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Patterns and processes of diversification in a widespread and ecologically diverse avian group, the buteonine hawks (Aves, Accipitridae)

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ABSTRACT

Buteonine hawks represent one of the most diverse groups in the Accipitridae, with 58 species distributed in a variety of habitats on almost all continents. Variations in migratory behavior, remarkable dispersal capability, and unusual diversity in Central and South America make buteonine hawks an excellent model for studies in avian evolution. To evaluate the history of their global radiation, we used an integrative approach that coupled estimation of the phylogeny using a large sequence database (based on 6411 bp of mitochondrial markers and one nuclear intron from 54 species), divergence time estimates, and ancestral state reconstructions. Our findings suggest that Neotropical buteonines resulted from a long evolutionary process that began in the Miocene and extended to the Pleistocene. Colonization of the Nearctic, and eventually the Old World, occurred from South America, promoted by the evolution of seasonal movements and development of land bridges. Migratory behavior evolved several times and may have contributed not only to colonization of the Holarctic, but also derivation of insular species. In the Neotropics, diversification of the buteonines included four disjunction events across the Andes. Adaptation of monophyletic taxa to wet environments occurred more than once, and some relationships indicate an evolutionary connection among mangroves, coastal and *várzea* environments. On the other hand, groups occupying the same biome, forest, or open vegetation habitats are not monophyletic. Refuges or sea-level changes or a combination of both was responsible for recent speciation in Amazonian taxa. In view of the lack of concordance between phylogeny and classification, we propose numerous taxonomic changes.

1. Introduction

Hawks, eagles and Old World vultures comprise the fourth largest non-passerine family (Accipitridae, 237 species; Thiollay, 1994) and represent one of the most successful avian radiations. Among the main characteristics of these diurnal raptors are extreme flight capability, high morphological diversity, widespread migratory behavior, and worldwide distribution in all available terrestrial habitats except Antarctica (Ferguson-Lees and Christie, 2001). Such attributes make the Accipitridae an excellent model for investigations of avian evolution.

Among the classic groups of accipitrids are the buteonine hawks (sensu Amadon, 1982). Defined by some authors as a subfamily (Buteoninae, e.g. Friedmann, 1950; Grossman and Hamlet, 1964), buteonine hawks consist of the widely distributed genus *Buteo*, which contains 28 species and occurs on all continents except Antarctica and Australia (Thiollay, 1994), and several closely related species in a group called "sub-buteonine hawks". The latter was formerly composed of the mainly Neotropical genera *Buteogallus*, *Parabuteo*, *Asturina*, *Leucopternis*, *Busarellus*, *Geranoaetus*, *Geranospiza* and *Harpyhaliaetus*, as well as the Old World genera *Butastur* and *Kaupifalco* (Amadon, 1982). It now contains *Ictinia* and *Rostrhamus* as well, but not *Kaupifalco* (Griffiths et al., 2007; Lerner and Mindell, 2005; Lerner et al., 2008; Riesing et al., 2003).

Most buteonine hawk species are found in the New World, especially in the Neotropics, occupying ecologically diverse habitats including forest (*e.g.,* most species currently included in *Leucopternis*), river edge (*e.g., Rostrhamus* and *Busarellus*), mangrove (*e.g., Buteogallus aequinoctialis)*, savannah (*e.g., B. meridionalis)* and semi-arid habitats (*e.g., Parabuteo unicinctus).* Some species are endemic to oceanic (*Buteo galapagoensis,* Galapagos, and *B. solitarius,* Hawaii) and continental islands (*B. ridgwayi,* Hispaniola; Ferguson-Lees and Christie, 2001). Buteonine hawks are also well

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known for their migratory behavior, which is displayed by several species both in the Old and New World. Various theories on the evolution of migration have been proposed for raptors (e.g. Bildstein, 2004), but these remain untested in a well-founded phylogenetic framework. Such a framework is essential to the understanding of the evolution of avian migration (Chesser and Levey, 1998; Kondo and Omland, 2007; Zink, 2002), as well as its role in raptor diversification.

In recent years, the phylogeny of buteonines has been explored using molecular methods (Amaral et al., 2006; Griffiths et al., 2007; Lerner and Mindell, 2005; Lerner et al., 2008; Riesing et al., 2003). However, a complete picture of the diversification of the group has been hampered by the lack of comprehensive comparisons and resolution of parts of the tree. Here we present a well resolved phylogeny based on more than 6000 base pairs of mitochondrial and nuclear DNA sequences of most buteonine species. Coupling this phylogeny with divergence time estimates and an ancestral state reconstruction of migratory behavior provides insight into the diversification of this speciose group on both a local and global scale. It provides particular focus on the buteonine center of diversity, in the Neotropics. In addition, because a large proportion of the mitochondrial data consisted of rDNA sequences, we were also able to evaluate the influence of RNA secondary structure on character interdependence and, thus, on phylogenetic reconstruction and divergence time estimates.

2. Materials and methods

2.1. Taxon sampling

We sampled 105 specimens of 54 accipitrid species, covering all buteonine species except *Butastur liventer, Buteogallus gundlachii, Buteo oreophilus*, *B. brachypterus* and *B. archeri*, and one outgroup (online appendix 1). We recognized as buteonines the genera *Buteo*, *Asturina* (recently moved to *Buteo*; Remsen et al., 2009), *Busarellus, Buteogallus*, *Leucopternis*, *Geranoaetus*, *Geranospiza*, *Harpyhaliaetus*, *Parabuteo*, *Rostrhamus*, *Ictinia,* and *Butastur* (Amadon, 1982; Lerner et al., 2008; Riesing et al., 2003). Names of New World species follows the American Ornithologists Union (1998) and Remsen et al. (2009), with modifications by Banks et al. (2006, 2007), except that we consider *Buteogallus subtilis* as a full species (Thiollay, 1994). Old World species nomenclature follows Thiollay (1994), except that we consider *Buteo japonicus* and *B. refectus* to be full species (Kruckenhauser et al., 2004; Riesing et al., 2003). *Helicolestes hamatus*, considered by some authors as closely related to *Rostrhamus sociabilis* (Amadon, 1964), and thus a potential buteonine hawk, also was not included. Voucher skins are available for most samples (76), as well as material rich in mitochondrial DNA (viscera, feathers or muscle, 82). Two or more individuals were sampled for most species. *Haliaeetus leucocephalus* was used as outgroup, as this species belongs to the buteonine sister group (Griffiths et al., 2007; Lerner and Mindell, 2005; Lerner et al., 2008).

We obtained complete DNA sequences of the mitochondrial genes 12S rRNA (12S, approximately 970 bp), valine tRNA (tRNA Val, approximately 71 bp), 16S rRNA (16S, approximately 1600 bp), ATP synthase F0 subunits 8 and 6 (ATP86, 842 bp), NADH subunits 6 (ND6, 519 bp) and 2 (ND2, 1041 bp), as well as almost complete sequences of cytochrome *b* (CYTB, 1077 bp) and intron 5 of the nuclear gene beta-fibrinogen (FIB5, approximately 500 bp). These markers taken together totaled more than 6000 bp. We complemented our genetic sampling with selected sequences from other studies (Amaral et al., 2006; Haring et al., 2001; Lerner et al., 2008; Riesing et al., 2003 – see online appendix 1).

2.2. DNA extraction and sequencing

DNA was extracted from samples of muscle, liver, feathers, toe pads or blood using the DNeasy kit (Qiagen Inc.) according to the manufacturer's protocol, or using a modified version of the phenol-chloroform method of Bruford et al. (1992) as described by Tavares et al. (2006). Fragment amplification was performed in 25 μl reactions, containing buffer 1× (Pharmacia), dNTPs (0.32 μM), 0.5 U of *Taq*-polimerase (Pharmacia), 0.5 μM of each primer and 25–50 ng of DNA. Amplifications were performed using primers described in online appendix 2, in several different combinations. Sequencing was performed using the same PCR primers.

