Mitogenomic analysis of extant condor species provides insight into the molecular evolution of vultures

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The evolution of large vultures linked to mountainous habitats was accompanied by extreme physiological and behavioral specializations for energetically efficient flights. However, little is known on the genetic traits associated with the evolution of these obligate soaring scavengers. Mitochondrial DNA plays a vital role in regulating oxidative stress and energy production, and hence may be an important target of selection for flight performance. Herein, we characterized the first mitogenomes of the Andean and California condors, the world’s heaviest flying birds and the only living representatives of the Vultur and Gymnogyps genus. We reconstructed the phylogenetic relationships and evaluated possible footprints of convergent evolution associated to the life-history traits and distributional range of vultures. Our phylogenomic analyses supported the independent evolution of vultures, with the origin of Cathartidae in the early Paleogene (~ 61 Mya), and estimated the radiation of extant condors during the late Miocene (~ 11 Mya). Selection analyses indicated that vultures exhibit signals of relaxation of purifying selection relative to other accipitrimorph raptors, possibly indicating the degeneration of flapping flight ability. Overall, our results suggest that the extreme specialization of vultures for efficient soaring flight has compensated the evolution of large body sizes mitigating the selection pressure on mtDNA.

Identification of the processes influencing genetic variation is critical to understand complex phylogenetic patterns, and essential for an accurate assessment of the conservation status of threatened species1–3. Previous studies revealed that the high aerobic capacity of the flight muscles of birds is modulated through the oxidative phosphorylation capacity of the mitochondria4. Thus, mtDNA may be an important target of natural selection, especially in high altitude birds exposed to cold temperatures and low oxygen pressure. For instance, selective analyses in Andean sparrows (Zonotrichia capensis) showed an elevational cline related to mt-haplotype frequency, but not with nuclear markers5, while a single mtDNA mutation was found to contribute to the exceptional ability of the bar-headed geese (Anser indicus) to migrate over the Himalayas6 and recent mitogenomic analysis revealed evidence of polygenic selection related to mitochondrial efficiency in high altitude Galliforms6. Despite the extreme specialization for highly efficient flights and the fact that many vultures inhabit high mountain areas, the role of mitochondrial adaptation in this guild remains unexplored.

Vultures constitute the only obligate vertebrate scavengers, exhibiting a wide array of adaptations that allows them to cover vast territories in search of patchy and unpredictable carrion resources7. Given their striking similarities, the phylogenetic relationships of this group have been challenging for many years, traditionally placing New World (NW) vultures (Cathartidae) along with Old World (OW) vultures (Accipitridae) in the order

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Falconiformes or in their own order7,8. Moreover, relationships within Cathartidae are still unclear largely due to incomplete taxonomic sampling and limited genetic resolution9. Recent nuclear genomic analyses showed that Falconiformes excluded the Turkey vulture (Cathartes aura), and suggested that Cathartidae split from a common ancestor of their sister group Accipitriformes at some point between the late Cretaceous and early Paleogene or even later10,11. However, nuclear genomic data is still scarce and the inclusion of more taxa is needed to test this hypothesis and to provide more precise dating. The use of mitogenomic data, on the other hand, has provided increased taxon sampling, including several OW vultures, resulting in powerful phylogenetic inferences12-14. However, with only one species annotated, NW vultures are still underrepresented in their availability of mitogenomic resources, leading to their exclusion from phylogenomic studies due to long branch attraction problems12,15,16.

