Multi-locus phylogenetic inference among New World Vultures (Aves: Cathartidae)

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Abstract

New World Vultures are large-bodied carrion feeding birds in the family Cathartidae, currently consisting of seven species from five genera with geographic distributions in North and South America. No study to date has included all cathartid species in a single phylogenetic analysis. In this study, we investigated the phylogenetic relationships among all cathartid species using five nuclear (nuc; 4060 bp) and two mitochondrial (mt; 2165 bp) DNA loci with fossil calibrated gene tree (27 outgroup taxa) and coalescent-based species tree (2 outgroup taxa) analyses. We also included an additional four nuclear loci (2578 bp) for the species tree analysis to explore changes in nodal support values. Although the stem lineage is inferred to have originated ~69 million years ago (Ma; 74.5-64.9 credible interval), a more recent basal split within Cathartidae was recovered at ~14 Ma (17.1-11.1 credible interval). Two primary clades were identified: 1) Black Vulture (*Coragyps atratus*) together with the three *Cathartes* species (Lesser *C. burrovianus* and Greater *C. melambrotus* Yellow-headed Vultures, and Turkey Vulture *C. aura*), and 2) King Vulture (*Sarcoramphus papa*), California (*Gymnogyps californianus*) and Andean (*Vultur gryphus*) Condors. Support for taxon relationships within the two basal clades were inconsistent between analyses with the exception of Black Vulture sister to a monophyletic *Cathartes* clade. Increased support for a yellow-headed vulture clade was recovered in the species tree analysis using the four additional nuclear loci. Overall, these results are in agreement with cathartid life history (e.g. olfaction ability and behavior) and contrasting habitat affinities among sister taxa with overlapping geographic distributions. More research is needed using additional molecular loci to further resolve the phylogenetic relationships within the two basal cathartid clades, as speciation appeared to have occurred in a relatively short period of time.

Key words: Cathartidae; Miocene; vulture; condor; scavenger; species tree

1. Introduction

Cathartidae is a small family of large-bodied carrion feeding birds that occur throughout North and South America. There are seven recognized extant cathartid species, organized into five genera, although the family previously enjoyed greater diversity (e.g. Miller 1942; Fisher 1944; Emslie, 1988a, 1988b; Stucchi and Emslie, 2005; Snyder and Fry, 2012) that included several Old World representatives in the mid- to late-Paleogene (Rich, 1983), rendering the common name of the family (i.e. New World Vultures) a misnomer. Extant species are often divided into two groups based on size: the large California (*Gymnogyps californianus*) and Andean (*Vultur gryphus*) Condors and intermediate King Vulture (*Sarcoramphus papa*), and the smaller vultures that include the Black Vulture (*Coragyps atratus*), Turkey Vulture (*Cathartes aura*), and Greater (*Cathartes melambrotus*) and Lesser (*C. burrovianus*) Yellow-headed Vultures.

Cathartids are distributed over a large geographic area extending throughout North, Central, and South America, but the range of each species varies based largely on habitat preference and availability. Prior to European settlement, the geographic distribution of the California Condor once extended from southwest Canada to northwest Mexico along large cliffs for nesting and roosting, but today is greatly reduced and restricted to highly managed populations dependent on the release of captive-reared condors in southern and central California, northern Arizona, and southern Utah, USA, and Baja California, Mexico (Finkelstein et al., 2015). The Andean Condor range is restricted to cliffs for nesting and roosting and open country for foraging in western South America along the Andes Mountains (Venezuela and Colombia, where the species is fairly rare, to Tierra del Fuego in southern Chile and Argentina; Ferguson-Lees and Christie, 2001). The King Vulture is also observed in South America, but with a range extending from southern Mexico where the species is less common to northern

Argentina and southern Brazil, preferring lowland forested habitat including savannas and grasslands (Ferguson-Lees and Christie, 2001).

Both the Black and Turkey Vultures are abundant and have large geographic distributions including North, Central, and South America. The Black Vulture range extends throughout much of southern U.S. and Central and South America to central and southern Chile and Argentina, preferring open areas for foraging, including urban and refuse dumps, and forested habitat for nesting and roosting (Buckley, 1999). The Turkey Vulture has the largest geographic distribution of cathartid species extending from southern Canada through the continental U.S. and Central and South America to Tierra del Fuego and the Falkland Islands, often found in mixed farmland and forested habitat (Kirk and Mossman, 1998). The Lesser Yellow-headed Vulture is observed often in seasonally flooded lowland savannas, marshes, and coastal swamp and mangrove habitat ranging from southeast Mexico south through Brazil and northeast Argentina. The range of the Greater Yellow-headed Vulture consists of the Amazon Basin in South America, preferring tropical moist lowland primary forest habitat (Ferguson-Lees and Christie, 2001; Griffiths and Bates, 2002).