Amplification of mitochondrial fragments was performed using a touchdown program with the following thermal cycling conditions: an initial denaturation step of 95 °C for 5 min, followed by 10 cycles of 95 °C (30 s), 60 °C decreasing 1° per cycle (30 s) and 72 °C (40 s), and then 30 cycles using the same conditions as the previous ones, except for a fixed annealing temperature of 50 °C. Amplification of FIB5 was performed using a total of 40 cycles with times and temperatures identical to the touchdown program except for a constant annealing temperature of 50 °C.

PCR products were checked using electrophoresis on agarose gels, and single band amplifications were purified using polyethylene glycol (PEG) precipitation. Purified PCR products were directly sequenced using Big Dye terminator 3.0 cycle sequencing kit (Applied Biosystems), according to the manufacturer's protocol. Sequences were read with an ABI 377 or 3100 automated sequencer. Both strands were sequenced for each region studied.

All mitochondrial marker amplifications, with exception of ND6, were performed in multiple fragments with at least 50 bp overlap in variable regions, and most DNA extractions were performed from mitochondrial-rich muscle, viscera or feathers, as a strategy to minimize chance of *numt* amplification. Because of degradation of some samples (mostly tissue samples from the Academy of Natural Sciences of Philadelphia and material from study skins), complete sequences could not be obtained for some specimens.

2.3. Phylogenetic analyses

Eletropherograms were carefully inspected and assembled in contigs using Codoncode Aligner (Codoncode Inc.). Heterozygous positions in FIB5 were coded using the IUPAC code. Multiple sequences were aligned using Clustal X 1.83 (Thompson et al., 1997) set to default parameters. Indels and regions of ambiguous alignment of FIB5 and non-coding mitochondrial markers (e.g., long C stretches in RNA markers) were removed using Bioedit 7.0.9 (Hall, 1999). Alignment of RNA markers was performed to reflect available models of secondary structure, which were also applied to phylogenetic analyses (see below). Coding sequences were translated and checked for stop codons using MEGA 4 (Tamura et al., 2007). All markers were tested for significant deviations of base frequencies in PAUP 4b10 (Swofford, 2003). We defined the following partitions: 12S + tRNA Val + 16S, ATP8 + ATP6, ND6, CYTB, ND2 and FIB5. Sequences of ATP8 and ATP6, as well as 12S, tRNA Val and 16S were analyzed in single partitions to decrease the stochastic error resulting from model selection based on short markers (tRNA Val and ATP8), as well as to avoid overparametrization. Maximum likelihood and Bayesian analyses were performed in both single and combined partitions. Models of evolution were selected using Akaike Information Criteria (AIC) as implemented in MODELTEST v3.7 (Posada and Crandall, 1998). Incongruence among partitions was identified based on the existence of a strongly supported node (posterior probabilities of 0.95 or higher and bootstrap replicates of 70 or higher) in a topology which was in disagreement with a well-supported node in another topology.

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In such cases, statistical significance of the conflict was evaluated in PAUP, using Shimodaira-Hasegawa tests (Shimodaira and Hasegawa, 1999) with 1000 replicates of RELL bootstrapping. Using the latter test, we compared trees with conflicting nodes to constrained trees in congruence with relationships from other partitions. When conflicts were not statistically significant, partitions were combined.

Maximum likelihood heuristic and bootstrap analyses using 500 replicates were performed using Garli v0.951 (Zwickl, 2006). Base frequencies, gamma distribution α parameters, and proportions of invariant sites were estimated during runs, except during constrained analyses for the Shimodaira-Hasegawa test, in which identical parameters obtained in unconstrained heuristic searches were used. All Garli searches were automatically stopped using default parameters and repeated twice to check for likelihood and topological congruence. Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), applying independent models of evolution for each partition. All analyses were performed twice using four million generations, tree sampling at each 100 generations and a conservative burn-in of 1500 trees.

We evaluated the impact of site interdependence of RNA markers by performing a second set of partitioned Bayesian analyses of the combined mtDNA and mtDNA + FIB5 data applying for the RNA partition a *doublet* model + I + G for stems, and GTR + I + G for loops. Character pairs were assigned based on models of RNA secondary structure as follows: for 12S, Espinosa de los Monteros (2003), excluding stems 2, 8, 18, 19, 36 and 39, which could not be reliably identified; for tRNA Val, an inference performed in tRNAscan SE (Lowe and Eddy, 1997); and for 16S, an adaptation of a core mammalian model (Burk et al., 2002). Sites containing non-canonical pairing in all species were left unstructured.

2.4. Bayesian estimates of divergence time

We performed divergence time estimates using the relaxed clock method of Thorne et al. (1998) and Kishino et al. (2001) as implemented in the MULTIDISTRIBUTE package, which includes the programs MULTIDIVTIME, base2paml and estbranches (http://statgen.ncsu.edu/thorne/multidivtime.html). Estimates were obtained using the F84 model with four rate categories. The parameters for the model were calculated in PAML 4 (Yang, 1997). Branch lengths and a matrix of variance-covariance were then obtained using estbranches. The results from estbranches were used to infer divergence times in MULTIDIVTIME, using one million generations, sampling at each 100th generation after a burn-in of 300000 generations. Divergence time estimates were computed only from the mitochondrial data, because nuclear data were not available for all taxa. We compared only one individual per species (or lineage, in cases of paraphyletic species). We used the combined phylogenetic analysis ML topology as input tree, with nodes with bootstrap and posterior probabilities lower than 70 and 0.95 collapsed. Calibration was enabled by the occurrence of two endemic species on well dated volcanic archipelagos, Galápagos (*Buteo galapagoensis*) and Hawaii (*B. solitarius*). We considered the oldest exposed rocks on each island as the maximum age of those species: no more than 4 millions of years ago (Ma) based on the eastern islands of the Galápagos (White et al., 1993), and 5.1 Ma according to K-Ar estimates of the oldest exposed rocks of Kauai (Fleischer and McIntosh, 2001). A third calibration point was provided by the oldest fossil specimen attributed to the osprey family, Pandionidae (Harrison and Walker, 1976). This served as the minimum age between Accipitridae and Pandionidae. To use this date, we compared sequences of *Pandion haliaetus* (GenBank DQ780884), and set the minimum divergence time between both families at 38 Ma, as suggested by Ericson et al. (2006). Since MULTIDIVTIME requires an additional outgroup, we used sequences of *Falco peregrinus* of the family Falconidae (GenBank AF090338). ND6 sequences of *Falco peregrinus* and *Pandion haliaetus* included indels not present in the buteonines. These indels, together with the high overall divergence among *Falco*, *Pandion*, and the buteonines, made it difficult to align a small section of the ND6 gene. Thus, we removed 24 bases (corresponding to codons 106–113 in *Pandion*) from the ND6 alignment. A conservative mean of the distribution for the time separating the ingroup root from present (rttm, divergence of *Pandion* from the rest of ingroup) and its standard deviation (rttmsd) was set as 69 Ma and 31 millions of years (Myr), respectively. This range includes an additional 30 Myr beyond the confidence interval estimated previously (Brown et al., 2008). Additional parameters for MULTIDIVTIME runs were obtained as suggested in the software manual, as follows: rtrate (mean deviation of prior distribution for the rate of molecular evolution at the ingroup node) = median of the amount of evolution for the different tips/rttm; rtrate standard deviation (rtratesd) = rtrate; mean and standard deviation of prior distribution of "nu" (browmean and brownmeansd, respectively) = 2/rttm; minab (prior for the time of the interior nodes given the time of the root) = 1; bigtime (number that is absolutely positively bigger than the age of any node in the data set) = 150 Myr. All MULTIDIVTIME runs were performed twice to check for convergence of the MCMC chains. We tested the potential effects of site interdependence in RNA genes on divergence time estimates by performing the analysis twice: first with the complete mitochondrial dataset, and again including only one site on each RNA stem position.

