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Molecular systematics of the caracaras and allies (Falconidae: Polyborinae) inferred from mitochondrial and nuclear sequence data

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The Polyborinae is the most diverse subfamily of the Falconidae in terms of both morphology and behaviour, and includes falconet-shaped birds (*Spiziapteryx*), arboreal omnivores (*Daptrius*, *Ibycter*), as well as terrestrial generalists and scavengers (*Caracara*, *Milvago* and *Phalcoboenus*). The Polyborinae are endemic to the New World, with all but one species (*Caracara cheriway*) being restricted to Central and South America. Using over 7300 bp of mitochondrial and nuclear sequence data, we aim to clarify the taxonomy and biogeography of the Polyborinae. The genus *Milvago* was unexpectedly found to be polyphyletic, with Chimango Caracara *Milvago chimango* being related to the genus *Phalcoboenus* and Yellow-headed Caracara *Milvago chimachima* being sister to *Daptrius*. Furthermore, very low genetic divergence was found among the four species of the genus *Phalcoboenus*, with the lowest divergence being between White-throated Caracara *Phalcoboenus albogularis* and Mountain Caracara *Phalcoboenus megalopterus*. Our divergence time analyses revealed that the Polyborinae started to diversify in the Miocene, at about 14 Ma, and that the generalist/scavenger behaviour in the Falconidae appeared between 14 and 6.6 Ma. All speciation events within the caracaras occurred during the Pleistocene. This situation differs from the general pattern described for forest birds, in which most diversification events are older, occurring primarily in the Pliocene and Miocene.

Keywords: foraging behaviour, historical biogeography, *Milvago* polyphyly, South America.

The avian order Falconiformes (11 genera and 65 species; International Ornithological Committee 2011) represents a broadly distributed lineage of raptorial birds with diverse behaviours, encompassing both the aerial swift hunting falcons and the neotropical generalists and carrion-eating caracaras. The Falconidae have traditionally been divided into two subfamilies: the Polyborinae (forest falcons, Laughing Falcon *Herpetotheres cachinnans* and caracaras) and Falconinae (Spot-winged Falconet *Spiziapteryx circumcincta*, Old World falconets and falcons) (White *et al.* 1994, Dickinson 2003). Recent molecular analyses have suggested that the Polyborinae are paraphyletic with the forest falcons (*Micrastur*) and Laughing Falcon (Herpetotherinae) being the sister group of the Polyborinae *sensu stricto* (caracaras) and the Falconinae clade (Griffiths 1999, Griffiths *et al.* 2004). Furthermore, the genus *Spiziapteryx*, traditionally assigned to the Falconinae due to its overall similarity to Old World falconets (*Microhierax* and *Polihierax*) (White *et al.* 1994, Dickinson 2003), has been associated with the Polyborinae as an early diverging lineage and thus as sister to the other lineages. This result is supported by both morphology (Griffiths 1999) and mitochondrial and nuclear DNA sequence data (Griffiths *et al.* 2004).

The numbers of species assigned to higher-level taxa within the Falconidae vary considerably: the Herpetotherinae (Laughing Falcon and forest

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falcons) includes two genera and eight species endemic to the Neotropics, the Polyborinae (caracaras) includes six genera and 12 species endemic to the New World, and the Falconinae (falcons) includes three genera and 45 species with a global distribution. As suggested by the number of genera, the subfamily Polyborinae is the most diverse in morphology and behaviour, with falconet-shaped birds (*Spiziapteryx*), chicken-like arboreal omnivores (*Daptrius*, *Ibycter*) and more terrestrial generalists and scavengers (*Caracara*, *Milvago* and *Phalcoboenus*). The core of the distribution for the Polyborinae is Central/South America (11 endemic species) with the remaining species, the Northern Crested Caracara *Caracara cheriway*, extending to southern North America. The subfamily includes several cases of disputed taxonomy, some of which have been resolved recently (e.g. Griffiths *et al.* 2004), while others await further study.

For example, caracaras, the clade including all large omnivorous species, have traditionally been divided into four genera: *Daptrius* (variously including the genus *Ibycter*), *Caracara*, *Milvago* and *Phalcoboenus* (e.g. Brown & Amadon 1968, Stresemann & Amadon 1979). By contrast, Vuilleumier (1970) divided the caracaras into two genera, *Daptrius* including the two species of forest caracaras (Red-throated Caracara *Ibycter americanus* and Black Caracara *Daptrius ater*) and *Caracara*, in which he included all species from the now recognized genera *Milvago*, *Caracara* and *Phalcoboenus*. Recent molecular data suggested that the two forest Polyborinae species are not directly related: *D. ater* is thought to be the sister-group of Yellow-headed Caracara *Milvago chimachima* and *Daptrius* (*Ibycter*) *americanus* sister to the *D. ater/Milvago/Phalcoboenus* group (Griffiths *et al.* 2004). Further, the genus *Caracara* is thought to be the sister-group of the clade formed by the remaining caracara genera, suggesting that the two genera delimited by Vuilleumier (1970) are polyphyletic.

To date, the monophyly of the two Polyborinae genera has not been tested. The genus *Milvago* includes two species that are largely parapatric, Chimango Caracara *Milvago chimango* and *Milvago chimachima*, which have been considered a superspecies by some (Brown & Amadon 1968), although differences in morphology and ecology suggest that the two species are distinct, with no documented hybridization (Vuilleumier 1970). The second genus, *Phalcoboenus*, includes four parapatric species that are distributed along the Andes above 2000–3000 m in the tropics and in the open lowlands of Patagonia. Three *Phalcoboenus* species (Mountain Caracara *Phalcoboenus megalopterus*, Carunculated Caracara *Phalcoboenus carunculata* and White-throated Caracara *Phalcoboenus albogularis*) are thought to be closely related and have been considered a superspecies or subspecies from a single species (Vuilleumier 1970). The latter decision was based on specimens with intermediate plumage between *caruncalata* and *megalopterus* and between *megalopterus* and *albogularis*. The Mountain and White-throated Caracara differ by plumage characteristics (black or white throat) and habitat preference (scrub vs. *Nothofagus* forest, respectively). Here we aim to further resolve the phylogenetic relationships and biogeographical history within the Polyborinae using a combination of several mitochondrial and nuclear markers.

