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Molecular phylogenetics of the bee-eaters (Aves: Meropidae) based on nuclear and mitochondrial DNA sequence data

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Abstract

The bee-eaters (family Meropidae) comprise a group of brightly colored, but morphologically homogeneous, birds with a wide variety of life history characteristics. A phylogeny of bee-eaters was reconstructed using nuclear and mitochondrial DNA sequence data from 23 of the 25 named bee-eater species. Analysis of the combined data set provided a well-supported phylogenetic hypothesis for the family. *Nyctiornis* is the sister taxon to all other bee-eaters. Within the genus *Merops*, we recovered two well-supported clades that can be broadly separated into two groups along geographic and ecological lines, one clade with mostly African resident species and the other clade containing a mixture of African and Asian taxa that are mostly migratory species. The clade containing resident African species can be further split into two groups along ecological lines by habitat preference into lowland forest specialists and montane forest and forest edge species. Intraspecific sampling in several of the taxa revealed moderate to high (3.7–6.5%, ND2) levels of divergence in the resident taxa, whereas the lone migratory taxon showed negligible levels of intraspecific divergence. This robust molecular phylogeny provides the phylogenetic framework for future comparative tests of hypotheses about the evolution of plumage patterns, sociality, migration, and delayed breeding strategies.

Keywords: Bee-eater; Meropidae; Systematics; *Merops*; *Nyctiornis*; *Meropogon*

1. Introduction

The bee-eaters (family Meropidae) comprise a group of 25 species (Dickinson, 2003) of brightly colored, but morphologically homogeneous, birds. As their common name suggests, bee-eaters feed primarily on bees and other Hymenoptera, which are usually plucked from the air in gracefully aggressive foraging forays. Bee-eaters generally possess a conserved body plan with a long decurved bill, long slender wings, a long tail (sometimes with elongated central rectrices), and usually bold green or red base coloration. The low level of morphological diversity in the family is in stark contrast to their high diversity in ecological and life history traits. Bee-eaters are distributed throughout the Old World, found in habitats ranging from the driest of deserts to the wettest of rainforests. The family spans a spectrum ranging from sedentary tropical rainforest species to species that migrate between the temperate zone and the tropics for breeding and wintering. Some species of bee-eaters nest solitarily, whereas others nest in large breeding colonies. The young of some species delay breeding in their first year to serve as "helpers" at the nests of their parents (Fry, 1972; Hegner et al., 1982). The remarkable level of heterogeneity of behaviors (migratory, social, and breeding) among the bee-eaters is ideal for comparative study, which requires a well-resolved phylogeny. However, until now there has been only a single formal analysis of bee-eater phylogenetic relationships (Burt, 2004) and no quantitative assessment of support for relationships.

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Most authors recognize three genera in the family: *Nyctiornis*, which includes two species of humid forest specialists inhabiting the rainforests of India and SE Asia, *Meropogon*, a monotypic genus inhabiting forest edge and clearings in Sulawesi, and *Merops*, which includes 22 species distributed across a variety of habitats throughout the Old World. Two of the most thorough investigations of the relationships among bee-eater species are those of von Boetticher (1951) and Fry (1969). Both studies were based primarily on plumage (feather structure and color pattern) and morphometric features, although Fry (1969, 1984) also considered ecology and biogeography in producing his hypothesis of relationships. Although generic names applied in these revisions differed, the classifications were largely congruent, differing primarily in the placement of the African forest species (*Merops muelleri*, *M. gularis*, and *M. breweri*). von Boetticher (1951) considered *M. breweri*, a large and mostly solitary species, to be closely related to the SE Asian *N. amicta*, *N. athertoni*, and *Meropogon forsteni* and placed all four taxa in the genus *Nyctiornis*. The remaining African forest specialists (*M. gularis* and *M. muelleri*) were placed in a separate genus *Meropiscus*. Fry (1969), using primarily biogeographic information, treated *M. breweri* as a member of *Merops*, but later conceded that *M. breweri* may indeed be more closely related to *Meropogon* (Fry, 1984). Fry (1969, 1984) remained hesitant to group all of the lowland forest taxa into *Nyctiornis*. These early hypotheses of relationships among bee-eater species, based on phenetic analyses of morphological characters and life history data, were recently revisited by Burt (2004) in a comprehensive cladistic analysis of 30 plumage-based and morphometric characters. The relationships inferred from Burt's (2004) analysis were generally consistent with previous studies, but added *M. hirundineus* and *M. boehmi* to the list of taxa with enigmatic relationships. Burt (2004) found support for a sister relationship between the African *M. hirundineus* and the Australian *M. ornatus*, a relationship not supported by either Fry (1969, 1984) or von Boetticher (1951); both of whom place *M. hirundineus* in a superspecies consisting of other African bee-eaters (*M. oreobates*/*pusillus*/*variegatus*). In Burt's (2004) four equally parsimonious trees, *M. boehmi* exhibited sister relationships with *M. superciliosus*/*philippinus* or *M. viridis*/*leschenaulti*, whereas the earlier treatments placed *M. boehmi* close to the widespread *M. orientalis* (Fry, 1969) or the African *M. albicollis* (von Boetticher, 1951). The plumage characters used by Burt (2004) did not lend themselves to quantitative analysis such as bootstrapping, and as a result there is no measure of support for the nodes depicted in his phylogenetic hypothesis. Furthermore, plumage characters, such as those used by Burt (2004), are known to evolve rapidly in other birds (Omland and Hofmann, 2006) and a number of studies (Johnson, 1999; Crochet et al., 1999; Johnson and Lanyon, 2000; Omland and Lanyon, 2000; Armenta et al., 2005; Weckstein, 2005) have found high levels of homoplasy in plumage coloration patterns, which suggests that the phylogenetic signal in coloration characters may be weak.

