299 | EF486758 | EF468306 | EF471825 | EF472839 | EF484096 |
| *Acridotheres fuscus* | AMNH-9618 | T | Malaysia, Kuala Lumpur | EF468166 | EF484196 | EF484296 | EF486755 | EF468304 | EF471823 | EF472837 | EF484094 |
| *Acridotheres ginginianus* | CLOFBP-AKD04 | F | Captive bird (PAC) | EF468167 | EF484197 | EF484297 | EF486756 | — | — | — | — |
| *Acridotheres tristis* | UWBM-42794 | T | Cook Islands, Mangaia, Lake Tiriara | EF468170 | EF484200 | EF484300 | EF486759 | EF468307 | EF471826 | EF472840 | EF484097 |
| *Leucopsar rothschildi* | UWBM-CHC001 | T | Captive bird (WPZ) | EF468176 | EF484205 | EF484305 | EF486764 | EF468311 | EF471831 | EF472843 | EF484102 |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sturnus nigricollis* | AMNH-105473 | T | Captive bird (WCS) | EF468174[d] | | | | | | | |
| *Sturnus nigricollis* | NMNH-B5709 | T | Myanmar, Sagaing Division, Kan Blu, Kyat Thin | EF468173 | EF484203 | EF484303 | EF486762 | EF468309 | EF471829 | EF472842 | EF484100 |
| *Sturnus contra* | AMNH-409725 | S | China, Dalu | EF468175 | | | | | | | |
| *Sturnus philippensis* | AMNH-790490 | S | Phillippines, Mt. Calaviti | EF468180[d] | | | | | | | |
| *Sturnus philippensis* | CLOFBP-BTK4 | F | Captive bird (PAC) | EF468179 | EF484208 | EF484308 | EF486767 | EF468314 | EF471834 | EF472846 | EF484105 |
| *Sturnus sinensis* | CLOFBP-AKD07 | F | Captive bird (MNHNPC) | EF468183[d] | | | | | | | |
| *Sturnus sinensis* | CLOFBP-BTK3 | F | Captive bird (PAC) | EF468184 | | | | | | | |
| *Sturnus malabaricus* | NMNH-B5708 | T | Myanmar, Sagaing Division, Kan Blu, Kyat Thin | EF468178 | EF484207 | EF484307 | EF486766 | EF468313 | EF471833 | EF472845 | EF484104 |
| *Sturnus albofrontatus* | AMNH-265248 | S | Sri Lanka, Newara Eliya | EF468244 | | | | | | | |
| *Sturnus pagodarum* | LSUMNS-B37263 | T | Captive bird (PAC) | EF468187 | EF484213 | EF484313 | EF486772 | EF468319 | EF471839 | EF472851 | EF484110 |
| *Sturnus roseus* | UWBM-46226 | T | Kazakhstan, Almaty Oblysy, Alma Ata | EF468181 | EF484209 | EF484309 | EF486768 | EF468315 | EF471835 | EF472847 | EF484106 |
| *Sturnus sericeus* | CLOFBP-BTK1 | F | Captive bird (PAC) | EF468182 | EF484210 | EF484310 | EF486769 | EF468316 | EF471836 | EF472848 | EF484107 |
| *Sturnus cineraceus* | UWBM-47190 | T | Russia, Khabarovskiy Kray, Khurmuli | EF468177 | EF484206 | EF484306 | EF486765 | EF468312 | EF471832 | EF472844 | EF484103 |
| *Sturnus vulgaris* | CUMV-44167 | T | USA, New York, Ithaca | EF468186 | EF484212 | EF484312 | EF486771 | EF468318 | EF471838 | EF472850 | EF484109 |
| *Sturnus unicolor* | CLOFBP-3251760 | B | Spain, Madrid Prov., Collado Villalba | EF468185 | EF484211 | EF484311 | EF486770 | EF468317 | EF471837 | EF472849 | EF484108 |
