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A complete species-level molecular phylogeny for the "Eurasian" starlings (Sturnidae: *Sturnus*, *Acridotheres*, and allies): Recent diversification in a highly social and dispersive avian group

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Abstract

We generated the first complete phylogeny of extant taxa in a well-defined clade of 26 starling species that is collectively distributed across Eurasia, and which has one species endemic to sub-Saharan Africa. Two species in this group—the European starling *Sturnus vulgaris* and the common Myna *Acridotheres tristis*—now occur on continents and islands around the world following human-mediated introductions, and the entire clade is generally notable for being highly social and dispersive, as most of its species breed colonially or move in large flocks as they track ephemeral insect or plant resources, and for associating with humans in urban or agricultural landscapes. Our reconstructions were based on substantial mtDNA (4 kb) and nuclear intron (4 loci, 3 kb total) sequences from 16 species, augmented by mtDNA NDII gene sequences (1 kb) for the remaining 10 taxa for which DNAs were available only from museum skin samples. The resulting mitochondrial gene tree embedded within a multilocus framework shows that the well-studied taxa *S. vulgaris*/*unicolor* are the sister lineage to the remaining members of the radiation, from which other relatively early lineages gave rise to forms that are now nomadic or locally migrant in Africa (*Creatophora*) and western Asia (*Pastor*). The remaining taxa form a clade with a complicated biogeographic history primarily in central and eastern Asia; this group contains a range of sedentary to highly migratory taxa, as well as widely distributed species and single-island endemics such as the highly endangered Bali myna (*Leucopsar*). Several groups of species in the genus *Acridotheres* have low magnitudes of within-group divergence and likely diversified via their respective colonization of islands. The taxonomy of this entire group has remained highly volatile over the past century; we propose dividing these 26 species among 11 reciprocally monophyletic genera (*Acridotheres*, *Poliopsar*, *Temenuchus*, *Sturnornis*, *Leucopsar*, *Gracupica*, *Agropsar*, *Pastor*, *Creatophora*, and *Sturnus*).

Keywords: Sturnidae; Starling; *Sturnus vulgaris*; Phylogeny; Taxonomy

1. Introduction

Starlings have radiated impressively throughout much of Eurasia, Africa, and the eastern Pacific. Recent phylogenetic work has defined the taxonomic and geographic distributions of the six major clades within the starling family Sturnidae (Zuccon et al., 2006; Lovette and Rubenstein, 2007). One of these sub-groups, which we informally term the "Eurasian" starlings, is particularly notable for comprising species that are nearly all disturbed habitat specialists, many of which now occur as human commensals in urban or agricultural landscapes (Feare and Craig, 1999). Nearly all of the 26 currently recognized extant species (Dickinson, 2003) in this Eurasian clade are likewise at least partially nomadic or migratory, and most breed colonially and/or flock during the non-breeding season (Feare and Craig, 1999). This combination of high dispersal

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potential and the simultaneous movements of groups of individuals has likely facilitated the natural geographic expansion of this group across Eurasia and (by one species) throughout much of Africa, giving this group a greater geographic breadth than that of any equivalent sub-clade within the Sturnidae. The distribution of the Eurasian starling clade has been expanded further by the extensive human-mediated introduction of two species (the European starling *Sturnis vulgaris* and the common myna *Acridotheres tristis*) to all non-polar continents and to hundreds of islands in both hemispheres (Feare and Craig, 1999). This clade of starlings is further important because *Sturnus vulgaris* has long been the subject of extensive ecological, physiological, and behavioral research (Feare, 1984), and placing such studies in a comparative context requires phylogenetic information on how *vulgaris* is related to allied taxa.

The taxonomy of this group of starlings has been highly volatile over the past century, particularly at the genus-level (Table 1), owing largely to the lack of known morphological synapomorphies within the group. Several workers with extensive systematic expertise with starlings have commented on the confusing relationships among these taxa (Amadon, 1943; Beecher 1978; Feare and Craig, 1999). Beecher (1978) generated a dendogram of starling genera based on his studies of jaw musculature, in which the genera in our Eurasian clade cluster together. A terminal branch in the DNA–DNA hybridization studies of Sibley and Ahlquist (1984, 1990) similarly groups together the four Eurasian species they investigated. More recently, one to three species in the Eurasian clade have been included in a number of molecular phylogenetic studies of deeper songbird relationships (Barker et al., 2004; Voelker and Spellman, 2004; Spicer and Dunipace, 2004; Ericson and Johansson, 2003; Cibois and Cracraft, 2004; Alström et al., 2006), and one recent study (Zuccon et al., 2006) included eight. Kang et al. (1994) used cytochrome *b* sequences to examine relationships among three closely allied *Acridotheres* species.