METHODS

Sampling and data collection

We sampled at least one individual from all recognized species assigned to the Polyborinae with the exception of the extinct Guadalupe Caracara *Caracara lutosus* (Table 1, Dickinson 2003), which has been considered a subspecies of the *Caracara cheriway/plancus* species group (Bierregaard 1994a). Representatives of all subfamilies within the Falconidae (Herpetotherinae and Falconinae) were also included. Recent studies identified the Passeriformes to be among the closest relatives of the Falconidae (Hackett *et al.* 2008), whereas traditional hypotheses suggest a close relationship between Accipitridae and Falconidae (Accipitriformes), supported by both morphological and molecular analyses (e.g. Pereira & Baker 2006, Livezey & Zusi 2007). Therefore, trees were rooted with sequences from both Passeriformes (Northern Raven *Corvus corax*) and Accipitridae (Red-tailed Hawk *Buteo jamaicensis*).

DNA was extracted from fresh tissues (muscle, liver, kidney) using the Qiagen DNeasy extraction kit (Valencia, CA, USA) following the manufacturer's protocol. We extracted DNA from two toe-pad samples of *P. albogularis* in a room dedicated to historical DNA using a phenol-chloroform extraction protocol with the addition of 20 μL of dithiothreitol (DTT, 0.1 M) to facilitate the digestion.

We analysed DNA sequences from eight independent loci: a mitochondrial fragment of *c.* 2.4 kb

Table 1. List of taxa studied (following Dickinson 2003), and tissue/voucher number information.

Genus	Species	Tissue/voucher no.	Source
Caracara	*cheriway*	LSUMNS B-8513*	USA
Caracara	*cheriway*	AMNH DOT7696*	USA
Caracara	*cheriway*	LSUMNS B-49353*	USA
Caracara	*cheriway*	UWBM 69063*	Nicaragua
Caracara	*cheriway*	UWBM 81394*	Mexico
Caracara	*plancus*	NRM 937176*	Paraguay
Ibycter	*americanus*	LSUMNS B-1019*	Bolivia
Daptrius	*ater*	AMNH DOT8775*	Venezuela
Daptrius	*ater*	LSUMNS B-4185*	Peru
Falco	*columbarius*	CAS 91441*	USA
Falco	*peregrinus*	CAS 90669*	USA
Herpetotheres	*cachinnans*	KUNHM 90147*	Paraguay
Micrastur	*ruficollis*	NRM 937326	Paraguay
Micrastur	*semitorquatus*	LSUMNS B-11298*	Peru
Microhierax	*caerulescens*	AMNH DOT10891*	Unknown
Milvago	*chimachima*	NRM 947028	Paraguay
Milvago	*chimachima*	KUNHM 90184*	Paraguay
Milvago	*chimango*	USNM 635931*	Uruguay
Milvago	*chimango*	USNM 614585*	Argentina
Phalcoboenus	*albogularis*	UMMZ 204744* (TP)	Argentina
Phalcoboenus	*albogularis*	KUMNH 78323* (TP)	Argentina
Phalcoboenus	*australis*	AMNH DOT14660*	Argentina (Falklands)
Phalcoboenus	*carunculata*	CAS ESM-10	Ecuador
Phalcoboenus	*megalopterus*	CAS WOB-3	Captive (World of Birds Wildlife Sanctuary)
Phalcoboenus	*megalopterus*	LSUMNS B-22907*	Bolivia
Phalcoboenus	*megalopterus*	CAS HUA-02	Peru
Polihierax	*semitorquatus*	FMNH 391014*	Captive
Spiziapteryx	*circumcincta*	LSUMNS 18642*	Bolivia
Spiziapteryx	*circumcincta*	LSUMNS 18584*	Bolivia
Outgroup			
Buteo	*jamaicensis*	CAS89962*	USA
Corvus	*corax*	CAS90612*	USA

AMNH, American Museum of Natural History, New York, NY, USA; CAS, California Academy of Sciences, San Francisco, CA, USA; FMNH, Field Museum of Natural History, Chicago, IL, USA; KUNHM, University of Kansas, Natural History Museum, Lawrence, KS, USA; LSUMNS, Louisiana State University Museum of Natural Sciences, Baton Rouge, LA, USA; NRM, Swedish Museum of Natural History, Stockholm, Sweden; UWBM, University of Washington, Burke Museum, Seattle, WA, USA; UMMZ, University of Michigan Museum of Zoology, Ann Arbor, MI, USA; WOB, World of Birds, Houtbay, South Africa. Asterisks indicate tissue with voucher specimens. TP refers to individuals for which the DNA source was a toe-pad sample.

(encompassing the tRNa-Leu, ND1, trNA-Ile, tRNA-Gln, tRNA-Met and ND2 regions) and seven autosomal loci (myoglobin intron-2-MB-, β-fibrinogen intron-5-FGB-, transforming growth factor beta 2 intron-5-TGFB2-, phosphoenol pyruvate carboxykinase intron-9-PEPCK-, vimentin intron-8-VIM-, period homolog 2 intron-9, -PER- and recombination activating gene 1-RAG1). The primer sequences used for PCR-amplification and sequencing are detailed in Supporting Information Table S1a,b. We PCR-amplified the mitochondrial region in one fragment with the primers L3827 and H613 using TaKaRa LA Taq (TaKaRa Co. Ltd, Tokyo, Japan). The thermocycling conditions for the mitochondrial fragment included a hotstart at 94 °C, an initial denaturation at 94 °C for 3 min, followed by 40 cycles at 94 °C for 40 s, 56 °C for 40 s and 72 °C for 3 min, and was completed by a final extension at 72 °C for 15 min. The thermocycling conditions for the nuclear introns included a hotstart at 94 °C, an initial denaturation at 94 °C for 3 min, followed by 35–40 cycles at 94 °C for 40 s, 55–60 °C for 30–45 s and 72 °C for 30–45 s, and was completed by a final extension at 72 °C for 10 min. Nuclear introns were PCR-amplified using the Recombinant Invitrogen Taq (Invitrogen Co., Carlsbad, CA, USA). Purified PCR products were cycle-sequenced using Big Dye terminator

chemistry (AB, Applied Biosystems, Foster City, CA, USA) in both directions with the same primers as used for PCR-amplification, and run on an automated AB 3100 DNA sequencer. Heterozygous sites in the nuclear loci were coded using the appropriate IUPAC code. Individuals showing length-polymorphism at particular loci were cloned using the TOPO TA cloning kit with pCR2.1 vector and Mach1 cells (Invitrogen), following the manufacturer's protocol. Between four and 10 clones were sequenced per individual. All sequences have been deposited in GenBank (accession numbers JN650302–JN650437).