| *Creatophora cinerea* | CLOFBP-DRRWS1 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468172[d] | | | | | | | |
| *Creatophora cinerea* | UWBM-70373 | T | South Africa, Free State, Springfontein | EF468171 | EF484201 | EF484301 | EF486760 | EF468308 | EF471827 | EF472841 | EF484098 |
| *Lamprotornis nitens* | CLOFBP-4A14817 | B | Namibia, Tandala Ridge, Windpoort Farm | EF468122[d] | | | | | | | |
| *Lamprotornis nitens* | UWBM-70405 | T | South Africa, KwaZulu/Natal Prov., Ulundi | EF468121 | EF484151 | EF484252 | EF486710 | EF468261 | EF471780 | EF472798 | EF484051 |
| *Lamprotornis chalybaeus* | CLOFBP-04208 | B | Kenya, Eastern Prov., Lewa Wildlife Cons. | EF468112[d] | | | | | | | |
| *Lamprotornis chalybaeus* | CLOFBP-04222 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468111[d] | | | | | | | |
| *Lamprotornis chalybaeus* | CLOFBP-09564 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468113 | EF484143 | EF484244 | EF486702 | EF468254 | EF471773 | EF472792 | EF484043 |
| *Lamprotornis chloropterus* | AMNH-764912 | S | Uganda, Lendju, Mt. Matagi, Lake Albert | EF468232 | | | | | | | |
| *Lamprotornis chalcurus* | NMK-4913 | S | Uganda, Yalogi Gulu | EF468240[d] | | | | | | | |
| *Lamprotornis chalcurus* | CUMV-30003 | S | Nigeria, Northern Region, Kishi | EF468238 | | | | | | | |
| *Lamprotornis splendidus* | FMNH-385397 | T | Uganda, Southern Prov., Ngoto Swamp | EF468128 | EF484158 | EF484259 | EF486717 | EF468267 | EF471786 | EF472803 | EF484057 |
| *Lamprotornis ornatus* | AMNH-266276 | S | Sao Tome and Principe, Principe | EF468229 | | | | | | | |
| *Lamprotornis iris* | CLOFBP-981868 | F | Captive bird (DAK) | EF468119[d] | | | | | | | |
| *Lamprotornis iris* | LSUMNS-B20774 | T | Captive bird (PAC) | EF468118 | EF484148 | EF484249 | EF486707 | EF468259 | EF471778 | EF472796 | EF484048 |
| *Lamprotornis purpureus* | CLOFBP-AKD01 | F | Captive bird (MNHNPC) | EF468124 | EF484154 | EF484255 | EF486713 | EF468263 | EF471782 | EF472799 | EF484053 |
| *Lamprotornis purpuroptera* | CLOFBP-04217 | B | Kenya, Rift Valley Prov., Lake Bogoria | EF468125[d] | | | | | | | |
| *Lamprotornis purpuroptera* | ZMUC-122452 | T | Uganda, Queen Elizabeth | EF468126 | EF484156 | EF484257 | EF486715 | EF468265 | EF471784 | EF472801 | EF484055 |
| *Lamprotornis caudatus* | LSUMNS-B19352 | T | Captive bird (SAZ) | EF468110 | EF484140 | EF484241 | EF486699 | EF468251 | EF471770 | EF472790 | EF484040 |
| *Lamprotornis regius* | CLOFBP-AKD02 | F | Captive bird (DAK) | EF468127 | EF484157 | EF484258 | EF486716 | EF468266 | EF471785 | EF472802 | EF484056 |
| *Lamprotornis mevesii* | CUMV-32262 | S | Botswana, Bechuanaland, Tuli Block | EF468239[d] | | | | | | | |