2.5. Ancestral states reconstruction of migratory behavior

We evaluated evolutionary patterns of migratory behavior via parsimony optimization of migratory character states onto the ML heuristic topology inferred from the combined dataset. Optimization was performed with Mesquite v. 2.5 (Maddison and Maddison, 2008). Migratory behavior was coded as a multistate character, as this approach has been shown to be more informative than use of binary characters in studies of evolution of migration (Kondo and Omland, 2007). Species were coded as sedentary, partial migrant or complete migrant, according to several sources (Bildstein, 2004, 2006; Bildstein and Zalles, 2005; Ferguson-Lees and Christie, 2001; Thiollay, 1994). Only latitudinal seasonal movements were considered in this characterization. Although most data were congruent across sources, in a few cases conflicting categorizations were found, and we had to make decisions based on a variety of data. For example: (1) *Buteo albicaudatus*: Recent data suggest potential migratory movements, as in Bolivia (Olivo, 2003). However, those observations have to be considered with caution since that area is part of the migration route of *B. swainsoni*, which may confound identification (Bildstein, 2004). In the absence of independent corroboration, we coded this species as non-migratory; (2) *B. albigula*: Although Ferguson-Lees and Christie (2001) do not consider this species migratory, recent data (Pavez, 2000) suggest seasonal movements in Chile. Thus, it was considered a partial migrant, as in Bildstein (2004); (3) *Geranoaetus melanoleucus*: Little is known about the migratory behavior of this species, so contrary to Bildstein (2004), we consider it sedentary.

3. Results

3.1. Sequence characteristics

Sequence and partition characteristics, details of each evolutionary model, and ranges of uncorrected distances are described in Table 1. The combined data totaled 6677 bp, of which 6411 bp

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Table 1
Sequence characteristics and evolutionary models selected by MODELTEST.

Marker	Sites included	Range of *P* distances	Partition	Sites included	Variable sites	Parsimony informative sites	Model of evolution
12S	926	0.000–0.086	12S + tRNAVal + 16S	2433	596	507	GTR + I + G (stems + loops)
tRNA Val	62	0.000–0.129					GTR + I + G (loops only)
16S	1445	0.000–0.072					
ATP8	168	0.000–0.208	ATP8 + ATP6	842	376	334	TrN + I + G
ATP6	684	0.000–0.140					
ND2	1041	0.000–0.150	ND2	1041	475	419	GTR + I + G
ND6	519	0.000–0.160	ND6	519	224	206	TVM + I + G
CYTB	1077	0.000–0.123	CYTB	1077	400	360	GTR + + I + G
FIB5	499	0.000–0.030	FIB5	499	52	31	TVM
			Combined mtDNA	5912	2071	1826	TVM + I + G
			Total dataset	6411	2123	1857	TVM + I + G

were analyzed after removal of indels and ambiguous sections (12S + tRNA + 16S, 258 bp; FIB5, 8 bp). According to the assumed models of secondary structure of RNA, 1000 bp (corresponding to 500 pairings) were identified as RNA stems. Six substitution-rate models with invariable site correction and gamma distribution were selected by MODELTEST for the mitochondrial partitions and the combined data, while a six-rate model without invariant sites or gamma distribution was selected for FIB5. All coding sequences lacked unexpected stop codons, and most variation was found in third codon positions. No significant deviation of base composition was found in any marker or partition ($p > 0.05$). We believe that our mitochondrial sequences are authentic because: (1) most sequences were obtained from mitochondrial-rich tissue; (2) overlapping fragments had identical sequences; (3) sequences were easily aligned to other avian sequences available in GenBank; (4) there were no unexpected stop codons; and (5) mitochondrial trees obtained from different partitions were completely congruent. Evidence of saturation was found in all mitochondrial coding genes, but not in RNA and nuclear markers.

3.2. Phylogenetic analyses

Topologies inferred from single mitochondrial partitions were generally poorly resolved (with exception of the RNA partition), and no incongruence was detected among partitions. For this reason, mitochondrial markers were combined and analyzed as a single dataset. These combined data produced a single maximum likelihood tree (−ln 40768.5763, Fig. 1) in which most nodes were supported with high posterior probability ($\geqslant$0.95) and maximum likelihood bootstrap values ($\geqslant$70).

Trees based on FIB5 (Fig. 2) were poorly resolved but generally congruent with mitochondrial topologies. The only clade with high nodal support that conflicted with the mtDNA tree was the inclusion of *Leucopternis lacernulatus* in a clade composed of *L. schistaceus*, *Buteogallus anthracinus* and *B. aequinoctialis*. However, the maximum likelihood heuristic topology (−ln 1065.2313) was not statistically different from a tree enforced to reflect the mitochondrial topology (−ln 1079.4400; Shimodaira-Hasegawa test, $P = 0.1$). For this reason, we combined the mitochondrial and FIB5 data.

The maximum likelihood tree derived from the combined data (−ln 42102.7565, Fig. 3) was almost identical to the mitochondrial topology (Fig. 1), except for differences in nodal support. The combined and mtDNA Bayesian trees were completely congruent. No incongruence in highly supported nodes was detected between Bayesian analyses using standard or doublet models for RNA markers (support values in Fig. 1 and Fig. 3). Five main clades of buteonine hawks were recovered: (1) sampled species of *Butastur*, (2) *Ictinia*, (3) *Busarellus, Rostrhamus* and *Geranospiza,* (4) *Leucopternis plumbeus*, *Harpyhaliaetus*, *Leucopternis lacernulatus*, *Leucopternis schistaceus* and all species of *Buteogallus*, and (5) *Buteo, Geranoaetus, Parabuteo* and the remaining *Leucopternis* species. Old World *Buteo* species (*B. rufofuscus, B. augur, B. auguralis, B. rufinus, B. buteo vulpinus, B. buteo buteo, B. hemilasius, B. japonicus* and *B. refectus*) comprised a monophyletic group nested in the latter clade. Two species, *Buteo jamaicensis* and *Leucopternis albicollis,* were not monophyletic.

3.3. Divergence times estimates

Divergence time estimates based on datasets containing complete or partial RNA stems were similar in point estimates and 95% confidence intervals (Table 2, Fig. 4). The estimates suggest a long period of diversification, which may have begun in the Miocene, and which extended through the Pleistocene. Separation between *Pandion* and buteonine hawks plus *Haliaeetus leucocephalus* may have occurred between 38 and 78 Ma. This node has been omitted from Fig. 4 to allow a larger scale for the buteonines (but see table 2, node 79).

3.4. Ancestral states reconstruction of migratory behavior

The most parsimonious reconstruction of migration yields 17 steps, indicating at least seven independent gains of migratory behavior during buteonine diversification (Fig. 5). Transitions occurred from sedentary to partially migratory, partially migratory to completely migratory, and partially migratory to sedentary. Migratory behavior occurred in most or all species in three clades: (1) *Butastur* species, (2) *Ictinia* species, and (3) predominantly Nearctic and Old World *Buteo* species.

