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A comprehensive molecular phylogeny for the hornbills (Aves: Bucerotidae)

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ABSTRACT

The hornbills comprise a group of morphologically and behaviorally distinct Palaeotropical bird species that feature prominently in studies of ecology and conservation biology. Although the monophyly of hornbills is well established, previous phylogenetic hypotheses were based solely on mtDNA and limited sampling of species diversity. We used parsimony, maximum likelihood and Bayesian methods to reconstruct relationships among all 61 extant hornbill species, based on nuclear and mtDNA gene sequences extracted largely from historical samples. The resulting phylogenetic trees closely match vocal variation across the family but conflict with current taxonomic treatments. In particular, they highlight a new arrangement for the six major clades of hornbills and reveal that three groups traditionally treated as genera (*Tockus*, *Aceros*, *Penelopides*) are non-monophyletic. In addition, two other genera (*Anthracoceros*, *Ocyceros*) were non-monophyletic in the mtDNA gene tree. Our findings resolve some longstanding problems in hornbill systematics, including the placement of '*Penelopides exharatus*' (embedded in *Aceros*) and '*Tockus hartlaubi*' (sister to *Tropicranus albocristatus*). We also confirm that an Asiatic lineage (*Berenicornis*) is sister to a trio of Afrotropical genera (*Tropicranus* [including '*Tockus hartlaubi*'], *Ceratogymna*, *Bycanistes*). We present a summary phylogeny as a robust basis for further studies of hornbill ecology, evolution and historical biogeography.

1. Introduction

The hornbills and ground-hornbills comprising the family Bucerotidae are charismatic land-birds that have long been the focus of research attention. Amongst evolutionary biologists, they are well known for their elaborate bill casques, cooperative breeding systems, and the remarkable strategy of self-incarceration, the females of many species sealing themselves into tree-holes for several weeks by plastering the entrances of their nest cavities (Moreau, 1934; Kemp, 2001). Amongst ecologists, the vital contribution of hornbills as long-distance seed dispersers has led to them being viewed as keystone species (Trail, 2007), and implicated in the historical expansion of Palaeotropical forests (Viseshakul et al., 2011). They also play an important role in tribal cultures from South Africa to East Asia (Bennett et al., 1997). Unfortunately, as a corollary of their large size and need for extensive foraging areas, many hornbills are highly sensitive to hunting and habitat fragmentation, making them one of the most threatened components of tropical ecosystems (Kinnaird and O'Brien, 2007). Over a third of hornbill species are considered to be of conservation concern globally, including 62% (20/32) of Asiatic species (see Table A1), some of which (e.g. *Anthracoceros montani*, *Aceros waldeni*) are close to extinction. Because of these attributes, hornbills are becoming increasingly prominent as study systems in ecology (e.g. Holbrook and Smith, 2000; Holbrook et al., 2002; Kitamura, 2011) and conservation biology (e.g. Sethi and Howe, 2009; Lenz et al., 2011).

The evolutionary history of the family has received less attention, although the basic outline of hornbill systematics is now well established. Several anatomical features—including fused upper vertebrae (atlas and axis), long flattened upper eyelashes, and bilobular kidneys (Kemp, 2001)—are unique to hornbills, suggesting that they form a relatively distinct clade. Their apparent divergence from related families has led to some authors separating them into their own order, Bucerotiformes (e.g. Kemp, 1995). Relationships within the family have been estimated on the basis of a qualitative assessment of characters such as phenotype, vocalizations and breeding behavior (Kemp and Crowe, 1985; Kemp, 1988), culminating in the publication of a consensus cladogram built using 26 such characters (Kemp, 1995). This tree has proved to be a useful framework for hornbill systematics, particularly because its coverage (54 of 61 taxa) is reasonably comprehensive (Kinnaird and O'Brien, 2007).

Several quantitative assessments of hornbill relationships have been undertaken using molecular techniques, but all have been

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based on highly incomplete datasets. The first steps involved karyological studies focused on seven species (Belterman and de Boer, 1984, 1990), and a 17-taxa tree constructed using DNA–DNA hybridization (Sibley and Ahlquist, 1990). These were followed by phylogenetic approaches generating partial cytochrome *b* (cyt *b*) sequences (189 bp) for 11 taxa (Morin et al., 1994; Srikwan and Woodruff, 1998). The results of these analyses agreed on the placement of the genus *Bucorvus* (ground-hornbills) as a highly divergent sister clade to all other hornbills, perhaps warranting designation as a separate family (Kemp, 1995). They also suggested that *Tockus* hornbills were an ancient lineage sharing a common ancestor with the rest of the Bucerotidae. Further sequencing led to expanded mitochondrial DNA (mtDNA) phylogenies for hornbills, first including 22 species (Hübner et al., 2003), then more recently all 34 species for which molecular data are currently available, i.e. 56% of species diversity in the family (Viseshakul et al., 2011).

The phylogeny published by Viseshakul et al. (2011) provided the most informative assessment of the historical relationships between major clades of hornbills, particularly as it contained at least one member from each genus. However, many nodes had low confidence in terms of bootstrap values, presumably because tree topology was based on variation in one mitochondrial gene (cyt *b*) across a limited set of species. Moreover, most species were only represented by partial sequences (400–1043 bp), whereas complete gene sequences (1143 bp) were only available for 15 species, i.e. 25% of species diversity in the family. Viseshakul et al. (2011) noted that a fuller understanding of phylogenetic relationships within the clade, as well as a better grasp of the timing of evolutionary events, could only be resolved by more comprehensive sampling of lineages and loci.

To address this issue we conducted the first complete species-level phylogenetic analysis for hornbills, based on both nuclear and mtDNA sequences. We found that well-preserved hornbill tissue was relatively rare in collections, and we therefore mainly extracted genetic material from captive individuals or museum samples. Sequencing from this material is challenging, and potentially prone to error (Mundy et al., 1997), so we also tested whether our results were consistent with phenotypic variation. Specifically, we focused on variation in vocal signals, which are often informative about evolutionary history in birds. Because vocal signals are often less labile than morphological traits, they are widely considered to be more useful indicators of phylogenetic relationships (Lanyon, 1969; McCracken and Sheldon, 1997; Price and Lanyon, 2002; Rheindt et al., 2008). This pattern holds true for non-passerine families that do not learn their songs (Weckstein, 2005; Patané et al., 2009; Wink et al., 2009), suggesting that vocal signals are likely to be informative in hornbills.

Our main goal was to provide a robust evolutionary tree, supported by independent datasets. An important component of this task was to clarify the position of certain lineages (e.g. *Berenicornis comatus*, *Tockus hartlaubi*, *T. camurus*, the genus *Ocyceros* and all Philippine taxa) that remained either unsampled or unresolved by Viseshakul et al. (2011). The provision of similar comprehensive phylogenetic frameworks has opened up multiple research avenues in a number of avian study systems (e.g. Lovette and Rubenstein, 2007; Lovette et al., 2010), and is considered a vital step towards resolving questions relating to speciation, biogeography and evolution in the Bucerotidae (Kinnaird and O'Brien, 2007).

2. Materials and methods

2.1. Taxon sampling, DNA extraction and sequence alignment

We were able to sample directly from 59 of 61 currently recognized hornbill species, and the remaining two missing taxa (*Tockus kempi* and *T. damarensis*) were added by downloading sequences from GenBank (see Table 1). Direct sampling involved the extraction of genomic DNA from contemporary material (i.e. captive and wild-trapped individuals) and historical material (i.e. museum samples collected over the last 160 years). Types and sources of material are given in Table B1.

For contemporary material, DNA was extracted from molted flight feathers and plucked pin-feathers (the latter preserved in 70–90% ethanol) following Morin et al. (1994). For historical material, we extracted DNA from toe-pads following Mundy et al. (1997). Historical samples were processed in a separate laboratory following standard extraction and polymerase chain reaction (PCR) controls, and using stringent protocols to avoid cross-contamination with modern avian DNA (Lerner and Mindell, 2005). In all cases, short fragments of genes (200–500 bp) were amplified to improve recovery of degraded DNA. Amplification was mainly conducted using a set of 17 newly designed primers developed from existing GenBank sequences using the program PRIMER3 v.04.0 (Rozen and Skaletsky, 2000). We also used two previously published primers (Shapiro and Dumbacher, 2001). For full details of primers see Table S1 (Supplementary material).

PCR amplification was performed using pre-optimized Qiagen HotStarTaq Master Mix in ABI 2720 thermal cyclers (Applied Biosystems, Foster City, CA) and purified using the Qiagen Mini-elute kit. The PCR profile followed for AK1 intron 5 was a touchdown of 15 min at 95 °C, followed by 45 cycles of 95 °C for 45 s, 54 °C for 60 s, and 72 °C for 60 s, and a final extension phase at 72 °C for 10 min. The equivalent profile for cyt *b* was a touchdown of 15 min at 95 °C, followed by 45 cycles of 94 °C for 30 s, 52 °C for 45 s, and 72 °C for 60 s, and a final extension phase at 72 °C for 10 min. Cycle sequencing reactions were run using the Big Dye Sequencing kit and analyzed in the ABI Prism Genetic Analyzer 377. Gene sequence contigs were assembled and edited using SEQUENCHER v4.2 (Gene Codes Corp., Ann Arbor, MI) and BIOEDIT v7.0.5.3 (Hall, 1999). Validity of sequences was assessed using BLAST (Altschul et al., 1990), and the raw contig files were scrutinized to ensure that we did not include any contaminated sequences, mis-called bases, or pseudogenes. We were particularly stringent with nuclear genes, mismatches amongst trees, or any sequence producing unexpectedly long branch-lengths. Cytochrome *b* sequences were aligned using CLUSTALW v.2.0 (Larkin et al., 2007) and truncated following a prescribed start codon (ATG) and termination codon (TAA/TAG). MUSCLE (Edgar, 2004) was used to align AK1 intron 5 and concatenate nuclear-mtDNA datasets manually in MEGA v.5.03 (Tamura et al., 2011). Final alignments in FASTA and NEXUS format are available on request from the authors.

Complete mtDNA cyt *b* genes (1143 bp) were generated for 59 hornbill species, including multiple representatives of most lineages. We also generated complete or partial sequences (500–703 bp) of a nuclear gene, cytosolic adenylate kinase 1 intron 5 (AK1 intron 5: Shapiro and Dumbacher, 2001), for 54 species. The combined length of nuclear and mitochondrial loci used in this study was 1846 aligned nucleotides. The final dataset contained 214 genetic sequences, with 1–4 sequences per species (mean = 3.492, ±SD = 0.744; see Table B1). Overall, 164 (77%) sequences for 57 species were historical, including 39 nuclear sequences and 125 mtDNA sequences. Sampling of individuals differed between gene partitions, with nuclear DNA sequences for 1 individual, and mtDNA sequences for a mean of 2.623 (±SD = 0.522) individuals, per species. The limited number of nuclear sequences reflects the relative difficulty of recovering nuclear genes from historical material.

Our nuclear genes represent the first AK1 intron 5 DNA sequences available for any hornbill species, and the most comprehensive such dataset for the family to date. Previous studies have

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Table 1
Taxonomy and nomenclature of all hornbill species included in this study, and comparison with six taxonomic treatments of this group. Classification follows Gill and Donsker (2012), which contained updates for Bucerotidae based on Kemp and Delport (2002) and Viseshakul et al. (2011).