Although the monophyletic status of Cathartidae has never been seriously contested, phylogenetic relationships within the family remain unclear largely due to incomplete taxonomic sampling, limited resolution from few informative characters (e.g., Avise, 1994; Seibold and Helbig, 1995; Wink, 1995; Wink and Sauer-Gürth, 2004), and morphological trait convergence due to similar feeding habits (Hertel 1994) or poor preservation (Graves 2016). Several previous phylogenetic analyses of large genetic matrices have involved cathartid exemplars (e.g. Ericson et al., 2006; Brown et al., 2008; Hackett et al., 2008; McCormack et al., 2013; Jarvis et al., 2014; Prum et al., 2015), but those studies were more concerned with the relationship of Cathartidae

relative to other avian families, rather than relationships within the family itself. Phylogenetic placement for Cathartidae has been a long-standing question in avian systematics (see review in Brown and Mindell, 2009), with some studies purporting a close relationship between cathartid vultures and storks (Aves: Ciconiiformes).

Originally suggested from morphological data (Garrod, 1874; Ligon, 1967), the cathartid-stork hypothesis appeared to be supported by nuclear DNA-DNA hybridization (Sibley and Ahlquist, 1990) and mitochondrial (mtDNA) cytochrome b sequences (Avisé et al., 1994); however, both studies had significant methodological problems (see Mindell, 1992; Avisé and Nelson, 1995; Hackett et al., 1995; Helbig and Seibold, 1996). Although a few studies continue to assume a close cathartid-stork relationship (e.g. Tagliarini et al., 2009; Song et al., 2015; see also Houston, 1994), no recent phylogenetic analyses with broad taxon sampling and informative characters have recovered a close cathartid-stork relationship (e.g. Ericson et al., 2006; Livezey and Zusi, 2007; Brown et al., 2008; Hackett et al., 2008; Kimball et al., 2013), and a cathartid sister relationship with Accipitridae (hawks, eagles, and kites) appears well resolved in recent analyses (McCormack et al., 2013; Jarvis et al., 2014; Prum et al., 2015).

The family-level focus of the above studies has meant that Cathartidae has been poorly sampled, and consequently, cathartid genera and species-level relationships have been largely overlooked, which is surprising given the most recent taxonomic revision of the family was more than 50 years ago (Wetmore, 1964; but see Amadon, 1977 and Rich, 1983). Although based on incomplete taxonomic sampling (i.e., no phylogenetic studies to date have included *C. melambrotus*), previous analyses using mitochondrial cytochrome b (Cyt-b) and maximum parsimony have tended to group the Turkey and Lesser-headed Vultures as sister taxa (Wink, 1995; Wink and Sauer-Gürth, 2004) and the Black Vulture with the California Condor (Wink,

1995). Other cathartid species-level relationships remain untenable in previous studies due to low statistical support or incomplete reporting. Here, we use a multi-locus DNA sequence dataset to identify the phylogenetic relationships and timing of divergence among all extant species of New World Vultures.

2. Material and methods

2.1 Taxon sampling, DNA extraction, and sequencing

Between one and four individuals from each of seven recognized extant species of Cathartidae were sampled (n=15 total) as well as 28 outgroup taxa from Neognathae (Table S1). All but one of the outgroup taxa was used to represent a diverse set of taxa for fossil calibration purposes in the chronogram analysis, and two outgroup taxa (*Daptrius ater* and *Gyps bengalensis*) were used in the species tree analysis. *G. bengalensis* was used given the close affinity of Accipitridae and Cathartidae in recent phylogenetic analyses using much broader taxonomic sampling (Hackett et al., 2008; Kimball et al., 2013; Jarvis et al., 2014; Prum et al., 2015), and *D. ater* represented a non-Accipitriformes taxon. Total genomic DNA was extracted from blood, feathers, or muscle tissue using a DNeasy Qiagen Tissue Extraction Kit (Qiagen Inc., Valencia, CA, USA).