(*continued on next page*)

**Appendix A** (*continued*)

| Taxon | Museum source[a] and sample no. | Type[b] | Locality [c] | Mitochondrial coding genes | | | | Nuclear intron loci | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NDII[d] | COI | COII | ATPases | Fib-5 | Fib-7 | Rho-1 | TGFB2-4 |
| *Lamprotornis mevesii* | CLOFBP-NB1154 | T | Namibia, Kunene Region | EF468120 | EF484150 | EF484251 | EF486709 | EF468260 | EF471779 | EF472797 | EF484050 |
| *Lamprotornis australis* | CLOFBP-056487 | B | Namibia, Otjiwarongo District, Utsig Farm | EF468108[d] | | | | | | | |
| *Lamprotornis australis* | CLOFBP-5772 | T | Namibia, Otjiwarongo District, Utsig Farm | EF468109 | EF484139 | EF484240 | EF486698 | EF468250 | EF471769 | EF472789 | EF484039 |
| *Lamprotornis acuticaudus* | AMNH-347958 | S | Zambia, Kasempa | EF468221 | | | | | | | |
| *Lamprotornis corruscus* | NMK-16136 | S | Kenya, Kipende, Wenje, Tana River | EF468231[d] | | | | | | | |
| *Lamprotornis corruscus* | ZMUC-119491 | T | Kenya, Malindi, Sokoke Forest | EF468114 | EF484144 | EF484245 | EF486703 | EF468255 | EF471774 | EF472793 | EF484044 |
| *Lamprotornis superbus* | CLOFBP-04209 | B | Kenya, Eastern Prov., Lewa Wildlife Cons. | EF468130[d] | | | | | | | |
| *Lamprotornis superbus* | CLOFBP-41313 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468129 | EF484159 | EF484260 | EF486718 | EF468268 | EF471787 | EF472804 | EF484058 |
| *Lamprotornis hildebrandti* | CLOFBP-04206 | B | Kenya, Eastern Prov., Lewa Wildlife Cons. | EF468117[d] | | | | | | | |
| *Lamprotornis hildebrandti* | CLOFBP-04224 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468116 | EF484146 | EF484247 | EF486705 | EF468257 | EF471776 | EF472795 | EF484046 |
| *Lamprotornis shelleyi* | NMK-15450 | S | Kenya, E. Tenar, Tsavo | EF468215 | | | | | | | |
| *Lamprotornis pulcher* | AMNH-822528 | S | Mali, Timbuktu | EF468233[d] | | | | | | | |
| *Lamprotornis pulcher* | CLOFBP-HOU1 | F | Captive bird (HZG) | EF468123 | EF484153 | EF484254 | EF486712 | — | — | — | — |
| *Lamprotornis purpureiceps* | CLOFBP-NMK29 | S | Uganda, Bwamba Forest, Mongiro | EF468214[d] | | | | | | | |
| *Lamprotornis purpureiceps* | NMK-15364 | S | Uganda, Bwamba, Makitengya | EF468225 | | | | | | | |
| *Lamprotornis cupreocauda* | CUMV-15304 | S | Ghana, Gold Coast, Winnebah | EF468230 | | | | | | | |
| *Lamprotornis unicolor* | NMK-15473 | S | Tanzania, Dodoma | EF468241[d] | | | | | | | |
| *Lamprotornis unicolor* | CLOFBP-AKD03 | F | Tanzania, Tarengire National Park | EF468131 | EF484161 | EF484262 | EF486720 | — | — | — | — |
| *Lamprotornis fischeri* | CLOFBP-04216 | T | Kenya, Eastern Prov., Shaba | EF468115 | EF484145 | EF484246 | EF486704 | EF468256 | EF471775 | EF472794 | EF484045 |
| *Cinnyricinclus femoralis* | NMK-4889 | S | Kenya, Chyulu | EF468217 | | | | | | | |
| *Cinnyricinclus leucogaster* | UWBM-72577 | T | Malawi, Mwanza District Mwanza | EF488683[d] | | | | | | | |