4. Discussion

4.1. Phylogenetic relationships

The buteonine phylogeny reconstructed in this study is the most comprehensive and best resolved of its kind. It highlights previous discoveries of paraphyly in *Buteo*, *Buteogallus* and *Leucopternis* (Amaral et al., 2006; Lerner et al., 2008; Riesing et al., 2003), resolves several traditionally difficult nodes (especially deeper nodes), and also presents novel relationships. These newly resolved relationships include: a clade containing *Rostrhamus sociabilis*, *Busarellus nigricollis* and *Geranospiza caerulescens*, which is sister group to the remaining buteonines (excluding *Ictinia* and *Butastur*); the configuration of *Buteo solitarius*, *Buteo albigula*, *Buteo galapagoensis*, *Buteo brachyurus* and *Buteo swainsoni*; affinities among almost all species in clade H (Fig. 3, with exception of the uncertain exact branching of the *L. lacernulatus*/*B. meridionalis* clade), as well as the connection between this group and the rest of the buteonines; and the position of *Leucopternis princeps* relative to *Buteo magnirostris* (first split of clade G). Unfortunately, some other relationships remain poorly resolved, such as the exact position of *Buteo nitidus*; part of the nodes in clade B (Fig. 3);

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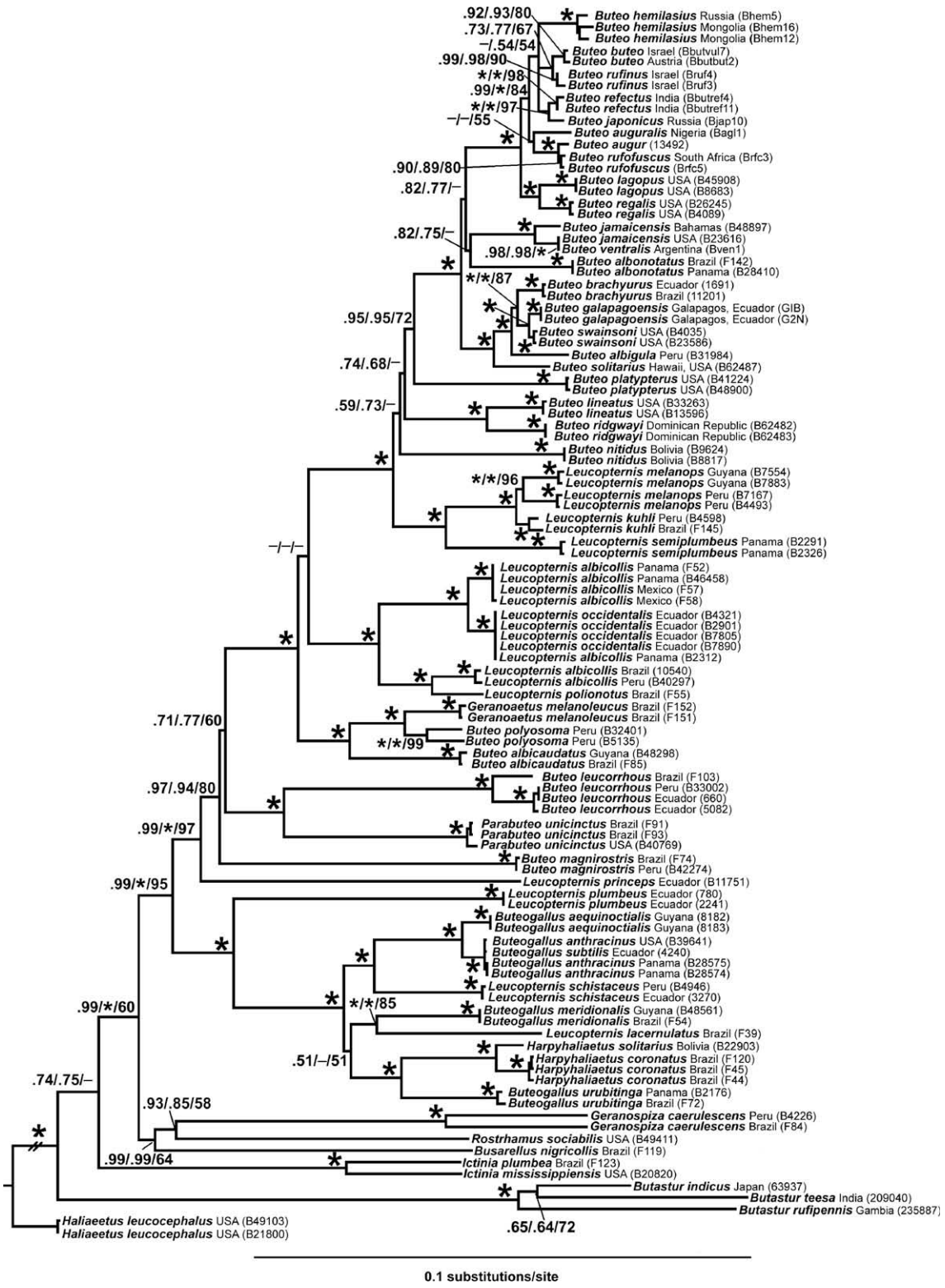

Fig. 1. Maximum likelihood tree inferred from combined mtDNA sequences. Numbers near nodes indicate Bayesian posterior probabilities using standard models of evolution, Bayesian posterior probabilities using a doublet model for RNA markers, and maximum likelihood bootstrap proportions, respectively. Maximum values of each measure of nodal support are represented by small stars, while maximum values for all measures are indicated by large stars. Dashes indicate values below 50 (bootstrap) or .50 (posterior probabilities). Origins and sample numbers are indicated after species names. The branch leading to the outgroup was shortened for illustrative purposes.

relationships among *Rosthramus*, *Geranospiza* and *Busarellus*; splits prior to nodes D and F (Fig. 3). Rapid speciation events may preclude complete resolution even with much larger datasets. Although the sister relationship of *Ictinia* to all remaining Buteonines except *Butastur* has been recovered with low support in the present study, this same configuration has been found with high nodal support in a previous study (Lerner et al., 2008).

Relationships among the distal *Buteo* species in Fig. 3 (clade A) are less well resolved than those in the rest of the phylogeny. In addition to a higher proportion of species with missing data in this

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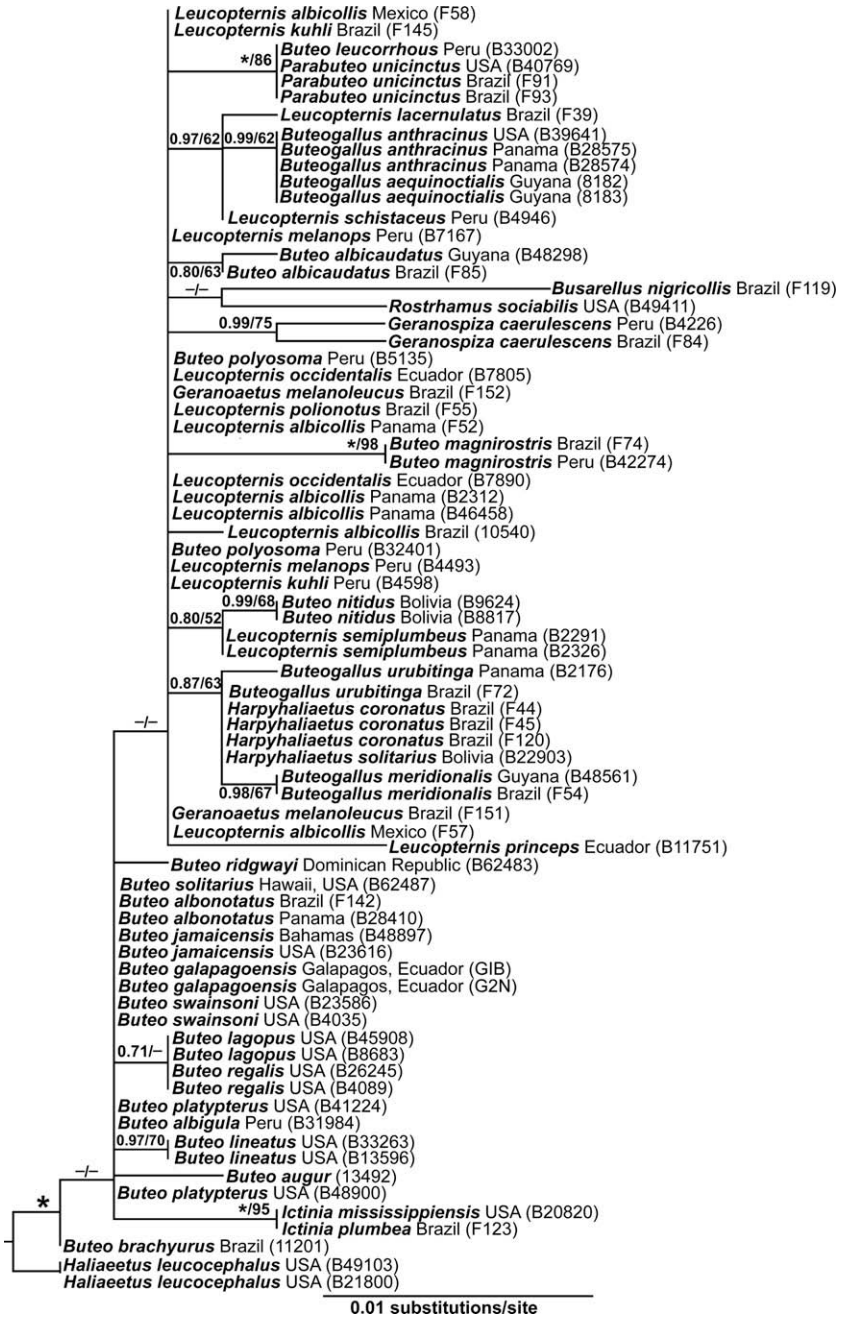