Phylogenetic analyses

Phylogenetic analyses including estimation of individual gene trees and a concatenated approach were conducted using maximum likelihood (ML) and Bayesian inference, as implemented in RAXML v.7.0.4 (Stamatakis 2006, Stamatakis *et al.* 2008), MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003) and BEAST 1.6 (Drummond *et al.* 2002, 2006, Drummond & Rambaut 2007). The most appropriate models of nucleotide substitution were determined with TOPALI v.2.5 (Milne *et al.* 2009) and the Bayesian information criterion (BIC). Maximum likelihood and Bayesian analyses under the concatenated approach were performed allowing substitution model parameters to differ among loci (Nylander *et al.* 2004). The relevance of partitioning the protein coding genes by codon position was assessed using Bayes Factors (B_F; Nylander *et al.* 2004). A value greater than 4.6 for $\ln B_F$ was considered to be strong evidence against the simpler model (Jeffreys 1961). For MRBAYES 3.1.2, we used default priors for the base frequency and substitutions models. For the gene tree analyses, we ran several preliminary analyses by changing the branch-length prior, from *unconstrained: exp (10)* to *unconstrained: exp (150)*, and the temperature from 0.2 to 0.1. Better mixing was always achieved using a temperature of 0.1 instead of the 0.2 default value. For the gene tree analyses, we also observed better mixing of the chains and higher likelihood using the *unconstrained: exp (10) branchlength* prior for the mtDNA data and *unconstrained: exp (100)* or *unconstrained: exp (50)* for nuclear data. Analyses of the concatenated data were performed using an *unconstrained: exp (50) prior* for the branch length. In all MRBAYES analyses, four to six Metropolis-coupled Markov Chain Monte Carlo (MCMC) runs, one cold and three to five heated, were conducted for 20 million iterations, each with trees sampled every 1000 iterations. Two independent Bayesian runs initiated from random starting trees were performed for each dataset, and the log-likelihood values and posterior probabilities (PP) were checked to ascertain that the chains had reached stationarity. We ensured that the potential scale reduction factor (PSRF) approached 1.0 for all parameters and that the average standard deviation of split frequencies converged towards zero.

As an alternative to the traditional concatenated approach (see Degnan & Rosenberg 2006, Kubatko & Degnan 2007 for caveats regarding concatenation), we estimated the species tree using the coalescent method in STARBEAST (Heled & Drummond 2010) implemented in BEAST 1.6 (Drummond *et al.* 2002, 2006, Drummond & Rambaut 2007) with traditional species delineations. We assumed an uncorrelated lognormal molecular clock model for all loci and used the best-fit model, as inferred from the BIC, for each locus. Each locus had its own specific substitution (Supporting Information Table S2) and clock model. We assumed a Yule speciation process for estimation of the species tree. We ran the chains for 100 million iterations and excluded the first 20 million iterations as burnin. Two independent runs were performed to help assess convergence.

We used TRACER v.1.5 (Rambaut & Drummond 2007) to ensure that our sampling of the posterior distribution had reached a sufficient effective sample size (ESS > 200) for meaningful parameter estimation. The species tree was summarized as a Maximum Clade Credibility tree using TREEANNOTATOR (Drummond & Rambaut 2007).

Divergence time estimates

We used BEAST 1.6 to estimate divergence times within the Falconidae, assuming an uncorrelated lognormal clock model and the best-fit substitution models for each locus. We assumed a Yule speciation process for the tree prior. The split between the Falconidae and the Polyborinae was used as a calibration point, estimated at least 16.3 Ma ago based on the earliest Falconinae fossil *Pediohierax ramenta* (Wetmore 1936) from the Late Hemingfordian (20.43–16.3 Ma) to the Early Barstovian (16.3–13.6 Ma) (Becker 1987). We used a lognormal distribution (zero set off 16.3, lognormal

mean: 0.8, lognormal standard deviation: 0.61); the 95% credibility interval of the prior distribution was 17–23.0 Ma ago. The latter value corresponds to the beginning of the Miocene epoch (International Commission on Stratigraphy 2010). We specified uniform priors (0, 10) for the substitutions matrices for the three mitochondrial partitions, uniform priors for each of the rate heterogeneity parameters (Γ) and default priors for the remaining parameters. MCMC chains were run for 5×10^7 steps and were sampled every 1000 steps. We ran the analyses twice and discarded the first 5×10^6 steps as the burnin. TRACER v.1.5 was used to visualize the posterior distributions for all parameters.

RESULTS

Mitochondrial DNA

We obtained the complete mitochondrial fragment (tRNA-Leu to ND2) for all individuals included in this study. The length of the sequenced fragment differed among species due to length variation in the intergenic segments and insertion-deletions in tRNAs and ND1. A codon insertion was observed in *Spiziapteryx* ND1 sequences, inducing differences in the specific stop codon utilized: AGG/AGA was found for non-Falconidae and Herpetotherinae, whereas TAA was found in the Polyborinae and Falconinae (resulting in a 3-bp deletion in the two latter subfamilies). Further, the last two guanine nucleotides of ND1 overlapped with the neighbouring tRNA-Ile or non-coding sequences in the outgroup taxa and Herpetotherinae. The complete ND1 sequence was 975 bp long for the majority of ingroup taxa and 978 bp long for *Spiziapteryx*, the Herpetotherinae and outgroups.