Genus	Species	Peters (1945)	Sanft (1960)	Sibley and Monroe (1990)	Kemp (1995)	Kemp (2001)	Dickinson (2003)	Clements (2007)
Bucorvus[a]	*abyssinicus*	–	–	–	–	–	–	–
Bucorvus[a]	*leadbeateri*	–	*cafer*	–	–	–	–	–
Tockus	*ruahae*[b]	NC	NC	NC	NC	NC	NC	NC
Tockus	*kempi*[b]	NC	NC	NC	NC	ssp *erythrorhynchus*	ssp *erythrorhynchus*	ssp *erythrorhynchus*
Tockus	*damarensis*[b]	ssp *erythrorhynchus*	ssp *erythrorhynchus*	NC	ssp *erythrorhynchus*	ssp *erythrorhynchus*	ssp *erythrorhynchus*	ssp *erythrorhynchus*
Tockus	*rufirostris*[b]	ssp *erythrorhynchus*	ssp *erythrorhynchus*	NC	ssp *erythrorhynchus*	ssp *erythrorhynchus*	ssp *erythrorhynchus*	ssp *erythrorhynchus*
Tockus	*erythrorhynchus*	–	–	–	–	–	–	–
Tockus	*monteiri*	–	–	–	–	–	–	–
Tockus	*deckeni*	–	–	–	–	–	–	–
Tockus	*jacksoni*	–	*syn deckeni*	–	ssp *deckeni*	ssp *deckeni*	–	–
Tockus	*leucomelas*	ssp *flavirostris*	ssp *flavirostris*	–	–	–	–	–
Tockus	*flavirostris*	–	–	–	–	–	–	–
Tockus	*fasciatus*	–	–	–	–	–	–	–
Tockus	*hemprichii*	–	–	–	–	–	–	–
Tockus	*nasutus*	–	–	–	–	–	–	–
Tockus	*pallidirostris*	–	–	–	–	–	–	–
Tockus	*bradfieldi*	–	–	–	–	–	–	–
Tockus	*alboterminatus*	–	–	–	–	–	–	–
Tockus	*camurus*	–	–	–	–	–	–	–
Tockus	*hartlaubi*	–	–	–	–	–	–	–
Tropicranus	*albocristatus*	*Berenicornis*	–	–	–	–	–	–
Berenicornis	*comatus*	–	–	*Aceros*	*Aceros*	–	–	*Aceros*
Ceratogymna	*atrata*	–	–	–	–	–	–	–
Ceratogymna	*elata*	–	–	–	–	–	–	–
Bycanistes	*fistulator*	ssp *bucinator*	–	*Ceratogymna*	*Ceratogymna*	–	–	*Ceratogymna*
Bycanistes	*bucinator*	–	–	*Ceratogymna*	*Ceratogymna*	–	–	*Ceratogymna*
Bycanistes	*cylindricus*	–	–	*Ceratogymna*	*Ceratogymna*	–	–	*Ceratogymna*
Bycanistes	*albotibialis*	ssp *cylindricus*	ssp *cylindricus*	*Ceratogymna*	ssp *cylindricus*	ssp *cylindricus*	ssp *cylindricus*	*Ceratogymna*
Bycanistes	*subcylindricus*	–	–	*Ceratogymna*	*Ceratogymna*	–	–	*Ceratogymna*
Bycanistes	*brevis*	–	–	*Ceratogymna*	*Ceratogymna*	–	–	*Ceratogymna*
Buceros	*rhinoceros*	–	–	–	–	–	–	–
Buceros	*bicornis*	–	–	–	–	–	–	–
Buceros	*hydrocorax*	–	–	–	–	–	–	–
Rhinoplax	*vigil*	–	–	*Buceros*	*Buceros*	–	–	*Buceros*
Anorrhinus	*tickelli*	*Ptilolaemus*	*Ptilolaemus*	–	–	–	–	–
Anorrhinus	*austeni*[c]	ssp *tickelli*	ssp *tickelli*	ssp *tickelli*	–	–	ssp *tickelli*	–
Anorrhinus	*galeritus*	–	–	–	–	–	–	–
Anthracoceros	*marchei*	–	–	–	–	–	–	–
Anthracoceros	*albirostris*[d]	*'malabaricus'*	ssp *coronatus*	–	–	–	–	–
Anthracoceros	*coronatus*	–	–	–	–	–	–	–
Anthracoceros	*montani*	–	–	–	–	–	–	–
Anthracoceros	*malayanus*	–	–	–	–	–	–	–
Ocyceros	*griseus*	*Tockus*	*Tockus*	–	–	–	–	–
Ocyceros	*gingalensis*	ssp *griseus*	ssp *griseus*	–	–	–	–	–
Ocyceros	*birostris*	*Tockus*	*Tockus*	–	–	–	–	–
Aceros	*nipalensis*	–	–	–	–	–	–	–
Aceros	*waldeni*	ssp *leucocephalus*	ssp *leucocephalus*	–	–	–	–	–
Aceros	*leucocephalus*	–	*Rhyticeros*	–	–	–	–	–
Aceros	*corrugatus*	–	*Rhyticeros*	–	–	–	–	–
Aceros	*cassidix*	–	*Rhyticeros*	–	–	–	–	–
Rhyticeros	*plicatus*	*Aceros*	–	*Aceros*	*Aceros*	–	–	*Aceros*
Rhyticeros	*narcondami*	*Aceros*	–	*Aceros*	*Aceros*	–	–	*Aceros*
Rhyticeros	*undulatus*	*Aceros*	–	*Aceros*	*Aceros*	–	–	*Aceros*
Rhyticeros	*everetti*	*Aceros*	–	*Aceros*	*Aceros*	–	–	*Aceros*
Rhyticeros	*subruficollis*	ssp *plicatus*	*syn undulatus*	*Aceros*	*Aceros*	–	–	*Aceros*
Penelopides	*manillae*[e]	ssp *panini*	ssp *panini*	–	–	–	ssp *panini*	–
Penelopides	*mindorensis*[e]	ssp *panini*	ssp *panini*	–	–	–	ssp *panini*	–
Penelopides	*affinis*[e]	ssp *panini*	ssp *panini*	–	–	–	ssp *panini*	–
Penelopides	*samarensis*[e]	ssp *panini*	ssp *panini*	–	ssp *affinis*	ssp *affinis*	ssp *panini*	–
Penelopides	*exarhatus*	–	–	–	–	–	–	–
Penelopides	*panini*	–	–	–	–	–	–	–

Definitions: – indicates congruence with IOC World Bird List ver 2.11 (Gill and Donsker, 2012); ssp: treated as a subspecies of the named species (i.e. ssp *affinis* means subspecies of *affinis*); syn: synonym of the named species (i.e. syn *deckeni* means synonym of *deckeni*); NC: not considered.

[a] Sometimes treated as separate family, Bucorvidae.

[b] Recently described taxa (Tréca and Érard, 2000; Kemp and Delport, 2002).

[c] Split from *A. tickelli* (Kemp, 1995; Rasmussen and Anderton, 2005).

[d] Previously treated by Peters (1945) as *A. malabaricus* and *A. coronatus convexus* (see Frith and Frith, 1983).

[e] Split from *P. panini* (Kemp, 1995, 2001).

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only sequenced nuclear genes (β Fibrinogen, RAG-1, c-*myc*, PCBD1) for four species of hornbills: *Tockus erythrorhynchus*, *T. camurus*, *T. flavirostris* and *Bucorvus abyssinicus* (Ericson et al., 2006; Hackett et al., 2008). We also produced the first cyt *b* sequences for 23 species, including both African (e.g. *Tockus hemprichii*, *T. bradfieldi*, *Bycanistes fistulator*, *B. cylindricus*, *Ceratogymna elata*) and rare Asiatic taxa (e.g. *Penelopides mindorensis*, *Anthracoceros montani*, *Rhyticeros everetti*, *R. narcondami*). In all, 27 species were added to the previous mtDNA phylogeny because Viseshakul et al. (2011) did not include genes available on GenBank for an additional four species (*T. rufirostris*, *T. damarensis*, *T. monteiri*, and *Aceros waldeni*). Table B1 gives GenBank accession numbers for all sequences used in this study.

For outgroup samples, we included eight lineages varying from the closest relatives of hornbills to more distant orders. Inclusion of closely related outgroups is crucial for accurate phylogenetic reconstruction, while the inclusion of more distant relatives increases the accuracy of branch length calculations and dating of nodes. We do not apply these techniques here, but hope that this information will be useful for future studies (dates for priors can be supplied with nexus files on request). Six species were selected from related coraciiform families: *Phoeniculus purpureus* (Phoeniculidae), *Upupa epops* (Upupidae), *Coracias caudata*, *Eurystomus orientalis*, *E. glaucurus* (Coraciidae) and *Todiramphus sanctus* (Alcedinidae). For more distantly related taxa, we selected *Rallus longirostris* (Rallidae) and *Morphnus guianensis* (Accipitridae), because of the availability of AK1 intron 5 for both these lineages. Cyt *b* sequence data were downloaded from GenBank for all outgroup species, and we also sequenced AK1 intron 5 for *Phoeniculus purpureus* using methods described below. All reconstructions were rooted to outgroup taxa, but these are not shown in the trees.

2.2. Phylogenetic analysis and tree construction

Preliminary phylogenetic reconstruction revealed that sequences from conspecific samples had very high similarity (see Fig. S2). As we are primarily concerned with interspecific relationships, we therefore selected a single representative of each species to include in phylogenetic trees or analyses to reduce computation times and to simplify tree topology. In all cases, we included the longest sequence available to maximize information content. Sequences selected for phylogenetic analysis are highlighted in Table B1.

Focusing on this dataset, we compared one nuclear DNA sequence with one mtDNA sequence for 54 ingroup species with both mitochondrial and nuclear genes available. We used MEGA5 (Tamura et al., 2011) to estimate base composition, transition (Ts) and transversion (Tv) bias, and the substitution matrix (Table S2). We then used the partition homogeneity test (ILD statistic; Farris et al., 1994) as implemented in PAUP (version 4.0b10; Swofford, 2002) to compare phylogenetic signal and to test for incongruence between data partitions. This was run using Maximum Parsimony (MP) with the tree bisection/reconnection (TBR) branch-swapping algorithm. To visualize relative rates of evolution, and to assess potential saturation in our genetic markers, we conducted a pairwise comparison of nuclear and mtDNA divergence (*p*-distance).

We conducted separate phylogenetic analyses for the cyt *b* and AK1 intron 5 datasets using MP, ML and Bayesian inference (BI). In each case, selection of best-fit models was implemented in MEGA5 and MRMODELTEST v.2.3 (Nylander, 2004), using least scores of the Akaike Information Criterion (AIC) and ML values ($\ln L$) (see Table S3). Tree reconstruction with MP was conducted in PAUP with TBR branch swapping. The ML trees were reconstructed with PHYML v.3.0 (Guindon and Gascuel, 2003) using the approximate Likelihood-ratio test (aLRT; Anisimova and Gascuel, 2006) to calculate branch support. For both MP and ML searches, we also estimated robustness of clades using non-parametric bootstrapping with 1000 pseudoreplicates (Felsenstein, 1985). BI was implemented in MRBAYES v.3.1 (Ronquist and Huelsenbeck, 2003) using default parameters and priors for each dataset. Two independent Markov-chain Monte Carlo (MCMC) runs with four chains of 20 million generations were sampled every 500 increments.

We also reconstructed phylogenetic relationships based on a combined dataset of mtDNA and nuclear DNA sequences. We assumed that partitions were compatible when no significant incongruence was detected and when evolutionary models were similar. Following numerous studies, we ran model-based analyses (ML and BI) by fitting an evolutionary model to the combined dataset, and constructing trees using the methods outlined above.

