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Phylogenetic relationships of the bulbuls (Aves: Pycnonotidae) based on mitochondrial and nuclear DNA sequence data

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Abstract

Bulbuls (Aves: Pycnonotidae) are a fairly speciose (ca. 130 sp.) bird family restricted to the Old World. Family limits and taxonomy have been revised substantially over the past decade, but a comprehensive molecular phylogeny for the family has not been undertaken. Using nuclear and mitochondrial DNA sequences, we reconstructed a well-supported phylogenetic hypothesis for the bulbuls. Three basal lineages were identified: a large African clade, a large Asian clade that also included African *Pycnonotus* species, and the monotypic African genus *Calyptocichla*. The African clade was sister to the other two lineages, but this placement did not have high branch support. The genus *Pycnonotus* was not monophyletic because three species (*eutilotus*, *melanoleucos*, and *atriceps*) were highly diverged from the other species and sister to all other Asian taxa. Additional taxon sampling is needed to further resolve relationships and taxonomy within the large and variable *Hypsipetes* complex.

Keywords: Bulbul; Pycnonotidae; Systematics; Africa; Asia

1. Introduction

Bulbuls (Pycnonotidae) are small to medium sized passerine birds that forage on a variety of invertebrates and fruit. They are broadly distributed across Africa and Asia, with distinct peaks of species diversity in the tropical forests of sub-Saharan Africa and Southeast Asia. Several species are found in the Philippines, but the family's distribution is severely truncated by Wallace's line; a single species (*Thapsinillas affinis*) is native to the Moluccas and some small islands off Sulawesi. Throughout Africa and Asia, a few species of bulbuls (especially in the genus *Pycnonotus*) tend to be common garden birds, and so the family is generally familiar to local people. Several species have also been introduced to areas outside their natural distribution and become well established (e.g. Hawaii, Florida, and Australia).

Delacour (1943) provided a synopsis of previous work on familial limits and taxonomic relationships among pycnonotid genera, and synthesized the available data into his own hypothesis of bulbul relationships. He divided the bulbuls into 13 genera and four major groups: the *Pycnonotus* group (*Pycnonotus*, *Baeopogon*, *Ixonotus*, *Spizixos*, and *Calyptocichla*), the *Phyllastrephus* group (*Phyllastrephus*, *Bleda*, and *Nicator*), the *Criniger* group (*Criniger*, *Setornis*, and *Microscelis*), and the *Chlorocichla* group (*Chlorocichla* and *Thescelocichla*). His ideas were summarized in a figure depicting the inter-generic relationships, not in the manner of a modern cladogram but rather one showing links between genera. For example, *Criniger* was depicted on a line between *Setornis* and *Chlorocichla*, rather than all three being terminals connected by a bifurcating network. He also assessed the relationships of several taxonomically enigmatic species that had previously been considered pycnonotids including, among others, the bulbuls on Madagascar and the African genus *Nicator*.

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Recent analyses of DNA sequence data have provided new insights into the relationships of these enigmatic taxa. Four of the five species on Madagascar classically treated as pycnonotids were shown to be part of an endemic radiation of Malagasy taxa that is unrelated to bulbuls and likely related to the Sylviidae (Cibois et al., 2001). A fifth Malagasy species, *Xanthomixis tenebrosus*, formerly treated as a member of the genus *Phyllastrephus*, was not included in their analysis. The affinities of the genus *Nicator* (three species endemic to tropical Africa) have long been in question. Based on skeletal features, Olson (1989) surmised that the genus was more appropriately placed with the bush-shrikes (Malaconotidae), but DNA–DNA hybridization analysis (Sibley and Ahlquist, 1990) placed it sister to the rest of the bulbuls. Recent analyses of nuclear DNA sequence data showed that *Nicator* did not belong in the bulbuls, but also was not a malaconotine (Beresford et al., 2005).

Modern studies dealing with bulbul taxonomy and systematics are few and have primarily dealt with population-level questions (Smith et al., 1997, 2005), species limits (Chappuis and Erard, 1993), and relationships within a genus (Roy, 1997). Relationships among pycnonotid genera are still largely unresolved. The most complete investigation of bulbul relationships to date (Pasquet et al., 2001) used mitochondrial DNA sequences to address the systematics of the genus *Criniger*. Their results showed that African and Asian clades of *Criniger* were not each other's closest relatives, supporting taxonomic treatments that separated the Asian species into the genus *Alophoixus* (e.g. Hall and Moreau, 1970; Inskipp et al., 1996). Although Pasquet et al. (2001) focused on *Criniger*, their taxon sampling allowed general inferences about the family as a whole. All the African species except *Pycnonotus barbatus* (the only African *Pycnonotus* species included) formed a clade, as did all of the Asian species (including *P. barbatus*). Their data also indicated that *Hypsipetes* was paraphyletic, but that conclusion lacked support from bootstrap resampling.