The two extant species of condors represent both the most iconic and threatened vultures of the New World. The Andean (Vultur gryphus; wingspan of 3.2 m and weigh up to 16 kg) and California condor (Gymnogyps californianus; wingspan of 2.9 m and weigh up to 14 kg), are the World’s heaviest flying birds and the only living representatives of their genus17,18. While the latter has been the subject of intense, and so far successful, conservation efforts to prevent its extinction through the use of reproduction programs19,20, the former continues in steep decline. Recently, the Andean condor has been classified globally as Vulnerable and is Critically Endangered in their northern distribution due to persistent human persecution19,20. Previous analysis using historical samples of California condors from the nineteenth century revealed that the remarkable low levels of mtDNA diversity found in extant individuals are a direct consequence of recent human action21. Unlike the California condor, however, recent studies of the Andean condor including museum samples collected across their historical range almost two centuries ago revealed that although this species lost significant genetic variation during the early twentieth century, low mtDNA diversity is mostly ancient22. Notwithstanding, Andean condors from the southern Andes still retain normal levels of nuclear genetic variation23,24, suggesting that evolutionary constraints on genetic diversity only affected the mitochondrial genome.

It has been argued that the evolution of the body mass of vultures reflects the physiological limits imposed by the flight conditions in each habitat. Thus, the largest vultures occur in mountainous landscapes where the intense winds provide strong updrafts, while smaller ones inhabit lowlands with weak kinetic energy25,26. Condors, for example, are highly dependent on high mountain slopes to soar and are predicted to experience one of the highest metabolic costs of flapping flight27. California condors can fly > 1,000 m above ground level in the mountains of central California (up to 3,000 masl), while Andean condors can fly > 5,000 masl across the high Andes, and both species can move 350 km in a single day28,29. In addition, male condors dominate over females, possibly forcing them to schedule poorly efficient routines, resulting in higher selective pressures for metabolic efficiency in the latter30. Indeed, female Andean condors fly at higher altitudes31, and experience greater physiological costs associated with longer flights, resulting in shorter telomere lengths than males32. Similarly, the Himalayan Griffon (Gyps himalayensis; wingspan of 2.89 m and weigh up to 12 kg) and the Cinereus vulture (Aegypius monachus; wingspan of 3 m and weigh up to 13 kg), which might be considered as condor’s OW counterparts due to its body size and mountainous distribution, experience important selection pressures related to flight performance. The Himalayan Griffon, for example, can fly at extremely high elevations of up to 6,500 masl, employing behavioral adaptations33, while the Cinereus vulture exhibit a specialized hemoglobin alpha34 subunit of high oxygen affinity35. However, whether flight adaptations of large vultures involved the selection of mitochondrial variants is unknown.

Herein, we characterized the first complete mitochondrial genomes of the Andean and California condor. We investigated the phylogenetic relationships and divergence times across raptor species and evaluated whether the observed low mtDNA diversity in the Andean condor is the result of genetic constraints or a lack of characterization of informative mt-loci. We also performed selection analyses in the mitogenome of large species of NW and OW vultures to assess possible footprints of convergent evolution associated to their extreme life-history traits and mountainous distribution.

Methods
Sampling and sequencing. Total DNA was extracted from blood cell samples from a captive male Andean condor (ID: 01C5-218A), used as a primary genetic founder in the breeding program of the Buenos Aires Zoo (Ecoparque BA). The individual was originally from the Andean province of Mendoza (Argentina), a region encompassing the highest mountains in the Americas (up to 6,961 masl). We followed a standard phenol:chloroform extraction protocol for high-molecular-weight DNA and sequenced using a combination of short and long reads, employing the Illumina HiSeq4000 platform (2 × 151 bp, insert size = 350 bp) and Oxford Nanopore Technology MinION (two flow-cells; mean length: ~ 10 kbp) at the Centre National d’Analisi Genómica (Barcelona, Spain). We mapped our sequences against the reference mitogenome of the Turkey vulture (Cathartes aura; Accession: KX379719) and the uninformed method of UniCycler 0.4.834, which takes advantage of the long reads in combination with the short reads of the Andean condor. Given the reported conserved gene order