In all methods, convergence of runs was verified using Tracer v.1.5 (Rambaut and Drummond, 2007). We assumed that replicate analyses converged when the average standard deviation of split frequencies (ASDSF) across independent runs was smaller than 0.1, and all parameters met benchmark effective sample size values (>200). Values of potential scale reduction factor (PSRF) for branch

Table 2
Estimated model parameters for relative substitution rates, nucleotide frequencies and overall mean pairwise genetic distances calculated using Maximum Likelihood (ML). Analyses were based on all 54 species for which both mtDNA and nuclear genes were sequenced.

	Nuclear intron locus (AK1 intron 5)	mtDNA coding gene (cyt *b*)	Concatenated loci (AK1 intron 5 + cyt *b*)
Relative substitution rates[a]			
Total positions (gaps eliminated)	608 (187)	1143 (852)	1751 (1039)
Overall mean distance (*d*) ± SE	0.13 ± 0.07	0.17 ± 0.01	0.16 ± 0.01
Nucleotide frequencies (ML)			
Adenine (A)	0.19	0.28	0.25
Cytosine (C)	0.31	0.36	0.34
Guanine (G)	0.31	0.14	0.19
Thymine (T)	0.19	0.23	0.22
Overall transition/transversion bias (ML)	0.93	2.45	2.12
Maximum log likelihood (ML)	−5171.67	−16471.47	−22086.37
Nucleotide substitution pattern (ML)			
A–C (Tv)	0.07	0.05	0.06
A–G (Tr)	0.16	0.12	0.15
A–T (Tv)	0.05	0.04	0.04
C–G (Tv)	0.07	0.02	0.03
C–T (Tv)	0.10	0.13	0.13
Γ statistic	−1.151	−3.899	−1.857
Treeness	0.422	0.281	0.339

[a] Relative substitution rates and statistics: Tr = transitional substitution rate; Tv = transversional substitution rate; Γ = Pybus–Harvey gamma statistic; SE = standard error.

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Table 3
Comparison of tree topologies with alternative phylogenetic hypotheses using Shimodaira–Hasegawa (SH) and Approximately Unbiased (AU) tests. Δ $-\ln L$: difference in tree likelihood compared to the 'best' tree. Significant statistical differences ($p < 0.05$) are highlighted in bold.

Tree topology[a]	PAUP			CONSEL		
	$-\ln L$[b]	Δ $-\ln L$[b]	SH-test p Value	$-\ln L$[b]	AU-test p Value	PP[c]
MP concatenated tree	31145.94	(best)	–	−11.8	0.77	1.00
ML concatenated tree	31164.89	18.95	0.73	18.9	0.32	0.00
BI concatenated tree	31165.62	19.68	0.71	19.7	0.33	0.00
Kemp (1995) cladogram	31775.06	629.11	**<0.001**	629.1	**<0.001**	0.00
Viseshakul et al. (2011)-BI tree	31289.64	143.70	**0.01**	143.7	**<0.001**	0.00
Viseshakul et al. (2011)-ME tree	31344.37	198.42	**<0.001**	198.4	**<0.001**	0.00
Gill and Donsker (2012) topology	31401.47	255.52	**<0.001**	255.5	**<0.001**	0.00

[a] Tree topologies: MP = Maximum Parsimony; ML = Maximum Likelihood; BI = Bayesian Inference; ME = Minimum Evolution;
[b] $\ln L$: Log likelihood.
[c] PP: posterior probability calculated by Bayesian Information Criterion approximation.

lengths ranged from 1.00 to 1.072 across all datasets, with values close to 1 indicating convergence. After runs had reached stationary distribution, as evaluated by the stability of log-likelihood plots, the first 25% was discarded as burn-in. We then visualized the 50% majority rule consensus tree for each dataset in FIGTREE v.1.3.1. Following previous studies (e.g. Muellner et al., 2008), we treated 0.90–0.98 PP and 70–89% bootstrap values (BS) as moderate support, and >0.98 PP and 90–100% BS as strong support.

2.3. Tree topology

To assess congruence between our molecular datasets (nuclear DNA, mtDNA and concatenated) we used PAUP to implement one-tailed Shimodaira–Hasegawa (SH) tests (Shimodaira and Hasegawa, 1999), with likelihood scores computed using bootstrapping and full optimization in 1000 replicates. We also used CONSEL v.0.1i (Shimodaira and Hasegawa, 2001) to conduct Approximately Unbiased (AU) tests based on multi-scale bootstrap resampling (Shimodaira, 2002). These approaches determine whether each tree is supported significantly less by the data than alternative phylogenetic hypotheses, which are specified *a priori* (see Goldman et al., 2000). Although both SH and AU are likelihood-based methods, they are routinely used to compare amongst phylogenies generated by parsimony, Bayesian approaches or morphology (e.g. Leaché and Reeder, 2002; Grau et al., 2005; Marks et al., 2007; Pereira and Wajntal, 2008).

We used the same SH and AU tests to compare our results against four alternative phylogenetic hypotheses (Table 3): a cladogram taken from Kemp (1995), two mtDNA gene trees (BI and Minimum Evolution) constructed by Viseshakul et al. (2011), and a tree topology adapted from the most recent taxonomic list in Gill and Donsker (2012). Alternative tree topologies were developed using MacClade v.4.08a (Maddison and Maddison, 2005) and tested against the MP consensus tree (Fig. 3). These comparisons were straightforward when alternative trees had the same sample size (e.g. Gill and Donsker, 2012), or differed only in minor splits (e.g. Kemp, 1995). To compare against the smaller trees published by Viseshakul et al. (2011), we made the minimum number of node changes required to match the previous topology, retaining the full sample of 61 species. This is a highly conservative approach as it assumes that all nodes unsampled in the earlier tree were identical to our consensus tree.

2.4. Phylogenetic signal of vocalizations

We compiled a descriptive dataset of hornbill vocalizations from primary literature and online sound archives (see Table A1 for descriptions and sources). We then used phylogenetic independent contrasts (PIC) to estimate the fit of vocal trait data to a Brownian motion model of trait evolution based on the MP phylogeny of our concatenated dataset. We assigned vocal traits to categories (1–10), based on terms used by Kemp (2001): booming, whistling, clucking, hooting, nasal wail, resonant honk, shrill cackle, raucous cackle, harsh bark and staccato bark. Outgroups were arbitrarily assigned to a separate category. The phylogenetic signal of traits was assessed by comparing the observed (actual) PIC variance of the trait with a null distribution from randomly simulated data. If the observed value is less than 95% of values in the null distribution, then trait evolution can be assumed to be a good fit to the tree topology (Winger et al., 2011). We also calcu-

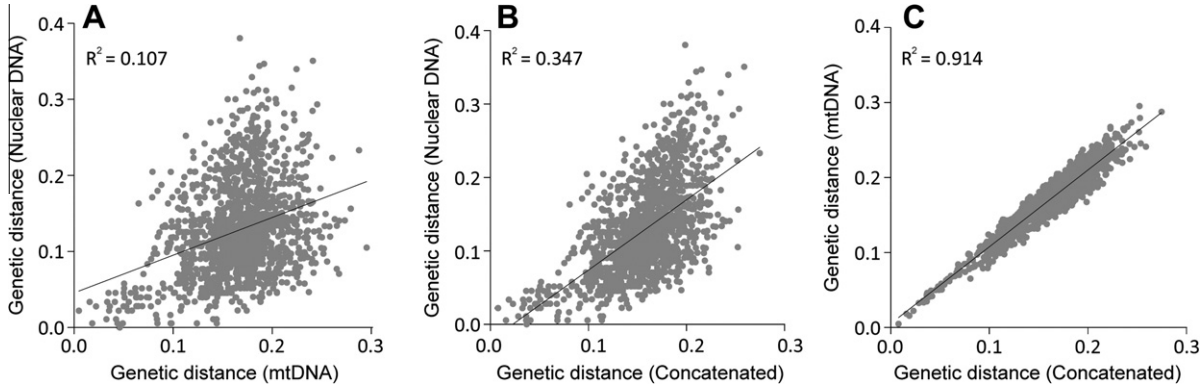

Fig. 1. Relative divergence compared between different loci based on pairwise distances among 54 hornbill species. Comparisons were made between (A) divergence in the nuclear locus AK1 intron 5 and the protein-coding mtDNA cyt *b* gene, and then between (B) AK1 intron 5 and (C) cyt *b* against the concatenated nuclear-mtDNA dataset. All comparisons were based on maximum likelihood distances calculated using locus-specific substitution models.

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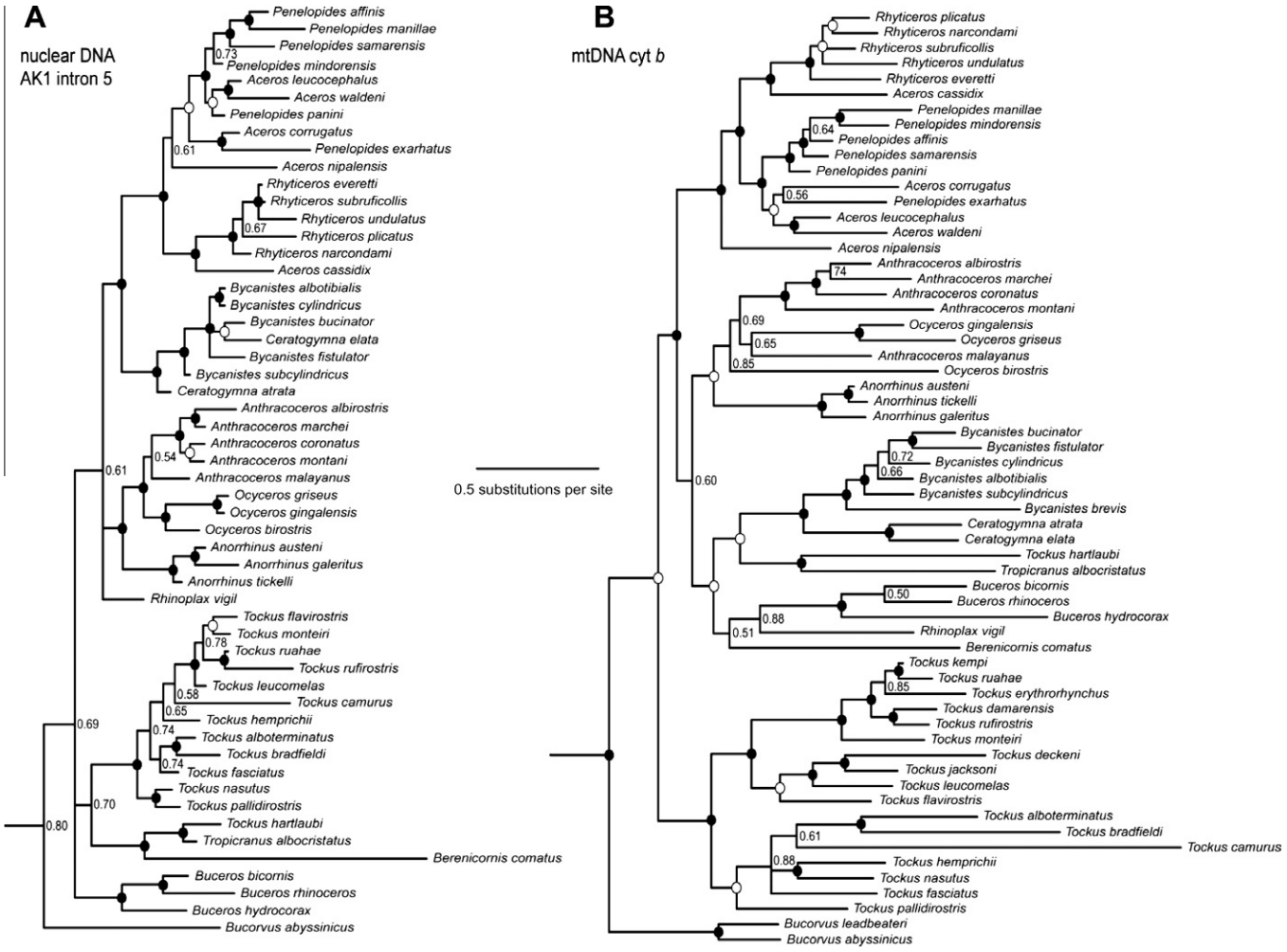

Fig. 2. Bayesian consensus trees of hornbills (90–100% species coverage) derived from aligned sequences of (A) nuclear loci AK1 intron 5 and (B) mtDNA cyt *b* (C). Numbers and circles on nodes indicate posterior probabilities (PP), with black circles indicating strong support at ⩾0.98 PP, and open circles indicating moderate support at ⩾0.90–0.97 PP. Support values <0.90 PP are labeled on nodes. Shifting the threshold to <0.95 PP only downgrades two nodes in the AK1 intron 5 tree (*P. affinis–P. manillae*; *B. comatus–T. hartlaubi/T. albocristatus*); and two nodes in the cyt *b* tree (*A.corrugatus/P.exarhatus–A. leucocephalus/A.waldeni*; *A. nipalensis*).

lated the *K* statistic (Blomberg et al., 2003) using the 'picante' package (Kembel et al., 2010) implemented in R (R Development Core Team, 2012) to assess the phylogenetic signal of vocal traits across the same concatenated dataset. The *K* statistic compares the observed signal in a trait to the Brownian model of trait evolution with the phylogeny using ML estimation. If $K > 1$, then traits are regarded as conserved, whereas $K < 1$ indicates that traits are labile.