The most extensive previous phylogenetic sampling of the Eurasian starlings was in a recent mtDNA and intron based study by our group (Lovette and Rubenstein, 2007) that included 20 Eurasian clade species along with robust sampling of all other Sturnidae clades and genera. Our previous work was directed largely towards defining the various major clades within the deeper Sturnidae radiation, and it provided unambiguous support for the in-group considered here, as the basal internode leading to the Eurasian clade was long and universally highly supported across datasets and analysis methods (Lovette and Rubenstein, 2007). Other relevant findings from our previous study include: (i) the likely position of *Sturnus vulgaris/unicolor* as the basal lineage within the Eurasian starling clade; (ii) the inclusion of the African species *Creatophora cinerea*

Table 1
Taxonomic recommendations for "Eurasian" starlings based on their molecular phylogenetic affinities, and comparisons to five recent taxonomic treatments of this group

Proposed here		Amadon (1962)	Wolters (1982)	Sibley and Monroe (1990)	Feare and Craig (1999)	Dickinson, (2003)
Genus	Species					
Acridotheres	*grandis*[a]	—	*Aethiopsar*	—	—	—
Acridotheres	*cristatellus*[a]	—	*Aethiopsar*	—	—	—
Acridotheres	*albocinctus*[a]	—	*Aethiopsar*	—	—	—
Acridotheres	*javanicus*[b]	ssp. *fuscus*	ssp. *fuscus*	—	—	—
Acridotheres	*cinereus*[b]	*Sturnus*	*Aethiopsar*	ssp. *fuscus*	—	—
Acridotheres	*fuscus*[b]	—	*Aethiopsar*	—	—	—
Acridotheres	*melanopterus*	*Sturnus*	*Leucopsar*	*Sturnus*	—	—
Acridotheres	*burmannicus*	—	*Leucopsar*	—	*Sturnus*	—
Acridotheres	*ginginianus*	—	—	—	—	—
Acridotheres	*tristis*	—	—	—	—	—
Poliopsar	*sericeus*	*Sturnus*	*Sturnopastor*	*Sturnus*	*Sturnus*	*Sturnus*
Poliopsar	*cineraceus*	*Sturnus*	*Sturnopastor*	*Sturnus*	*Sturnus*	*Sturnus*
Temenuchus	*sinensis*	*Sturnus*	*Sturnia*	*Sturnus*	*Sturnia*	*Sturnus*
Temenuchus	*malabaricus*	*Sturnus*	—	*Sturnus*	*Sturnia*	*Sturnus*
Temenuchus	*erthropygius*	*Sturnus*	—	*Sturnus*	*Sturnia*	*Sturnus*
Temenuchus	*pagodarum*	*Sturnus*	—	*Sturnus*	—	*Sturnus*
Sturnornis	*albofrontata*	*Sturnus*	*Temenuchus*	*Sturnus*	—	*Sturnus*
Leucopsar	*rothschildi*	—	—	—	—	—
Gracupica	*nigricollis*	*Sturnus*	—	*Sturnus*	—	*Sturnus*
Gracupica	*contra*	*Sturnus*	*Sturnopastor*	*Sturnus*	—	*Sturnus*
Agropsar	*sturninus*	*Sturnus*	—	*Sturnus*	*Sturnia*	*Sturnus*
Agropsar	*philippensis*	*Sturnus*	—	*Sturnus*	*Sturnia*	*Sturnus*
Pastor	*roseus*	*Sturnus*	—	*Sturnus*	—	*Sturnus*
Creatophora	*cinerea*	—	—	—	—	—
Sturnus	*vulgaris*[c]	—	—	—	—	—
Sturnus	*unicolor*[c]	—	—	—	—	—

[a,b,c] Superscripts denote three clades within which species are separated by very low levels of genetic differentiation.

as the sole non-Eurasian member of the clade, which is consistent with previous suggestions based on its morphological and behavioral similarities to the remaining species in this group (Amadon, 1943, 1956; Feare and Craig, 1999; Fry et al., 2000); and (iii) a lack of close correspondence between the DNA based reconstructions and any previous genus-level classification of these taxa.

Here, we reconstruct relationships among all 26 extant species in the Eurasian starling group. High-quality genetic materials were available from 16 species, and for these taxa we have substantial mitochondrial DNA and nuclear intron information. For 10 species, however, we have only mitochondrial sequences derived from museum skin samples. The combined analysis of these nested datasets allows us to place the mtDNA-only gene tree within the more robust phylogenetic framework provided by the more extensive, multilocus markers, and we discuss the opportunities and caveats associated with this combination of markers. The resulting reconstructions are the first complete phylogenies for this clade of extant starlings; they provide a formal phylogenetic basis for revised genus-level taxonomy and allow us to examine the evolution of the jaw musculature traits that have formed the primary basis for the existing taxonomy of this group.