Fig. 2. Maximum likelihood tree inferred from FIB5 sequences. Numbers near nodes indicate Bayesian posterior probabilities and maximum likelihood bootstrap proportions, respectively. Maximum values of each measure of nodal support are represented by small stars, while maximum values for all measures are indicated by large stars. Dashes indicate values below 50 (bootstrap) or .50 (posterior probabilities). Origins and sample numbers are indicated after species names. The branch leading to the outgroup was shortened for illustrative purposes.

part of the tree, the diversification in the Old World *Buteo* species has been relatively rapid, making it difficult to parse the exact branching pattern (see Kruckenhauser et al., 2004). The sister species relationship between *Buteogallus meridionalis* and *Leucopternis lacernulatus* (as in Amaral et al., 2006) contrasts with the arrangement found by Lerner et al. (2008): (*L. lacernulatus* (*B. urubitinga* + *Harpyhaliaetus*)). The incongruence probably reflects the use of fewer buteonine species and mostly fast-evolving genes by Lerner et al. (2008).

Intra-specific studies are warranted for some taxa. For example, we discovered relatively large genetic distances between individuals in a variety of species (ND2 uncorrected distances are used except when indicated): *Geranospiza caerulescens* from Peru and Brazil have diverged by 5.7%; two individuals of Peruvian *Buteo polyosoma* have diverged by 1.4%; specimens of *Leucopternis melanops* from Peru and Guyana have diverged by 1.3%; and Atlantic and Andean individuals of *Buteo leucorrhous* have diverged in ATP8 + 6 by 2.0% (corroborating Riesing et al., 2003). On the other hand, we also found remarkably little difference between haplotypes of *Buteogallus anthracinus* and *B. subtilis* (ND2 divergence <0.1%). Furthermore, *Buteo jamaicensis* is paraphyletic in relation to *B. ventralis* (as in Riesing et al., 2003), and *trans*-Andean individuals of *Leucopternis albicollis,* which represent the subspecies *L. a. ghiesbreghti* and *L. a. costaricensis*, are closer to *Leucopternis occidentalis* than *to cis*-Andean samples of *L. albicollis*, which represent subspecies *L. a. albicollis* (as in Lerner et al., 2008).

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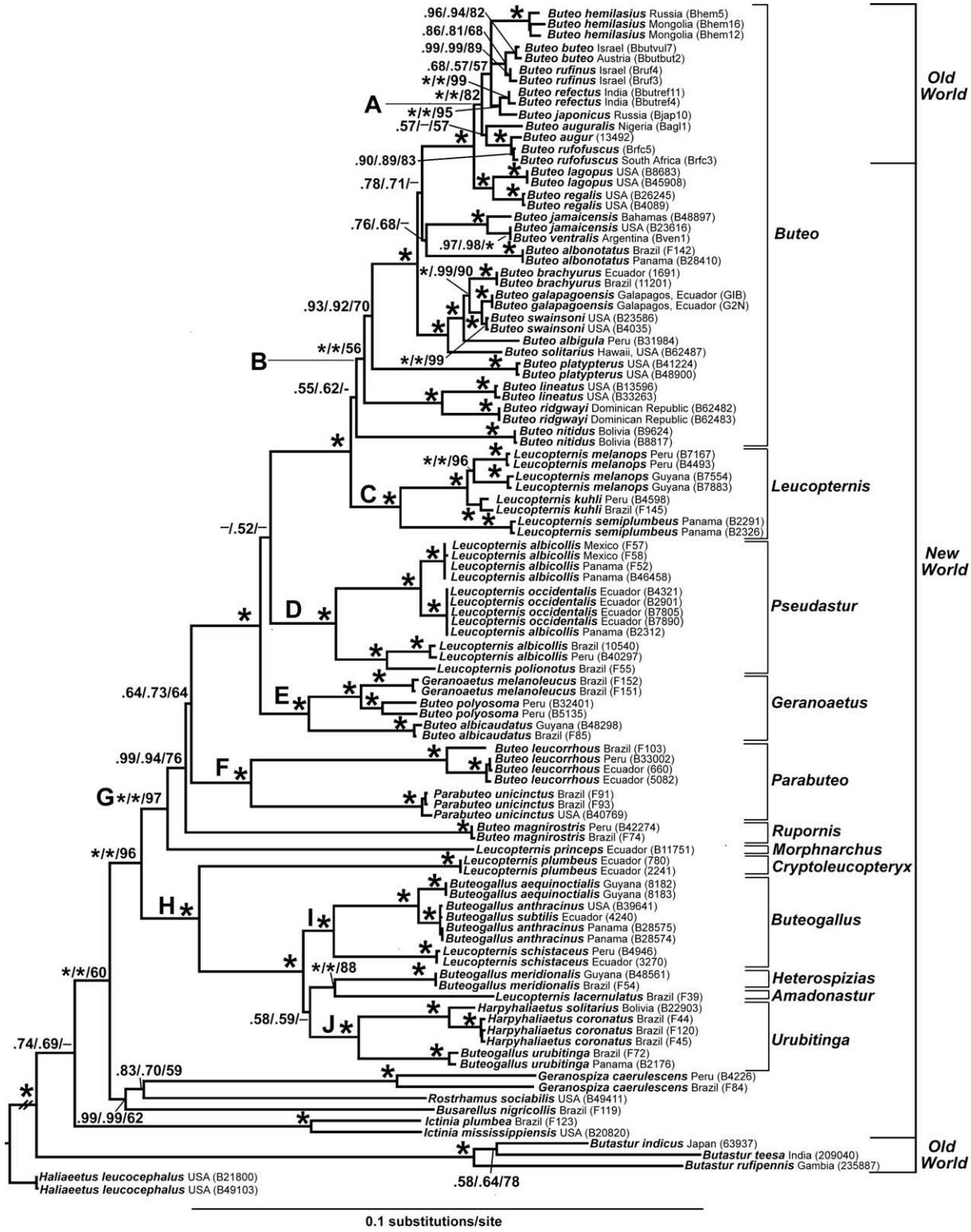

Fig. 3. Maximum likelihood tree inferred from combined mtDNA and FIB5 sequences. Numbers near nodes indicate Bayesian posterior probabilities using standard models of evolution, Bayesian posterior probabilities using a doublet model for RNA markers, and maximum likelihood bootstrap proportions, respectively. Maximum values of each measure of nodal support are represented by small stars, while maximum values for all measures are indicated by large stars. Dashes indicate values below 50 (bootstrap) or .50 (posterior probabilities). Origins and sample numbers are indicated after species names. Letters represent clades discussed in the text. Proposals of taxonomic changes are indicated at the right of the tree. Although *Buteo lagopus* also occurs in the Old World, it was included in the New World category since only North American samples were used. The branch leading to the outgroup was shortened for illustrative purposes.