3. Results

3.1. Sequence attributes and comparison of genes

The proportion of potentially informative nucleotide sites differed between nuclear loci AK1 intron 5 and the mtDNA cyt *b* gene (Table S3, see Supplementary material). AK1 intron 5 sequences exhibited 319 (53%) variable sites, with 188 (31%) being parsimony-informative; cyt *b* sequences exhibited 748 (65%) variable sites, with 551 (48%) being parsimony-informative. In the concatenated dataset, 1087 (62%) of sites were variable, of which 745 (43%) were informative. Base composition was biased to adenine (A) and cytosine (C) in the cyt *b* dataset, but biased to guanine (G) and cytosine in the nuclear dataset (Table 2). Composition was more A–C rich in the concatenated dataset, consistent with patterns found in other birds (e.g. Moyle and Marks, 2006; Marks et al., 2007). Relative substitution rates, empirical base frequencies and nucleotide composition bias varied little between the three datasets. Patterns of transitions (Ts) and transversions (Tv) were relatively similar in nuclear and mtDNA, although overall Ts/Tv bias was higher in cyt *b* (Table 2).

The relationship between nuclear and mtDNA divergence (*p*-distance) was weak (Fig. 1A), indicating heterogeneity in rates of molecular evolution between AK1 intron 5 and cyt *b*, in line with previous studies (e.g. Shapiro and Dumbacher, 2001; Allen and Omland, 2003). When we compared divergence in the concatenated dataset with divergence at nuclear (Fig. 1B) and mitochondrial (Fig. 1C) loci, we found much stronger congruence with mtDNA divergence, representing 62% (1143 of 1846 bp) of the combined sequence. Accordingly, the topology of the phylogenetic tree based on the concatenated dataset was less congruent with that based on nuclear DNA (Fig. 2A) than mtDNA (Fig. 2B), indicating that the final topology is primarily driven by the signal in the mitochondrial data partition.

Several parsimony-informative indels (insertions/deletions) were recovered in AK1 intron 5, with a total of 13 insertions and 5 deletions across the different clades (Fig. 3; Table S4, see Supplementary material). Five independent insertions differentiate *Bucorvus* from the rest of the hornbills (Bucerotinae), and the Bucerotinae were defined by two further independent insertions. Long-tailed forest hornbills (*Berenicornis*, *Tropicranus*) were united by a 2 bp insertion and 1 bp deletion, and the large Afrotropical

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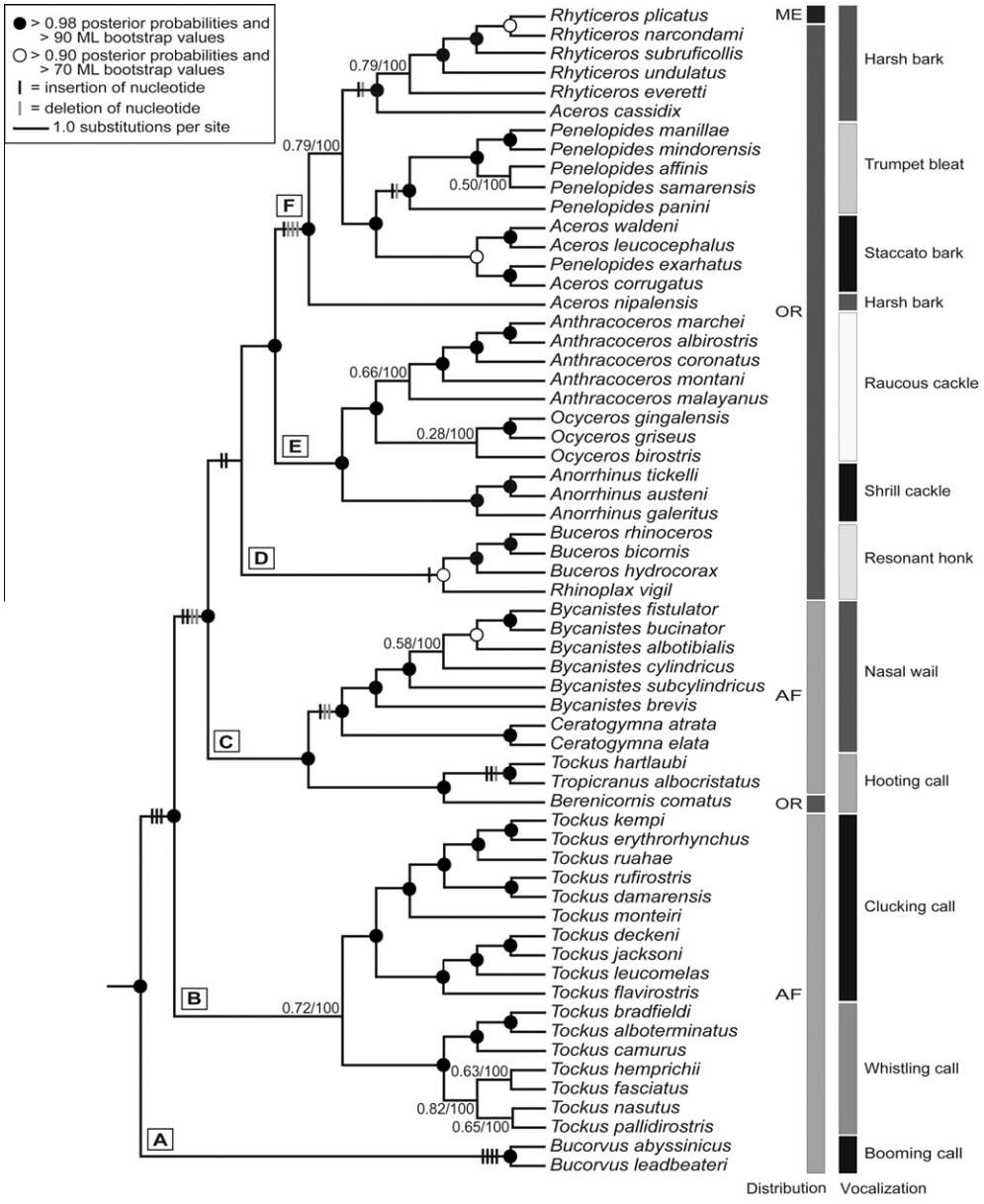

Fig. 3. Maximum Parsimony 50% majority rule bootstrap consensus of hornbills (100% species coverage) from the combined analysis of mtDNA (cyt *b*) and nuclear DNA (AK1 intron 5). Squares indicate major clades: A (*Bucorvus* clade); B (*Tockus* clade); C (*Berenicornis* clade); D (*Rhinoplax* clade); E (*Anorrhinus* clade); F (*Aceros* clade). Vertical slash indicates insertions and deletions for the nuclear locus. Circles and numerical values at nodes correspond to support values. Shaded bars on right refer to distribution in biogeographical regions (ME: Melanesian; OR: Oriental; AF: Afrotropical) and vocalizations (see Table A1 for source of vocal data).

hornbills (*Ceratogymna–Bycanistes*) were united by a single insertion. Notably, all the Philippine *Penelopides* were unified with 1 bp insertion, as was the *Rhinoplax* clade. All cyt *b* sequences for the 61 hornbill species had the same start codon (ATG), but varied in their terminal codons (TAA/TAG). The initiation codon for AK1 intron 5 (GTG/GCA) was similar to *Gallus gallus* (511 bp), but differed in the termination codon, which was CTG/CTC rather than AAG (Suminami et al., 1988).

3.2. Inconsistency between gene partitions

There were minor inconsistencies between clade-level topologies in the nuclear DNA tree (Fig. 2A) and the mtDNA tree (Fig. 2B). Specifically, Bucerotinae was subdivided into 6–7 clades in the nuclear tree (Fig. 2A) and slightly simplified to 5 prominent clades in the mtDNA and concatenated trees (Fig. 2B, Fig. 3). We refer to these 5 clades henceforth by their ancestral lineage: B = *Tockus* clade, C = *Berenicornis* clade, D = *Rhinoplax* clade, E = *Anorrhinus* clade, F = *Aceros* clade (see Fig. 3 and Table A1 for constituent species).

Conflict between partitions was restricted to 18 mismatched nodes, resulting in the inconsistent placement of taxa such as *Rhyticeros everetti*, *Penelopides panini*, *Tockus flavirostris*, *Ceratogymna elata*, *Berenicornis comatus* and *Rhinoplax vigil*. These inconsistencies resulted in only minor topological changes and were poorly supported, with one receiving strong support (*T. nasutus–T. pallidirostris*). Similar minor disparities between nuclear and mitochondrial gene partitions are frequently recovered in multilocus phylogenies, and can reflect a number of different factors (see Section 4). Most nodes were consistent across gene trees, and an ILD test revealed no significant conflict between data partitions ($p = 0.65$). In addition, we identified GTR+Γ+I as the best substitution model for both gene partitions (Fig. S3). Therefore, on the grounds of congruence in topology and evolutionary mode, we

combined cyt *b* and AK1 intron 5 data sets for phylogenetic analyses.

3.3. Tree topology

Using the concatenated dataset, we generated an MP consensus tree (Fig. 3), an ML majority-rule consensus tree (Fig. S1B) and a BI maximum clade credibility tree (Fig. S1C). These reconstructions produced congruent tree topologies, with consistent composition of major clades and placement of key taxa. All trees consistently placed the genus *Bucorvus* as sister to the rest of the hornbills (Bucerotinae), recovered monophyly of *Tockus*, *Anorrhinus*, *Rhinoplax* and *Aceros* clades, and agreed on topologies for clucking *Tockus*, *Ceratogymna–Bycanistes*, *Rhyticeros*, and Philippine *Penelopides*.