2. Methods

2.1. Taxon sampling and laboratory methods

For this study, we sampled at least one representative of all extant "Eurasian" Sturnidae taxa that have been considered full species in any of five recent classifications of the family (Table 1). Twenty of these taxa were included in our previous study of Sturnidae phylogenetics (Lovette and Rubenstein, 2007) and the corresponding sample sources and sequence data are described in detail there. Samples new to this analysis are described in Table 2. In all cases, these new samples were derived from toe-pad tissues taken from existing museum skin materials. Toe-pad skin samples were taken by shaving a narrow band of skin from a single toe (usually the hallux), using a new, sterile scalpel. These skin samples were placed dry into sterile 1.5 mL tubes, sealed, transferred to our degraded-DNA laboratory, and stored at room temperature until DNA extraction. We also attempted to generate sequences from the extinct Reunion Island taxon *Fregilupus varius*, which has some morphological similarities to the *Sturnus* starlings (Amadon, 1956), but which has generally uncertain affinities and may not be a starling (Miller 1941; Berger, 1957); we were unable to amplify PCR products using Sturnidae-specific primers from toe-pad skin samples of a *Fregilupus* specimen collected some time before 1840 that is now in the collection of the Harvard University Museum of Comparative Zoology. We also did not include *Necropsar leguati*, a taxon described as a starling but now known to be based on a fraudulently labeled specimen (Olson et al., 2003).

From most of the samples included in our previous study, we have sequences spanning the NDII, COI, COII, ATPase8, and ATPase6 mitochondrial genes (total length 4118 bp), and sequences from four introns totaling nearly 3 kb, including indels after alignment: rhodopsin intron 1 (Primmer et al., 2002), intron 5 of transforming growth factor β-2 intron 5 (Primmer et al., 2002), and the closely linked β-fibrinogen introns 5 (F.K. Barker, personal communication) and 7 (Prychitko and Moore, 1997). From some additional taxa included previously, and from all samples new to the present analysis, we obtained only

Table 2
Samples included in this study that have not been reported previously and GenBank accession numbers for the associated NDII sequences

Taxon	Source[a]	Specimen	Year[b]	Locality	GenBank No.
Acridotheres albocinctus	AMNH	347176	1945	Myanmar (Burma)	EU403586
Acridotheres albocinctus	AMNH	409723	1935	India: Assam, Dalu	EU403585
Acridotheres cinereus	AMNH	299902	1931	Indonesia: Celebes, Lombasang	EU403589
Acridotheres cinereus	AMNH	299898	1930	Indonesia: Sulawesi, Makassar	EU403590
Acridotheres javanicus	AMNH	666486	1927	Indonesia: Java, Mt. Ohojoeno	EU403592
Acridotheres javanicus	AMNH	666493	1927	Indonesia: Java, Mt. Ohojoeno	EU403593
Acridotheres melanopterus	AMNH	417390	1937	Captive bird (New York Zoo)	EU403595
Acridotheres melanopterus	AMNH	666360	1927	Indonesia: Java	EU403594
Acridotheres burmannicus	MVZ	156076	1965	Vietnam: Saigon	EU403587
Acridotheres burmannicus	MVZ	156077	1965	Vietnam: Saigon	EU403588
Acridotheres ginginianus	AMNH	304811	1931	Pakistan: Punjab, Lahore	EU403591
Sturnornis albofrontata	AMNH	666278	1876	Sri Lanka: "Powanala"	EU403596
Temenuchus erythropygius	AMNH	666245	1907	Nicobar Island	EU403597
Temenuchus erythropygius	AMNH	666249	1907	Nicobar Island	EU403598
Agropsar sturninus	AMNH	666153	1910	Malaysia: Perak	EU403600
Agropsar sturninus	AMNH	666149	1918	Indonesia: Sumatra, Deli	EU403599

All samples below were derived from toe-pads taken from museum skin specimens.

[a] Museum specimen sources: AMNH, American Museum of Natural History, New York, NY, USA; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, USA.

[b] Year of specimen collection.

NDII sequences. Laboratory methods for the generation of these sequences are detailed in Lovette and Rubenstein (2007).

All samples new to this study were processed in a laboratory dedicated to degraded-DNA extraction and PCR-set up that has physical and air-handling isolation from our general-use laboratory. Toe-pad extractions and PCR reactions were assembled and conducted within a laminar-flow clean bench with ISO class V air filtering, and we frequently sterilized surfaces and equipment in the degraded-DNA room with intense 254 nm UV irradiation and 10% sodium hypochlorite (chlorine bleach) solutions. To enhance the probability of detecting any contaminated reactions, we interspersed negative control reactions at both the extraction (1 control:1 tissue-containing extraction) and PCR (also 1:1) stages. When sequencing multiple toe-pad samples from one species, we separated the processing of those different specimens by several weeks to months, over which interval many other samples were processed using the same laboratory facility, equipment, primers, and reagents. Degraded-DNA PCR amplifications targeted short (100–500 bp), overlapping regions of the NDII gene. We employed numerous primers flanking or within the NDII coding region, including many designed specifically for the taxa included here. Although the detection of preferentially or co-amplified nuclear homologs of mtDNA sequences is more difficult in studies involving degraded sample sources than it is when working with high-quality samples, we found no evidence of nuclear copies in the chromatogram data or overlapping regions amplified in separate PCR reactions.