| *Cinnyricinclus leucogaster* | LSUMNS-B22550 | T | Captive bird (HZG) | EF488682 | EF484136 | EF484237 | EF486695 | EF468247 | EF471766 | EF472788 | EF484036 |
| *Spreo bicolor* | UWBM-70392 | T | South Africa, Free State, Harrismith | EF468143 | EF484173 | EF484274 | EF486732 | EF468281 | EF471800 | EF472815 | EF484071 |
| *Spreo albicapillus* | CLOFBP-06001 | T | Kenya, Eastern Prov., Kalacha | EF468141 | EF484171 | EF484272 | EF486730 | EF468279 | EF471798 | EF472813 | EF484069 |
| *Onychognathus morio* | CLOFBP-C7837 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468133[d] | | | | | | | |
| *Onychognathus morio* | UWBM-71314 | T | South Africa, KwaZulu/Natal Prov., Melmoth | EF468132 | EF484162 | EF484263 | EF486721 | EF468270 | EF471789 | EF472805 | EF484060 |
| *Onychognathus tenuirostris* | FMNH-356559 | T | Uganda, Western Prov., Rwenzori Mts. | EF468135 | EF484165 | EF484266 | EF486724 | EF468273 | EF471792 | EF472807 | EF484063 |
| *Onychognathus fulgidus* | AMNH-827360 | S | Uganda, Bwamba Forest, Ntotoro | EF468224[d] | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| *Onychognathus fulgidus* | CUMV-33750 | S | Uganda, Kibale Forest, Fort Portal | EF468223 | | | | | | | |
| *Onychognathus walleri* | FMNH-439575 | T | Malawi, Wilindi Forest, Chitipa | EF468137 | EF484167 | EF484268 | EF486726 | EF468275 | EF471794 | EF472809 | EF484065 |
| *Onychognathus blythii* | AMNH-669364 | S | Somalia, Sheilem | EF468245[d] | | | | | | | |
| *Onychognathus blythii* | AMNH-669371 | S | Somalia, Golis, Gidial Valley | EF468243 | | | | | | | |
| *Onychognathus frater* | AMNH-669276 | S | Socotra, Hornbill | EF468246[d] | | | | | | | |
| *Onychognathus frater* | AMNH-669278 | S | Socotra, Celilo Pass | EF468235 | | | | | | | |
| *Onychognathus tristamii* | CLOFBP-ISR2 | B | Israel, Southern District, Masada | EF468136 | EF484166 | EF484267 | EF486725 | EF468274 | EF471793 | EF472808 | EF484064 |
| *Onychognathus nabouroup* | CUMV-32851 | S | Namibia, Erongo, Homeb | EF468222 | | | | | | | |
| *Onychognathus salvadorii* | CLOFBP-04211 | T | Kenya, Eastern Prov., Shaba | EF468134 | EF484164 | EF484265 | EF486723 | EF468272 | EF471791 | EF472806 | EF484062 |
| *Onychognathus albirostris* | CUMV-15258 | S | Ethiopia, Lenafe | EF468236 | | | | | | | |
| *Poeoptera stuhlmanni* | NMK-18046 | S | Kenya, Nandi Forest | EF468218 | | | | | | | |
| *Poeoptera kenricki* | ZMUC-123520 | T | Tanzania, Udzungwa Forest, Iringa | EF468138 | EF484168 | EF484269 | EF486727 | EF468276 | EF471795 | EF472810 | EF484066 |
| *Poeoptera lugubris* | AMNH-10691 | T | Central African Rep., Sanga-Mbare, Bayanga | EF468139 | EF484169 | EF484270 | EF486728 | EF468277 | EF471796 | EF472811 | EF484067 |
| *Pholia sharpii* | FMNH-356553 | T | Uganda, Western Prov., Rwenzori Mts. | EF468140 | EF484170 | EF484271 | EF486729 | EF468278 | EF471797 | EF472812 | EF484068 |
| *Grafisia torquata* | AMNH-162929 | S | Cameroon, Pawa | EF468234[d] | | | | | | | |