The results of SH and AU topology tests indicated that there were no significant differences between trees (Table 3). Although all the trees are equally valid, the top-ranked tree according to the site-likelihoods calculated by these tests is the MP consensus tree. This tree was strongly supported at most nodes, with only 20% of nodes having weaker support (<70% BS and <90% PP). It closely matches the topology of an alternative MP tree that we generated using MEGA5, following the closest-neighbor interchange option (Fig. S2). It is also highly congruent with our expanded BI tree (Fig. S2) constructed from a concatenated nuclear-mtDNA dataset of all 162 hornbill sequences (Table B1). The results of further SH and AU tests (Table 3) revealed that the MP consensus tree was significantly different from all published tree topologies for the hornbills (Kemp, 1995; Viseshakul et al., 2011; Gill and Donsker, 2012).

3.4. Phylogenetic signal of vocalizations

Vocal traits of hornbills have high phylogenetic signal according to two analytical approaches using the MP consensus tree. First, observed PIC variance was significantly lower than that extracted from a null model for all vocal traits and for individual vocalizations (Table S5, see Supplementary material). Second, the calculated *K* statistic for all vocal traits was extremely high at 9.73, and with *K* values for individual vocalizations ranging from 1.51 to 6.63 (Table S5). The high value of *K* indicates that vocal traits exhibit a very strong phylogenetic signal in our dataset (Fig. 3, Table A1).

4. Discussion

4.1. A phylogenetic framework for the Bucerotidae

We have presented the first phylogenetic analysis for all hornbill species, producing trees with high topological support for most nodes. The maximum parsimony reconstruction of combined nuclear and mitochondrial datasets (Fig. 3) represents our best hypothesis of evolutionary relationships in hornbills. We recommend the use of this topology as the most complete framework for future studies of this Palaeotropical radiation, including phylogenetic comparative analyses, tests of biogeographic hypotheses and models of trait evolution.

The topology of our proposed tree differs significantly from all previous phylogenies, and provides new insights into the historical patterns of diversification in hornbills. One example is *Berenicornis comatus*, which Viseshakul et al. (2011) left as enigmatic because different analyses disagreed whether it was sister to a clade containing Asiatic and Afrotropical genera (*Ocyceros*, *Tropicranus*, *Ceratogymna*, *Bycanistes*) or to the Asiatic *Rhinoplax–Buceros* lineage. Our sequencing of nuclear genes strengthens support for the placement of *Berenicornis*—an Asiatic omnivorous species—as sister to a clade of Afrotropical insectivores (*Tropicranus*) and frugivores (*Ceratogymna*, *Bycanistes*). Moreover, where Viseshakul et al. (2011) tentatively grouped Asiatic *Ocyceros* with African *Tropicranus*, *Ceratogymna* and *Bycanistes*, our analyses revealed this genus to be allied to *Anthracoceros* in an exclusively Asiatic clade. Our results also help to resolve the previously uncertain placement of *Tockus hartlaubi*, *T. camurus*, and several other species absent from previous analyses. These findings are summarized and placed in context in the following sections, which focus on each of the five major clades of the Bucerotinae identified by our analyses.

4.2. Phylogenetic relationships within major clades

4.2.1. Tockus clade

Tockus is currently the largest genus in the family Bucerotidae with 18 species, several of which were previously treated as *T. erythrorhynchus* until being proposed as species by Kemp and Delport (2002). Our nuclear and mtDNA trees (Fig. 2) provide some support for these taxonomic changes by confirming substantial genetic divergence among lineages in this complex. Similar levels of divergence are also consistent with previous taxonomic proposals in yellow-billed hornbill (split into *T. leucomelas* and *T. flavirostris*; Kemp and Crowe, 1985) and Von der Decken's hornbill (proposed split into *T. deckeni* and *T. jacksoni*; see Kemp, 2001).

Both nuclear and mtDNA sequences indicate that the genus *Tockus* as currently defined is subdivided by a deep phylogenetic split into two major groups, each representing different vocal types ('whistlers' and 'cluckers'). These findings support the splitting of *Tockus* into two genera, as first suggested by Hübner et al. (2003), with *Rhynchaceros* being revived for the 'whistlers'. This arrangement is also consistent with the evidence of DNA–DNA hybridization, morphology, and behavior (e.g., nest-lining, hop/walk locomotion, etc.) (Kemp, 1995).

Our results also help to clarify the position of *Tockus camurus*, a contentious species previously placed in a subclade separate from the 'whistlers' and 'cluckers' (Kemp, 1995). In the AK1 intron 5 tree (Fig. 2A), *T. camurus* is sister to all whistling *Tockus*, supporting the suggestion of Kemp (1979) that they are derived from a smaller-bodied, finer-billed, *Phoeniculus*-like ancestor. However, a slightly different topology was recovered in our combined tree, with *T. camurus* as sister to *T. alboterminatus* and *T. bradfieldi*. This is also intuitive based on phenotype, as an examination of *T. camurus* suggests it to be a dwarf relative of *T. alboterminatus* (Elliot, 1882; Kemp, 1976).

More unexpectedly, our analyses reveal that *Tockus hartlaubi* only superficially resembles *Tockus*, and instead is sister to *Tropicranus albocristatus*, in the *Berenicornis* clade. This placement makes sense on the basis of phenotype, as examination of museum specimens indicates that *T. hartlaubi* and *Tropicranus albocristatus* share several diagnostic characters (e.g., crest structure, graduated tail, etc.). Kemp (1995) noted that *T. hartlaubi* had uncertain affinities, but he still placed the taxon in a subclade of *Tockus*. Thus, our findings indicate that *Tockus* is polyphyletic, although no previous study has explicitly questioned the monophyly of the genus.

4.2.2. Berenicornis clade

This clade contains three subclades with a heterogeneous mix of taxa, including Asiatic *Berenicornis* and Afrotropical *Tropicranus* (both primarily faunivorous) and Afrotropical *Ceratogymna* and *Bycanistes* (frugivorous). *Berenicornis* is a problematic lineage previously subsumed within *Aceros* on the basis of morphological features (Kemp, 1995) and genetic data (Hübner et al., 2003). However, our findings support the tentative suggestion of Viseshakul et al. (2011) that it should be reunited in a clade with Afrotropical *Tropicranus*, as first proposed by Peters (1945) on the basis of their shared crests and long, graduated tails. We also show that *Tockus hartlaubi* is sister to *Tropicranus* in all analyses.

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These largely faunivorous lineages (*Berenicornis*, *Tropicranus* and *Tockus hartlaubi*) gave rise to two genera of Afrotropical frugivores (*Ceratogymna* and *Bycanistes*). The consensus tree provides strong support for a pair of sibling species (*Ceratogymna elata* and *C. atrata*) being sister to all *Bycanistes*, as found by Viseshakul et al. (2011). However, our evidence supports a revised topology for *Bycanistes*, with *B. fistulator* and *B. bucinator* representing the most recent split. The remaining taxa (*brevis*, *subcylindricus*, *cylindricus*, *albotibialis*) form a clade, and equate to the group previously proposed as the subgenus *Baryrhynchodes* (Sanft, 1960; Kemp, 1995).

4.2.3. Rhinoplax clade

The *Rhinoplax* clade represents an early branch of the Asiatic lineage that arose from African hornbills (Kemp, 1995; Viseshakul et al., 2011). It contains four large forest frugivores in the genera *Rhinoplax* and *Buceros*, and is sister to the large Asiatic radiation comprised of *Anorrhinus* and *Aceros* clades. *Rhinoplax* is sister to *Buceros* in nearly all topologies, and our analyses provide novel evidence that *Buceros hydrocorax* is sister to a clade including both *B. bicornis* and *B. rhinoceros*. These four species collectively exhibit the distinctive strategy of cosmetic coloration using uropygial gland secretions (Delhey et al., 2007), a feature shared with wrinkled hornbills (*Aceros*).

4.2.4. Anorrhinus clade

Our results confirm a close association between *Anorrhinus* and *Anthracoceros*, in contrast with the early phylogeny based on DNA–DNA hybridization (Sibley and Ahlquist, 1990), but in agreement with previous molecular phylogenies (Srikwan and Woodruff, 1998; Viseshakul et al., 2011). Unlike previous studies, however, we show that the *Anorrhinus* clade is sister to a combined *Ocyceros–Anthracoceros* clade. Our phylogram topologies (Fig. 2) reveal that there is only minor genetic divergence between *Anorrhinus galeritus* and *A. austeni*/*tickelli* (previously treated as *Ptilolaemus*), thus supporting the merger of *Ptilolaemus* into *Anorrhinus* (Kemp, 1995, 2001). We note that, as *Ptilolaemus* is distinctive in a number of features, including bill color, casque shape, and plumage, it may warrant treatment as a subgenus.

The recent mtDNA tree of Viseshakul et al. (2011) suggested that Asiatic gray (*Ocyceros*) and Asiatic pied (*Anthracoceros*) hornbills were distantly related, but their only *Ocyceros* sequence (*O. gingalensis*) did not align well with any of our eight *Ocyceros* sequences (from three species), and we consider it likely to be erroneous. All our analyses identify a clade formed by *Ocyceros* and *Anthracoceros*, with strong support for the ancestral node. This is the first molecular evidence for a close affinity between *Ocyceros* and *Anthracoceros*, although a similar arrangement had previously been suspected on the basis of plumage details (Kemp, 1979, 1988). We note that the boundaries of these genera remain uncertain. Our nuclear (Fig. 2A) tree suggests that *Ocyceros* and *Anthracoceros* are reciprocally monophyletic, whereas our mtDNA tree (Fig. 2B) recovered polyphyly of both genera. Concatenated trees were similarly inconsistent, with the 'best' tree (Fig. 3) recovering monophyly, while all other analyses of the combined dataset (Fig. S1) suggested polyphyly. Further sampling of loci is needed to resolve phylogenetic relationships between *Ocyceros* and *Anthracoceros*.

We maintain *Anthracoceros malayanus* within *Anthracoceros*, as sister to the other members. These other 'pied hornbills' are monophyletic, with the earliest split being between *A. montani* and other members of the genus (*A. coronatus*, *A. albirostris*, *A. marchei*). This finding resolves the uncertainty surrounding the placement of this critically endangered hornbill (Kemp, 2001; Kinnaird and O'Brien, 2007): *A. montani* is a black-billed 'pied hornbill' (i.e. closely allied to *A. marchei*, *A. albirostris*, and *A. coronatus*) rather than a white-tailed 'black hornbill' (i.e. not related to *A. malayanus*).

4.2.5. Aceros clade

Aceros was once considered to be a diverse genus containing at least 10 species (Table 1), yet our results reveal the complex evolutionary history, and threefold polyphyly, of this earlier grouping. In effect, the name *Aceros* is only valid for the type species, *Aceros nipalensis*, which is sister to the rest of the *Aceros* clade. The 'wreath-billed' hornbills separate into the genus *Rhyticeros*, as proposed by Viseshakul et al. (2011). Although the structure of this genus differs between different gene partitions, the final concatenated tree suggests that *R. everetti* is sister to a quartet of species (*undulatus*, *subruficollis*, *plicatus*, and *narcondami*). Our data also shed light on the uncertain evolutionary relationships of *R. subruficollis* (Rasmussen, 2000), a taxon once thought to be the juvenile of *R. undulatus* (Sanft, 1960), and often considered a subspecies of *R. plicatus* (e.g. Deignan, 1963; Elbel, 1969). Our analyses place *R. subruficollis* as a divergent lineage somewhat intermediate between *R. plicatus* and *R. undulatus*, but closer to *R. plicatus* in the concatenated tree. Meanwhile, *R. plicatus* and *R. narcondami* were consistently recovered as a sister pair, confirming the close affinities suggested by earlier treatments (Kemp, 2001; Dickinson, 2003).