Two mtDNA protein-coding genes, cytochrome b (Cyt-b) and NADH dehydrogenase subunit 2 (ND2), and five sets of nuclear (nucDNA) introns (eukaryotic elongation factor 2, introns 5 & 6 (EEF2), glyceraldehyde-3-phosphate dehydrogenase, intron 11 (GAPDH), nonhistone chromosomal protein in high mobility group 17, introns 2 & 3 (HMG2), rhodopsin intron 1 (RHOD), and transforming growth factor beta 2, intron 5 (TGFb2)) were sequenced for all sampled individuals and species (hereafter referred to as the “5nuc/2mt DNA” dataset; Table S2). An additional four nucDNA introns (β -fibrinogen intron-5 (FGB), myoglobin intron-2

(MB), phosphoenol pyruvate carboxykinase intron-9 (PEPCK), and vimentin intron-8 (VIM); Table S2) were sequenced for a subset of samples including all sampled individuals (n=15) for the cathartid ingroup taxa and two outgroup taxa (Accipitridae: *Gyps bengalensis*, and Falconidae: *Daptrius ater*; hereafter the “9nuc/2mt” dataset) to augment the species tree analysis. All DNA sequences generated for this study were deposited in Genbank (accession numbers: KX534411-KX534745, KX534750).

Nuclear DNA sequences were aligned using MUSCLE v3.8.31 (Edgar, 2004) using the recommended default parameters. The protein-coding mtDNA contained no INDELS. The aligned 5nuc/2mt dataset consisted of 6225 bp for 34 taxa (42 individuals). The 9nuc/2mt matrix included an additional 2578 bp of nuclear sequence for a total of 8239 bp, but for only 17 taxa (see Table 1). PHASE v2.1.1 (Stephens et al., 2001), as implemented in DNASP v5.0 (Librado and Rozas, 2009), was used to infer the alleles for each nuclear locus, where results were compared across triplicate analyses. The recombination model was used with iterations of the final run 10 times longer than the initial runs. The output of the long final PHASE run was considered the best estimate of alleles. Alignment manipulations (concatenation and format conversion) were performed using phyx v0.1 (Brown et al. 2016).

2.2 Species tree analysis

Species trees were reconstructed using the coalescent-based model implemented in *BEAST v1.8.2 (Drummond et al., 2012) using both the 5nuc/2mt and 9nuc/2mt phased datasets with two outgroup taxa (*Gyps bengalensis* and *Daptrius ater*). The two mtDNA loci were partitioned by codon for all analyses. An uncorrelated lognormal relaxed clock was applied separately for loci, and best-fit substitution models and their respective partitions as determined using

Partitionfinder v1.1.1 (Lanfear et al., 2012; see Table S3). Clock models and trees were unlinked for all partitions, with the exception of mitochondrial partitions where topologies were linked. The Yule model of speciation with a piecewise linear and constant root population size model was used for each dataset. Reported mean rates of sequence evolution and associated 95% confidence intervals following a normal distribution were used to aid in convergence with mitochondrial codon partitions (1st, mean = 0.63%, std dev = 0.05%, or 95% CI 5.3-7.3 x 10⁻³ substitutions/site/lineage/Ma; 2nd, mean = 0.16%, std dev = 0.02%, or 95% CI 1.2-2.0 x 10⁻³ substitutions/site/lineage/Ma; 3rd, 5.8%, std dev = 0.3%, or 95% CI 5.2-6.4 x 10⁻² substitutions/site/lineage/Ma; Lerner et al., 2011), and the nuclear introns (mean = 0.128%, std dev = 0.001%, or 95% CI 1.1-1.5 x 10⁻³ substitutions/site/lineage/Ma; Ellegren, 2007).

Three independent runs with 4 x 10⁸ generations were performed for each analysis, sampling parameters every 10³ generations. For each data set, samples were pooled across runs with a 25% burnin using phyx (Brown et al., 2016). Convergence was assessed using Tracer v1.6.0 (Rambaut et al., 2014), judged as acceptable when runs resulted in similar posterior distribution curves for each parameter and all effective sample sizes (ESS) values were greater than 200. An additional set of runs was conducted assuming a strict clock for each locus partition to verify consistent topology and nodal support values. Maximum clade credibility species trees were calculated using TreeAnnotator v1.8.2 (Drummond et al., 2012).