To test these proposed relationships among bee-eaters, we reconstructed a molecular phylogeny for the family based on mitochondrial and nuclear DNA sequence data. In the process, we resolve many phylogenetic and taxonomic uncertainties in the family and provide a phylogenetic framework for future comparative tests of hypotheses about the evolution of plumage patterns, sociality, migration and delayed breeding strategies.

2. Materials and methods

2.1. Taxon and character sampling

Ingroup sampling for our study (Table 1) included representatives of 23 out of the 25 named species (Dickinson, 2003), missing only *Nycitornis athertoni* and *Merops revolii*. We also sampled two subspecies each for four of the species (Table 1). Tissue samples were available for most taxa, but for some taxa, only museum study-skin toepads or blood samples were available (Table 1). Outgoup taxa were drawn from other Coraciiform families; kingfishers, motmots and todies. Genomic DNA was extracted from muscle tissue using the Qiagen DNeasy tissue kit following the manufacturer's protocol (Qiagen, Valencia, California). Protocols for DNA extraction from museum skins generally followed those for fresh tissues, with the addition of 10 μl of 1 M dithiothreitol solution. The protocols also required special handling of samples and prevention of contamination, as described by Mundy et al. (1997). We extracted, amplified, and sequenced all bee-eater tissue samples in the molecular labs at the LSU Museum of Natural Science and the Pritzker Laboratory at The Field Museum, whereas the toepad samples were extracted, amplified, and sequenced in the Cullman Molecular Laboratory at the American Museum of Natural History.

From the tissue samples we collected DNA sequence data from the entire second subunit of the mitochondrial nicotinamide adenine dinucleotide dehydrogenase gene (ND2, 1041 bp) and two nuclear introns: the fifth intron of the nuclear β-fibrinogen gene (BFib5, 604 bp) and the fifth intron of the transforming growth factor, β2 gene (TGFb2, 566 bp). For toepad samples we sequenced the entire second subunit of the ND2 gene (primers available upon request). We used primers L5215 (Hackett, 1996), H6313, L5758, and H5766 (Johnson and Sorenson, 1998) to amplify and sequence the ND2 gene, primers FIB5L and FIB5H (Driskell and Christidis, 2004) to amplify and sequence the Fib5 intron, and primers TGF5 and TGF6 (Primmer et al., 2002) to amplify and sequence the TGFb2 intron. We purified PCR products with Perfectprep PCR cleanup kits (Eppendorf) or with Exonuclease and Shrimp Alkaline Phosphotase enzymatic reactions (United States Biochemical). Sequencing of purified PCR products was performed with BigDye Terminator Cycle Sequencing Kits (Applied Biosystems). Primers used for PCR were also used

Table 1
Voucher information for species included in the study

Genus	Species	Subspecies	Distribution	Habitat	R/M[a]	Voucher information
Nyctiornis	*amicta*		Asia	Forest	R	LSUMNS B47037
Meropogon	*forsteni*		Asia	Forest	R	AMNH 299316[b]
Meropogon	*forsteni*		Asia	Forest	R	AMNH 299315[b]
Merops	*leschenaulti*		Asia	Forest/savanna	R	AMNH 409286[b]
Merops	*leschenaulti*		Asia	Forest/savanna	R	AMNH 812115[b]
Merops	*boehmi*		Africa	Savanna	R	ZMUC 114552
Merops	*gularis*	*gularis*	Africa	Forest	R	LSUMNS B39368
Merops	*gularis*	*australis*	Africa	Forest	R	ANSP 11535
Merops	*pusillus*	*pusillus*	Africa	Savanna	R	LSUMNS B39280
Merops	*pusillus*	*meridionalis*	Africa	Savanna	R	MBM 11049
Merops	*viridis*	*viridis*	Asia	Forest/savanna	R	LSUMNS B51022
Merops	*viridis*	*americanus*	Asia	Forest/savanna	R	FMNH 358335
Merops	*persicus*	*persicus*	Africa/Asia/Europe	Savanna	M	LSUMNS B46361
Merops	*apiaster*		Africa/Asia/Europe	Savanna	M	LSUMNS B35153
Merops	*apiaster*		Africa/Asia/Europe	Savanna	M	MBM 11515
Merops	*muelleri*	*mentalis*	Africa	Forest	R	LSUMNS B45318
Merops	*muelleri*	*muelleri*	Africa	Forest	R	ANSP 11434
Merops	*bullocki*	*bullocki*	Africa	Savanna	R	LSUMNS B39305
Merops	*albicollis*		Africa	Forest	M	LSUMNS B39423
Merops	*ornatus*		Australia	Forest	M	ANSP 11226
Merops	*nubicus*	*nubicoides*	Africa	Savanna	M	UWBM 57051
Merops	*superciliosus*	*superciliosus*	Europe/Africa	Savanna	M	FMNH 384686
Merops	*oreobates*		Africa	Forest	R	FMNH 384825
Merops	*variegatus*	*loringi*	Africa	Forest/savanna	R	FMNH 346214
Merops	*bullockoides*		Africa	Savanna	M	MBM 11950
Merops	*hirundineus*		Africa	Savanna	M	MBM 8013
Merops	*phillipinus*		Asia	Savanna	M	C-40531[c]
Merops	*malimbicus*		Africa	Savanna	M	NMNH B16241
Merops	*orientalis*		Africa/Asia	Savanna	R	NMNH B5669
Merops	*breweri*		Africa	Forest	R	NMNH B16221
Actenoides	*concretus*					LSUMNS B36383
Momotus	*momota*					AMNH 12359
Todus	*angustirostris*					AMNH 6949

Each species is broadly categorized by distribution, habitat preference (forest or savanna), and as either resident (R) or migratory (M). Taxonomy follows Dickinson (2003).