Despite the findings of Pasquet et al. (2001), many aspects of bulbul systematics remain unresolved. The affinities of several small genera have never been addressed with molecular data or modern systematic methods. Some of these genera have morphological (*Spizixos*) or habitat (*Setornis* and *Thescelocichla*) specializations. It is unknown if these specializations reflect distant relationships to more speciose bulbul genera, or recent adaptations that obscure close affinities with more generalized clades. Relationships between major clades in the family are also poorly known. Although Pasquet et al. (2001) found evidence of two large clades in the family, the four basal nodes in the Asian clade were not well supported. Any attempt to study the biogeographic history or historical ecology of bulbuls requires resolution of basal nodes and placement of the smaller genera within a phylogenetic framework.

To further clarify bulbul systematics, we used DNA sequences to reconstruct relationships within the family. In this study, we more than doubled the taxon sampling of Pasquet et al. (2001) and utilized both nuclear and mitochondrial genes. This data set enabled us to clarify several major regions of uncertainty in the bulbul phylogeny, such as the placement of small enigmatic genera and the basal structure within the major clades.

2. Methods

Taxon sampling (Table 1) included individuals from 57 species representing 20 of the 25 genera (Dickinson, 2003 plus *Alophoixus*). Outgroup taxa were drawn from babblers (Timaliidae), swallows (Hirundinidae), and thrushes (Turdidae), all close relatives of bulbuls in recent higher-level studies of passerine birds (Barker et al., 2004; Beresford et al., 2005). Genomic DNA was extracted from pectoral muscle tissue using proteinase K digestion following the manufacturer's protocol (Dneasy tissue kit, Qiagen). Corresponding vouchers (skins or skeletons) for all specimens in the study have been deposited in museum collections.

We sequenced the entire second and third subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (ND2 and ND3) and the seventh intron of the nuclear gene Beta-fibrinogen (Fib7). Primers for the ND3 gene were L10755 and H11151 (Chesser, 1999). Primers for the ND2 gene were L5215 (Hackett, 1996), H6313, L5758, and H5766 (Johnson and Sorenson, 1998). The intron was amplified and sequenced with the primers FIB-BI7L, FIB-BI7U (Prychitko and Moore, 1997), FIB-BI7Lb, and FIB-BI7Ub (Sheldon et al., 2005). We were able to obtain robust direct sequence reads for the majority of species for Fib7, however, due to a homopolymer run, we were unable to get reliable direct reads from some of the species. For these species, we cloned PCR products into PCR®2.1-TOPO® vector (Invitrogen) and transformed the recombinant vector into TOP10 chemically competent *Escherichia coli* following the manufacturers recommendations (TOPO TA Cloning Kit, Invitrogen). Four positive colonies were selected from each plate and used as a template for PCR amplification to check the size of the insert. Colonies with inserts of the anticipated size were then purified using the QIAprep Spin Miniprep Kit (Qiagen) and sequenced in both directions using the M13 forward and reverse primers.

We purified PCR products with Perfectprep PCR cleanup kits (Eppendorf). Sequencing of purified PCR products was performed with BigDye Terminator Cycle Sequencing Kits (Applied Biosystems). Primers used for PCR were also used for cycle sequencing reactions, resulting in bi-directional sequence for all taxa. Cycle sequencing products were run on an ABI Prism 3100 automated DNA sequencer (Perkin-Elmer Applied Biosystems). The computer program Sequencher 4.1 (Genecodes) was used to reconcile chromatograms of complementary fragments and align sequences across taxa.

Congruence between phylogenetic signal in the genes was tested with the incongruence length difference test (Farris et al., 1994, 1995), implemented in PAUP*4.0b10 (i.e. partition homogeneity test; Swofford, 2002). The test