Aceros corrugatus forms a separate lineage from *A. nipalensis*, and distinct from the *Rhyticeros* and *Penelopides* clades, thus supporting the preliminary results of Viseshakul et al. (2011). We add to previous results by confirming that the other 'wrinkled' hornbills—*A. waldeni* and *A. leucocephalus*—also belong in this subclade. More unexpectedly, we found that *Penelopides exarhatus* is a fourth member of the lineage, providing the first evidence that *Penelopides* is polyphyletic. Unlike the other three 'wrinkled' hornbills, *P. exarhatus* lacks a knob-like casque and is a cooperative breeder (Kemp, 1995, 2001), producing a superficial similarity to *Penelopides*. With the repositioning of *P. exarhatus* in the 'wrinkled' *Aceros*, all Philippine *Penelopides* form a recent monophyletic offshoot of the *Aceros* clade. This separation of the 'wrinkled' *Aceros* subclade supports placement in a distinct genus, and thus the resurrection of *Cranobrontes* (Riley, 1921).

4.3. Disparity between nuclear and mtDNA

Given the observed conflict in topology between our nuclear (Fig. 2A) and mtDNA gene trees (Fig. 2B), it is important to consider the factors underlying these differences and whether they may bias the findings described above. One possibility is that our data are affected by contamination or amplification errors. This is highly unlikely in our mtDNA data, as mitochondrial genes are relatively easy to sequence from toe-pads and in most cases we generated multiple sequences per species for cross-checking (Table B1). We also made every effort to minimize common problems with nuclear DNA, including designing effective primers, meticulously checking contigs, and repeating the extraction of uncertain sequences. Thus, while we cannot rule out the possibility of laboratory errors, we consider them unlikely to explain deviations between our nuclear and mtDNA trees.

Nuclear and mtDNA have different evolutionary origins and modes of inheritance, and thus mismatches in topology are common for a number of 'natural' reasons. In contrast to nuclear genes, mitochondrial genes have (1) smaller effective population size, (2) faster evolution, and (3) an absence of recombination (Edwards and Beerli, 2000). Such factors can promote heterogeneity in rates of evolution across lineages when comparing between nuclear and mtDNA. In addition, hybridization can cause partial introgression in the mitochondrial genomes of some species, leading to disparity in gene trees (Irwin et al., 2009; Hailer et al., 2012). These sources of incongruence may explain some or all of the mismatched nodes in our gene partitions.

Differences in topology raise the question of which dataset is 'correct'. It is often argued that mtDNA provides a more accurate

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Table A1
Taxonomic recommendations, casque design, vocal type, and conservation status of hornbills.

Clade	Taxonomic recommendation[a]	Casque design[b]	Vocalizations	Status[c]	Sources[d]
A – *Bucorvus*	*Bucorvus abyssinicus*	High cowl-like curve	Booming call	LC	1, 2, 3
	Bucorvus leadbeateri	Low ridge at base	Booming call	VU	1, 2, 3
B – *Tockus*	*Tockus ruahae*	Slight ridge	Clucking call	LC	1, 2, 3
	Tockus kempi	Slight ridge	Clucking call	LC	1, 2, 3
	Tockus damarensis	Slight ridge	Clucking call	LC	1, 2, 3
	Tockus rufirostris	Slight ridge	Clucking call	LC	1, 2, 3
	Tockus erythrorhynchus	Slight ridge	Clucking call	LC	1, 2, 3
	Tockus monteiri	Low grooved ridge	Clucking call	LC	1, 2, 3
	Tockus deckeni	Low ridge (complete)	Clucking call	LC	1, 2, 3
	Tockus jacksoni	Low ridge (complete)	Clucking call	LC	1, 2, 3
	Tockus leucomelas	Low ridge (complete)	Clucking call	LC	1, 2, 3
	Tockus flavirostris	Low ridge (complete)	Clucking call	LC	1, 2, 3
	Rhynchaceros bradfieldi	Low ridge (attenuated)	Whistling call	LC	1, 2, 3
	Rhynchaceros alboterminatus	High ridge (attenuated)	Whistling call	LC	1, 2, 3
	Rhynchaceros fasciatus	High ridge (attenuated)	Whistling call	LC	1, 2, 3
	Rhynchaceros hemprichii	Low ridge (attenuated)	Whistling call	LC	1, 2, 3
	Rhynchaceros nasutus	Low ridge (attenuated)	Whistling call	LC	1, 2, 3
	Rhynchaceros camurus	Low ridge (attenuated)	Whistling call	LC	1, 2, 3
	Rhynchaceros pallidirostris	Low ridge (attenuated)	Whistling call	LC	1, 2, 3
C – *Berenicornis*	*Bycanistes fistulator*	Projecting low cylinder	Nasal wail	LC	1, 2, 3
	Bycanistes bucinator	Projecting low cylinder	Nasal wail	LC	1, 2, 3
	Bycanistes cylindricus	Projecting high cylinder	Nasal wail	NT	1, 2, 3
	Bycanistes albotibialis	Projecting high cylinder	Nasal wail	LC	1, 2, 3
	Bycanistes subcylindricus	Projecting high cylinder	Nasal wail	LC	1, 2, 3
	Bycanistes brevis	Projecting high cylinder	Nasal wail	LC	1, 2, 3
	Ceratogymna atrata	Projecting high cylinder	Nasal wail	LC	1, 2, 3
	Ceratogymna elata	Curved high cylinder	Nasal wail	NT	1, 2, 3
	Tropicranus hartlaubi	Low ridge (attenuated)	Hooting call	LC	1, 2, 3
	Tropicranus albocristatus	High ridge (attenuated)	Hooting call	LC	1, 2, 3
	Berenicornis comatus	High ridge (attenuated)	Hooting call	NT	2, 3, 6
D – *Buceros*	*Buceros rhinoceros*	Cylindrical block	Resonant honk	NT	2, 3, 6
	Buceros bicornis	Bifurcated block	Resonant honk	NT	2, 3, 5, 6
	Buceros hydrocorax	Pointed block	Resonant honk	NT	2, 3, 4
	Rhinoplax vigil	Broad ridged block	Resonant honk	NT	2, 3, 6
E – *Anorrhinus*	*Anthracoceros marchei*	Cylinder with blade	Raucous cackle	VU	2, 3, 4
	Anthracoceros albirostris	Cylinder with blade	Raucous cackle	LC	2, 3, 5, 6
	Anthracoceros coronatus	Cylinder with blade	Raucous cackle	NT	2, 3, 5
	Anthracoceros montani	Cylinder with blade	Raucous cackle	CR	2, 3, 4
	Anthracoceros malayanus	Cylinder with blade	Raucous cackle	NT	2, 3, 6
	Ocyceros griseus	Low ridge	Raucous cackle	LC	2, 3, 5
	Ocyceros gingalensis	Low ridge	Raucous cackle	LC	2, 3, 5
	Ocyceros birostris	Pointed blade	Raucous cackle	LC	2, 3, 5
	Anorrhinus tickelli	Low ridge	Shrill cackle	NT	2, 3, 6
	Anorrhinus austeni	Low ridge	Shrill cackle	NT	2, 3, 5, 6
	Anorrhinus galeritus	Low ridge	Shrill cackle	LC	2, 3, 6
F – *Aceros*	*Aceros nipalensis*	Low ridge	Harsh bark	VU	2, 3, 5, 6
	Rhyticeros plicatus	Low wreath	Harsh bark	LC	2, 3,6
	Rhyticeros narcondami	Low wreath	Harsh bark	EN	2, 3, 5
	Rhyticeros undulatus	Low wreath	Harsh bark	LC	2, 3, 5, 6
	Rhyticeros everetti	Low wreath	Harsh bark	VU	2, 3, 6
	Rhyticeros subruficollis	Low wreath	Harsh bark	VU	2, 3, 6
	Rhyticeros cassidix	High wrinkled knob	Harsh bark	LC	2, 3, 6
	Cranobrontes waldeni	High wrinkled ridge	Staccato bark	CR	2, 3, 4
	Cranobrontes leucocephalus	High wrinkled ridge	Staccato bark	NT	2, 3, 4
	Cranobrontes exarhatus	Low grooved ridge	Staccato bark	LC	2, 3, 6
	Cranobrontes corrugatus	High wrinkled ridge	Staccato bark	NT	2, 3, 6
	Penelopides manillae	Low half ridge	Trumpet bleat	LC	2, 3, 4
	Penelopides mindorensis	Low half ridge	Trumpet bleat	EN	2, 3, 4
	Penelopides affinis	Low half ridge	Trumpet bleat	LC	2, 3, 4
	Penelopides samarensis	Low half ridge	Trumpet bleat	LC	2, 3, 4
	Penelopides panini	Low half ridge	Trumpet bleat	EN	2, 3, 4

[a] Taxonomic treatment recommended on the basis of new data present in this paper, to compare with traditional hypotheses (Table 1).
[b] Descriptions of the distinctive casque situated on top of hornbill beaks described on basis of key literature.
[c] Conservation status of hornbills according to the IUCN Red List assessments: LC, Least Concern; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; assignment to categories follows the IUCN Red List (data accessed from www.iucnredlist.org on 26 February 2012).
[d] Sources: (1) Fry et al., 1998; (2) Kemp, 1995; (3) Kemp, 2001; (4) Kennedy et al., 2000; (5) Rasmussen and Anderton, 2005; (6) Robson, 2009.

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Table B1
List of 171 samples used in this study, representing 61 hornbill (ingroup) species and eight outgroup species, with details of museum registry or source material, geographic origin, gene regions, and GenBank accession numbers.