2.2. Phylogenetic analysis

Phylogenetic reconstructions were rooted to the distant ougroup *Buphagus africanus*, but we also included in all analyses one representative of each of the non-Eurasian Sturnidae clades and one representative Mimidae species; these additional taxa were *Cinnyricinclus leucogaster*, *Lamprotornis superbus*, *Onchognathus morio*, *Rhabdornis inornatus*, and *Mimus polyglottos* (corresponding sequences all from Lovette and Rubenstein, 2007).

To reconstruct phylogenies, we used Bayesian methods as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Because of the heterogeneous composition of our dataset, we ran two separate phylogenetic analyses: the first on all data available and the second restricted to the NDII gene sequence subset which we had from all 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 reached stationarity within the first 1×10^6 generations but were run for 5×10^6 generations; samples from the initial 4×10^6 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; in no case did topologies conflict or parameter values substantially differ. The multilocus analysis included five independent partitions, one for the mitochondrial sequences and one for each of the four intron loci; parameters were estimated for each under the GTR+G+I model. In the NDII-only analysis, all data were treated as a single partition. An ultrametric NDII topology was generated in PAUP* 4.0b10 (Swofford, 2002) using the 50% majority-rule consensus topology from the NDII-only Bayesian analysis, with maximum likelihood branch lengths calculated using the mean model parameters from the 1×10^6 sampled generations (empirical base frequencies; alpha rate parameter = 0.765; proportion of invariable sites = 0.376; relative substitution rates: A–C = 0.35, A–G = 11.18, A–T = 0.30, C–G = 0.32, C–T = 4.55, G–T = 1.00).

3. Results

3.1. Phylogenetic hypotheses and the systematics of the Eurasian starling clade

We obtained complete NDII gene sequences (1041 bp) for all samples listed in Table 2. In all cases, NDII sequences from conspecific samples were identical, or were separated by the low level of divergence typical of within-species comparisons among different geographic sampling locations: the largest within-species NDII difference (among two of the three *Acridotheres javanicus* samples) was 1.1% uncorrected divergence. All novel sequences have been deposited in GenBank (Table 2). In all reconstructions, the Eurasian clade considered here was always recovered with 100% posterior probability support for a long basal internode separating this in-group from the non-Eurasian representatives of the Sturnidae radiation.

We conducted separate analyses of (i) all data from all taxa, and (ii) solely the NDII gene sequences that were part of the data subset common to all samples (Fig. 1). Somewhat surprisingly given the much higher information content of the larger dataset, the resulting reconstructions were almost topologically identical and had nearly equivalent resolution, as indexed by the number and identity of nodes receiving $\geqslant$95% posterior probability scores. Excluding 14 nodes involving very shallow terminal sister taxa (usually conspecific replicates) that all received $\geqslant$95% scores in both trees, only 2 of the remaining 19 nodes with $\geqslant$95% support in the all data tree received lower support in the NDII-only reconstruction. Those two variably supported nodes both involve the relationships among *Sturnopastor sericeus*, *Sturnopastor cineraceus*, and the *Acridotheres* group: in the complete data reconstruction, *sericeus* and *cineraceus* are well-supported sister taxa at the two relevant nodes, whereas in the NDII-only tree *cin-*