Table 1
Samples included in the study

Species	Sample #	Source	Locality
Ingroup			
Spizixos semitorques	B20554	LSUMNS	Captive
Pycnonotus atriceps	B36320	LSUMNS	Borneo
Pycnonotus barbatus	B39494	LSUMNS	Ghana
Pycnonotus brunneus	B36341	LSUMNS	Borneo
Pycnonotus cyaniventris	B36425	LSUMNS	Borneo
Pycnonotus erythrophthalmus	B36378	LSUMNS	Borneo
Pycnonotus eutilotus	B36313	LSUMNS	Borneo
Pycnonotus goavier	B36351	LSUMNS	Borneo
Pycnonotus nigricans	B34228	LSUMNS	South Africa
Pycnonotus plumosus	B23354	LSUMNS	Borneo
Pycnonotus zeylanicus	B23321	LSUMNS	Borneo
Pycnonotus leucogenys	F347847	FMNH	Pakistan
Pycnonotus melanoleucos	B50353	LSUMNS	Borneo
Pycnonotus melanicterus	B51038	LSUMNS	Borneo
Andropadus curvirostris	F384875	FMNH	Uganda
Andropadus gracilis	F396569	FMNH	Ghana
Andropadus virens	F384863	FMNH	Uganda
Andropadus ansorgei	11736	ANSP	Equatorial Guinea
Calyptocichla serina	11614	ANSP	Equatorial Guinea
Baeopogon indicator	F391689	FMNH	Uganda
Baeopogon clamans	F429455	FMNH	Central African Republic
Chlorocichla flavicollis	F391690	FMNH	Uganda
Thescelocichla leucopleura	11476	ANSP	Equatorial Guinea
Pyrrurhus scandens	F389336	FMNH	Gabon
Phyllastrephus albigularis	B39597	LSUMNS	Ghana
Phyllastrephus icterinus	B27097	LSUMNS	Cameroon
Phyllastrephus placidus	B21133	LSUMNS	Tanzania
Phyllastrephus debilis	F356727	FMNH	Tanzania
Phyllastrephus flavostriatus	F384938	FMNH	Uganda
Phyllastrephus hypochloris	F384936	FMNH	Uganda
Phyllastrephus fisheri	F384931	FMNH	Uganda
Phyllastrephus terrestris	F390098	FMNH	South Africa
Phyllastrephus xavieri	F357212	FMNH	Democratic Republic of Congo
Bernieria madagascariensis	F393262	FMNH	Madagascar
Xanthomixis apperti	F393159	FMNH	Madagascar
Xanthomixis cinereiceps	F393283	FMNH	Madagascar
Xanthomixis tenebrosus	F393278	FMNH	Madagascar
Xanthomixis zosterops	F356646	FMNH	Madagascar
Bleda canicapilla	B39389	LSUMNS	Ghana
Bleda eximia	B39580	LSUMNS	Ghana
Criniger barbatus	F389346	FMNH	Gabon
Criniger ndussumensis	F357235	FMNH	Democratic Republic of Congo
Criniger calurus	B27104	LSUMNS	Cameroon
Criniger chloronotus	B27094	LSUMNS	Cameroon
Alophoixus bres	B38567	LSUMNS	Borneo
Alophoixus ochraceus	B38614	LSUMNS	Borneo
Alophoixus phaeocephalus	B38568	LSUMNS	Borneo
Setornis criniger	B23359	LSUMNS	Borneo
Tricholestes criniger	B38601	LSUMNS	Borneo
Iole olivacea	B51043	LSUMNS	Borneo
Ixos philippinus	F392263	FMNH	Philippines
Ixos malaccensis	B51130	LSUMNS	Borneo
Microscelis amaurotis	B16985	LSUMNS	Japan
Hemixos flavalus	B38659	LSUMNS	Borneo
Hyptipetes madagascariensis	F393308	FMNH	Madagascar
Nicator chloris	F391705	FMNH	Uganda
Nicator vireo	F430360	FMNH	Central African Republic
Outgroup:			
Progne subis	B25082	LSUMNS	
Hirundo rustica	B14102	LSUMNS	
Trichastoma bicolor	B36396	LSUMNS	
Stachyris plateni	C37768	CMNH	
Enicurus leschenaulti	B36452	LSUMNS	

Taxonomy follows Dickinson (2003) except for the inclusion of *Alophoixus*. Louisiana State University Museum of Natural Science (LSUMNS), Field Museum of Natural History (FMNH), Academy of Natural Sciences, Philadelphia (ANSP), and Cincinnati Museum of Natural History (CMNH).