| *Grafisia torquata* | AMNH-162928 | S | Cameroon, Pawa | EF468226 | | | | | | | |
| *Speculipastor bicolor* | NMK-16455 | S | Kenya, Kekerongole | EF468219[d] | | | | | | | |
| *Speculipastor bicolor* | CLOFBP-06004 | T | Kenya, Eastern Prov., Kalacha | EF468144 | EF484174 | EF484275 | EF486733 | EF468282 | EF471801 | EF472816 | EF484072 |
| *Neocichla gutturalis* | NMK-15433 | S | Tanzania, Itigi | EF468242[d] | | | | | | | |
| *Neocichla gutturalis* | NMK-15432 | S | Tanzania, Itigi | EF468216 | | | | | | | |
| *Buphagus erythrorhynchus* | NMK-15490 | S | Kenya, Eastern Prov., Archer's Post | EF468213 | | | | | | | |
| *Buphagus africanus* | CLOFBP-DRR4 | B | Kenya, Rift Valley Prov., Mpala Res. Centre | EF468188 | EF484214 | EF484314 | EF486773 | EF468320 | EF471840 | EF472852 | EF484111 |
| *Dumatella carolinensis* | STRI-BHDCA4 | T | Bahamas, Grand Bahama Island | EF468192 | EF484216 | EF484317 | EF486776 | EF468323 | EF471844 | EF472856 | EF484115 |
| *Melanoptila glabrirostris* | LSUMNS-B0081 | T | Mexico, Quintana Roo, Isla Cozumel | EF468197 | EF484221 | EF484322 | EF486781 | EF468328 | EF471849 | EF472861 | EF484120 |
| *Mimus polyglottos* | LSUMNS-B21369 | T | USA, California, San Bernardino | EF468202 | EF484226 | EF484327 | EF486786 | EF468333 | EF471854 | EF472866 | EF484125 |
| *Mimus gilvus* | STRI-CCMGI1 | T | Trinidad, Chacachacare Island | EF468196 | EF484220 | EF484321 | EF486780 | EF468327 | EF471848 | EF472860 | EF484119 |
| *Mimus gundlachi* | STRI-JAMGU1 | T | Jamaica, Portland Ridge | EF468198 | EF484222 | EF484323 | EF486782 | EF468329 | EF471850 | EF472862 | EF484121 |
| *Mimus thenca* | MNHCN-2676 | T | Chile | EF468204 | EF484228 | EF484329 | EF486788 | EF468335 | EF471856 | EF472868 | EF484127 |
| *Mimus longicaudatus* | LSUMNS-B5229 | T | Peru, Lambayeque | EF468200 | EF484224 | EF484325 | EF486784 | EF468331 | EF471852 | EF472864 | EF484123 |
| *Mimus saturninus* | CUMV-50582 | T | Argentina, Buenos Aires Prov. | EF468203 | EF484227 | EF484328 | EF486787 | EF468334 | EF471855 | EF472867 | EF484126 |
| *Mimus patagonicus* | CUMV-50577 | T | Argentina, Jujuy Prov. | EF468201 | EF484225 | EF484326 | EF486785 | EF468332 | EF471853 | EF472865 | EF484124 |
| *Mimus triurus* | CUMV-MACH14 | T | Argentina, Buenos Aires Prov. | EF468205 | EF484229 | EF484330 | EF486789 | EF468336 | EF471857 | EF472869 | EF484128 |
| *Nesomimus parvulus* | | | Arbogast et al. (2006) | AY311587 | | | | | | | |
| *Nesomimus trifasciatus* | | | Arbogast et al. (2006) | AY311551 | | | | | | | |
| *Nesomimus macdonaldi* | | | Arbogast et al. (2006) | AY311566 | | | | | | | |
| *Nesomimus melanotis* | | | Arbogast et al. (2006) | AY311577 | | | | | | | |
| *Oreoscoptes montanus* | LSUMNS-B19513 | T | USA, California, Barstow | EF468206 | EF484230 | EF484331 | EF486790 | EF468337 | EF471858 | EF472870 | EF484129 |
| *Mimodes graysoni* | | | Barber et al. (2004) | AY758199 | | | | | | | |