The 50% majority rule consensus tree recovered from the Bayesian analyses (seven partitions, harmonic mean: −14 370.47) was well resolved (Fig. 1). The monophyly of the Polyborinae received moderately high support in the likelihood but not in the Bayesian analyses (ML Bootstrap: 89%, PP: 0.82), and the genus *Spiziapteryx* was recovered as the sister-group of all other Polyborinae genera with maximum support (Bootstrap: 100%, PP: 1.0). The genus *Caracara* was the first genus to branch-off within the Polyborinae (Bootstrap: 74%, PP: 0.88), followed by the genus *Ibycter* (Bootstrap: 100%, PP: 1.0). The genus *Milvago* was polyphyletic: *M. chimachima* was sister to the genus *Daptrius* (Bootstrap: 96%, PP: 1.0) whereas *M. chimango* was sister to *Phalcoboenus* (Bootstrap: 52%, PP: 0.61). Within *Phalcoboenus*, *P. carunculata* and Striated Caracara *P. australis* were recovered as sister species (Bootstrap: 71%, PP: 0.75) and in turn were sister to the species pair *P. megalopterus* and *P. albogularis* (Bootstrap: 98%, PP: 1.0). We found very low genetic differentiation between two species pairs: Northern Crested Caracara *C. cheriway* and Southern Crested Caracara *C. plancus* (uncorrected p-distance: 0.5%) and *P. megalopterus* and *P. albogularis* (uncorrected p-distance: 0.08%), with *P. albogularis* nested within *P. megalopterus*.

Nuclear sequence data

The number of base pairs analysed for all individual loci, as well as the best-fit model utilized, are indicated in Table S2. With the exception of the FGB sequence for *P. australis*, we obtained sequence data for all loci for fresh samples. For the two *P. albogularis* toe-pad samples, we obtained a complete sequence for MB and VIM and a partial sequence from TGFb2 and FGB. No PCR product was obtained from PER, PEPCK or RAG1 for *P. albogularis*.

The individual gene trees for each of the nuclear loci were similar in terms of topology and support values (Supporting Information Figs S1–S7). The phylogenetic relationships consistently recovered for each nuclear locus were: (1) *Spiziapteryx* positioned at the base of Polyborinae, (2) a basal divergence between *Caracara* and the *Ibycter/Daptrius/Milvago/Phalcoboenus* clade, (3) a sister-group relationship between *Ibycter* and *Daptrius/Milvago/Phalcoboenus*, and (4) non-monophyly of the genus *Milvago*. The topology resulting from the analyses of the concatenated nuclear dataset (5008 bp, harmonic mean: −30 466.35, Fig. 2) was nearly identical to the mitochondrial topology, differing only in the relative position of *Phalcoboenus carunculatus* with respect to *P. australis* and *P. megalopterus/P. albogularis*.

Concatenated dataset

The concatenated alignment was 7316 bp long (mitochondrial: 2308 bp, nuclear: 5008 bp). The resulting topology from the traditional concatenated approaches (RAXML and MRBAYES) was

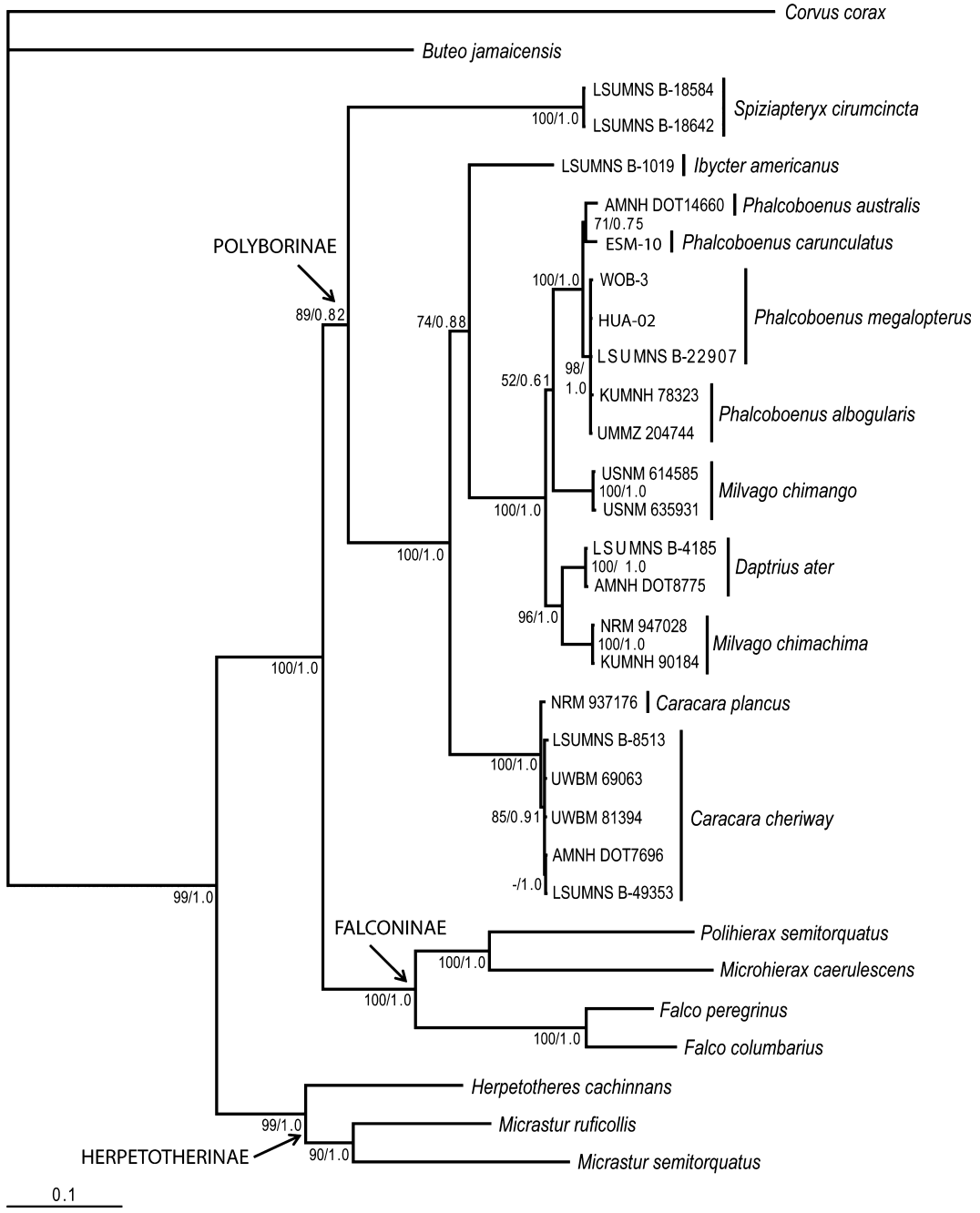

Figure 1. The 50% majority rule consensus tree resulting from the Bayesian analyses of the mitochondrial dataset (seven partitions). Numbers close to the nodes are maximum likelihood bootstrap support values/posterior probabilities

well resolved and supported (harmonic mean, −ln = −16 000.25), with > 70% bootstrap support or > 0.95 posterior probability for all but one node involving interspecific taxa. The node that was not supported centres on the relationship between *P. albogularis* and *P. megalopterus* (data not shown).