excluded constant characters and ran for 1000 bootstrap repetitions. PAUP*4.0b10 was also used to test the base composition of each gene using a χ^2 analysis of base frequencies across taxa. Maximum likelihood (ML) and maximum parsimony (MP) analyses were performed for each gene as well as the combined data 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 Modeltest 3.5 (Posada and Crandall, 1998) to determine the appropriate model of evolution and parameter estimates. Support for nodes in the maximum likelihood tree was assessed by non-parametric bootstrapping (Felsenstein, 1985) and reanalysis of the data (100 replicates). MrBayes 3.0 (Huelsenbeck and Ronquist, 2001) was used to estimate model parameters from the data and to evaluate support for specific relationships in the phylogeny. For the combined data set, a mixed model approach was implemented to account for the potential difference in evolutionary model parameters between data partitions (nuclear and mitochondrial DNA). A general time reversible (Yang, 1994a) model framework, with γ-distributed rates among sites (Yang, 1994b) and invariant sites, was used for both partitions (from Model test). All parameters (except topology) 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 help evaluate stationarity, the condition in which parameter estimates (and likelihood scores) have converged on a value and the Markov chain is sampling trees according to their posterior probability. All samples prior to reaching stationarity were discarded. 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.

3. Results

3.1. Sequence attributes

The mitochondrial DNA matrix contained 57 ingroup taxa and 1392 bp (1041 ND2 and 351 ND3) of which 772 (55.4%) were parsimony informative. A clean sequence of the fibrinogen intron could not be produced for some of the taxa, so a combined data set with slightly sparser taxon sampling was created. The combined data matrix contained 49 ingroup taxa and 2311 bp (1041 ND2, 351 ND3, and 919 Fib7), of which 979 were parsimony informative (571 ND2, 182 ND3, and 226 Fib7). All sequences are available through the GenBank database (http://www.ncbi.nlm.nih.gov, Accession Nos. DQ402186–DQ402359). Aligned ND2 and ND3 sequences appeared to be of mitochondrial origin, rather than nuclear copies. Sequences contained no stop codons, overlapping fragments contained no conflicts, base composition was relatively homogenous across taxa, codon positions contained expected relative divergences (3 > 1 > 2), and resulting relationships contained no highly suspect arrangements.

Base composition in the mitochondrial data was biased, with an excess of adenine and cytosine (A = 0.302, C = 0.342, G = 0.116, and T = 0.240). Base composition in the intron data was biased in favor of adenine and thymine (A = 0.314, C = 0.186, G = 0.166, and T = 0.334). These levels of bias are consistent with levels found in these genes in other bird groups (e.g. Marks et al., 2002; Moyle, 2004, 2005; Sheldon et al., 2005). Models of evolution chosen for all likelihood analyses accounted for base composition bias. A χ^2 test for homogeneity of base frequencies across taxa detected no significant differences for the combined data ($p = 0.98$) or individual genes (ND2, $p = 0.59$; ND3, $p = 1.00$; FIB7, $p = 1.00$).

Aligned FIB7 sequences contained several inferred insertions or deletions (indels), but alignment of the sequences was straightforward. Placement of indels was generally unambiguous 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 facilitated alignment and default placement of indels.

3.2. Phylogenetic relationships

Maximum likelihood and Bayesian analyses of the combined data set produced a well-resolved hypothesis of bulbul relationships (Fig. 1). Due to outgroup sampling, two sets of taxa, *Nicator* and the Malagasy *Bernieria* and *Xanthomixis*, previously shown not to be part of the Pycnonotidae (Fjeldsa et al., 1999; Cibois et al., 2001; Beresford et al., 2005) could not be placed with any certainty. Aside from these taxa, bulbul monophyly was well supported. The basal node in the radiation received high bootstrap support and Bayesian posterior probability. Within the bulbul radiation, three lineages diverged from the base of the crown clade, but relationships among them lacked significant support. One lineage gave rise to a clade (clade A, Fig. 1) containing all of the Asian species sampled, as well as two African species, *Pycnonotus barbatus* and *Pycnonotus nigricans*, which were embedded well inside the Asian radiation. A second lineage (clade B, Fig. 1) included only African species, but lacked the two species mentioned above and the Golden Greenbul, *Calyptocichla serina*. This African forest species was the sole taxon in the third basal lineage of the bulbul phylogeny. Analyses placed *C. serina* sister to the Asian clade, but this relationship lacked significant support (Bayesian posterior probability = 0.90 and bootstrap proportion = 60) and was contradicted by the mtDNA results (Fig. 2).