Taxon	Institutional source[a]	Type[B]	Locality	cyt *b*	AK1 intron 5
In-group					
Aceros cassidix	BMNH 1969.32.18	S	Indonesia, Sulawesi	KC754753[d]	KC754899[d]
	BMNH 88.10.30.140	S	Indonesia, Sulawesi	KC754754	-
	UMZC 25/Buc/1/a/1	S	Indonesia, Sulawesi	KC754755	-
Aceros corrugatus	OUMNH B05362	S	Malaysia, Borneo, Sarawak	KC754758[d]	KC754900[d]
	NUS 3.11111	S	Indonesia, Sumatra	KC754757	-
	NEZS ACA1	F	United Kingdom, Captive bird	KC754756	-
Aceros leucocephalus	LWPRC P45	F	Philippines, Captive bird	KC754759[d]	-
	OPAV P08	F	Philippines, Captive bird	-	KC754901
	MSUIL 56487	S	Philippines, Dinagat	KC754760	-
	NFEFI P05	F	Philippines, Captive bird	KC754761	-
Aceros nipalensis	BMNH 1941.12.1.827	S	Myanmar, Hmu-Chanka	KC754762[d]	KC754902[d]
	UMZC 25/Buc/1/f/1	S	India, Darjeeling	KC754764	-
	BMNH 87.9.1.202	S	India, Sikkim	KC754763	-
Aceros waldeni	UPLB 2103	S	Philippines, Negros	KC754767[d]	-
	WVSU P19	F	Philippines, Panay	KC754765	KC754903[d]
	BMNH 96.4.15.98	S	Philippines, Guimaras	KC754766	-
Anorrhinus austeni	BMNH 1904.7.24.1	S	India, Assam	KC754769[d]	-
	BMNH 1932.5.14.31	S	Laos, Phou-Kong-Ntoul	KC754768	KC754904[d]
Anorrhinus galeritus	OUMNH B05356	S	Malaysia, Borneo, Sabah	KC754771[d]	-
	UMZC 25/Buc/2/a/6	S	Malaysia, Borneo, Sarawak	KC754772	KC754905[d]
	OUMNH B05359	S	Malaysia, Borneo, Sarawak	KC754770	-
Anorrhinus tickelli	BMNH 1924.12.22.202	S	Thailand, Sawan	KC754773[d]	KC754906[d]
	BMNH 83.4.54	S	Myanmar, Tenasserim	KC754774	-
	GenBank[c]		Thailand	GU257907	-
Anthracoceros albirostris	BMNH 1949.25.878	S	India, Doon Valley	KC754775[d]	-
	OUMNH B05374	S	Malaysia, Pahang	KC754776	KC754907[d]
	OUMNH B05375	S	Malaysia, Borneo, Sabah	KC754777	-
Anthracoceros coronatus	BMNH 1948.57.16	S	Sri Lanka, Uva Province	KC754779[d]	KC754908[d]
	BMNH 1926.12.23.1494	S	India, Karnataka	KC754778	-
	OUMNH B05371	S	India, Hindostan	KC754780	-
Anthracoceros malayanus	BMNH 1921.10.24.1	S	Indonesia, Sumatra	KC754781[d]	-
	ZSL AMA1	F	United Kingdom, Captive bird	KC754783	KC754909[d]
	OUMNH B05370	S	Malaysia, Borneo, Sarawak	KC754782	-
Anthracoceros montani	AMNH 802255	S	Philippines, Tawi-Tawi	KC754787[d]	-
	DMNH 27721	S	Philippines, Batu-batu	KC754788	KC754911[d]
	MSUM Sulu	S	Philippines, Sanga-sanga	KC754789	-
Anthracoceros marchei	MGR PHB1	F	Philippines, Captive bird	KC754785[d]	KC754910[d]
	NMP 014884	S	Philippines, Calamianes	KC754786	-
	DMNH 37064	S	Philippines, Balabac	KC754784	-
Berenicornis comatus	BMNH 1935.10.22.163	S	Malaysia, Borneo, Sarawak	KC754791[d]	-
	BMNH 1882.7.24.12	S	Indonesia, Sumatra	KC754792	KC754912[d]
	AMNH 644968	S	Indonesia, Sumatra	KC754790	-
Buceros bicornis	BMNH 1925.12.23.1493	S	India, Karnataka	KC754794[d]	-
	NEZS BBA1	F	United Kingdom, Captive bird	KC754793	KC754913[d]
	NUS 3.11132	S	Indonesia, Sumatra	KC754795	-
Buceros hydrocorax	PAWB P47	F	Philippines, Captive bird	KC754797[d]	-
	SMNP BHSA1	F	Philippines, Luzon	KC754796	KC754914[d]
	UPD 043	S	Philippines, Luzon	KC754798	-
Buceros rhinoceros	NEZS BRA1	F	United Kingdom, Captive bird	KC754801[d]	KC754915[d]
	AMNH 122436	S	Indonesia, Java	KC754800	-
	BMNH 88.10.30.207	S	Indonesia, Sumatra	KC754799	-
Bucorvus abyssinicus	ZSL BAB1	F	United Kingdom, Captive bird	KC754803[d]	KC754916[d]
	ZSL BAA2	F	United Kingdom, Captive bird	KC754802	-
Bucorvus leadbeateri	BMNH 1932.5.5.356	S	Botswana, Mochaba river	KC754804[d]	-

(*continued on next page*)

Table B1 (*continued*)

Taxon	Institutional source[a]	Type[B]	Locality	cyt *b*	AK1 intron 5
	GenBank[c]		-	NC015199	-
Bycanistes albotibialis	BMNH 1951.34.661	S	Nigeria, Kumba River	KC754805[d]	KC754917[d]
	OUMNH B016444	S	Cameroon	KC754806	-
Bycanistes brevis	BMNH 1942.12.1.3	S	Ethiopia, Dilla	KC754807[d]	-
	GenBank[c]		Germany, Captive bird	AF346915	-
	GenBank[c]		-	NC015201	-
Bycanistes bucinator	UMZC 25/Buc/7/b/1	S	Zambia, Serenje	KC754808[d]	KC754918[d]
	GenBank[c]		Germany, Captive bird	AF346920	-
Bycanistes cylindricus	BMNH 1977.20.473	S	Liberia, Mt Nimba	KC754809[d]	KC754919[d]
	BMNH 1934.3.16.68	S	Ghana, Ashanti	KC754810	-
Bycanistes fistulator	BMNH 1948.22.10	S	Nigeria ,Yankari	KC754811[d]	-
	BMNH 1926.8.8.163	S	Cameroon, North Yaounde,	KC754812	KC754920[d]
Bycanistes subcylindricus	BMNH 1955.59.1195	S	Nigeria, Ahoada	KC754813[d]	KC754921[d]
	BMNH 1939.10.2.32	S	Sudan, Iloma plateau	KC754814	-
	GenBank[c]		Germany, Captive bird	AF346924	-
Ceratogymna atrata	OUMNH B16445	S	Cameroon	KC754815[d]	KC754922[d]
	GenBank[c]		Germany, Captive bird	AF346912	-
Ceratogymna elata	BMNH 1934.3.16.66	S	Ghana, Ashanti	KC754816[d]	-
	BMNH 1951.34.663	S	Nigeria, Kumba River	KC754817	KC754923[d]
Ocyceros birostris	BMNH S/2002.22.1	T	United Kingdom, Captive bird	KC754819[d]	KC754924[d]
	AMNH 257649	S	India, Bombay	KC754818	-
	OUMNH B05558	S	India, Saharimpur	KC754820	-
Ocyceros gingalensis	BMNH 1940.12.3.159	S	Sri Lanka, Uva Province	KC754822[d]	KC754925[d]
	BMNH 1940.12.3.162	S	Sri Lanka, Uva Province	KC754821	-
Ocyceros griseus	BMNH 1949.Whl.1.16521	S	India, Karnataka	KC754824[d]	KC754926[d]
	AMNH 344282	S	India, Mysore	KC754823	-
	UMZC 25 Buc/12/i/1	S	India, Madras	KC754825	-
Penelopides affinis	UPLB 2081	S	Philippines, Mindanao	KC754828[d]	-
	PAWB WA0010	F	Philippines, Captive bird	KC754827	KC754927[d]
	MSUIL 53258	S	Philippines, Mindanao	KC754826	-
Penelopides exarhatus	BMNH 1888.10.30.146	S	Indonesia, Sulawesi	KC754829[d]	KC754928[d]
	BMNH Y3.5.12.2423	S	Indonesia, Sulawesi	KC754830	-
	UMZC 25 Buc/9/a/1	S	Indonesia, Sulawesi	KC754831	-
Penelopides manillae	SMNP PMSA1	F	Philippines, Luzon	KC754834[d]	-
	SMNP PMC01FT	F	Philippines, Luzon	KC754833	KC754929[d]
	DMNH 70334	S	Philippines, Palaui	KC754832	-
Penelopides mindorensis	CWRC P12	F	Philippines, Mindoro	KC754835[d]	KC754930[d]
	NMP 0-9600	S	Philippines, Mindoro	KC754836	-
	UPD PSO-402	S	Philippines, Mindoro	KC754837	-
Penelopides panini	BMNH 1896.4.15.70	S	Philippines, Masbate	KC754838[d]	-
	WVSU P25	F	Philippines, Panay	-	KC754931[d]
	NMP 0-06363	S	Philippines, Negros	KC754839	-
	UMZC 25 Buc/9/b/1	S	Philippines, Guimaras	KC754840	-
Penelopides samarensis	BMNH 673.97.5.13.492	S	Philippines, Samar	KC754841[d]	-
	SBAV P01	F	Philippines, Leyte	-	KC754932[d]
	NMP 0-12255	S	Philippines, Samar	KC754842	-
	UPLB 2073	S	Philippines, Leyte	KC754843	-
Rhinoplax vigil	OUMNH B05388	S	Malaysia, Borneo, Sarawak	KC754847[d]	-
	OUMNH B05387	S	Malaysia, Borneo, Sarawak	KC754846	KC754933[d]
	NUS 3.11140	S	Malaysia, Pahang	KC754845	-
Rhyticeros everetti	BMNH 1898.12-8.71	S	Indonesia, Sumba	KC754850[d]	KC754934[d]
	AMNH 346740	S	Indonesia, Sumba	KC754848	-
	AMNH 346744	S	Indonesia, Sumba	KC754849	-
Rhyticeros narcondami	UMZC 25/Buc/1/e/1	S	India, Narcondam	KC754853[d]	-
	BMNH 1920.10.29.1	S	India, Narcondam	KC754851	KC754935[d]
	BMNH 1920.10.29.2	S	India, Narcondam	KC754852	-

Table B1 (*continued*)

Taxon	Institutional source[a]	Type[B]	Locality	cyt *b*	AK1 intron 5
Rhyticeros plicatus	BMNH 1911.12.20.891	S	Indonesia, West Papua	KC754855[d]	-
	OPAV P13	F	Philippines, Captive bird	KC754856	KC754936[d]
	AMNH 300745	S	Indonesia, Waigen	KC754854	-
Rhyticeros subruficollis	BMNH 1888.10.30.119	S	Myanmar, Tonghoo	KC754857[d]	-
	OUMNH B05366	S	Malaysia, Borneo, Sarawak	-	KC754937[d]
	BMNH 1887.9.1.243	S	Myanmar, Mandalay	KC754858	-
	GenBank[c]		Thailand	GU257914	-
Rhyticeros undulatus	BMNH 1887.9.1.224	S	India, Manipur	KC754860[d]	-
	BMNH 57.6.10.7	S	Indonesia, Java	KC754859	KC754938[d]
	OUMNH B05366	S	Malaysia, Borneo, Sarawak	KC754861	-
Tockus alboterminatus	OUMNH B16328	S	Kenya	KC754864	-
	UMZC 25/Buc/12/a/3	S	Zimbabwe, Umtali	KC754862	KC754939[d]
	OUMNH B02061	S	South Africa	KC754863[d]	-
Tockus bradfieldi	BMNH 1950.50190	S	Botswana, Ngamiland	KC754866[d]	-
	BMNH 1944.10.26.7	S	Botswana, Ngamiland	KC754865	KC754940[d]
Tockus camurus	BMNH 1977.20.3088	S	Liberia, Mt Nimba	KC754867[d]	KC754941[d]
	BMNH 1951.34.260	S	Cameroon, Kumba	KC754868	-
Tockus damarensis	GenBank[c]		Namibia, Damaraland	AY054443[d]	-
	GenBank[c]		Namibia, Damaraland	AY054443	-
Tockus deckeni	BMNH 1940.1.12	S	Tanzania, Shinyanga	KC754869[d]	-
	GenBank[c]		Germany, Captive bird	AF345931	-
Tockus erythrorhynchus	ZSL TE1	F	United Kingdom, Captive bird	KC754870[d]	-
	ZSL TE2	F	United Kingdom, Captive bird	KC754871	-
Tockus fasciatus	OUMNH B16442	S	Cameroon	KC754873[d]	KC754942[d]
	OUMNH B16441	S	Cameroon	KC754872	-
	BMNH 1977.20.451	S	Liberia, Mt Nimba	KC754874	-
Tockus flavirostris	BMNH 1923.8.7.5793	S	Somalia, Waisangli	KC754876[d]	-
	BMNH 1945.40.56	S	Sudan, East district	KC754875	KC754943[d]
Tockus hartlaubi	BMNH 1977.20.460	S	Liberia, Mt Nimba	KC754878[d]	KC754944[d]
	BMNH 1977.20.456	S	Liberia, Mt Nimba	KC754879	-
	BMNH 1975.6.2	S	Uganda, Ituri	KC754877	-
Tockus hemprichii	BMNH 1946.5.1288	S	Ethiopia, Yavello	KC754881[d]	-
	BMNH 1939.10.1.453	S	Sudan, Didinga hills	KC754880	KC754945[d]
Tockus jacksoni	BMNH 1971.16.16	S	Kenya, Kangetet	KC754882[d]	-
	GenBank[c]		Germany, Captive bird	AF346930	-
Tockus kempi	GenBank[c]		Gambia	AY423372[d]	-
Tockus leucomelas	OUMNH B18556	S	Zimbabwe	KC754884[d]	KC754946[d]
	BMNH 1911.12.18.176	S	Angola, Marro da Cruz	KC754883	-
	UMZC 25 Buc/12/h/1	S	Zimbabwe, Sabi Valley	KC754885	-
Tockus monteiri	BMNH 1957.35.43	S	Angola, Sa de Bandeira	KC754887[d]	-
	BMNH 1950.50.188	S	Namibia, Damaraland	KC754886	KC754947[d]
Tockus nasutus	OUMNH B18098	S	Tanzania	KC754890[d]	-
	UMZC 25/Buc/12/n/2	S	Zimbabwe, Nuanetsi	KC754889	KC754948[d]
	UMZC 25/Buc/12/n/1	S	Namibia, Damaraland	KC754888	-
Tockus pallidirostris	BMNH 1957.35.42	S	Angola, Teixeira de Sousa	KC754892[d]	-
	UMZC 25/Buc/12/o/1	S	Zambia, Kalulushi	KC754891	KC754949[d]
Tockus ruahae	OUMNH B17998	S	Tanzania, Serengeti	KC754894[d]	KC754950[d]
	OUMNH B17997	S	Tanzania, Serengeti	KC754893	-
	GenBank[c]		Tanzania, Ruaha National Park	AY423370	-
Tockus rufirostris	UMZC 25/Buc/12/f/3	S	Malawi, Hewe River	KC754895[d]	KC754951[d]
	GenBank[c]		Zimbabwe	AF082071	-
Tropicranus albocristatus	BMNH 1977.20.3087	S	Liberia, Mt Nimba	KC754896[d]	KC754952[d]
	OUMNH B16443	S	Cameroon	KC754897	-
	BMNH 1935.7.17.23	S	Ghana, Ashanti	KC754898	-
Out-group					
Phoeniculus purpureus	NEZS PP1	F	United Kingdom, Captive bird	KC754844[d]	KC754953[d]