The tree resulting from the species tree approach (STARBEAST; Fig. 3) was identical to the nuclear topology (Fig. 2). All but two nodes

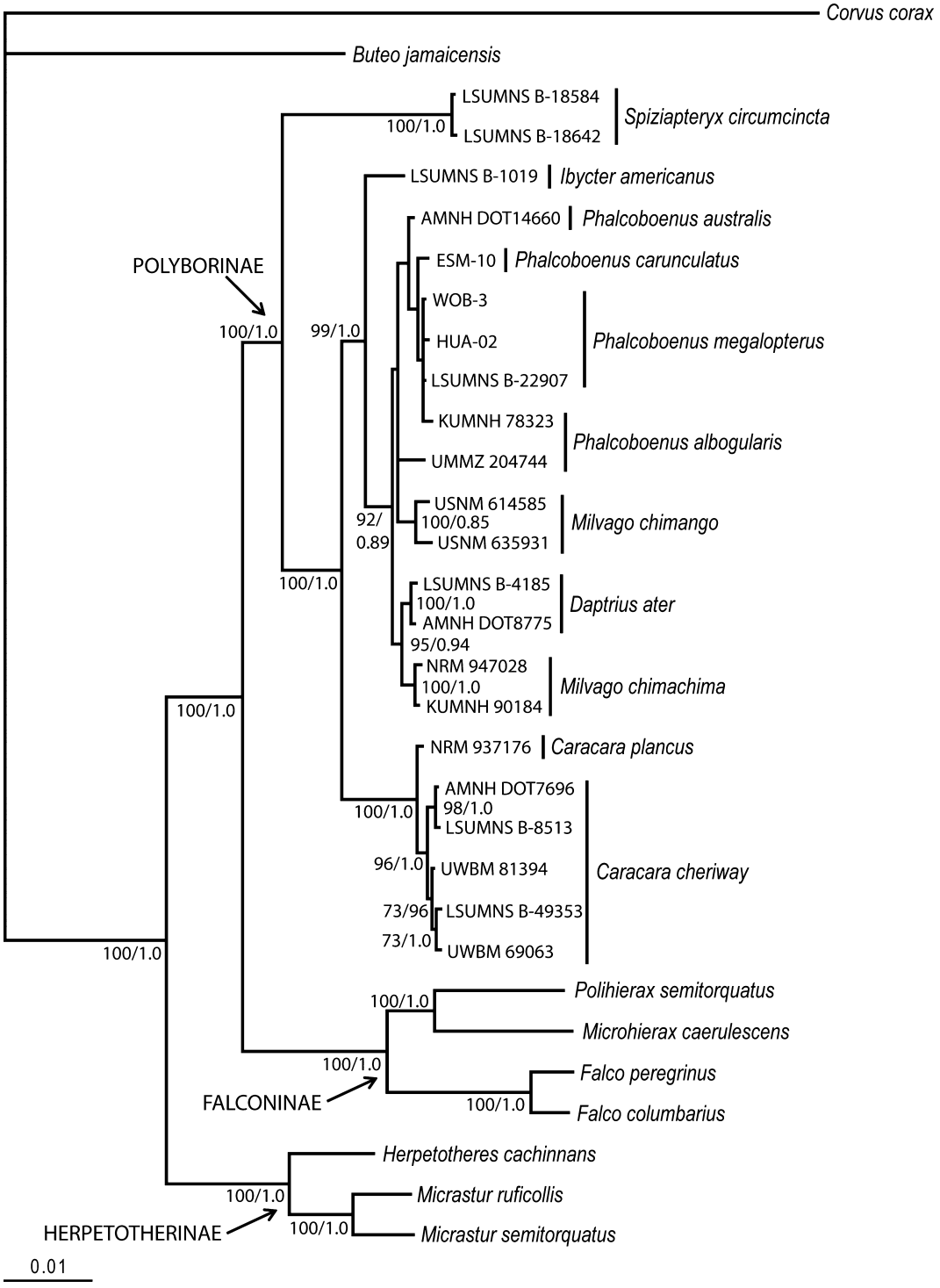

Figure 2. The 50% majority rule consensus tree resulting from the Bayesian analyses of the nuclear dataset. Numbers close to the nodes are maximum likelihood bootstrap support values/posterior probabilities

received posterior probabilities of 1.0. The two nodes with values < 1.0 recovered *M. chimango* as the sister-group of the genus *Phalcoboenus* (PP 0.85) and *P. carunculatus* as the sister-group relative to *P. australis* and *P. megalopterus/P. albogularis* (PP 0.84).