Note: AMNH, American Museum of Natural History; ANSP, Academy of Natural Sciences Philadelphia; FMNH, Field Musuem of Natural History; LSUMNS, Louisiana State University Museum of Natural Science; MBM, Marjorie Barrick Museum of Natural History UNLV; NMNH, Smithsonian Institution National Museum of Natural History; UWBM, University of Washington Burke Museum; ZMUC, Zoological Museum University of Copenhagen.

[a] R/M—R, resident; M, migratory.

[b] DNA sample collected from study-skin toe pad.

[c] No voucher, blood sample.

for cycle sequencing reactions, resulting in bi-directional sequence for all taxa. Cycle sequencing products were run on an ABI Prism 3100 or 3730 automated DNA sequencer (Perkin-Elmer Applied Biosystems). The computer program Sequencer 4.1 (Genecodes) was used to reconcile chromatograms of complementary fragments and align sequences across taxa. All of the sequence data generated in this study have been submitted to GenBank (Accession Nos. EU21508–EU21595).

2.2. Phylogenetic analysis

We used the partition homogeneity test (ILD statistic, Farris et al., 1994, 1995) as implemented in PAUP* (version 4.0b10; Swofford, 2002) to compare phylogenetic signal and to test for incongruence between data partitions. We ran three different ILD tests including one between ND2 and the two nuclear loci combined, one between the three separate partitions (ND2, BFib5, and TGFb2), and one between the two nuclear partitions (BFib5 and TGFb2). For each of these ILD tests, we removed taxa for which we only have ND2 data and we excluded all characters that were not parsimony informative from the analyses (Cunningham, 1997). PAUP*4.0b10 was used to test the base composition of each gene using a χ^2 analysis of base frequencies across taxa. To visualize the degree of divergence between ingroup and outgroup taxa, the extent of saturation, and relative substitution rates between the nuclear and mitochondrial data, we plotted the uncorrected distance (*p*-distance) and maximum likelihood transformed distances of ND2 and the faster of the two nuclear genes, TGFb2 against those of FIB5 for all pairwise comparisons of taxa.

Maximum likelihood (ML) and maximum parsimony (MP) analyses were performed for the combined data set using PAUP* 4.0b10 (Swofford, 2002). Heuristic searches employed TBR branch-swapping and 100 random taxon addition sequences (10 addition sequences for ML searches). For each likelihood analysis, we used the Akaike Information Criterion in Modeltest 3.5 (Posada and Crandall, 1998) to determine parameter estimates and the best fit model of molecular evolution. Support for nodes in the ML and MP trees were assessed by analysis of 100 non-parametric bootstrap replicates (Felsenstein, 1985). For the combined data set, we implemented a mixed model approach to account for the potential difference in evolutionary model parameters between data partitions (Nylander et al., 2004). We used MrModeltest2.2 (Nylander, 2004) to determine the evolutionary model appropriate for each partition and chose among partitioning schemes using Bayes factors (see Brandley et al., 2005 for a thorough discussion of the methodology and rationale). Bayes factors were calculated using the harmonic mean from the sump command within MrBayes. A difference of 2 ln Bayes factor >10 was used as the minimum value to discriminate between partitioning schemes. MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003) was used to estimate model parameters from the data and to evaluate support for specific relationships in the phylogeny. All parameters (except topology and branch lengths) were unlinked between partitions. We ran four Markov chains for 10 million generations as well as two shorter 2 million generation runs. The shorter runs were used to ensure that our analyses were not stuck in local optima (Huelsenbeck and Bollback, 2001) and to evaluate stationarity, the condition in which trees are being sampled according to their posterior probabilities. Stationarity was judged by visually inspecting plots of likelihood scores. Markov chains were sampled every 1000 generations, yielding 10,000 parameter point estimates. These subsamples, minus the burn-in generations, were used to create 50% majority-rule consensus trees. We took a cautious approach and removed more samples than indicated by visual inspection of the likelihood plots to ensure that burn-in runs were not included in our consensus trees (Leache and Reeder, 2002).

In this study, we sequenced both mitochondrial and nuclear DNA regions with the hope that the nuclear DNA would help resolve the deeper nodes in the tree where the mitochondrial data were saturated and potentially misinformative (see Fig. 1, Springer et al., 2001; Moyle, 2004). We were unable to obtain the nuclear intron data for the four samples we extracted from museum study skins (Table 1), and therefore those samples have less than half the characters of all other species in the matrix. The effects of such incomplete matrices are difficult to predict, but simulations suggest the amount of missing data for a given taxon may be less important, in terms of accurately placing it on a phylogenetic tree, than the type and quality of the existing data (Wiens, 2003). To assess the effect that the missing nuclear data had on the resultant topology and node support, we ran two separate Bayesian analyses. One analysis was run with all taxa, including the four taxa with missing data (complete data set). The second Bayesian analysis excluded these taxa and included only species with complete mtDNA and nuclear intron sequences (reduced data set). A likelihood analysis was run only with the reduced data set.