2.3 Concatenated gene tree and divergence time analyses

As a corroborative analysis, we performed maximum likelihood (ML) phylogenetic reconstructions using RAxML v8.2.0 (Stamatakis, 2014) on the 34 taxon concatenated 5nuc/2mt dataset. An 8-partition model (three mtDNA codons and five individual nuclear genes) was

employed, with each partition allocated a GTR+gamma substitution model. Model optimization precision was set to 0.001 log-likelihood units. Node support was assessed through 100 non-parametric bootstrap analyses.

To add a temporal perspective to the study of cathartid relationships, a time-calibrated phylogeny was estimated using the concatenated 34 taxon 5nuc/2mt dataset with the program BEAST v1.8.2 (Drummond et al., 2012). The rate of molecular evolution was modelled assuming a lognormal distribution of uncorrelated branch-specific substitution rates (Drummond et al., 2006), with topology and divergence times estimated simultaneously. Similar to the species tree analysis, mtDNA was partitioned by codon, and each nuclear intron was partitioned separately. We allowed different loci to have unique patterns of branch rate heterogeneity by jointly estimating separate lognormal distributions for each locus. All mtDNA partitions were assigned to a single lognormal distribution describing mtDNA branch rate heterogeneity, although sub-partitions (codon positions) were allowed to have unique relative rates. In order to avoid confounding intra- and inter-specific rates of molecular evolution (Ho et al., 2005) we included only one individual per species.

To allow for the placement of temporal constraints and increase the efficiency of chronogram reconstruction, three nodes were constrained to be monophyletic. These constrained nodes are justified by the results below and published results from much larger data sets (Hackett et al., 2008; Kimball et al., 2013; Jarvis et al., 2014; Prum et al., 2015). First, the clade Galloanseres (containing all Galliformes and Anseriformes) was constrained to allow placement of the temporal constraint derived from the 66 million year (Ma) fossil duck *Vegavis iaai* (Clarke et al., 2005). Second, the clade uniting Procellariiformes and Sphenisciformes was constrained to allow placement of the temporal constraint derived from the 62 Ma fossil penguin *Waimanu*

manneringi (Slack et al., 2006). Third, the clade uniting Pandionidae and Accipitridae was constrained to allow placement of the temporal constraint derived from the 37 Ma fossil osprey *Palaeocircus cuvieri* (Harrison and Walker, 1976). Because of the limited amount of material from *P. cuvieri*, we conducted chronogram inferences with and without this constraint. We employed standard lognormal distributions to all temporal constraints with mean = standard deviation = 0.3, with the offset positioned to the age of the respective fossil because insufficient data exist regarding the extent of ‘ghost lineages’ in most lineages of birds. These distributions lend more credence to the fossil record than do conventional Boolean minimum constraints, but are broad enough to accommodate divergence time estimates from previous molecular studies.

Although several stem group fossil representatives exist for Cathartidae (Rich, 1983), many of these are of limited material and/or controversial taxonomic affinities; we therefore refrained from including these as calibration constraints. The age of Neognathae (the root of our tree) is unclear (e.g., Ericson et al., 2006; Brown et al., 2007, 2008; Brocklehurst et al., 2012; Jarvis et al., 2014). We therefore employed a broad uniform prior on the root spanning 130-70 Ma; this prior allows for results concordant with either molecular- or fossil-inferred timescales. Nodes not directly involved in temporal constraints were modeled using a birth-death prior. For both temporal calibration regimes (i.e. those with and without *P. cuvieri*), two replicate analyses were run for 10^8 generations, sampling every 2.5×10^3 generations. For each calibration regime, all samples following a 25% burnin were pooled using phyx (Brown et al., 2016) prior to parameter summary.

3. Results and discussion

Phylogenetic analyses identified a basal split within extant Cathartidae forming two clades with full nodal support. The monophyletic genus *Cathartes* [Lesser (*C. burrovianus*) and Greater (*C. melambrotus*) Yellow-headed Vultures, and Turkey Vulture (*C. aura*)] form a clade with the Black Vulture (*Coragyps atratus*), and the Andean (*Vultur gryphus*) and California (*Gymnogyps californianus*) Condors and the King Vulture (*Sarcoramphus papa*) form the other clade (Figs. 1, 2, S1). Relationships within these two clades, however, were less conclusive when comparing the species tree (Fig. 1), chronogram (Fig. 2), and concatenated ML tree (Fig. S1) using the same set of nuclear and mtDNA loci (5nuc/2mt; see also Brown, 2010).