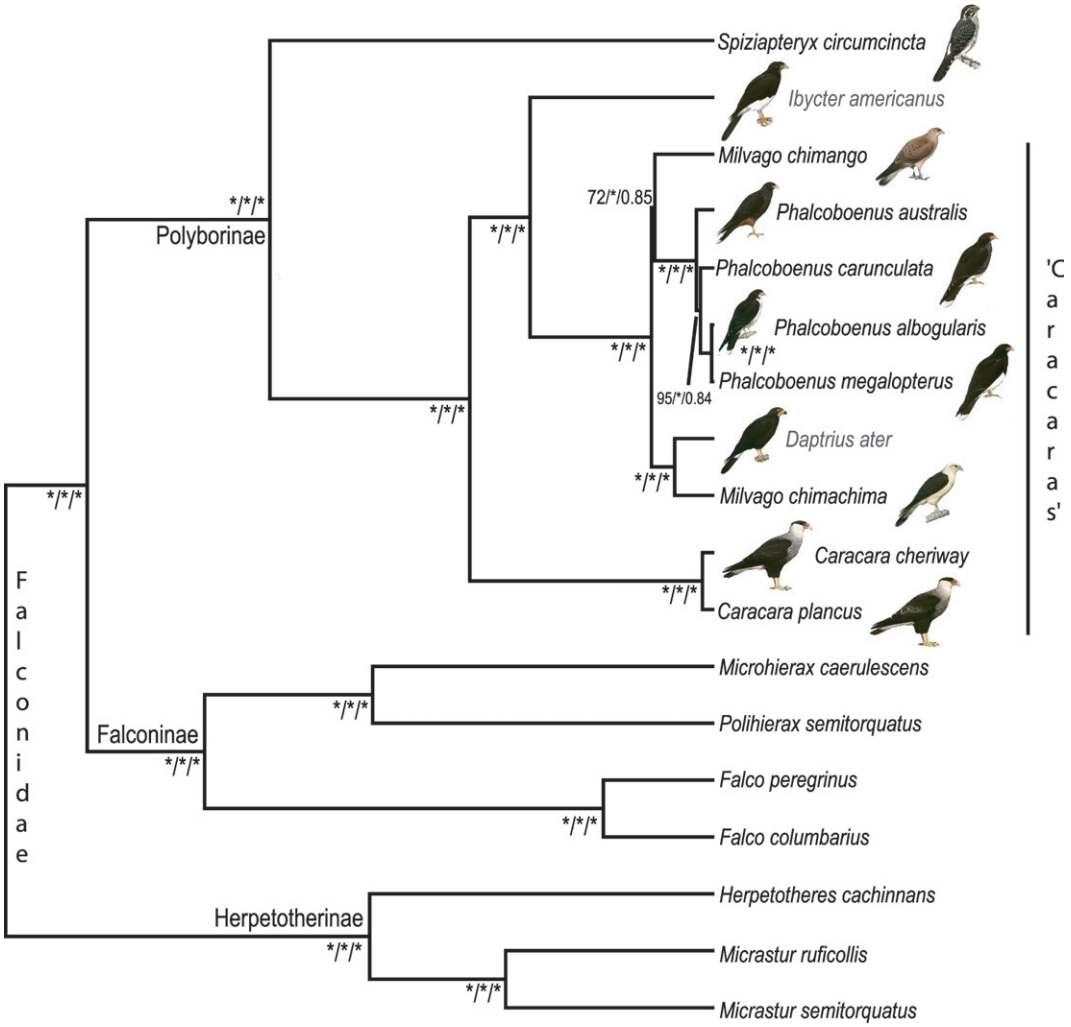

Figure 3. Species tree obtained using the coalescent approach implemented in STARBEAST. Values close to nodes represent support values (ML Bootstrap Concatenated Data/Posterior Probabilities Concatenated Data/Posterior Probabilities Species tree). Asterisks indicate maximum likelihood support values and posterior probabilities 100% and 1.0, respectively. Taxa in grey (*Daptrius* and *Ibycter*) are the caracara species that are found in more forested habitats. Illustrations were modified from Del Hoyo *et al.* (1994).

Divergence time estimates

Our analyses indicated that the Polyborinae started to diversify during the Miocene (14 Ma, 95% highest posterior density (HPD): 12.2–16.0 Ma, Supporting Information Table S3, Fig. 4), with the falconet genus *Spiziapteryx* splitting from the caracara genera. The 'caracara' morphotype, large raptorial birds with strong legs and a generalist diet, appeared between 14.0 Ma (95% HPD: 12.2–16.0 Ma) and 6.6 Ma (95% HPD: 5.4–7.8 Ma), corresponding to the period elapsed between the divergence of *Spiziapteryx*, a falconet-shaped genus, from all other Polyborinae, and of *Caracara* from the remaining caracara genera. The majority of speciation events for the caracaras occurred during the Pleistocene, from 1.9 Ma (95% HPD: 1.5–2.4 Ma) between *M. chimango* and the genus *Phalcoboenus*, to 0.3 Ma (95% HPD: 0.2–0.5 Ma) between *C. plancus* and *C. cheriway*.

DISCUSSION

Our analyses provide a well-supported phylogeny for the Polyborinae, yielding new insights into the systematics and biogeography of the group. All analyses confirmed that *S. circumcincta* was the first taxon to branch off within the Polyborinae, around 14 Ma. All other species traditionally referred to as caracaras form a monophyletic group with the first