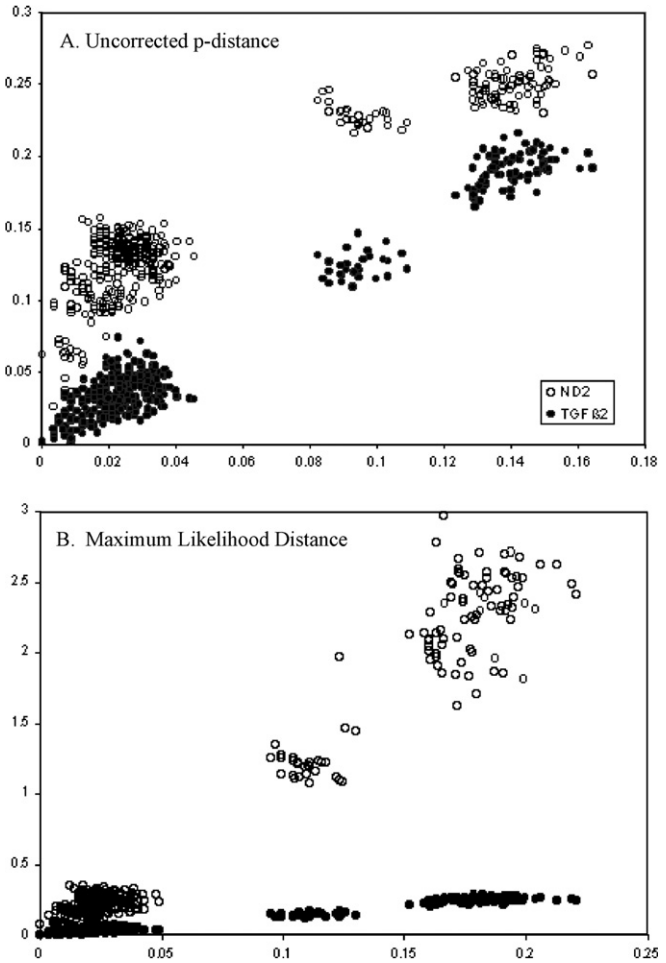

Fig. 1. Comparison of pairwise divergences (uncorrected *p*-distance and maximum likelihood distance) among nuclear and mitochondrial gene regions sequenced for this study. BFib5 distances are plotted on the *x*-axis, the mitochondrial ND2 and the nuclear TGFb2 are plotted on the *y*-axis. (A) Uncorrected proportional distances. (B) Maximum likelihood distances determined using the models chosen by Modeltest. ND2: Model = GTR + I + G, base frequencies = (A = 0.3362, C = 0.4093, G = 0.0743), rate matrix = (AC = 0.2419, AG = 16.2017, AT = 0.3733, CG = 0.3970, CT = 5.5763), $\gamma = 0.8346$, proportion invariable = 0.3895. BFib5: Model = GTR + G, base frequencies = (A = 0.3141, C = 0.1704, G = 0.2212), rate matrix = (AC = 1.0991, AG = 3.0395, AT = 0.5127, CG = 1.3404, CT = 3.6422), $\gamma = 1.0033$. TGFb2: Model = GTR + G, base frequencies = (A = 0.2419, C = 0.2152, G = 0.2068), rate matrix = (AC = 0.9510, AG = 4.6336, AT = 0.7160, CG = 1.4673, CT = 2.6666), $\gamma = 1.8795$.

To provide a statistical assessment of congruence between our molecular phylogeny and the morphological phylogeny of Burt (2004) we used the SH (Shimodaira and Hasegawa, 1999) test. The alternative tree topology was developed using MacClade v4.0 (Maddison and

Maddison, 2000). The SH test was used because of its conservative nature relative to the similar SOWH test which has been shown to be misleading under certain conditions (Buckley, 2002). The SH test was run with full approximation, 1000 bootstrap replicates, and the model of evolution identified by MODELTEST.

3. Results

3.1. Sequence attributes

The DNA sequence alignment included 33 taxa (30 ingroup and three outgroup) and 2211 bp (1041 ND2, 604 BFib5, and 566 TGFb2). Excluding the three outgroup taxa (*Momotus, Todus*, and *Actenoides concretus*), 1450 (65.5%) characters were constant, 316 (14.3%) were variable but uninformative, and 447 (20.2%) were parsimony informative. Aligned ND2 sequences appear to be of mitochondrial origin rather than nuclear copies. Sequences contained no stop codons, overlapping fragments contained no conflicts, base composition was homogenous across taxa (for ND2: $\chi^2 = 39.01$, df = 84, $p = 0.99$, TgG: $\chi^2 = 12.85$, df = 84, $p = 1.0$, and Fib5: $\chi^2 = 7.19$, df = 84, $p = 1.0$), codon positions contained expected relative levels of polymorphism (3 > 1 > 2). The aligned intron sequences (BFib5 and TGFb2) contained several inferred insertions or deletions (indels), but alignment of the sequences was straightforward for two reasons. First, indels were infrequent enough that they generally did not overlap, allowing homologous indels to be easily identified. Second, the nucleotide sequences themselves were not highly diverged, which allowed straightforward alignment by eye and unambiguous placement of indels. Table 2 and Fig. 1 summarize sequence attributes and relative levels of divergence for all three gene regions used in this study. Plots of pairwise divergences of the three gene regions (Fig. 1) indicate that as expected, ND2 is evolving faster than either of the nuclear introns and that among the two introns TGFb2 is evolving at a faster rate than BFib5. Bayes factor analysis provided strong evidence that a five-partition model (three codon positions and each intron) was more appropriate than one partition (all data together), two partition (nuclear vs mtDNA), or four partition (codon positions and nuclear DNA) models. Several other studies have found support for similar partitioning schemes that account for biologically meaningful differences in the sequence data (e.g., Brandley et al., 2005; Fyler et al., 2005; Moyle et al., 2006). MrModeltest determined a general time reversible model framework, with γ-distributed rates among sites and invariant sites (GTR + I + G) for each codon position and the same model, but lacking invariant sites (GTR + G), for each intron. None of the three ILD tests indicated that there was significant conflict among the data partitions (ND2 vs nuclear loci: $p = 0.06$; ND2 vs BFib5 vs TGFb2: $p = 0.58$; BFib5 vs TGFb2: $p = 0.35$). Therefore, we combined ND2, BFib5, and TGFb2 data sets for all phylogenetic analyses.