The species tree, chronogram, and concatenated ML tree using the 5nuc/2mt DNA dataset all supported a sister relationship between the Black Vulture and *Cathartes* spp. (Turkey and yellow-headed vultures), but lacked strong support for a yellow-headed vulture clade (*C. melambrotus* and *C. burrovianus*; Figs. 1, 2, S1). The California Condor (*G. californianus*) tended to group with the King Vulture (*S. papa*) in the chronogram and ML trees (Figs. 2, S1), but the relationship was not well resolved in the species tree analysis based on the 5nuc/2mt DNA dataset (Fig. 1). In contrast, there was a strong tendency in the species tree analysis for a sister relationship between the Greater and Lesser Yellow-headed Vultures with increased number of nuclear loci (i.e. 9nuc/2mt DNA), and a similar but weaker tendency for a sister relationship between King Vulture and Andean Condor (Fig. 1). These results therefore provide higher resolution for discerning cathartid phylogenetic relationships than previously reported based on a single mtDNA locus using maximum parsimony analysis (e.g., Wink, 1995; Helbig and Seibold, 1996). Topology and nodal support values did not change in the species tree analyses when a strict clock was used for each of the locus substitution rate parameters, nor

when different substitution rate priors were used for each mtDNA codon partition (results not shown).

Chronogram reconstruction revealed why phylogenetic inference within Cathartidae is difficult. Although the family has its origin in the Late Cretaceous (~69 Ma; 74.5-64.9 credible interval; Fig. 2) or early Paleogene (Jarvis et al., 2014; Prum et al., 2015), the crown clade is quite young diverging much later in the Miocene ~14 Ma (17.1-11.1 credible interval; Fig. 2), with multiple lineages arising within the two primary basal clades over a short period of time. Given this rapid radiation, morphological similarity amongst non-sister cathartid taxa, e.g., all cathartids lack a syrinx and intrinsic syringeal muscles and possess perforated nostrils, is understandable. Results for the ingroup taxa were insensitive to the inclusion/exclusion of the controversial 37 Ma fossil osprey (*P. cuvieri*) temporal constraint (Harrison and Walker, 1976), whereas age estimates for deeper nodes changed only slightly (< 2 Ma; only results using the constraint are presented in Fig. 2, but all results are available in the supporting information). The oldest putative stem-cathartid fossil material is *Paracathartes howardae* (Harrison and Walker, 1977) from Wyoming approximately 55 Ma (early Eocene). This taxon is somewhat contentious, as the material is represented by a single tibiotarsus and affinities are controversial. However, our results indicate that this fossil at least fits within our temporal timeframe for the family.

The timing of diversification among extant cathartid genera accords well with increased mammalian diversification, particularly large herbivores and predatory mammals in open environments, during the Mid-Miocene Climatic Optimum (Janis et al., 2000, 2004; Blois and Hadly, 2009). In fact, a much richer cathartid species diversity existed during that time period, increasing in numbers through the Plio-Pleistocene resulting in a large range of cathartid body sizes (Emslie, 1988a, 1988b) and presumed feeding habits (Alvarenga and Olson, 2004). In the

late Pleistocene, however, terrestrial mammalian megafauna diversity declined significantly, which most likely reduced the food supply for scavengers, contributing to the extinction of many cathartid species (Steadman and Martin, 1984; Koch and Barnosky, 2006).

Using stable isotope analyses, Fox-Dobbs et al. (2006) documented a strong correlation between dietary habits and cathartid species persistence in the late Pleistocene. Cathartid species that went extinct predominately possessed a terrestrial diet, or consumed animal carcasses that fed in a C_3 or C_4 grassland ecosystem. In contrast, cathartids that fed on a more marine diet were likely to persist (Fox-Dobbs et al., 2006) presumably because marine megafauna did not decline to the same magnitude as experienced by terrestrial megafauna during the late Pleistocene (Fox-Dobbs et al., 2006; Malhi et al., 2016). Being top consumers with an unpredictable and opportunistic food resource, reduced carrion availability on the landscape following the megafauna decline at the end of the Pleistocene suggests a possible contributing factor to the low species diversity observed within Cathartidae today (e.g. Steadman and Martin 1984; Emslie 1987).