4.2. Global biogeography

Divergence time estimates suggest a long evolutionary history of buteonines, starting possibly in the Lower/Middle Miocene and extending as late as the Pleistocene. Old World *Buteo* species comprise a relatively young group nested in a clade consisting predominantly of Nearctic *Buteo* species (clade B, Fig. 3). These two are included in a major Neotropical clade. This pattern suggests a

Table 2
Divergence time estimates, standard deviation (SD) and 95% confidence intervals in Ma, inferred from the mtDNA datasets containing total and partial RNA stems, respectively. Nodes are represented in Fig. 4.

Node	Total Stems				Partial Stems			
	Time	SD	95% Min.	95% Max.	Time	SD	95% Min.	95% Max.
44	4.17	1.05	2.73	6.77	4.20	1.06	2.78	6.90
45	11.94	2.74	8.36	18.85	11.84	2.72	8.36	18.86
46	1.02	0.29	0.60	1.72	1.02	0.29	0.60	1.72
47	3.88	0.95	2.58	6.27	3.83	0.94	2.57	6.19
48	4.72	1.14	3.17	7.59	4.65	1.12	3.17	7.49
49	0.93	0.27	0.54	1.57	0.95	0.27	0.55	1.63
50	4.29	1.04	2.87	6.90	4.19	1.02	2.83	6.81
51	5.55	1.31	3.81	8.84	5.44	1.28	3.76	8.76
52	9.56	2.22	6.65	15.06	9.50	2.20	6.68	15.14
53	8.20	1.95	5.59	13.13	8.24	1.96	5.68	13.27
54	2.36	0.61	1.54	3.85	2.31	0.59	1.50	3.80
55	4.47	1.09	3.00	7.13	4.40	1.08	3.00	7.07
56	2.00	0.52	1.28	3.27	1.99	0.52	1.28	3.30
57	3.88	0.96	2.58	6.23	3.89	0.96	2.61	6.29
58	1.02	0.29	0.61	1.72	1.04	0.29	0.62	1.76
59	3.47	0.85	2.30	5.57	3.50	0.86	2.36	5.74
60	2.07	0.53	1.33	3.39	2.08	0.53	1.35	3.42
61	0.34	0.13	0.12	0.65	0.35	0.14	0.13	0.66
62	0.90	0.25	0.54	1.52	0.89	0.25	0.53	1.51
63	1.27	0.33	0.80	2.10	1.26	0.34	0.79	2.11
64	1.82	0.46	1.18	2.97	1.81	0.46	1.19	2.98
65	0.93	0.27	0.55	1.57	0.93	0.27	0.54	1.60
66	1.17	0.31	0.72	1.94	1.15	0.31	0.72	1.93
67	1.49	0.39	0.95	2.46	1.49	0.39	0.95	2.47
68	3.10	0.74	2.12	4.97	3.10	0.74	2.13	5.02
69	4.55	1.08	3.11	7.21	4.57	1.08	3.16	7.34
70	4.88	1.15	3.34	7.73	4.91	1.16	3.40	7.87
71	5.18	1.22	3.58	8.21	5.21	1.22	3.61	8.32
72	7.09	1.66	4.92	11.22	7.04	1.65	4.93	11.30
73	10.01	2.31	6.99	15.83	9.93	2.29	7.03	15.90
74	10.65	2.45	7.48	16.79	10.65	2.45	7.56	17.05
75	11.50	2.64	8.07	18.11	11.46	2.62	8.13	18.27
76	12.61	2.88	8.87	19.80	12.52	2.86	8.89	19.96
77	13.53	3.09	9.52	21.16	13.38	3.08	9.51	21.38
78	17.32	3.94	12.25	27.11	17.19	3.90	12.27	27.27
79	49.87	10.72	38.36	77.63	49.64	10.56	38.36	77.66

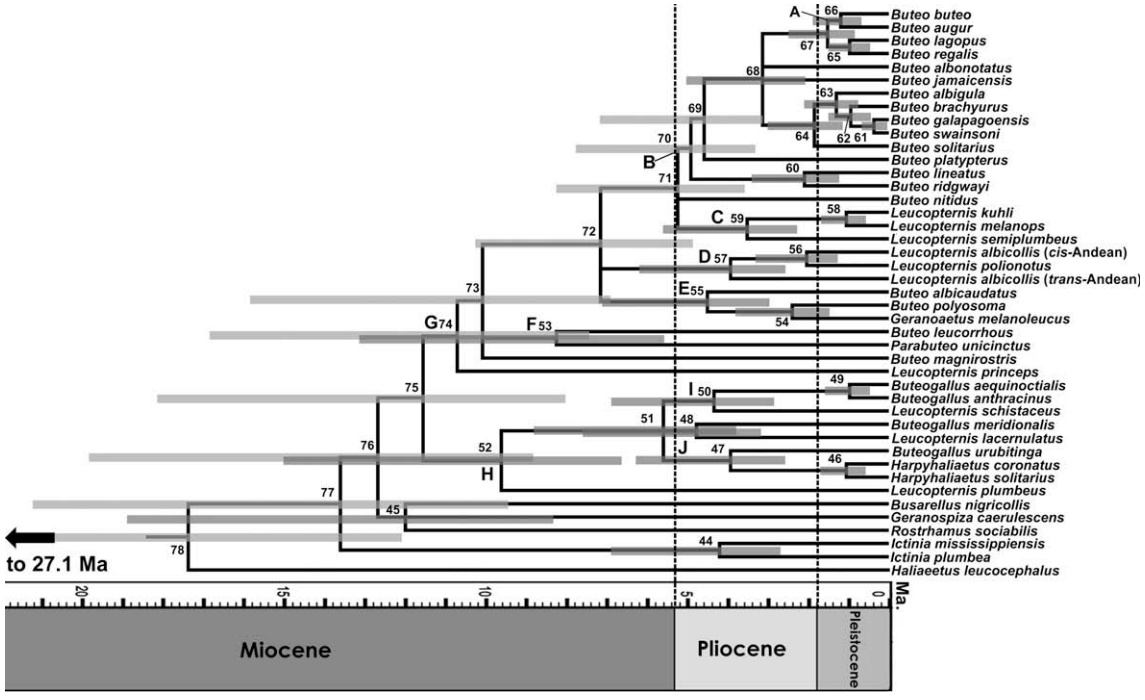

Fig. 4. Bayesian estimates of time of divergence in millions of years ago (Ma), based on combined mtDNA sequences including total RNA stems. Horizontal gray bars indicate 95% confidence intervals. Numbers at nodes represent estimates indicated in Table 2. Letters represent clades discussed in the text.