Table 2
Description of mitochondrial and nuclear gene sequences

Gene	% Characters		Maximum uncorrected sequence divergence	
	Variable	Informative	Within ingroup (%)	To outgroup (%)
ND2	53.7	42.8	23.8[a]	27.7[c]
TGFb2	44.5	20.3	13.2[a]	20.8[d]
BFIB5	34.4	13.7	10.2[b]	16.3[c]

[a] *Nyctiornis amicta/Merops pusillus meridonalis.*
[b] *Nyctiornis amicta/Merops malimbicus.*
[c] *Momotus momota/Merops malimbicus.*
[d] *Momotus momota/Merops philippinus.*

3.2. Phylogenetic results

The genus *Nyctiornis*, represented in this study by *Nyctiornis amicta*, received significant support in all analyses as the sister taxon of all other bee-eaters (Figs. 2 and 3). After the initial dichotomy between *Nyctiornis* and the rest of the bee-eaters, *Merops breweri*, a sister pairing of *bulocki* and *bullockoides*, and the monotypic genus *Meropogon* diverge in a basal polytomy at the base of the *Merops* radiation. Phylogenetic analysis of the reduced data set provided a topology (Fig. 2A) with higher basal support than the complete data set topology. The complete data set (Fig. 2B), which includes *Meropogon forsteni* and *Merops leschenaulti* without nuclear intron sequences, provides no basal support after the divergence of *Nyctiornis* and the rest of the family, resulting in a polytomy. In the reduced data set, when *Meropogon* is excluded, the support for the positions of *M. bulocki/bullockoides* and *M. breweri* dramatically improves.

A clade consisting entirely of African taxa (clade A, Figs. 2 and 3) was recovered in all analyses. Within this clade, the two African forest specialists *M. gularis* and *M. muelleri* are highly supported as sister taxa. This pair is sister to the African savanna and forest edge species (*M. hirundineus, M. oreobates, M. variegates*, and *M. pusillus*). Relationships within the savanna and forest edge clade were not fully resolved and are discussed below.

A second well-supported clade (clade B, Figs. 2 and 3) consisted primarily of Asian/Palearctic species that share a common ancestor with a grade of three intra-African migrants (*M. albicollis*, *M. nubicus*, and *M. malimbicus*). Within this clade are two highly supported subclades: one of which unites the widespread *M. orientalis* sister to the sedentary Southeast Asian *M. viridis* and *M. leschenaulti.* These species are sister to a clade of mostly migratory taxa, including the weakly supported sister taxa, *M. ornatus*, an intra-Australian migrant, and *M. apiaster*, a Palearctic migrant, which are sister to the *M. superciliosus* species complex (represented here by *M. superciliosus*, *M. persicus*, and *M. philippinus*).

Parsimony analysis (MP) recovered a single most parsimonious tree (TL = 2636, CI = 0.516, RI = 0.58; Fig. 3),