Assembly, annotation and comparative analysis. To assemble our mitochondrial sequences from de novo, we employed a combined strategy using alternative approaches. We used NOVOPlasty 4.232, seeded with the longest mitochondrial sequence available for each species (Andean condor: 2671 bp, Accession: AF173575.1; California condor: 2672 bp, Accession: AF173574), the multiple-seeded GetOrganelle 1.7.133 to include the partial sequence of their control region (Andean condor: 501 bp, Accession: AY129644.1; California condor: 569 bp, Accession: KX379719) and the de novo method of Unicycler 0.4.834, which takes advantage of the long reads in combination with the short reads of the Andean condor. Given the reported conserved gene order

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between Andean condors and chickens, we aligned our sequences to the reference mitogenome of *G. gallus*
in addition to *C. aura*, resulting in coherent consensus sequences. The assemblies were annotated with MITOS
using the vertebrate genetic code and RefSeq 89 Metzaoa as reference and visualized with OGDRAW 1.3.1
All genes (tRNAs, rRNAs and PCGs), as well as the control region (CR) were manually curated. We compared
the features of our assembled mitogenomes with all available vultures (*C. aura, G. fulvus, G. himalayensis, G.
crophotores* and *A. monachus*), including nucleotide identity similarity and relative amino-acid and synonymous
codon usage. In addition, we examined CG/AT skew rates, analyzed tRNAs secondary structures, and assessed
the characteristics of the CR (see details in Supplementary Material).

**Phylogenetic analysis and molecular clock.** To determine the phylogenetic position of NW vultures,
we used our assembled mitogenomes along with other 35 species/genera of birds available in the GenBank
database (Table S1): 26 representative taxons of raptors (Accipitridae, Cathartidae, Falconidae, Pandionidae,
Sagittariidae and Strigidae), and 9 outgroup species comprising Galliformes and Anseriformes. We used Phy-
loSuite v1.230 to conduct the bioinformatic pipeline with the help of several programs. The annotation of the
mitogenomes was standardized and ambiguously annotated tRNA genes were predicted with ARWEN v1.2.3.c39.
All sequences were aligned with MAFFT v7.48740 (using the codon and normal mode for PCGs and RNAs,
respectively) and PCG alignments were further refined with MACSE v2.041. Ambiguously aligned fragments
were removed in batches using GBLOCKS v0.9142 with a minimum length of a block of ten and allowing gap
positions. We used MODELLFINDER43 to find the best partition schemes and evolutionary models, employing
13 PCGs, and a control region (Fig. 1). As previously observed in partial mtDNA sequences35, NW vultures
showed a rela-

**Selection analysis.** We assessed signals of selection footprints in the PCGs by estimating the nonsynony-
mos-to-synonymous substitution rate ratio (ω = dN/dS). For this, we inferred a phylogenetic tree (as previously
described) including the available sequences from all raptor species included in the Afroaves clade (Table S1).
We used EasyCodeML 1.3149, employing the codon-aware filtered gene alignments to fit the models to our data.
Following recent studies50, we first performed an exploratory analysis estimating ω values for each terminal
branch using the free-ratios Model 1 to discard extreme results due to high saturation or low substitutions val-
ues. Because mt-genes tend to be conservative, we compared the fit of the Clade model C (CmC) with Likelihood
Ratio Tests (LRT) in order to detect subtle differences in site-specific selective constraints across the phylogeny51.
Our comparisons included: (A) vultures and the rest of the Accipitrimorphae; (B) NW and OW vultures; (C) All
large and small-sized vultures; (D) Large and small-sized species within OW vultures; and (E) within NW vul-
tures (see Fig. 4). We also performed correlation analyses between the maximum reported weight of each vulture
and our ω estimations for all PCGs. To further explore the data, we also tested the fitting of equivalent Branch
models (BM) and Branch-Site models (BSM, one species at a time) for genes exhibiting LRT
**Results and discussion Mitogenomic architecture.** The mitochondrial genome of the Andean and California condor was 16,808
and 16,870 bp in length, respectively, containing all 37 genes typical of vertebrates, including 22 tRNAs, 2 rRNAs,
13 PCGs, and a control region (Fig. 1). As previously observed in partial mtDNA sequences35, NW vultures
exhibited the ancestral avian gene order, while OW species differed from the standard arrangement due to the
presence of a pseudo control region14,52-54. The overall base composition of condors was similar (A = ~31%,
T = ~23%, C = ~32%, G = ~13%), showing a slight A + T bias for most regions, except ND6 that showed a marked
C + G bias, similar (r > 0.9; P < 0.001 in all cases) to other vultures (Figure S1). Our comparative identity analysis
revealed a clear phylogenetic pattern for both PCGs and rRNAs, except for COX1 and COX3 that showed a rela-
tively high similarity between OW and NW vultures, suggesting an important evolutionary constraint acting on
these regions. Most tRNA sequences were highly conserved across species, except for *trnR, S1, E, F, N* and *C*. The
The latter two showed an unusually high divergence, even within OW vultures, suggesting a possible pattern of selective relaxation (Fig. 2a). The tRNAs of condors displayed the typical cloverleaf of secondary structures found in other vultures, exhibiting larger structural differences with OW vultures (Figure S2).