(*continued on next page*)

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Table B1 (*continued*)

Taxon	Institutional source[a]	Type[B]	Locality	cyt *b*	AK1 intron 5
	GenBank[c]		-	EU167012[d]	-
	GenBank[c]		-	EU167030[d]	-
Upupa epops	GenBank[c]		United States, Captive bird	U89189	-
	GenBank[c]		-	EU344978[d]	-
Eurystomus orientalis	GenBank[c]		-	AF407452[d]	-
Eurystomus glaucurus	GenBank[c]		United States, Captive bird	CCU89184[d]	-
Coracias caudatus	GenBank[c]		New Zealand	EU410189 [d]	-
Todiramphus sanctus	GenBank[c]		South America	AY987269[d]	AJ601481[d]
Morphnus guianensis	GenBank[c]		North America	DQ485908[d]	AF307901[d]
Rallus longirostris	GenBank[c]				

[a] Institutional sources of samples: AMNH: American Museum of Natural History, New York, USA; BMNH: Natural History Museum, Tring, UK; CWRC: Calintaan Wildlife Rescue Centre, Occidental Mindoro, Philippines; DMNH: Delaware Museum of Natural History, Delaware, USA; LWPRC: Laguna Wildlife Park and Rescue Centre, Laguna, Philippines; MGR: Malagos Garden Resort, Davao City, Philippines; MSUIL: Mindanao State University Natural Science Museum, Iligan City, Philippines; MSUM: Mindanao State University Aga Khan Museum, Marawi City, Philippines; NFEFI: Negros Forests and Ecological Foundation Inc. Biodiversity Conservation Centre, Bacolod City, Philippines; NEZS: North of England Zoological Society, Cheshire, UK; NMP: National Museum of the Philippines, Manila, Philippines; SMNP: Northern Sierra Madre Natural Park, Isabela, Philippines; NUS: National University of Singapore, Raffles Museum Zoological Research Collection, Singapore; OPAV: Opol Aviary, Misamis Oriental, Philippines; OUMNH: Oxford University Museum of Natural History, Oxford, UK; PAWB: Protected Areas and Wildlife Bureau Wildlife Rescue Centre, Manila, Philippines; SHAV: Sabin Hotel Aviary, Ormoc, Leyte, Philippines; UMZC: Cambridge University Museum of Zoology, Cambridge, UK; UPD: University of the Philippines Diliman Institute of Biology Collection, Quezon City, Philippines; UPLB: University of the Philippines Los Baños Museum of Natural History, Laguna, Philippines; WVSU: West Visayas State University Mariit Wildlife Conservation Park, Iloilo, Philippines; ZSL: Zoological Society of London, London, UK.

[b] Sample types for sequences generated in this study: F = feather sample; T = preserved muscle tissue; S = skin shavings or toe-pad sample from museum skin. All F and T samples represent contemporary material, and all S samples represent historical material. All GenBank sequences were derived from contemporary samples except for *Tockus ruahae*.

[c] Genbank sequences derived from published phylogenetic analyses.

[d] Sequences selected to form a reduced matrix for phylogenetic tree building.

capture of recent events than nuclear DNA, specifically because of the three characteristics of the mitochondrial genome listed above (Edwards and Beerli, 2000). Moreover, our sampling was more extensive for mtDNA, with longer sequences of base pairs, and multiple individuals sampled per species. On the other hand, nuclear genes perform better in resolving deeper nodes (Hackett et al., 2008), or correcting for cases of mitochondrial introgression (Irwin et al., 2009; Hailer et al., 2012). We therefore assume that both datasets may contain useful information regarding the evolutionary history of hornbills.

It could be argued that our use of the combined dataset without partitioning under separate evolutionary models is inappropriate, potentially leading to inaccuracies. However, these problems only tend to arise when trees are highly incongruent (Wiens, 1998), whereas we detected no significant differences in topology or evolutionary model between nuclear and mitochondrial partitions. Moreover, our final tree is relatively stable, differing only slightly from trees reconstructed under independent partitioned analyses. Given the compatibility of sequence data from different loci, we therefore prefer to combine them in analyses, because this approach generally helps to overcome errors or introgression at one locus, and to increase explanatory power (Huelsenbeck et al., 1996; Edwards and Beerli, 2000; Nixon and Carpenter, 2005). Although we consider the concatenated tree to provide the best current representation of evolutionary history in hornbills, further analyses should attempt to verify our findings through additional sampling of loci and intraspecific lineages.

4.4. Congruence with vocal variation

A survey of vocal variation across the family revealed that our consensus tree groups hornbill vocalizations into distinct types, reflected in high phylogenetic signal for acoustic traits. Both ground-hornbills (*Bucorvus*) have distinct booming calls, while all members of *Tockus* divide cleanly into 'whistlers' and 'cluckers'. African forest hornbills (*Ceratogymna*, *Bycanites*) have distinctive wailing calls. In Asia, all *Aceros* hornbills share barking and bleating calls, while the members of *Ocyceros*, *Anthracoceros* and *Anorrhinus* have cackling calls. All hornbills in the *Rhinoplax* clade use resonant honks, and the uniquely complex 'song' of *Rhinoplax* itself reinforces the evolutionary divergence of this basal split from the genus *Buceros*.

We stress that this analysis is preliminary, being based on categorical assignments rather than quantitative acoustic measures. However, the fact that basic vocal variation maps closely onto the clade structure of our phylogeny provides additional support for the evolutionary relationships between hornbills recovered by our consensus tree (McCracken and Sheldon, 1997; Alström and Ranft, 2003). Mapping vocal variation in this way is no replacement for phylogenetic methods, as it provides little information about relationships within clades. Nonetheless, in some cases, vocal similarity between species provides additional evidence for systematic rearrangements suggested by our genetic data. For example, the novel grouping of *Berenicornis*, *Tropicranus* and *Tockus hartlaubi* is supported by their relatively similar polysyllabic hooting or piping calls.

4.5. Taxonomic implications

Our results shed light on hornbill systematics, and suggest several changes in taxonomy, particularly the revision of generic boundaries and relationships. In the *Berenicornis* clade, our results reveal that *Berenicornis* itself is not allied to *Aceros* (*contra* Kemp, 1995) but to *Tropicranus*. However, we do not propose a return to the subsuming of *Berenicornis* into *Tropicranus* first adopted by Peters (1945), particularly as these lineages are not monophyletic

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in the concatenated tree, and *Berenicornis* is divergent in size and ecology (Kemp, 2001). The genera *Ceratogymna* and *Bycanistes* are also retained owing to their genetic and phenotypic divergence (*contra* Kemp, 1995; Hübner et al., 2003). In the *Rhinoplax* clade, *Rhinoplax* is sister to *Buceros*, as suggested by Viseshakul et al. (2011), but is retained owing to its extreme vocal and morphological divergence. This treatment is also supported by highly divergent nuclear intron sequences (Fig. 2A).

Generic taxonomy in the *Anorrhinus* clade remains unclear, partly because of disparity between nuclear and mtDNA phylogenies. Judging from the AK1 intron 5 tree and the concatenated tree, both *Anthracoceros* Reichenbach 1849 and *Ocyceros* Hume 1873 are reciprocally monophyletic. However, the mtDNA tree implies that *Anthracoceros malayanus* forms a clade with *Ocyceros griseus/gingalensis*, and that *Ocyceros birostris* is divergent. As *birostris* is the type species of *Ocyceros* (assigned by Elliot, 1882), the *malayanus–griseus–gingalensis* trio could either be subsumed in *Anthracoceros* or placed in a separate genus. We maintain *Ocyceros* and *Anthracoceros* in their traditional format, but urge further sequencing to resolve their evolutionary relationship.

In the *Aceros* clade, *A. corrugatus*, *A. waldeni* and *A. leucocephalus* do not form a monophyletic grouping with either *A. nipalensis* or *A. cassidix*. They should thus be placed in the genus *Cranobrontes* Riley, 1921. We also show that *Cranobrontes* is actually a quartet of species, with the fourth being the Sulawesi endemic, *Penelopides exarhatus*. This lineage is so phenotypically divergent that it has been placed in its own genus (*Rhabdotorrhinus*), but this treatment is not supported by our analyses, which recover a sister relationship with *corrugatus*. Finally, our findings clarify that another Sulawesi endemic, *A. cassidix*, could either be grouped with the genus *Rhyticeros*, or separated into the monotypic genus *Cranorrhinus* (sister to *Rhyticeros*). This latter treatment emphasizes the divergent phenotype of *cassidix*, but we retain it with *Rhyticeros* pending further studies, particularly as the range of *cassidix* is geographically nested between other forms of *Rhyticeros* to the west and east. A full summary of taxonomic recommendations is given in Table A1.

4.6. Implications for biogeography

It has long been proposed that hornbills are essentially separated into African and Asiatic clades (Kemp and Crowe, 1985). One of the few instances of a biogeographical mismatch based on molecular evidence was the placement of Asiatic *Ocyceros* within African genera by Viseshakul et al. (2011). Our deeper sampling revealed that *Ocyceros* is related to other Asiatic species in the *Anorrhinus* clade, and thus fits with the traditional view of the historical biogeography of hornbills. The only remaining incongruity is the placement of *Berenicornis* in an African lineage. This result is intriguing and may suggest a number of different scenarios for hornbills, including either a double invasion of Asia or a recolonization of Africa. Further analyses are required to test these alternative hypotheses by reconstructing ancestral ranges.

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Appendix A

See Tables A1 and B1.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.02.012.

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