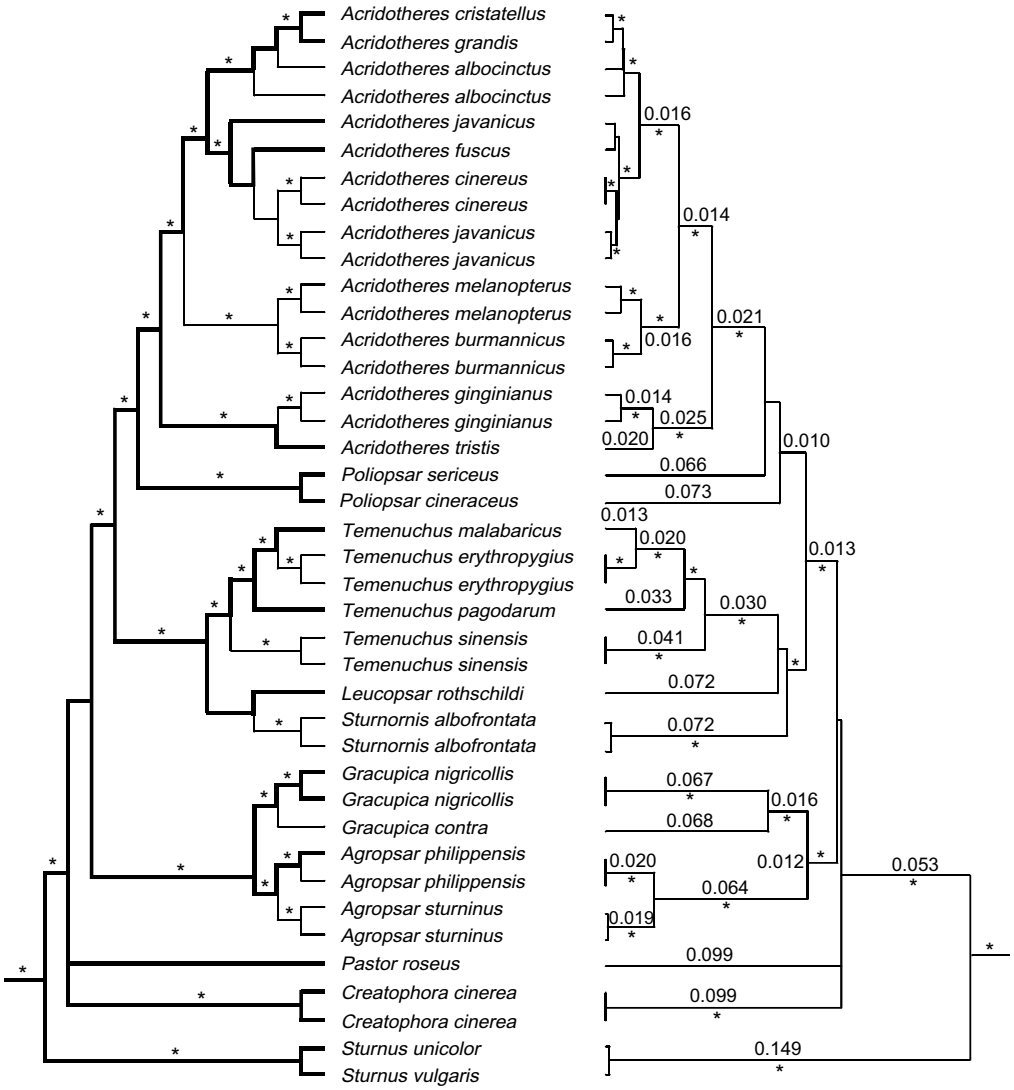

Fig. 1. Comparison of Bayesian likelihood reconstructions for Eurasian starling species. Topology at left is based on simultaneous analysis of taxa (thin branches) represented by mtDNA NDII gene sequences only (1041 bp), and taxa (thick branches) represented by more extensive mtDNA (4116 bp) and intron (2974 bp) sequences. Topology and branch lengths at right are based on analyses of only NDII sequences. Asterisks indicate internodes supported by $\geqslant$95% posterior probability values. Both analyses were rooted to *Buphagus africanus* and included representative taxa from other Sturnidae clades (not shown) outside the monophyletic group depicted here.

eraceus is basal (with 60% and 81% posterior probabilities for the two nodes in this alternative topology) to *sericeus* + *Acridotheres*. The only other topological difference between the reconstructions from the two datasets has *Leucopsar* sister to *Sturnia* in the complete data tree, and *Leucopsar* sister to *Temenuchus* in the NDII-only tree; in both trees, however, the relevant internode is very short and weakly supported. In no case, therefore, is a well-supported relationship in one tree in conflict with an alternative well-supported topology in the other tree. This high level of congruence may stem in part from the fortuitous distribution of robustly sampled taxa across the radiation: taxa that were included via NDII sequences alone were usually closely allied to taxa from which we have the full set of sequences from both mitochondrial and nuclear DNA (Fig. 1).

4. Discussion

4.1. Systematics of Eurasian starling genera

Although a few nodes in the phylogeny remain poorly resolved, the reconstructions presented here are consistent in their strong support for internodes in the deeper part of the radiation that can be used to define genera. In applying these phylogenetic hypotheses to inform a revised generic taxonomy for these 26 species of starlings, we follow the principle that genera correspond to monophyletic groups given the best available phylogenetic evidence. We also attempt to follow previous taxonomic designations where possible (while noting that this group has long suffered from a fairly dramatic nomenclatural inconsistency; Table 1). We

propose now dividing these 26 taxa among the 11 genera outlined below.

4.1.1. Acridotheres Vieillot 1816, and Poliopsar Sharpe 1888

The most species of our proposed genera is *Acridotheres*; all of its 10 specioses have been assigned to *Acridotheres* in most recent taxonomies (Table 2), and this genus is defined by a long and well-supported basal branch (Fig. 1). The probable sister-group to *Acridotheres* is comprised of two species (*sericeus* and *cineraceus*) which we assign to *Poliopsar*. This genus, for which *cineraceus* is the type, has generally been subsumed into *Sturnus* (Table 2). Our *Poliopsar* includes one migratory and one non-migratory species with seasonally overlapping ranges (Feare and Craig, 1999). Although the sister-relationship of *sericeus* and *cineraceus* was not recovered in the NDII-only reconstruction, both of these species were represented in our more robust mtDNA + intron dataset, and their sister-relationship was highly supported in the reconstructions based on those additional sequences and loci (Fig. 1). The monophyly of the taxa we include in *Acridotheres* + *Poliopsar* was also universally highly supported, and an alternative classification is to merge all 13 species into *Acridotheres*.