(*continued on next page*)

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**Appendix A** (*continued*)

| Taxon | Museum source[a] and sample no. | Type[b] | Locality [c] | Mitochondrial coding genes | | | | Nuclear intron loci | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NDII[d] | COI | COII | ATPases | Fib-5 | Fib-7 | Rho-1 | TGFB2-4 |
| *Toxostoma rufum* | LSUMNS-B0490 | T | USA, Louisiana, Cameron Parish | EF468209 | EF484233 | EF484334 | EF486793 | EF468340 | EF471861 | EF472873 | EF484132 |
| *Toxostoma cinereum* | LSUMNS-B6745 | T | Mexico, Baja California | EF468208 | EF484232 | EF484333 | EF486792 | EF468339 | EF471860 | EF472872 | EF484131 |
| *Toxostoma curvirostre* | | | Barber et al. (2004) | AY758201 | | | | | | | |
| *Ramphocinclus brachyurus* | STRI-SLRBR2 | T | St. Lucia | EF468207 | EF484231 | EF484332 | EF486791 | EF468338 | EF471859 | EF472871 | EF484130 |
| *Melanotis caerulescens* | LSUMNS-B0022 | T | Mexico, Puebla | EF468193 | EF484217 | EF484318 | EF486777 | EF468324 | EF471845 | EF472857 | EF484116 |
| *Melanotis hypoleucus* | CUMV-44026 | T | Mexico, Chiapas | EF468199 | EF484223 | EF484324 | EF486783 | EF468330 | EF471851 | EF472863 | EF484122 |
| *Allenia fusca* | STRI-DOMFU3 | T | Dominica, Springfield | EF468195 | EF484219 | EF484320 | EF486779 | EF468326 | EF471847 | EF472859 | EF484118 |
| *Margarops fuscatus* | STRI-BUMFT1 | T | Antigua and Barbuda, Barbuda | EF468194 | EF484218 | EF484319 | EF486778 | EF468325 | EF471846 | EF472858 | EF484117 |
| *Cinclocerthia ruficauda* | STRI-GUCRU1 | T | Guadaloupe | EF468191 | | | | | | | |
| *Bombycilla cedrorum* | CUMV-50897 | T | USA, New York, Tompkins County | EF468210 | EF484234 | EF484335 | EF486794 | EF468341 | EF471862 | EF472874 | EF484133 |
| *Catharus guttatus* | CUMV-50482 | T | USA, New York, Nassau County | EF468211 | EF484235 | EF484336 | EF486795 | EF468342 | EF471863 | EF472875 | EF484134 |
| *Myadestes townsendi* | LSUMNS-B20975 | T | USA, California, San Bernardino County | EF468212 | EF484236 | EF484337 | EF486796 | EF468343 | EF471864 | EF472876 | EF484135 |

[a] Institutional sources of samples: AM: Australian Museum, Sydney, Australia; AMNH: American Museum of Natural History, New York, NY, USA; CUMV: Cornell University Museum of Vertebrates, Ithaca, NY, USA; FMNH: Field Museum of Natural History, Chicago, IL, USA; LSUMNS: Louisiana State University Museum of Natural Science, Baton Rouge, LA, USA; NMK: National Museums of Kenya, Nairobi, Kenya; NMN: National Museum of Namibia, Windhoek, Namibia; NMNH: National Museum of Natural History, Washington, DC, USA; UWBM: University of Washington Burke Museum, Seattle, WA, USA; ZMUC: Zoological Museum University of Copenhagen, Copenhagen, Denmark.

[b] Sample types: B = blood; F = feather from live bird; T = frozen or buffer-preserved tissue; S = toe-pad shaving from museum skin.

[c] Avicultural collections abbreviated: BG: Bush Gardens, Tampa, FL, USA; DAK: Disney's Animal Kingdom, Lake Buena Vista, FL, USA; HZG: Houston Zoological Garden, Houston, TX, USA; JBP: Jurong Bird Park, Singapore; TZP: Taronga Zoological Park, Sydney, Australia; MNHNPC: Muséum National Histoire Naturelle, Parc de Clères, Clères, France; PAC: Private avicurtural collection; SAZ: San Antonio Zoo, San Antonio, TX, USA; SDZ: San Diego Zoo, San Diego, CA, USA; WCS: Wildlife Conservation Society, New York, NY, USA; WPZ: Woodland Park Zoo, Seattle, WA, USA.

[d] Replicated conspecific specimens for which NDII samples were included in preliminary analyses, but which were not included in the phylogenetic trees reported here.

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