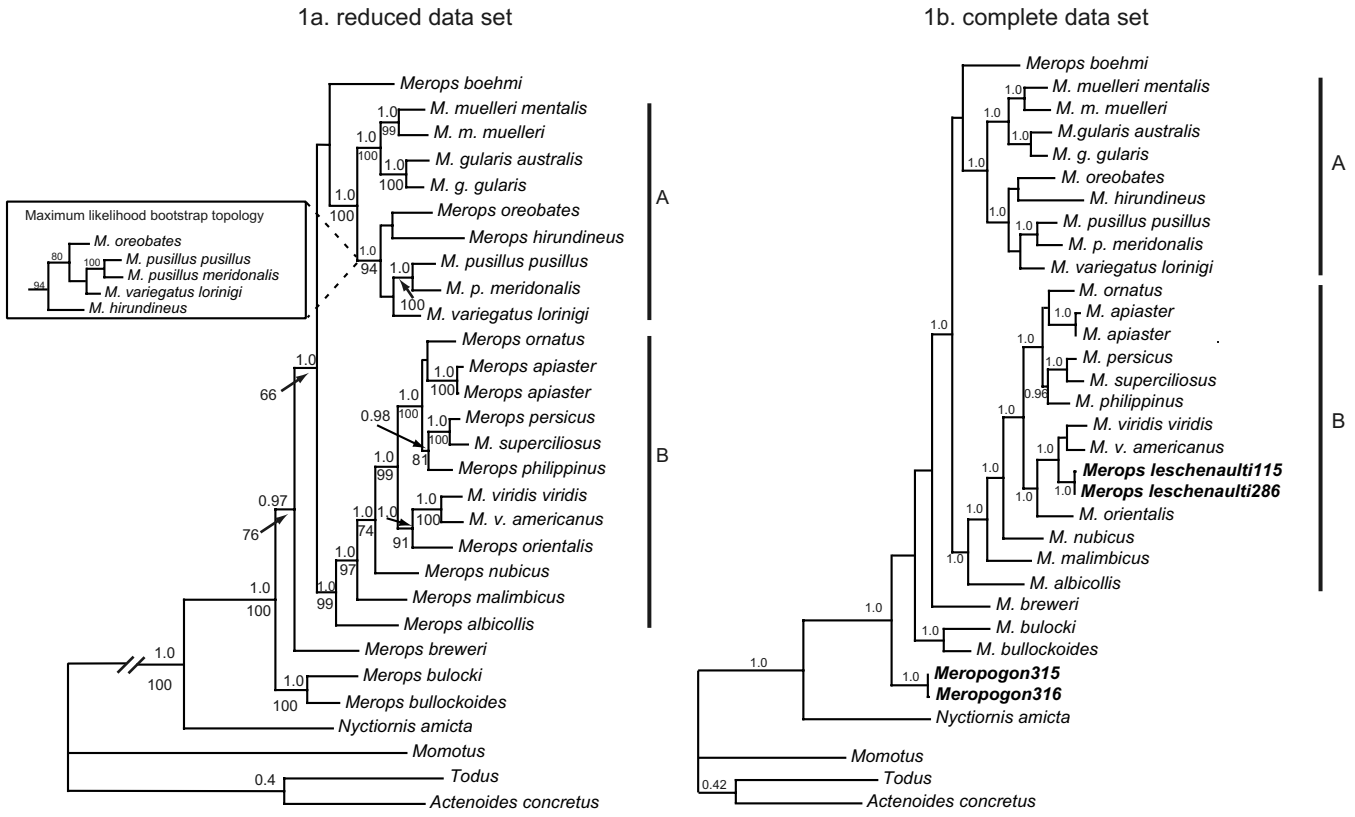

Fig. 2. Reduced and complete data set Bayesian consensus trees. Taxa shown in bold font in the complete data set tree are removed from the reduced data set. Bayesian probabilities are above the nodes, likelihood bootstrap proportions are below the nodes on the reduced data set consensus tree. Inset box shows likelihood bootstrapping proportions for an alternate arrangement of taxa.

which supports most of the same relationships seen in the Bayesian and ML analyses. Topology differences exist among some of the basal nodes, but the conflicting nodes were not supported by bootstrapping or posterior probabilities. One node received markedly different support indices from each method. Under the MP and ML criterion, a clade containing *M. boehmi* and clades A and B received low bootstrap support. In contrast, the same node was supported by a posterior probability of 1.0. There also is one case where Bayesian analysis failed to provide any support for a topology supported by MP and ML bootstrapping (Fig. 2A, see inset). This discrepancy involved support for nodes within the African savanna and forest edge clade including *M. pusillus, variegatus, oreobates*, and *hirundineus.* Parsimony and likelihood bootsrapping provided consistent support for a sister relationship between *M. hirundineus* and a polytomy including *M. oreobates, pusillus*, and *variegates*, whereas Bayesian analysis did not provide support for any structure within this clade.

We compared the Bayesian topology (Fig. 2B) with the strict consensus tree of Burt (2004, Fig. 3b) using the SH test to assess whether the plumage-based topology of Burt (2004) is significantly different from our Bayesian toplogy, given the molecular data. The molecular data from this study were able to reject ($p < 0.001$) the topology generated by the morphological characters of Burt (2004).

3.3. Intraspecific differentiation

Our sampling included multiple individuals (usually different subspecies) of several widespread species of bee-eaters, allowing us a first glance at levels of intraspecific variation. Within these species, uncorrected pairwise distances for the mitochondrial protein-coding ND2 gene ranged from 0.04% between two individuals of *M. apiaster* to 6.5% between two individuals of *M. muelleri* (*M. m. mentalis* from Ghana and *M. m. muelleri* from Equatorial Guinea). Between these two extremes were two subspecies of *M. pusillus*, including *M. p. pusillus* from Ghana and *M. p. meridionalis* from Malawi, which differ from one another by 4.8% uncorrected sequence divergence. Divergences within *M. gularis* and *M. superciliosus* are similar to those found for *M. pusillus*. *M. g. gularis*, from Ghana, and *M. g. australis* from Gabon differ by 3.7% uncorrected mtDNA sequence divergence. Finally, *M. supercilious* from Madagascar and *M. philippinus* from Taiwan, two taxa that have been alternately treated as subspecies and species, differ by 3.8% uncorrected mtDNA sequence divergence.

4. Discussion

Within the bee-eaters two well-supported clades can be broadly defined along ecological lines: one containing

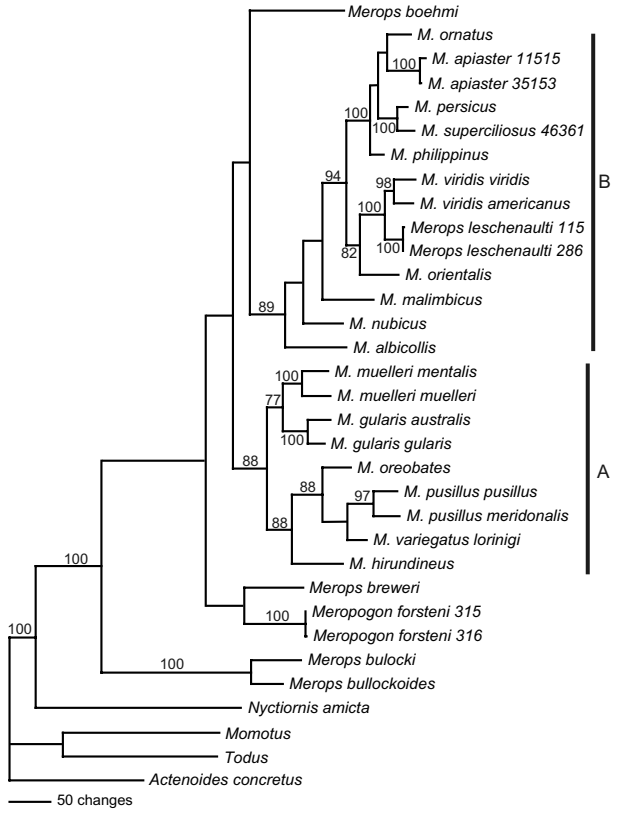

Fig. 3. Single most parsimonious tree (length = 2636, CI = 0.516, RI = 0.58) shown with bootstrap values from 100 bootstrap replicates above the nodes.