4.1.2. Temenuchus Cabanis 1815, Leucopsar Stresemann 1912, and Sturnornis Legge 1879

A second well-supported group includes taxa we assign to *Temenuchus* (4 species), *Leucopsar* (1 species), and *Sturnornis* (1 species). Within *Temenuchus*, the basal split separates the migratory *sinensis* of SE Asia from *pagodarum* (which is widespread on the Indian sub-continent) and *malabaricus* (broadly distributed from India to southeast Asia, overlapping with both *sinensis* and *pagodarum*). The fourth species in this group, *erythropygius*, is sister to *malabaricus*, and probably stems from a colonization of the remote Andaman/Nicobar archipelago in the eastern Indian Ocean by an early representative of the *malabaricus* lineage. The Bali myna, the sole species in *Leucopsar*, is restricted to the island of Bali, where it is critically endangered; this species is morphologically distinctive owing to its largely white plumage, and the phylogenetic results suggest that it has no close extant allies. The same is true for the monotypic *Sturnornis albofrontata*, which is a rare endemic in south–west Sri Lanka with a complicated nomenclatural history resulting from a misidentification of the original type specimen (see Mees, 1997). The pattern of relationship among these three genera was not well-resolved in our trees (Fig. 1), but their collective monophyly was highly supported, and an alternative taxonomic treatment would be to merge all six species into *Temenuchus*.

4.1.3. Gracupica Lesson 1831, and Agropsar Oates 1889

A third well-supported group contains two species each in *Gracupica* and *Agropsar*. Feare and Craig (1999) noted the morphological and behavioral similarities of *nigricollis* and *contra*, and we follow them in placing these two sister species in *Gracupica*. The two sister-species in *Agropsar*—*sturninus* and *philippensis*—have been previously assigned to various genera, but were separated into *Agropsar* by Wolters (1982). Both of these *Agropsar* species are long-distance migrants that breed allopatrically in northeastern Asia. An alternative taxonomic treatment would be to merge *Agropsar* into *Gracupica*.

4.1.4. Creatophora Lesson 1847, and Pastor Temminck 1815

The remaining taxa in the Eurasian radiation each have long branches that leads to low-diversity terminal groups. The monotypic genera *Creatophora* and *Pastor* have origins at the base of the sister-clade to *Sturnus* (Fig. 1). *Creatophora* likely represents an early colonization of sub-Saharan Africa: this species now occurs in semi-arid savannas throughout much of eastern and southern Africa, often in association with large mammals (Fry et al., 2000). *Creatophora* is widespread across Africa where it wanders nomadically in parts of its range and may be a local migrant in other areas (Feare and Craig 1999). Given the possibility that *Creatophora* colonized Africa early in the history of the Eurasian starling clade, it is somewhat surprising that it is now the only extant African lineage in this group. *Pastor* is another highly nomadic taxon that moves widely throughout western and central Asia in response to ephemeral foraging conditions, and that migrates to peninsular India for the winter (Feare and Craig, 1999).

4.1.5. Sturnus Linnaeus 1758

The basal split in the Eurasian starling clade separates *Sturnus vulgaris/unicolor* from the remaining taxa. The *Sturnus* lineage has the westernmost natural distribution of any Eurasian starling taxon, and *Sturnus* exhibits many of the behavioral attributes (such as colonial breeding, dense roosting and foraging flocks, and nomadic/migratory resource tracking) seen broadly among the species in the Eurasian clade, suggesting that these traits are ancestral to the entire group (Feare and Craig, 1999). The internode separating *Sturnus* from the remaining Eurasian starlings is notably long and well supported (Fig. 1). Our sample of *Sturnus vulgaris* (from North American) was nearly genetically identical to our sample of *S. unicolor* (from Europe), which is perhaps not surprising given that these species are known to hybridize in an area of recent contact (summarized in McCarthy, 2006). Nonetheless, *Sturnus vulgaris* has a broad geographic range and shows high geographic variation in body size, mensural characters, feather iridescence, and plumage spotting (Feare and Craig, 1999); although no detailed phylogeographic studies have yet been conducted on *Sturnus*, this genus may contain substantial diversity.

An advantage of the generic classification proposed here is that it recognizes genera with roughly similar magnitudes of genetic divergence, and hence similar periods of phylogenetic independence. A disadvantage is that it retains four monotypic genera and four genera that contain only two species. As noted above, a simpler taxonomy that also follows the monophyly criterion could lump our genera *Acri-*

dotheres/*Sternopastor*, *Temenuchus*/*Leucopsar*/*Sturnia*, and *Gracupica*/*Agropsar*, respectively.