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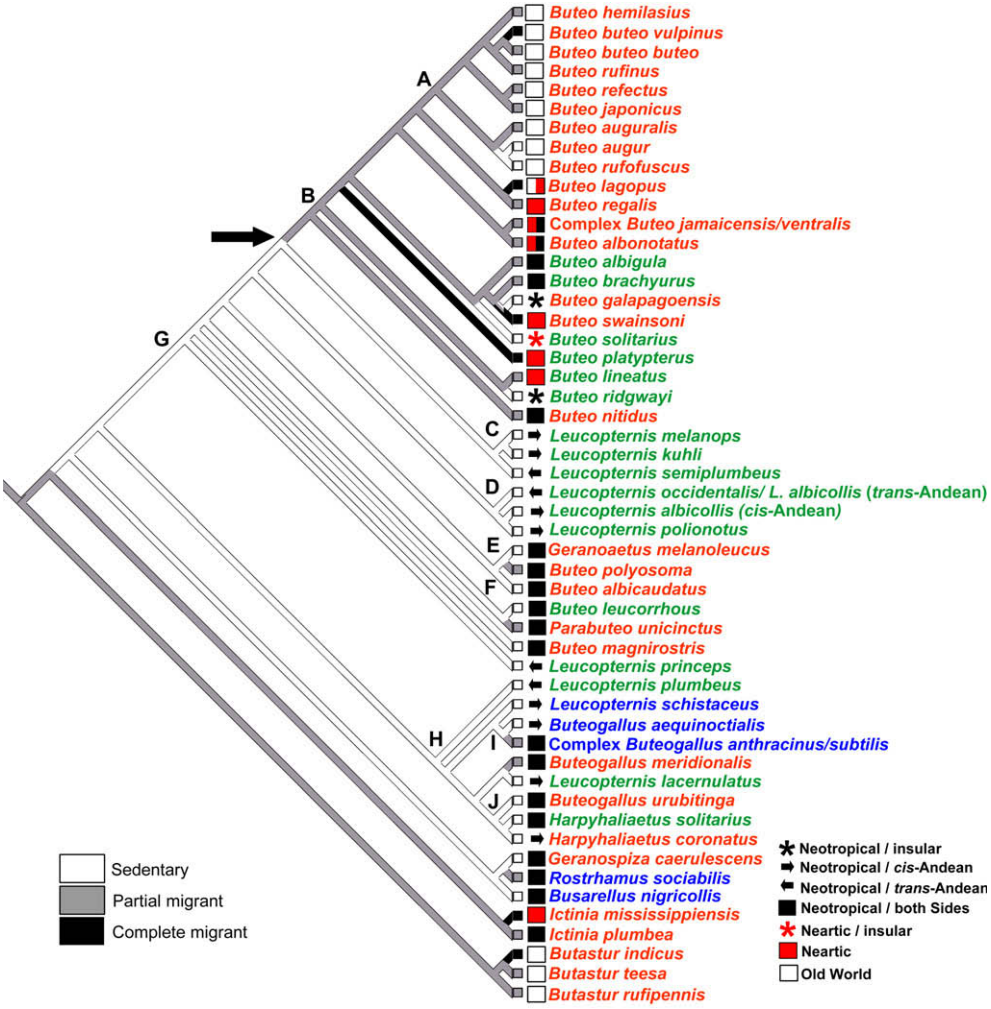

Fig. 5. Ancestral states of migratory behavior reconstructed by parsimony, and general breeding ranges and basic ecological characteristics of buteonines. Names in orange indicate species that occupy predominantly open vegetation and forest edge; in green, predominantly forest; and in blue, predominantly riparian, flooded and coastal habitats. The large black arrow at the left indicates the evolution of migratory behavior among *Buteo* species that breeds mostly in the Nearctic or in the Old World. The subspecies *B. b. buteo* and *B. b. vulpinus* were indicated because they differ in migratory behavior.

Neotropical origin for *Buteo* occupying North America, Asia, Europe and Africa (Amadon, 1982; Riesing et al., 2003; Voous and de Vries, 1978).

Diversification of *Buteo* species that breed mainly in the Nearctic (*B. regalis, B. jamaicensis, B. solitarius, B. platypterus* and *B. lineatus*) appears to be the result of at least one colonization event from South America between 7.7 and 3.3 Ma (Fig. 4, split B). This timing is congruent with the closing of the Panaman isthmus, which started approximately 15 Ma and finished 3.1–2.8 Ma (Coates and Obando, 1996). Colonization of Central and North America probably started before complete closure of the isthmus, as buteonine hawks are powerful fliers and thus excellent dispersers. Presence of Neotropical species in the otherwise mostly Nearctic *Buteo* clade (Fig. 5) suggests reinvasion and speciation of the Neotropics from the Nearctic. This possibility is especially true for *Buteo galapagoensis* and *B. ridgwayi* (see next subsection).

Pleistocene dispersal from North America led to the diversification of an exclusively Old World group of *Buteo* species (Fig 3. clade A, Fig. 4). The colonization was probably aided by climatic fluctuations that led to periodic exposure of Beringia (Marincovich and Gladenkov, 1999, 2001), as well as intermittency of suitable habitats due to development of extensive glaciers in colder epochs (Riesing et al., 2003). Diversification of Old World avian taxa from predominantly Neotropical groups is a pattern common in other avian families, such as Caprimulgidae (Barrowclough et al., 2006) and Falconidae (Griffiths et al., 2004). Although temperate *Buteo* species diversified mainly after the Upper Miocene, several "*Buteo*" fossils have been found in earlier periods of the Tertiary in North America and Europe, e.g., *B. grangeri,* Upper Oligocene, EUA (Wetmore and Case, 1934); *B. pusillus*, Middle Miocene, France (Ballman, 1969) and *B. spassovi*, Upper Miocene, Bulgary (Boev and Kovachev, 1998). These earlier taxa may represent extinct buteonine lineages or incorrectly identified fossils. Although estimating divergence times is controversial (see Arbogast et al., 2002; Brown et al., 2007), and extinction of early buteonine lineages probably occurred during the evolution of the group, allocation of fossils in *Buteo* is far from definitive (Olson, 1985). Detailed phylogenetic analysis of morphological characters, including both modern and extinct species, will be essential not only to confirm generic assignment of those species, but also to allow comparison of our estimates and fossil ages in a phylogenetic framework. Such studies will also be useful for understanding of the early history of the buteonines prior to Neotropical diversification, which remains unclear. The sister group relationship of *Butastur* to the buteonines may indicate an Old World origin, but fossil evidence coupled with a complete

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accipitrid phylogeny will be necessary to define the early origins of the buteonine hawks.

4.3. Evolution of migratory behavior and consequences for diversification

Ancestral state reconstruction indicated that migratory behavior developed multiple times during buteonine diversification (Fig. 5), as it has in other bird groups (Chesser, 2000; Joseph et al., 2003; Kondo and Omland, 2007; Outlaw et al., 2003). Because migration requires complex physiological interactions that are unlikely to evolve more than once, an alternative mechanism must explain the plasticity of this behavior. This is likely to be the "activation" and "suppression" of migration ability, which would have originated once, early in avian evolution (Zink, 2002).

Migratory behavior in buteonines appears to have evolved gradually, probably facilitated by ecological characteristics of the group. Complete migrants evolved from partial migrants, which in turn derived from sedentary species, thus conforming to a stepwise evolutionary model (Cox, 1985). The lifestyle of Neotropical buteonines may have predisposed them to migratory behavior as previously suggested for other birds (see Chesser and Levey, 1998; Levey and Stiles, 1992). Most species occupy mainly open habitats, forest edge and canopy, and track highly variable food resources. These are the predominant characteristics of buteonines in migratory lineages, whereas species that depend heavily on forest habitat (e.g., *Leucopternis*) are largely sedentary (Fig. 5). The lower migratory predilection in forest species probably has more to do with their restrictive habitat requirement than their dispersal capabilities, because all buteonines are excellent fliers.

Although migration evolved independently several times in the buteonines, one clade of *Buteo* is distinguished by having evolved migratory behavior only once, early during the Nearctic/Old World radiation (indicated by an arrow in Fig. 5). This pattern suggests that although dispersal into the Nearctic was promoted by formation of the Panaman isthmus, evolution of seasonal movements to southern latitudes may have been key to survival (and eventually diversification) in temperate environments. Migration also may have acted as a mechanism of speciation among island species. When a migratory species encounters a new environment, or simply stops at the edge of its migratory range, it has the potential for rapid divergence and speciation if individuals become isolated and sedentary in this new environment (Kondo et al., 2008; West-Eberhard, 2003). This hypothesis has been suggested for the evolution of island raptors (Bildstein, 2004). Our results support speciation by loss of migration among buteonine hawks in view of (1) the sister group relationship between the Galapagos endemic *Buteo galapagoensis* and the Nearctic-Neotropical complete migrant *B. swainsoni*, (2) the sedentary condition of the island endemics *B. ridgwayi* (Hispaniola) and *B. solitarius* (Hawaii), which according to the ancestral state reconstruction descended from migratory ancestors (Fig. 5), and (3) the recent speciation (Fig. 4; less than 1 Ma; Bollmer et al., 2006) and rapid suppression of migratory behavior in *B. galapagoensis* (Fig. 5).