Basal relationships within clade A were well resolved and supported paraphyly of the large genus *Pycnonotus*. The basal branch in the clade contained three *Pycnonotus* species (*atriceps*, *melanoleucos*, and *eutilotus*) isolated from all other Asian taxa by significant Bayesian posterior probability (0.99) and moderate bootstrap support (70). Signifi-

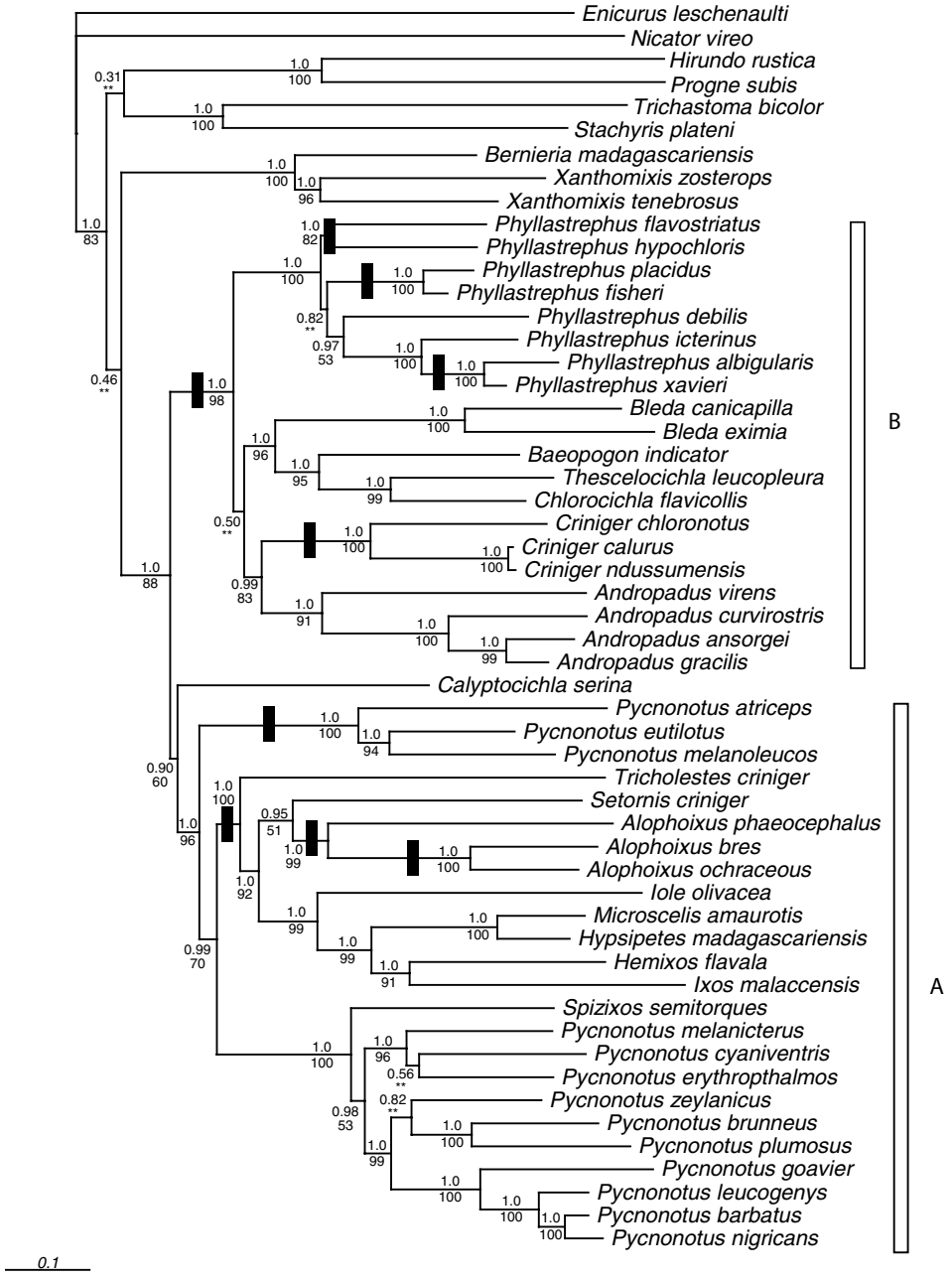

Fig. 1. Bayesian consensus tree of the combined ND2, ND3, and Fib7 data from mixed-model analysis. Numbers above nodes indicate Bayesian posterior probability/maximum likelihood bootstrap support. **Bootstrap support less than 50%. Open bars indicate two clades mentioned in the text. Black bars indicate the inferred occurrence of unambiguous indels in the intron.

cant posterior probability (1.0) supported monophyly of a clade including the other *Pycnonotus* species, and the Collared Finchbill (*Spizixos semitorques*). Within this clade, the two African species (*P. barbatus* and *P. nigricans*) were sister to a species distributed in the Middle East and Himalayas (*leucogenys*), and all three were sister to a common species in southern and Southeast Asia (*goavier*).