Although highly provisional owing to the negligible within-species sampling in our study, the low differentiation within several clusters of congeneric species suggests that these forms are recently diverged. Within *Acridotheres*, there are two sub-groups of three species each with notably low levels of within-group sequence divergence. The first of these comprises taxa (*cristatellus*/*grandis*/*albocinctus*) with parapatric distributions in continental Asia; information on reproductive isolation in any areas of overlap among these forms would be a useful contribution to their systematics. The second group includes one widespread continental form (the jungle myna *A. fuscus*) and two island taxa (*javanicus* and *cinereus*) that have often been lumped into *fuscus* (Table 1). *A. fuscus* and *A. javanicus* may now interbreed in areas of the Malay Peninsula where *javanicus* has recently been introduced (Kang et al. 1994). A final instance of extreme sequence similarity is represented by *Sturnus vulgaris* and *S. unicolor*, the latter also having often been treated previously as one of the many sub-species (Dickinson, 2003) of *vulgaris;* these two taxa have similarly low differentiation at allozyme loci (de la Cruz-Cardiel et al., 1997) and hybridize in a region of recent contact (summarized in McCarthy, 2006). In all three groups of closely allied taxa, our sampling of only 1–2 individuals/species precludes detailed analyses of the genetic distinctiveness of these complexes, but our results suggest that either the taxa within each of these groups are very recently derived from one another, or that they have experienced recent gene-flow. These results therefore provide marginal support for re-lumping these forms into fewer taxonomic species. Comprehensive analyses of phylogeographic patterns and geographic variation in behavior and morphology are required to test this possibility more rigorously.

4.2. Implications of nested gene and multilocus trees

The heterogeneous composition of our sequence dataset is an example of a likely trend in phylogenetic studies of birds and other taxa. Three factors make it probable that an increasing number of phylogenetic studies will be based on short mitochondrial sequences from some taxa, analyzed in combination with sequences from more diverse sets of loci from other samples. First, for taxa from which robust DNA samples are available, it has become standard to investigate phylogenetic relationships using multiple loci (often from both organellar and nuclear genomes), and we are beginning to be able to analyze these multilocus sequence data using powerful coalescent methods (e.g., Edwards et al., 2007; Ané et al., 2007), which will further encourage the generation of datasets targeting large numbers of independent loci. Second, it is also becoming routine (Wandeler et al., 2007) to obtain short sequences (usually mitochondrial) from old or degraded samples such as avian museum skin specimens that were not originally curated with DNA preservation in mind; in this study, for example, we added a number of starling taxa based solely on mtDNA sequences derived from toe-pads of avian study skins that ranged in age from several decades to more than a century (Table 2). The ability to use traditional museum specimens as a DNA source greatly enhances our ability to generate comprehensive phylogenies for entire clades because many groups of organisms are so broadly distributed that no individual worker could collect field samples from all species, and many species are likewise rare, endangered, extinct, or found only in politically or logistically inaccessible locations. The third factor that will likely drive the generation of heterogeneous DNA sequence datasets is the rapidly expanding generation of "DNA barcodes," typically short sequences of the mitochondrial COI gene, from most or all taxa in particular groups or from defined geographic regions (e.g., Hebert et al., 2004; Kerr et al., 2007). Although the information content of these short sequences may often render DNA barcodes sub-optimal for reconstructing phylogenetic relationships, they are already being broadly employed for this purpose. Placing DNA barcode samples from a subset of taxa within a topology based on a set of broader, more phylogenetically informative markers has the potential to greatly expand the phylogenetic utility of barcode sequences and databases.

As with all estimates of phylogenetic relationships based on individual loci it is important to distinguish between organismal histories and the gene trees derived from individual loci. In this study, the mitochondrial gene tree was highly similar to the multilocus topology for the species represented in both datasets, but this will not always be the case. It is important to keep in mind that a number of taxa and nodes in our multilocus reconstruction (thin branches in the left-hand tree in Fig. 1) are placed there solely via their mitochondrial gene tree. Distinguishing between single gene tree topologies and multilocus topologies will become increasingly challenging as heterogeneous datasets of this type expand to include large number of taxa and loci.

4.3. Trait variation and evolution

These Eurasian starlings figured prominently in Beecher's (1978) detailed comparative study of starling skull morphology, an investigation initially motivated by his observation that the jaw musculature and skull shape of the European starling (*Sturnus vulgaris*) are both highly modified for prying. The European starling typically forages by first forcing its bill into a turf mat then prying open the bill to create a window in the turf within which it scans for invertebrate prey (Feare, 1984). The force required to open the bill against pressure is opposite that faced by most other birds, for which the greatest force is required when closing the bill (as when cracking a seed or holding prey). Beecher (1978) found that the adductor muscles used to open the bill are correspondingly robust in the European starling, and that the anterior region of the skull is further

modified to facilitate forward vision along the axis of the bill, which is necessary for spotting prey in the hole held open by the bill. In extending this investigation to other starling genera, Beecher found that these modifications were absent in most starling taxa, but documented a graded series of specialization within the Eurasian starlings. Beecher placed the Eurasian starling species into three groups based on their degree of prying-related modification, and hypothesized that each of these groups had originated independently from a common, forest-dwelling (and non-prying) ancestor. Beecher's morphological groupings have influenced all later classifications of this group (e.g., Wolters, 1982; Sibley and Monroe, 1990; Dickinson, 2003).