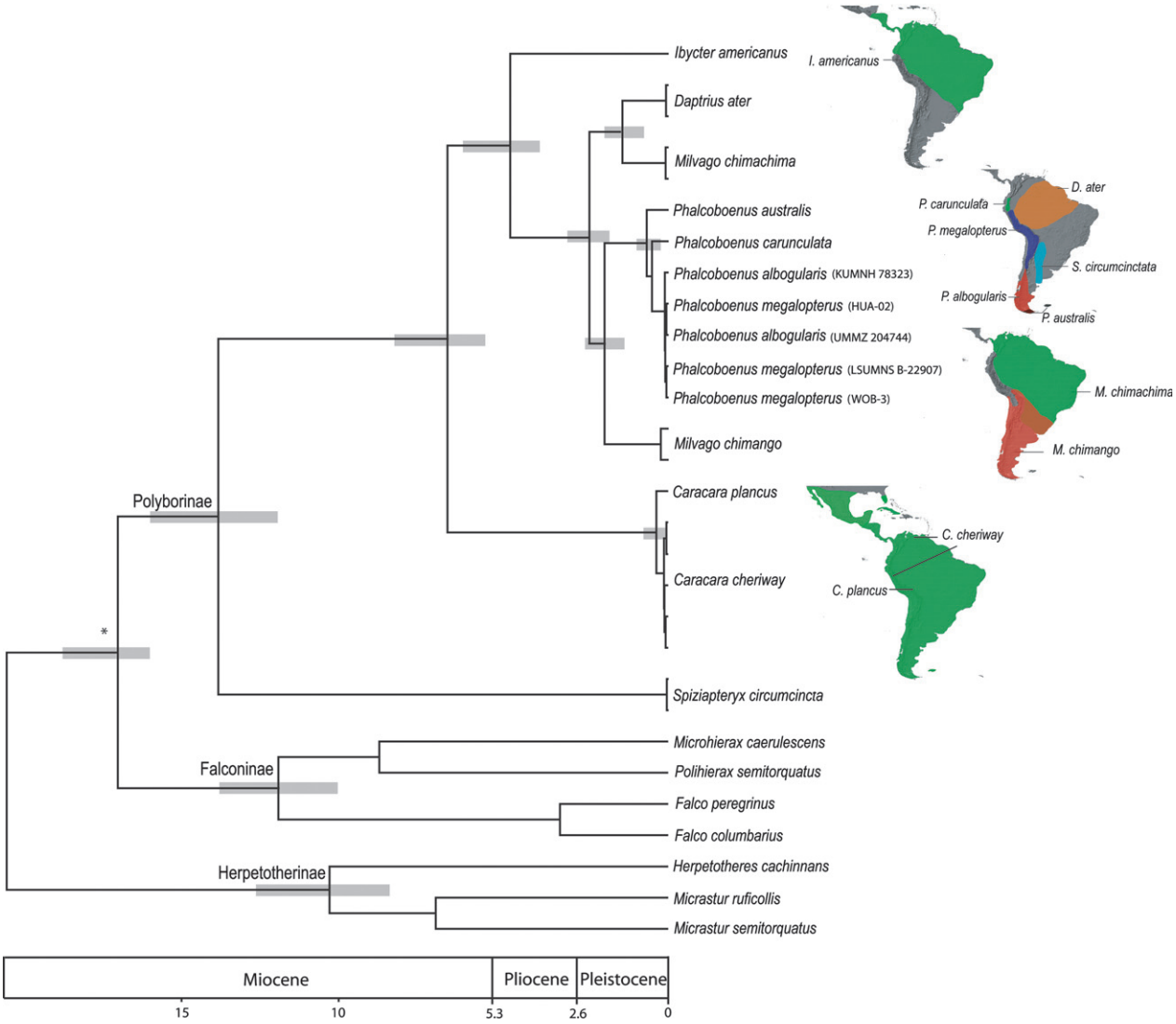

Figure 4. Divergence time estimates (in million years before present) for the primary lineages within the Falconidae using BEAST 1.6. Grey boxes represent the 95% HPD. Estimates were obtained using a fossil calibration point (*Pediohierax ramenta*; Becker 1987). All loci were allowed to have their own independent substitution matrix and an uncorrelated lognormal clock model was employed. The asterisk highlights the location of the calibration point. Distribution maps are derived from Del Hoyo *et al.* (1994).

genus to diverge being *Caracara*, about 6.7 Ma, suggesting that the generalist/scavenging-like behaviour in Falconidae appeared between 14 and 6.7 Ma. This pattern is paralleled by the diversification of the Cathartidae (New World Vultures), another lineage of opportunistic scavenger-like birds, where several genera appeared during the Miocene—Pliocene (Emslie 1988). Molecular data suggest that the extant Cathartidae initially diversified around 14 Ma (17.5–11.2 Ma) and subsequent diversification within the two primary clades occurred *c.* 10 Ma (Andean Condor *Vultur gryphus*, California Condor *Gymnogyps californicus* and King Vulture *Sarcoramphus papa*) and *c.* 2.7 Ma (*Cathartes* spp.; Brown 2010). Hence, several lineages of scavenging birds diversified in the New World during the last 14 Ma. This diversification may be linked to the aridification of the climate with accompanying expansion of open habitat enabling an increase in the abundance of mammalian grazers that could serve as potential food (Jacobs *et al.* 1999).

The majority of extant Polyborinae species originated during the last 2.6 Ma (Pleistocene; Fig. 4).

This situation differs from that found for another South American Falconidae genus, *Micrastur* (forest-falcons), where all speciation events occurred during the Miocene (23–5.3 Ma) and Pliocene (5.3–2.6 Ma) (Fuchs *et al.* 2011). The forest-falcons and caracaras differ in their habitat use, with forest-falcons being mostly found in humid and secondary growth forests, whereas caracaras generally inhabit open habitat and grassland (exceptions are the genera *Ibycter* and *Daptrius*, which are forest species). Hence, the differences in timing of diversification may reflect a more general pattern among vertebrates in South America, with forest species having diversified before the Pleistocene or at the Pliocene/Pleistocene boundary (Eberhard & Bermingham 2005, Ribas *et al.* 2005, 2007, 2009, Steiner *et al.* 2005, Brumfield & Edwards 2007, Santos *et al.* 2009, Fuchs *et al.* 2011), whereas open habitat and highland birds diversified later during the Pleistocene, with an increase in speciation rates for some lineages during the past million years (Weir 2006).

The two species of the genus *Caracara* differed by a mitochondrial uncorrected p-distance of 0.5%, which is one of the smallest divergences among Falconidae species based on similar sequence data (e.g. tRNA-Leu to ND2; the smallest being 0.08% between the Saker Falcon *Falco cherrug* and Gyrfalcon *Falco rusticolus*, J. Fuchs, J.A. Johnson, D.P. Mindell unpubl. data, as well as between *P. albogularis/P. megalopterus*, see below). Despite the low sequence divergence, the two *Caracara* taxa have been recognized as distinct species based on plumage characters (Dove & Banks 1999). Our samples of *C. cheriway* do form a monophyletic group with respect to the single *C. plancus* individual in the mitochondrial and in some nuclear trees; however, the individuals for the two species used in this study were sampled from the extremes of their geographical distributions. Dove and Banks (1999) suggested that biometric measurements are correlated with latitude for wing chord, bill length and bill depth. Hence, the pattern of molecular differentiation we found here could also be the result of isolation by distance. To enable more robust conclusions to be drawn concerning the taxonomic affinities within *Caracara*, further sampling is required, including samples from areas close to the Amazon River, where the distributions of *C. cheriway* and *C. plancus* overlap and where individuals with mixed plumage characteristics have been collected (Dove & Banks 1999).