Our analysis of PCGs in condors showed that COX3, ND4 and CYTB, share the same transcriptional exceptions of Turkey vultures, where TAA stop codon is completed by the addition of two 3' A residues to the mRNA. This exception has been reported for COX3, ND2 and ND4 in the Griffon (Gyps fulvus) and Cape (Gyps coprotheres) vultures, and for ND2 in the Cinereous vulture and Himalayan griffon. In addition, we found a frameshift in the ND3 gene of both condors, suggesting an alternative reading of mitochondrial mRNA. Previous studies have found that this feature is present in some birds, including some Cathartes species, the Cape and Griffon vultures, but seems to be absent in the Himalayan Griffon and Cinereous vultures, suggesting the existence of additional evolutionary constraints in the former taxa due to the maintenance of a secondary structure in the mRNA. Overall, we found that the relative amino-acid and synonymous codon usage in condors was similar to other vultures, exhibiting a frequent use of Leu, Thr, Ala, Ile, Gly, Ser, and Pro, with a common codon usage of CUA, ACA, GCC, AUC, GGA, UCA, and CCA, respectively (Figure S3).

The length of the CR of the Andean condor (1214 bp) was similar to the California condor (1260), Turkey vulture (1177 bp) and Gyps species (1201–1206 bp; A. monachus was excluded due to the poorly resolved CR). Our finding of high similarity of the Andean condor CR with that of the California condor (90%), Turkey vulture (88%) and Gyps species (~68%), similar to the observed in PCGs, is remarkable given that the CR of birds tends to evolve faster than PCGs. We detected common conserved regions in all three domains, following the same order in all vultures (Fig. 2b; Table S2). In the first domain, we found a Poly-C region usually observed in raptors, but exhibiting a TA island in the Andean condor, California condor, Turkey vulture and the Griffon vulture, and we also identified the adjacent extended termination-associated sequence ETAS1. The central domain contained short conserved blocks F, E, D, C and conserved sequence blocks CSBa, CSBb and the avian-specific Bird Box, while the third domain included CSB1 (Fig. 2b). However, we did not detect the presence of ETAS2, CSB2 or CSB3 in any species, suggesting that these sequences are not conserved among vultures. We detected a defined Short Tandem Repeat sequence (motif: AAC AAA C) at the 3' end of the third domain (Fig. 2b), in the Turkey vulture (14 repeats), the California (24 repeats) and Andean condor (20 repeats), previously reported as a source of length heteroplasmy in the latter, but not in OW vultures, where repeats are rich in T, and are located in the pseudo control region. The STRs of the Turkey vulture was able to form a stable stem-loop secondary structure, similar to those reported in some Strigiforms, while the stem of both condors was found in the terminal region of the third domain adjacent to the STRs (Figure S4).