mostly sedentary species and one containing mostly migratory species. Clade A (Figs. 2 and 3) is comprised entirely of sedentary African taxa. Its six species can be further separated into two groups by habitat preference into forest and savanna/forest edge species. The forest specialists *M. gularis* and *M. muelleri* were united into their own genus, *Meropiscus*, based on plumage and behavioral differences (von Boetticher, 1951). However, Fry (1969) considered the differences between *Meropiscus* and other *Merops* to be misleading due to convergence between the two forest specialists and retained them in *Merops.* Our data place *M. gularis* and *M. muelleri* as sister taxa embedded well inside the *Merops* radiation, providing no molecular basis for splitting these two taxa from the rest of *Merops*. The sister group to *M. muelleri* and *gularis* are savanna and forest edge species, four African taxa whose close relationships have long been suspected as they are often treated as a superspecies (Fry, 1969): *M. pusillus, hirundineus, oreobates* and *variegatus.* These species have overlapping ranges, but are rarely syntopic, occupying distinct habitats ranging from riparian grassland and woodland savanna to montane forests and grasslands. All analyses recover this clade although relationships within the clade are not well resolved.

In contrast to the sedentary African taxa in Clade A, the species in clade B are all migratory to some degree and are distributed widely across the Old World. Branching off in succession from the base of the clade are the intra-African migrants *M. albicollis, M. malimbicus*, and, *M. nubicus*. Sharing a common ancestor with these intra-African migrants is a group of Asian and Eurasian taxa. Within this clade there are three sub-clades. First is the sister pairing of the most widespread of the bee-eaters, *M. orientalis* with the Southeast Asian *M. viridis* and *M. leschenaulti*. Whereas Fry (1969) suspected that *M. orientalis* was sister to the African *M. boehmi*, he did propose a potential sister relationship between *M. leschenaulti* and *M. viridis*. von Boetticher (1951) recognized several similarities between *M. orientalis*, *M. viridis*, and M. *leschenaulti* and lumped all three into a subgenus *Phlothrus*. While our data provide strong support for the arrangement of von Boetticher (1951), apart from geographic proximity, we see no need to split these three species from *Merops*. The next well-supported sub-clade is the *M. persicus/superciliosus/philippinus* group; a species complex that has been treated a variety of ways including one widespread species with five subspecies, two species each with subspecific variation, and three species. Early authors treated it as a single widespread species with five distinct subspecies distributed across Africa, Madagascar, Asia, and New Guinea and its' surrounding islands. However, Marien (1950) noted that where *M. s. persicus* and *M. s. philippinus* nest sympatrically in India there is no hybridization, and thus concluded that the two taxa are specifically distinct. Subsequent treatments (e.g., Dickinson, 2003) follow this suggestion and further split *M. s. persicus* and *M. s. chrysocercus* from the Malagasy *M. s. superciliosus* yielding three species; *M. persicus, M. superciliosus*, and *M. philippinus*. Our taxon sampling included representatives from all three of the species-level taxa in this complex. *M. superciliosus* is sister to *M. persicus* with a roughly 2% uncorrected *p*-distance between the two. That sister pairing is roughly 4% divergent from its' sister taxon *M. philippinus*. The level of sequence divergence between the Malagasy *M. superciliosus* and asian *M. persicus* is lower than the level of divergence between many of the subspecific comparisons in our sample (see results and discussion below). Additional behavioral, morphological, and genetic work is needed to clarify the status of these taxa.

The two species of *Nyctiornis* are unique among bee-eaters in several ways (Fry, 1984). They differ morphologically in that they are the only bee-eaters: (1) with bi-colored beaks; (2) without a black eye-stripe; (3) with sexual dimorphism (*Nyctiornis amicta* only); and (4) with elongated pendant shaped throat feathers. They also differ from the rest of the family vocally by producing roller-like sounds including a gruff "quo-qua-qua-qua-qua", a deep "kwow, and hoarse guttural croaks and chuckles (Fry, 2001). All analyses provide strong support that *Nyctiornis* is sister to all other Meropidae, in agreement with earlier studies documenting its uniqueness among bee-eaters (Fry, 1969, 1984; von Boetticher, 1951; Burt, 2004).

After the splitting off of *Nyctiornis*, our data place several lineages in a polytomy at the base of the tree. Model-based analyses (Fig. 2B) recover four clades with little support for relationships among them: *Meropogon forsteni, Merops breweri, M. bullocki*/*M. bullockoides*, and a large clade including *M. boehmi* and clades A and B. The lack of resolution at the base of the tree seems to be due, at least in part, to the inclusion of *Meropogon* in the analysis. When the reduced matrix is analyzed, the basal nodes have much higher support (Fig. 2A). This suggests that the relationships of *Meropogon forsteni* were unsettled during Bayesian analysis and obscured the relationships of the remaining taxa. The difficulty in resolving the position of *Meropogon* may be due to the lack of nuclear data for that taxon.