The four *Phalcoboenus* species diverged about 1.9 Ma from their sister-group *M. chimango*, a species usually found in open areas in foothills but also sometimes recorded above 2500 m (Bierregaard 1994b). The last significant uplift of the Northern Andes occurred between 4 and 7 Ma and for the Central Andres at 6 Ma (Hooghiemstra & Van der Hammen 2004, Garzione *et al.* 2008, respectively). These results suggest that *Phalcoboenus* expanded into high-altitude habitats in the Andes rather than through parapatric differentiation across an altitudinal gradient resulting from the uplift of the Andes. A similar temporal colonization pattern of high-altitude habitat in the Andes has been highlighted for other lineages of birds (e.g. Brumfield & Edwards 2007, Parra *et al.* 2009, but see Burns & Naoki 2004, Ribas *et al.* 2007 and Sedano & Burns 2010 for counter examples) and plants (Hughes & Eastwood 2006). The four *Phalcoboenus* species that differ in plumage colour and extent of white on the belly and chest are currently distributed in allopatry along the Andes and in the Falkland Islands (Fig. 4). The phylogenetic relationships among the four species were not fully resolved in our analyses using over 7000 bp of DNA sequence data. The limited support for the position of *P. carunculatus* with respect to *P. australis* and *P. albogularis/P. megalopterus*, suggests a period of rapid diversification about 0.6 Ma within the genus. This time period experienced extensive glacial activity throughout much of the highland regions (Hooghiemstra & Van der Hammen 2004) and hence temporal fluctuations in habitat type and subsequent periods of allopatry may have contributed to the diversification of *Phalcoboenus* taxa.

Other avian taxa with similar geographical distributions and diversity exist along the Andean highlands. For example, the distributions of the suboscine passerines Slender-billed Miner *Geositta tenuirostris* and Short-billed Miner *Geositta antarctica* are very similar to those of *P. megalopterus* and *P. albogularis*, respectively. Yet, unlike *Phalcoboenus*, the two *Geositta* miners are not directly related to each other and belong to two distinct clades, suggesting an earlier divergence (Cheviron *et al.* 2005). Hence, these data suggest that the geographical communities where *Geositta* and *Phalcoboenus* currently reside did not develop from a single concurrent response to changes in the environment among existing lineages.

Although the four *Phalcoboenus* species have been considered a superspecies (Amadon & Bull

1988), the early split of *P. australis* in all our analyses agrees with Sibley and Monroe (1990) who considered *P. australis* to be sufficiently distinct from the remaining taxa to warrant species status. The status of *P. carunculatus* as part of the *P. megalopterus* superspecies is unclear, as its position within the genus is not well supported (Figs 1–3). Further, there is no evidence of hybridization between *P. carunculatus* and *P. megalopterus* (Poulsen 1993), suggesting that *P. carunculatus* may not be part of the *P. albogularis/P. megalopterus* superspecies. Molecular differentiation among the four species of the genus *Phalcoboenus* is limited, with the smallest genetic distance of 0.08% being between *P. megalopterus* and *P. albogularis*; the two lineages are not reciprocally monophyletic in either mitochondrial or nuclear data. These two taxa have variously been considered conspecific or split into two species based on plumage differences (black or white throat) and habitat affinities (scrub vs. *Nothofagus* forest), and birds with intermediate plumage have been described in Argentina (Vuilleumier 1970). From the present data, it is clear that a more thorough sampling of *P. megalopterus* and *P. albogularis* is necessary if we are to determine whether they form distinct species or should be recognized as different morphs or subspecies.

Systematic classification

Our results largely support the analyses of Griffiths (1999) and Griffiths *et al.* (2004) concerning the intergeneric relationships within the Polyborinae. This includes the relationships of *Spiziapteryx* with the Polyborinae, the early split of C*aracara* from other caracara genera and the lack of a sister relationship between *I. americanus* and *D. ater*. With increased taxonomic sampling in the present study, we are also able to demonstrate that the genus *Milvago* is polyphyletic, with *M. chimango* being sister to *Phalcoboenus*, whereas *M. chimachima* is most closely related to *D. ater* (Figs 1–4). The two *Milvago* species were considered a superspecies by some (Brown & Amadon 1968), whereas Vuilleumier (1970) suggested that differences in shape and ecology indicate that the two species did not diverge so recently. Wolters (1975–1982) also adopted this point of view and suggested that the two species belong to different subgenera, although he did not propose a genus name for *M. chimango*. The type species for the genus *Milvago* is *Milvago ochrocephalus* Spix 1824, which is a junior synonym of *Polyborus chimachima* Vieillot 1816. To maintain monophyletic genera, four options exist: (1) merging all species from the genera *Daptrius*, *Milvago* and *Phalcoboenus* into one genus, *Daptrius* Vieillot 1816; (2) having two genera *Daptrius* (including *ater* and *chimachima*) and *Phalcoboenus* Orbigny 1834 (including *chimango* and all species traditionally assigned to *Phalcoboenus*); (3) recognizing three genera, *Daptrius*, *Milvago* and *Phalcoboenus* (including *chimango*); or (4) recognizing four genera, *Daptrius*, *Milvago*, *Phalcoboenus* and naming a new genus for the species *chimango*. We recommend adopting option 3 (or three genera) because it maintains monophyletic genera while recognizing differences in overall shape, diet and habitat use between *M. chimachima* and *D. ater*.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. (a) List of primers used for amplification and sequencing of the fresh samples. (b) List of additional primers used for amplification and sequencing of the toe-pad samples (*Phalcoboenus albogularis*). All primers have been specifically designed for this study and primer combination amplified a 200–400 bp fragment. Only primers that successfully amplified a fragment are listed.

Table S2. List of substitution models selected under the Bayesian information criterion for each dataset.

Table S3. Divergence time estimates for the primary lineages within the Falconidae using BEAST 1.6.

Figure S1. 50% majority consensus rule tree resulting from the Bayesian analyses of myoglobin intron-2.

Figure S2. 50% majority consensus rule tree resulting from the Bayesian analyses of beta-Fibrino gen intron-5.

Figure S3. 50% majority consensus rule tree resulting from the Bayesian analyses of TGFb2 intron-5.

Figure S4. 50% majority consensus rule tree resulting from the Bayesian analyses of VIM intron-8.

Figure S5. 50% majority consensus rule tree resulting from the Bayesian analyses of PER intron-9.

Figure S6. 50% majority consensus rule tree resulting from the Bayesian analyses of PEPCK intron-9.

Figure S7. 50% majority consensus rule tree resulting from the Bayesian analyses of RAG1.

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