A second large clade within the Asian radiation (clade A) included *Setornis*, *Alophoixus*, and several genera often included in *Hypsipetes* (*Iole*, *Microscelis*, *Ixos*, *Tricholestes*, and *Hemixos*). Of these, *Tricholestes criniger* was strongly supported as the basal taxon. Three species of *Alophoixus* formed a clade sister to the monotypic *Setornis criniger*, but this node received low bootstrap support. *Hypsipetes* and four affiliated genera (*Iole*, *Ixos*, *Microscelis*, and *Hemixos*) comprised the final subclade of Asian species.

Within clade B, three sub-clades of African bulbuls were evident. Support for the monophyly of each subclade was strong, but little support existed for any particular arrangement among them. The first clade included all of the species of *Phyllastrephus* sampled (minus those species on Madagascar formerly treated as *Phyllastrephus*). In the second African sub-clade, *Thescelocichla* and *Chlorocichla* were sister taxa, with *Baeopogon* and *Bleda* branching successively

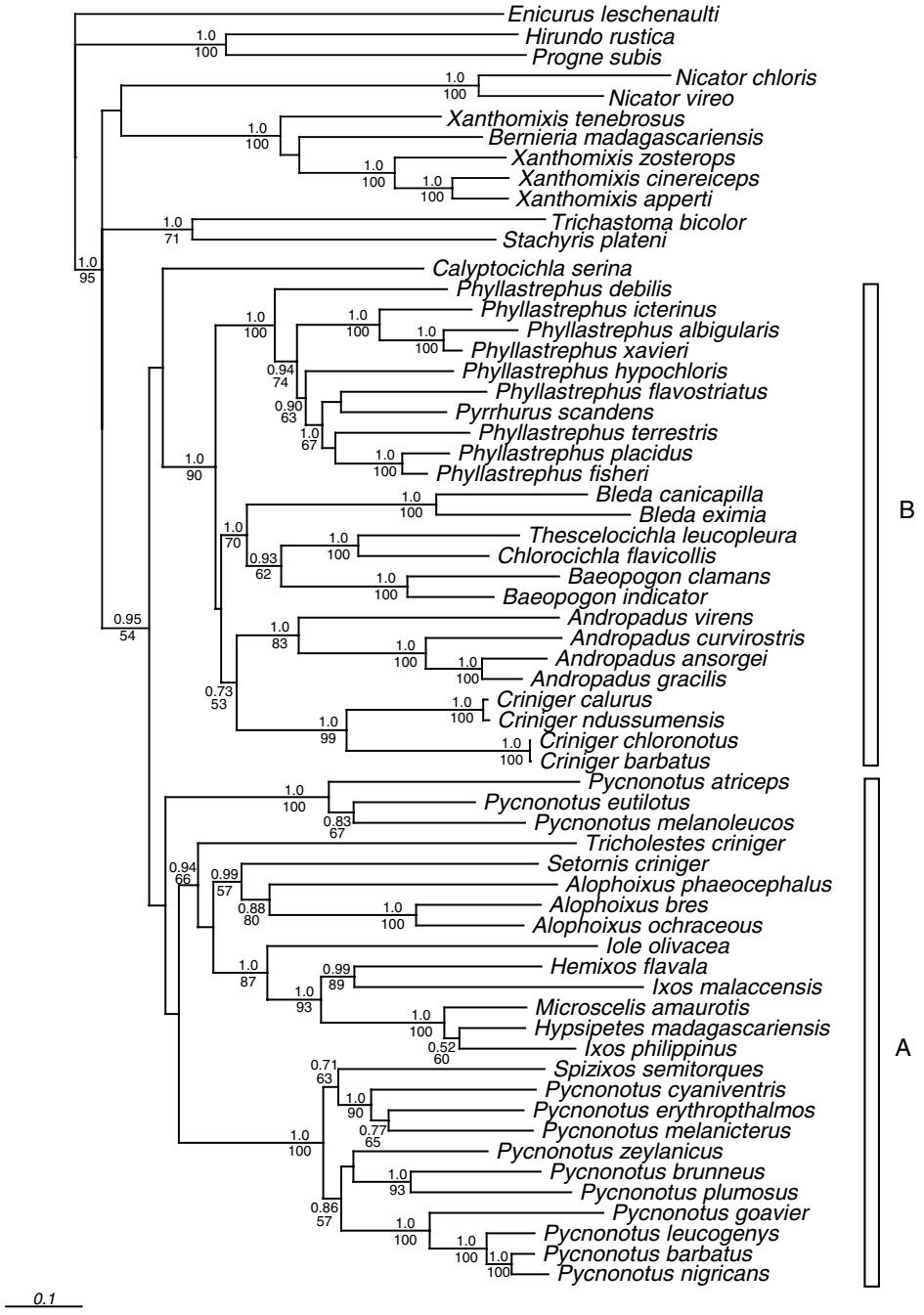

Fig. 2. Maximum likelihood tree from the combined ND2 and ND3 data. Numbers above nodes indicate Bayesian posterior probability/parsimony bootstrap proportions. Nodes without labels received less than 50% bootstrap support. Open bars indicate two clades mentioned in the text.