Maximum parsimony analysis places *Meropogon forsteni*, the monotypic Sulawesi endemic, sister to *Merops breweri* from Africa, although this relationship is not highly supported by bootstrapping. These two bee-eaters share many morphological and behavioral similarities. Both species are solitary inhabitants of forest edge. Morphologically, they share the same wing and tail colors and patterns, and both have elongated throat feathers that are often puffed out to form a beard or ruff during vocalizations (Fry, 1969). These ecological and morphological similarities have led previous authors to propose that they may be sister taxa (von Boetticher, 1951; Burt, 2004). Although this relationship seems biogeographically improbable, other examples exist of sister relationships between Southeast Asian and equatorial African taxa. For example, the River Martins *Pseudochelidon sirintarae* and *P. eurystomina* (Zusi, 1978; Sheldon et al., 2005), and the Peafowls *Afropavo* and *Pavo* (Kimball et al., 1997; Crowe et al., 2006), each have one species in equatorial African forests and other in SE Asian forests. However, despite the morphological and ecological similarities and the biogeographic precedent for the relationship, the molecular data do not strongly support this sister relationship with high bootstrap or Bayesian probabilities. More data are needed to unequivocally assess this sister relationship.

Our data also have difficulty placing the sedentary African savanna species *Merops bulocki* and *M. bullockoides*, which are well supported as sister taxa, but together are members of the basal polytomy. These taxa are allopatric, with the range of the northern *M. bulocki* approaching that of *M. bullockoides* at the southern end of its' distribution at Lake Albert in Uganda (Fry, 1969). Because they differ in plumage and overall size, most authors agree that these taxa are legitimate species (Irwin and Benson, 1966; Dickinson, 2003) High divergences in both our mtDNA and combined nuclear intron data sets (uncorrected *p*-distances of 7.9% and 1.9%, respectively) are consistent with recognizing *bulocki* and *bullockoides* as separate species.

Finally *M. boehmi*, an inhabitant of riparian woodlands centered around Lake Malawi, Africa, has uncertain phylogenetic affinities in our study. In taxonomic lists *Merops boehmi* has traditionally been linked to two other species. von Boetticher (1951) grouped *M. boehmi* and *M. albicollis* into a genus *Aerops* based on plumage similarities, whereas Fry (1969, 1984) believed that the plumage similarities between *M. boehmi* and *M. albicollis* were convergent and therefore phylogenetically misleading. Fry further suggested that *M. boehmi* originated as a parapatric offshoot of the widespread *M. orientalis* (Fry, 1969). Burt (2004) was unable to place *M. boehmi* sister to any other species with confidence. We recover two alternate phylogenetic placements for *M. boehmi* but neither received high support. Model-based analyses place *M. boehmi* sister to clade A (Fig. 3). Although this relationship is not well supported, high posterior probability unites *M. boehmi* with clades A and B, separating it from the more basal lineages in the family. In contrast, under maximum parsimony *M. boehmi* is sister to clade B, but several weakly supported nodes between it and the base of the tree mean that it must be considered part of the basal polytomy (Fig. 2). Although the specific relationships are uncertain, this suggests that *M. boehmi* is not most closely related to either *M. albicollis* or *M. orientalis*.

4.1. Intraspecific variation

The intercontinental migrant *M. apiaster* breeds throughout Europe and winters in Southern Africa. It displays very low levels of genetic differentiation between the two samples in our study; one each from Kuwait and Malawi (0.04% uncorrected *p*-distance). This is in contrast to the substantial intraspecific variation shown by the four non-migratory taxa. Four out of the five species for which we had multiple individuals are non-migratory. The average uncorrected pairwise divergence within these four species was 4.6%. The greatest divergence was between two individuals of *M. muelleri*, from Ghana and Equatorial Guinea. They were 6.5% divergent from one another, a degree of differentiation equal to that found in taxa that are treated as distinct species (e.g., *M. ornatus* to *M. apiaster* is 6.4%). In addition to the substantial genetic break, subspecies of *M. muelleri* are diagnosable by the length of the central rectrices, with *M.m. muelleri* having central rectrices up to 5 mm longer than the rest of the tail and *M. m. mentalis* with central rectrices at least 30 mm longer than the rest of the tail (Fry, 1969). Two specimens of *M. gularis* taken from the same localities as the two *M. muelleri* were only 3.7% divergent from one another, which may suggest that contact between subspecies of *M. gularis* occurred more recently than for *M. muelleri*. Another possibility is that the difference in the level of genetic divergence within the two species is demographically driven; for example by differences in population size. On a continental scale, the level of genetic differentiation between populations of these African forest taxa is similar to that found in some other species of lowland African rainforest birds (Beresford and Cracraft, 1999; Beresford, 2003; but see Roy, 1997; Bowie et al., 2004). The lone Asian taxon for which we have multiple

individuals is *Merops viridis*, an inhabitant of open country from southeast China through the Indonesian and Philippine archipelagos to Java. We compared one individual of each described subspecies. The *M. v. viridis* from Borneo and *M. v. americanus* from the Philippines are 3.8% divergent from one another, a comparable value to other subspecific comparisons.

We analyzed two individuals from the monotypic intercontinental migrant *M. apiaster*, which showed a negligible level of differentiation (0.04%) between birds collected in Kuwait and Malawi. Thus, the lone migratory taxon shows low levels of genetic divergence relative to the non-migratory taxa, a pattern seen in several other avian groups (McDonald et al., 1999; Korfanta et al., 2005). Taken together, these comparisons indicate that genetic diversity can be substantial within described species of bee-eaters, especially non-migratory ones, which is consistent with a higher species level diversity than is currently recognized. Denser taxon sampling and more intraspecific studies in general are needed to gain a better understanding of genetic structure at this level.

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