4.4. Patterns and processes of diversification in the Neotropics

4.4.1. Cis- and trans-Andean disjunctions: temporal patterns of evolution

Our results suggest that four disjunction events have occurred between sister lineages distributed on the eastern (*cis-*) and western (*trans-*) sides of the Andean cordillera. Two disjunctions occurred near the Miocene–Pliocene boundary (Fig. 3 and Fig. 4, splits C and D), and two in the Miocene (Fig. 3 and Fig. 4, splits G and H). These disjunctions differ in their structure; in two instances, monophyletic taxa are completely separated by the Andes (splits C and D), whereas in the remaining two (disjunctions G and H) some species or nested lineages occur in both sides of the Andes. Separation of sister species or lineages by the Andes can be explained by three different hypotheses: the Andean Uplift Hypothesis (Chapman, 1917), the Refuge Hypothesis (Haffer, 1969), and Dispersal Across the Andes Hypothesis (Chapman, 1917; Haffer, 1967). Although Andean orogeny was initiated more than 20 Ma, its final phase, when the Cordillera reached approximately its current elevation, occurred between 6 and 2.7 Ma (Gregory-Wodzicki, 2000). Thus, vicariance prior to 2.7 Ma is most likely explained by Andean uplift, whereas more recent separation is most likely the result of refugia or dispersal events (see Brumfield and Edwards, 2007). Given this logic, the Andean Uplift Hypothesis would explain disjunctions G and H, because they are considerably older than 2.7 Ma. Disjunction C and D, however, have wide confidence intervals that span 2.7 Ma and, thus, it is impossible to differentiate among the three potential bifurcating processes. Comparison of buteonine separation events to the timing of separation events estimated from other avian taxa with similar distributions (e.g. Brumfield and Edwards, 2007; Miller et al., 2008; Pereira and Baker, 2004; Ribas et al., 2005) suggests that separation of forest species on opposite sides of the Andes must have been caused by a variety of forces over long evolutionary time.

4.4.2. Diversification in wet environments

Five buteonine species (*Leucopternis schistaceus*, *Buteogallus anthracinus*, *B. aequinoctialis*, *Busarellus nigricollis* and *Rostrhamus sociabilis*) are largely restricted to flooded, riparian, or coastal habitats (Ferguson-Lees and Christie, 2001), thus presenting an opportunity to identify historical connections among those habitats. A sixth species, *Geranospiza caerulescens*, occurs in a variety of habitats, but mainly those associated with rivers (Ferguson-Lees and Christie, 2001). Although *Buteogallus subtilis* is considered a mangrove specialist, its lack of genetic differentiation from *B. anthracinus* brings into question its specific status (see online appendix 3).

Neotropical mangroves and coastal habitats share bird species with *várzea* forests in Amazonia, suggesting ecological similarity among those habitats (Sick, 1997). The monophyly of *Buteogallus aequinoctialis* (mangroves), *B. anthracinus* (mostly coastal), and *Leucopternis schistaceus* (*várzea* forests) (Fig. 3, clade I) indicates that those habitats may be connected not only ecologically but also historically. This connection may have been a result of predominance of *várzea* forests in a flooded western Amazonia (Aleixo and Rossetti, 2007; Lundberg et al., 1998; Rossetti et al., 2005) subject to marine trangressions until the end of the Miocene (Hoorn et al., 1995). Reorganization of the Amazonian fluvial system (Aleixo and Rossetti, 2007; Rossetti et al., 2005) and, more recently, dynamic changes in coastal habitats (Woodroffe and Grindrod, 1991) may have caused splitting of the *várzea* specialist (*Leucopternis schistaceus*) and the two mostly coastal species (*Buteogallus aequinoctialis* and *Buteogallus anthracinus*), respectively. On the other hand, diversification associated with wet habitats is also indicated by a clade that includes *Rostrhamus*, *Busarellus* and *Geranospiza*. Unlike the previous group, however, the age of this clade indicates that such diversification events started early in the evolution of the buteonines (Fig. 4).

4.4.3. The effect of the Amazon river in the diversification of the *Leucopternis melanops/L. kuhli* complex

Although expansion of riparian and flooded habitat may benefit *várzea* specialists, the development of rivers and their associated vegetation may isolate species restricted to *terra-firme* forests. The sister species *Leucopternis kuhli* and *L. melanops* have served as a classic example of disjunction north and south of the Amazon (Haffer, 1987), even though they occur in sympatry in several

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localities south of the Amazon River (Amaral et al., 2007; Barlow et al., 2002). Their sympatry suggests that the Amazon river in its current configuration does not represent a barrier to dispersal, at least in the case of *L. melanops* (Amaral et al., 2006). According to our divergence time estimates, these two species speciated in the Pleistocene, long after the development of the Amazon River (~8 MYA, Lundberg et al., 1998). Although it is possible that the Amazon posed as an effective barrier during periods of high sea-level (Marroig and Cerqueira, 1997), other explanations for the separation of *L. kuhli* and *L. melanops* are possible. The Refuge Hypothesis, for example, posits that forest fragments isolated by savannah (Haffer, 1969) or unsuitable forest types (Colinvaux, 1998) could have driven speciation. Another scenario combines elements of both an Amazon barrier and Refuge isolation, the River-Refuge hypothesis (Haffer, 1997).

4.5. Taxonomy

The need for a nomenclatural revision of buteonine hawks at the generic level is clear because *Leucopternis, Buteogallus* and *Buteo* as currently accepted (Remsen et al., 2009) are not monophyletic (Amaral et al., 2006; Lerner et al., 2008; Riesing et al., 2003). Taking into account phylogeny, plumage patterns and ecology, we propose a new arrangement based on reorganization of current genera and resurrection of the genera *Morphnarchus, Urubitinga, Pseudastur, Rupornis* and *Heterospizias* (Fig. 3, for details see online appendix 3). Because of distinction in ecology, morphology and genetics in relation to the other species of clade H (Fig. 3), we also propose two new monotypic genera to accommodate *Leucopternis lacernulatus* and *L. plumbeus*:

Amadonastur gen. nov.

Type species: *Falco lacernulatus* Temminck, 1827.

Diagnosis: Adults of *Amadonastur lacernulatus* are diagnosed by the combination of head and neck white; hind neck pale gray; mantle slate black; and underparts and underwing coverts plain white. The inner webs of the primaries are pure white and the same region of the secondaries and tertials are finely barred with black. The upper surface of the tail has a black band at the base. The lower surface has black streaks at the basal end and a white band and distinct subterminal black band at the apical end. *A. lacernulatus* is similar in plumage to the sympatric *Pseudastur polionotus*, but distantly related, and reflects the complex biogeographic history of the Atlantic forest. The plumage similarity may represent a case of mimetism.

Included taxon: *Amadonastur lacernulatus* (Temminck, 1827) gen. nov., comb. nov.

Etymology: We name this genus after Dean Amadon for his classic work on accipitrid systematics, which anticipated many of the relationships inferred in our study. From the Latin *astur*, meaning a hawk. Gender masculine.

Cryptoleucopteryx gen. nov.

Type species: *Leucopternis plumbea* Salvin, 1872.

Diagnosis: *Cryptoleucopteryx plumbea* is distinguished from all other buteonine hawks by the combination of an overall dark gray plumage, black tail with a single white medial band, and white underwings coverts.

Included taxon: *Cryptoleucopteryx plumbea* (Salvin, 1872) gen. nov., comb. nov.

Etymology: From the Greek *crypto*, meaning hidden, *leuco,* meaning white, and *pteryx,* meaning a wing. Gender feminine.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.07.020.

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