more basal. The final African sub-clade was a sister grouping of the monophyletic genera *Criniger* and *Andropadus*. Branch support indices within the three African sub-clades were high.

Analysis of the mtDNA alone (Fig. 2) resulted in a phylogenetic hypothesis that closely mirrored that of the combined data set but generally with lower branch support, particularly at basal nodes. This analysis allowed the inclusion of eight additional taxa for which clean FIB7 sequence could not be obtained. Of note, *Pyrrhurus* was embedded well within *Phyllastrephus*, rendering that genus paraphyletic. Also, *Ixos philippinus*, although in the same large clade as another *Ixos* species, was reconstructed as sister to *Hyptipetes madagascariensis*, rendering *Ixos* paraphyletic as well.

Insertions and deletions in the nuclear intron provided additional support for some nodes in the phylogeny. Indels can provide additional support for relationships either as characters used in the analysis (e.g. Prather et al., 2002) or as a qualitative assessment of nodes recovered by traditional analysis of nucleotide substitutions (e.g. Johnson et al., 2001; Moyle, 2004), which is the method used here. In

general, indels corresponded to strongly supported clades and could be mapped easily on the phylogeny (Fig. 1). For example, a large (ca. 230 bp) deletion compared to all other taxa in the study united three species of *Pycnonotus* (*melanoleucos*, *atriceps*, and *eutilotus*) corroborating the strong bootstrap support and Bayesian posterior probability for that clade. Other indels supported the large African clade (clade B, Fig. 1), the genus *Criniger*, and the genus *Alophoixus*. Not all indels could be mapped onto our estimate of phylogeny unambiguously. For example, a one base pair indel united three *Phyllastrephus* species (*debilis*, *flavostriatus*, and *hypochloris*). The three species do not form a clade in our tree (Fig. 1) but are separated by short internodes with low bootstrap support. A much larger indel (25 bp) unites *Spizixos* with seven *Pycnonotus* species (*goavier*, *leucogenys*, *barbatus*, *nigricans*, *zeylanicus*, *brunneus*, and *plumosus*). The *Pycnonotus* species form a clade, but *Spizixos* is separated from it by a single node that has significant posterior probability (0.98) but low bootstrap support (53).

4. Discussion

Our analysis of nuclear and mitochondrial DNA sequences yielded the first densely sampled phylogenetic hypothesis for bulbuls. Consistent with previous studies (Olson, 1989; Cibois et al., 2001; Beresford et al., 2005), two groups of taxa traditionally included in the bulbul family are not bulbuls. Cibois et al. (2001) found that four Malagasy taxa formerly placed in *Phyllastrephus* were not related to bulbuls at all, but instead were part of a sylvioid radiation endemic to Madagascar. Our data show that another Malagasy species not sampled by Cibois et al. (2001), the Dusky Bulbul (*X. tenebrosus*), is also in the clade of Malagasy endemics. Dickinson (2003) treated *tenebrosus* as a member of the genus *Xanthomixis*, but we recovered *Bernieria madagascariensis* within *Xanthomixis* (Fig. 2), suggesting that further work is necessary to identify the relationships within this clade. The African genus *Nicator* also is not part of the bulbul assemblage. Our outgroup sampling is not extensive enough to place this genus with any certainty, but nuclear DNA sequences showed it to be a basal lineage in the Sylvioidea (Beresford et al., 2005).