As expected, the first and third domains showed higher intergeneric variability between condors, than the second domain (Fig. 2b). Moreover, the intraspecific variation pattern reported in California condors (Fig. 2b) is consistent with the expectation that regions exhibiting the greatest interspecific variability tend to vary the most within species. However, we found that most of the intraspecific variability reported in the Andean condor.
was situated between the Bird Box and CSB1, while the third domain showed an unusually invariant pattern (Fig. 2b), suggesting the existence of functional constraints on this region. Our observation of high similarity between the CR of our NW vultures, with a divergent time as old as ~17 My (Fig. 3), along with a similar substitution pattern to those of PCGs, and the observation that STRs could be involved in secondary structures, suggest that the extremely low variability found in the Andean condor could be possibly explained by a slow evolutionary rate, especially in the third domain. Slow rates of evolution of the CR has been previously reported in some Galliforms, Gruiforms, Passeriforms and Charadriiforms1,56, while STRs found in the third domain of owls and gulls suggested that these sequences might be functionally essential in terminating mitochondrial genome replication1,57. Thus, we suggest that as in the case of the California condor, targeting the first domain of the Andean condor (between ETAS1 and F-BOX), might provide higher intraspecific variability for population genetic studies, especially when using degraded samples for ancient DNA approaches.

**Phylogenetic position and divergence times.** Our phylogenetic analyses were consistent across sampling schemes and between ML and BI methods, recovering the same phylogenetic positions for OW and NW vultures, except in the BI analysis of scheme 3 (all PCGs + nonPCGs), that failed to group NW vultures with Accipitriforms (Figure S5). Nodal supports obtained with PCGs (scheme 1) increased with the addition of
rRNAs (scheme 2), but decreased with the inclusion of tRNAs (scheme 3), probably due to the conservative nature of these short sequences (Fig. 1a). Thus, we estimated the maximum clade credibility chronogram using 13 PCGs + 2 rRNAs. Overall, our results were similar to recent analysis using whole nuclear genome sequences of raptors10,11, splitting our in-group into two basal branches: Afroaves, including Accipitridae (eagles, hawks, kites, harriers, buzzards and OW vultures), Pandionidae (ospreys), Sagittariidae (secretary birds), Cathartidae (NW vultures), Strigidae (owls); and Australaves, including Falconidae (falcons and caracaras; Fig. 3). Hence, the inclusion of mitogenomic data of condors supports the hypothesis that NW and OW vultures belong to Accipitriformes10,58 and that vultures are not closely related to Falconiforms as previously believed.

Conspicuous phenotypic differences between NW and OW species, such as the functional hind toe, lack of syrinx, internal separation of the nostrils, or the lack of squirting behavior on the legs in OW vultures, among others7,9, suggest that evolutionary constraints (parallel evolution sense neo-Gouldian59) are not driving their overall similarities. Moreover, NW vultures seem to be more sensitive to lead contamination than OW species20, while the striking tolerance to Diclofenac in the Turkey vulture (> 100 times than in OW vultures) suggests that NW vultures are less vulnerable to the toxic effects of non-steroidal anti-inflammatory drugs60. If true, components of genetic variation of the detoxification metabolism have diverged between NW and OW vultures. Future comparative analysis of the nuclear genome between these groups would help to understand to what extent NW and OW vultures exhibit divergent genetic pathways. This information will be critical not only to improve diagnosis and treatments, but also to assist in management plans aimed at maximizing adaptive genetic variability of threatened populations.