In mapping Beecher's categorization of each species onto our molecular tree (Fig. 2), we found that the skull traits he documented are poorly conserved. The few taxa with morphologies highly specialized for prying (*Sturnus vulgaris/unicolor* and *Poliopsar cineraceus*) are not close allies: *Sturnus* is the sister to the remaining Eurasian starlings, and *P. cineraceus* is embedded within a clade of taxa that all show only moderate prying specialization. This most extreme form of prying-related modification therefore likely evolved twice within this group. Likewise, the two taxa (*Temenuchus pagodarum* and *Pastor roseus*) that are similar to non-Eurasian starlings in exhibiting low skull specialization are not phylogenetically close, and each is allied to a group of taxa with moderate prying specialization (Fig. 2). These patterns suggest that these morphological traits have high evolutionary lability, and that they should be employed with caution as characters for phylogenetics and classification.

High lability in behavioral and life history traits has been shown previously in African starlings (Rubenstein and Lovette, 2007), but the Eurasian starlings have high conservation of some behavioral traits. All 26 Eurasian taxa are gregarious; most forage and roost in large flocks during the non-breeding season, and many breed colonially (Feare and Craig, 1999). Nearly all taxa are habitat generalists, occurring in a variety of disturbed habitats; only *Leucopsar*, and to a lesser degree *Sturnornis* and *Agropsar sturninus*, are restricted to non-disturbed forest habitats. More variation is present in movement behavior (Fig. 2): just under half of the Eurasian starling species are sedentary, whereas the rest show a gradient of movement types ranging from local migration, to periodic nomadicism, to obligate long-distance migration. There is low phylogenetic signal in the distribution of migratory behavior, as obligate migration appears to have arisen independently in four

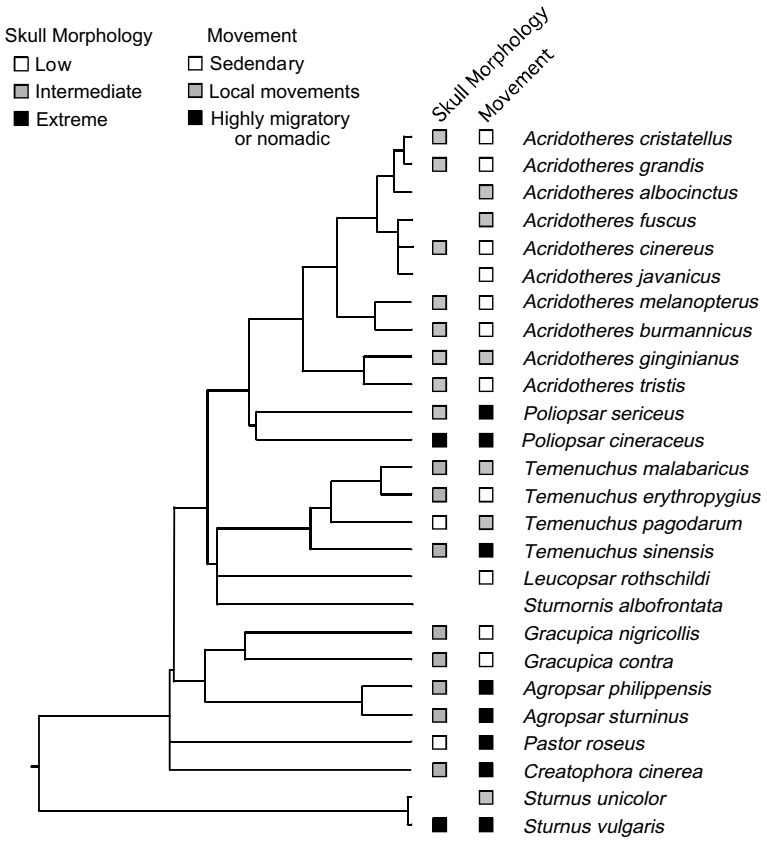

Fig. 2. Phylogenic distribution of variation in skull morphology and movement behavior among Eurasian starling species. Tree is a phylogram based on the reconstructions shown in Fig. 1. Specialization in skull morphology has historically contributed importantly to the classification of these taxa, and species are coded into three skull morphology classes (extreme, moderate, and low specialization for prying) following Beecher (1978). Information on species' tendency to be sedentary, locally, or more broadly migratory/nomadic is derived from Feare and Craig (1999). Species not studied by Beecher (1978) or with unknown movement behavior were not given boxes.

Eurasian starling lineages (Fig. 2). The lability of starling movement behavior is strikingly illustrated by the rapid evolution of migration in the European starling population of eastern North America following its introduction in 1890 (Kessel, 1953).

A combination of these traits seen broadly in the Eurasian clade—use of disturbed habitats, gregarious association, and behavioral lability—has likely contributed to the human-mediated spread of *Sturnus vulgaris* and *Acridotheres tristis* around the globe. Many other Eurasian clade species are expanding their geographic ranges as anthropogenic modification increases the amount of suitably disturbed habitat. It is notable and somewhat paradoxical that the European starling (which may now have the most expansive geographic range of any passerine bird) and its ecologically successful relatives fall within a recent radiation that also contains several highly endangered single-island endemics.

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