Three major lineages of bulbuls diverged from the base of the phylogeny, but the relationships among them could not be deciphered with any confidence. The three lineages produced a large entirely African clade (clade B, Fig. 1), a large mostly Asian clade (clade A, Fig. 1), and the monotypic African genus *Calyptocichla*. Relationships within clade A indicated that the large and morphologically heterogeneous genus *Pycnonotus* is not a natural group. Three species (*P. atriceps*, *P. melanoleucos*, and *P. eutilotus*) did not group with the other *Pycnonotus* species, but instead were sister to the entire Asian radiation. Strong bootstrap proportions, Bayesian posterior probability, and a large deletion in the intron data (ca. 230 bp) supported the close relationship of these three species. Significant posterior probability, but only moderate bootstrap support (70%), fixed the position of these three species at the base of the Asian clade. However, even if these three taxa were closer to the larger *Pycnonotus* clade the genus would not be monophyletic because the data unequivocally place *Spizixos* sister to, or within, the larger clade of *Pycnonotus* species. Bayesian and maximum likelihood analyses (Fig. 1) place *Spizixos* sister to the large *Pycnonotus* clade, but a large (25 bp.) indel common to *Spizixos* and seven *Pycnonotus* species (see Results), indicates that *Spizixos* may be imbedded within, rather than sister to, the large clade of *Pycnonotus* species. Pasquet et al. (2001) recovered a monophyletic *Pycnonotus*, but low bootstrap support linked *P. atriceps* to the other species, and *Spizixos* was not sampled.

Deignan (1960) and Delacour (1943) included the African and Asian bearded bulbuls in a single genus, *Criniger*. Hall and Moreau (1970) pointed out that morphological variation between the Asian and African species was substantial and warranted separate genera. Using molecular data, Pasquet et al. (2001) demonstrated that the Asian bearded bulbuls were not sister to the African bearded bulbuls (*Criniger*), and thus placing them in a separate genus, *Alophoixus*, was appropriate. Our data support this decision and suggest that the monotypic genus *Setornis* is sister to *Alophoixus*, but support for this relationship is low.

Our data corroborated many of the findings of Pasquet et al. (2001). Both studies recovered well-defined African and Asian clades (with African *Pycnonotus* species in the Asian clade). *Criniger* was found to be polyphyletic in both studies, supporting the suggestion to resurrect *Alophoixus* for the Asian bearded bulbuls. However, in some places our data produced a topology that was in conflict with that of Pasquet et al. (2001) at well-supported nodes. Pasquet et al. (2001) had strong support for all nodes within the African clade whereas we found little support for any relationship between three African sub-clades. Even allowing for our lack of resolution among the three African sub-clades, relationships among African genera remained a source of disagreement between the two studies. For example, Pasquet et al. (2001) inferred a sister relationship between *Andropadus* and *Phyllastrephus* with 90% bootstrap support whereas our data showed strong support for a sister relationship between *Andropadus* and *Criniger*. Second, Pasquet et al. (2001) recovered *Bleda* as the basal taxon in the African radiation, separated from the other African genera by a node with 89% bootstrap support. In contrast, our *Bleda* samples were a well-supported sister group of three other African genera (*Baeopogon*, *Chlorocichla*, and *Thesolocichla*). Taxon sampling differed between the two studies, but Pasquet et al. (2001) included the same *Baeopogon* species as in our study (*indicator*) and it was not sister to *Bleda*. In summary, not a single genus-level sister relationship in the African clade is shared between Pasquet et al. (2001) and our study. The high support indices for these differences preclude inferring stochastic differences in the phylogenetic signal of the genes used in each study. It is possible that differences in taxon sampling between the two studies

affected the phylogenetic resolution (see Pollock et al., 2002; Hillis et al., 2003). Additional taxon and character sampling is required to shed light on these inconsistencies.

4.1. Biogeography

Like many other bird groups (e.g. pittas, rollers, bee-eaters, hornbills, and alcedinine kingfishers), bulbuls are largely found in the tropics of Asia and Africa, and are absent from the New World. A basic question for biogeographers is where these groups originated, and subsequently how the biota of each region was assembled over time. Our hypothesis of bulbul relationships allows for some discussion of these fundamental biogeographic questions. Species in this family segregated largely by continent, with a large exclusively African clade and a large mostly Asian clade. The two exceptions to this pattern defined the only continental disjunctions in the phylogenetic tree. Two African species (*P. barbatus* and *P. nigricans*) were embedded within the Asian clade and were successively sister to a taxon in the Middle East (*P. leucogenys*) and then several taxa in southern and Southeast Asia. This pattern of relationships suggests that the African *Pycnonotus* were recently derived from Asian ancestors, and likely arrived in Africa via the Middle East rather than long distance dispersal across the Indian Ocean. Another African species (*C. serina*) does not have any close living relatives in the phylogeny but is reconstructed as basal to the Asian radiation, albeit with low support. If supported by additional data, this relationship may indicate an African origin for crown clade bulbuls. According to a higher-level study of passerine relationships (Barker et al., 2004), bulbuls are sister to a speciose clade of babblers and sylvioid warblers; a largely Asian radiation.

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