Our molecular dating of the origin of NW vultures in the early Paleogene (61.2 Mya; CI 72.5–50.3; Fig. 3) differs from the estimates of recent analysis using whole nuclear genome data of raptor species81, but is in agreement with previous studies including wider taxon sampling8,10,47,58. A partial explanation for this discrepancy is that...
Zhou et al. had a sole representative taxon of NW vultures (Turkey vulture) and lacked genomic resources for the extant representatives of Pandionidae (osprey) and Sagittariidae (secretary bird), resulting in a delayed estimate of the origin of Cathartidae (36 Mya). In fact, the fossil record indicates that Sagittariid, Accipitrid and Pandionid lineages split before the Oligocene, with the oldest putative representatives *Pterogyps schlosseri*, *Milvoides tempski*, and *Palaeocircus cuvieri*, occurring in the early Oligocene, middle and late Eocene, respectively. Therefore, our estimated divergence time of NW vultures agrees with the age of the oldest known fossils of the stem group of Cathartidae, *Diotropornis elioti* and *Paracathartes howardae*, around the early-mid Eocene, almost 40 to 55 Mya, respectively. Thus, the observed discrepancy between studies highlights the importance of using a combination of proper calibration information and broad taxon sampling. In addition, our determination of the diversification time of extant Cathartids between the mid and late Miocene (22–12 Mya; Fig. 3) is consistent with the earliest record of the condor-like vulture *Hadrogyps aigleus* from the Barstovian period (~ 16–13 Mya) of California. This result supports the early arising of this group, probably initiated by the paleo-climatic conditions and diversification of megafauna during the Miocene (24, 65–66). The historical record shows evidence that NW vultures were highly diverse during that period and harbored large populations, widely distributed until the massive loss of megafauna during the Pleistocene–Holocene transition. Andean and California condors persisted into the abrupt ecological changes of the Holocene, possibly due to their foraging flexibility to exploit marine mammals, small terrestrial carcasses, and human-related carrion resources. The oldest undisputed records of extant Andean condors from Pleistocene deposits found in Peru and Chile suggest the recent origin of the species linked to the Andean mountains. Notwithstanding, previous studies proposed that condants evolved from a smaller species in North America and expanded to the south crossing the ancient coastline (before the Isthmus of Panama), during the late Miocene—early Pliocene (but see). If true, the distinctive large body size of California and Andean condors may represent a case of rapid convergent evolution driven by the selection pressures of the respective environments. Although more efforts are still needed to elucidate the natural history of vultures, the use of mitogenomic data in conjunction with an increased taxon sampling seems a promising approach to untangle the long-standing controversial relationships within this group.

**Selective relaxation.** Our selection analyses suggest that vultures experienced similar levels of genetic constraints to the rest of accipitrimorphs, except for the selective relaxation detected in ND1 and ND4 codons in the former group (Fig. 4 and S6; Table S3). A possible explanation for these differences might be related to the contrasting flight abilities of these raptors. Our finding of selective relaxation in those genes is in line with previous studies that found larger substitution rates in ND genes in weak versus strong locomotive birds due to the stronger selective pressure to eliminate deleterious mutations in the latter. Thus, it is possible that the stronger purifying selection observed in non-vulture accipitrils is related to the active metabolism of flapping flight, while the specialization of soaring flight in vultures resulted in the relaxation of selective constraints on the energy metabolism. In fact, recent reports demonstrated that vultures are able to fly with a strikingly minimal amount of flapping, which in the Andean condor can be as low as 1% of the total flight time, a record for any living flying bird. In addition to the locomotive strategy, our results also suggested the implication of phylogenetic constraints influencing mitochondrial evolution. NW vultures showed unique and radical non-synonymous substitutions in several genes compared to the rest of Accipitrimorphs (Figure S7). Furthermore, our estimates for NW species were smaller, suggesting stronger purifying selection (Figure S6), while our CmC analysis showed that OW vultures exhibited significant relaxation in ND3 and ND6 codons (Fig. 4; Table S3). This is consistent with our observation of large numbers of non-synonymous substitutions fixed in these genes of OW vultures (Figure S7), and lower ω values in the terminal branches of NW species compared to OW vultures (0.04 and 0.28, respectively; P < 0.005 in both cases). Notwithstanding, a larger number of species is needed to confirm the phylogenetic inertia of substitution patterns. Our CmC analysis of selective signals according to the body size of vultures revealed that larger species exhibited significant relaxation in ND6 codons, while smaller species showed selective relaxation in ND1 codons (Fig. 4). However, our regression analyses showed no relation between the body mass of vultures and their ω values in any gene (P > 0.22 in all cases). Furthermore, after dis-aggregating the CmC analysis within OW and NW vultures, our results suggested that the observed relaxation pattern might be explained by the strong relaxation signal of large OW species, especially in ND6 (Fig. 4; Table S3). This was further confirmed by our BM and BSM analysis (P < 0.001), suggesting the involvement of positive selection acting on ND6 sites (Table S3). Thus, our results propose that the evolution of body size in this guild is not associated with a common or simple mitochondrial genetic basis. Given that large OW species inhabit higher mountains and fly at higher elevations than condors, our detection of positively selected ND6 sites suggests a possible signal of mitochondrial adaptation to hypoxia. Previous studies have shown that positive selection of the NADH dehydrogenase complex plays an important role in adaptation to high-altitude in mammals and in galliform and passeriform birds. If true, our observed selection footprint should be also present in smaller high-altitude vultures. Thus, the inclusion of the mitogenome of the Ruppell’s griffon (*Gyps rueppelli*) will be instrumental to test this hypothesis, as this medium-sized vulture (~ 7 kg) can fly as high as 11.300 masl and exhibit specialized haemoglobins with a higher oxygen affinity. Finally, our CmC and BM analyses focused on NW vultures revealed a selective relaxation signal in condor’s *CYTB* gene, respect the Turkey vulture’s ortholog (P = 0.028; Table S3). This result could be related to the migratory behaviour of Turkey vultures, which might exert higher selective pressures on mt-genes related to the energy metabolism of this small vulture.

Overall, our results suggest that the exceptional specialization of soaring flight of vultures has compensated the evolution of body mass without pressing directional changes on mtDNA. The energetic cost of flapping flight...
is expected to be extremely high compared to soaring or gliding flight, and lighter species tend to flap more
than large ones. Given that vulture species are among the largest flying birds and evolved the most extreme
use of soaring flight, the relaxation of selective constraints on mt-genes may indicate the degeneration of
flapping flight ability. Our results are consistent with previous studies that found small differences in the energy
consumption between flight and resting in large vultures, suggesting that low metabolic rates might have buffered the selection pressure on mitochondrial efficiency. Notwithstanding, nuclear genomic sequences and an increased number of species are needed to better understand how the evolution of the obligate scavenger lifestyle affected metabolic selection.

**Data availability**
Details of data analyses and accession numbers for sequences retrieved from GenBank have been uploaded as part of the Supplementary Material. Additionally, mitogenomic sequences generated in this study have been deposited in the GenBank database under accession No. MZ223429 and BK059163.

Received: 25 May 2021; Accepted: 31 July 2021
Published online: 24 August 2021
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Acknowledgements
We thank M. Martí-Renom, A. Navarro and T. Alioto for helping in this study, and also A. Najenson, C. Corio, J. Ibarra, C. Krenk and M.B. Ceballos for their assistance during sample and permit processing. This research was supported by the National Council of Scientific and Technical Research of Argentina (CONICET) and funded by the National Agency of Scientific and Technological Promotion (PICT 2016-0354). International mobility funds were provided by the Marie Curie IRSES Project DEANN (PIRSES-GA-2013-612583). The study was conducted according to the guidelines of the Consejo Nacional de Cuidado y Uso de Animales de Laboratorio (CICUAL) of the Facultad de Ciencias Veterinarias—Universidad de Buenos Aires, approved by resolution No. 2013/44. Sample permits were issued by the Argentinean Environmental Ministry, under CITES permit No. 1540531.

Author contributions
D.N.D.P., S.A.L. and J.P. conceived the ideas; G.W. collected the biological sample; D.N.D.P. and M.G. performed laboratory procedures; H.D., C.M. and I.G. provided bioinformatic resources; D.N.D.P. and J.P. analyzed the data; F.C.A provided a supporting role in the selection analysis; and D.N.D.P. and J.P. led the writing with contributions from S.A.L. All authors reviewed the final version of the manuscript and gave approval for publication.

Competing interests
The authors declare no competing interests.