In areas where cathartid species co-occur, competitive exclusion has also been suggested to restrict interactions among contemporary species due to limited food resources and possibly further limit cathartid species diversity. For example, a social dominance hierarchy based on body size often exists among cathartid species when feeding on large carcasses in open habitat (Wallace and Temple, 1987; Kirk, 1988; Hertel 1994; Buckley, 1997), yet abundance at the feeding site can also influence interspecific guild structure (Kirk, 1988; Carrete et al., 2010; see also Houston, 1994). Temporal segregation in feeding behavior based on differences in ability to locate carrion, particularly in forested habitat with visual obstructions and smaller-sized food items, has also been documented as a mechanism limiting cathartid species interactions

(Houston, 1988; Kirk, 1988; Lemon, 1991). A more developed olfactory sense among all *Cathartes* species, for example, provides an advantage for finding carrion (Stager, 1964; Houston, 1986; Lemon, 1991; Graves, 1992; see also Lisney et al., 2013), which may further reduce competition for limited resources among extant cathartid species that possess overlapping geographic distributions and similar dietary preferences.

Although the results from this study do not overturn our understanding of the systematic relationships of cathartids, they still provide important information about species phylogenetic relationships that may prove important to help set priorities for management and conservation. For example, high lead levels have been documented in tissue samples obtained from wild caught Black (Behmke et al., 2015) and Turkey Vultures (Martin et al., 2008; Kelly and Johnson 2011; Behmke et al., 2015) and California Condors (Cade, 2007; see also Golden et al., 2016). In fact, continued exposure to spent lead ammunition has prevented the California Condor from establishing a self sustaining population after near extinction (Finkelstein et al., 2012; Kelly et al., 2014). To what degree susceptibility to lead poisoning varies among cathartid species is not fully known, yet research does suggest a correlation between susceptibility and phylogeny. The two condor species possess a similar toxic and lethal response when lead is ingested (Pattee et al., 2006), while the Turkey Vulture has a higher tolerance to lead poisoning (Carpenter et al., 2003).

More work is needed to verify or further resolve sister taxon relationships and species limits within each of the two basal cathartid clades using additional molecular loci and increased sample sizes over a broader geographic distribution for each species. As shown with the species tree analyses, including additional loci could help identify sister taxa among cathartid species that remained unresolved in this study (i.e. King Vulture, California and Andean Condors).

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Table caption

Table 1. Sequence properties after excluding ambiguously-aligned sites for the 2 (species tree) and 27 (divergence time) outgroup alignments.

Figure captions

Figure 1. *BEAST maximum clade credibility cathartid species tree. Posterior nodal support values correspond with the (5nuc/2mt) / (9nuc/2mt) datasets; *, maximum nodal support. Species tree generated using an uncorrelated lognormal relaxed clock applied separately for each locus. Bird images modified from Houston (1994).

Figure 2. Maximum clade credibility chronogram using the concatenated 5nuc/2mt DNA dataset. The dashed vertical red line marks the K-Pg boundary, and error bars represent 95% posterior probability credibility intervals on inferred ages. Posterior node probabilities are shown only for ingroup nodes of probability < 1.0. An identical topology was recovered among cathartids in the RAxML analysis (see also Fig. S1), and bootstrap support values for the ingroup nodes <100 are shown. Galloanseres outgroup taxa not shown.

Figure 1

Figure 2

Table 1

Table 1 Sequence properties after excluding ambiguously-aligned sites for the 2 (species tree) and 27 (divergence time) outgroup alignments.

Locus (genome)	2 outgroup taxa			27 outgroup taxa		
	Aligned length (bp)	Variable sites (%)	Parsimony informative sites (%)	Aligned length (bp)	Variable sites (%)	Parsimony informative sites (%)
Cyt-b (mt)	992	305 (30.7)	221 (22.3)	1128	529 (46.9)	463 (41.0)
ND2 (mt)	1037	419 (40.4)	275 (26.5)	1037	632 (60.9)	564 (54.4)
All mtDNA 1 st	675	165 (24.4)	96 (14.2)	721	315 (43.7)	266 (36.9)
All mtDNA 2 nd	677	63 (9.3)	24 (3.5)	722	149 (20.6)	98 (13.6)
All mtDNA 3 rd	677	496 (73.3)	376 (55.5)	722	697 (96.5)	663 (91.8)
All (mtDNA)	2029	724 (35.7)	496 (24.4)	2165	1161 (53.6)	1027 (47.4)
EEF2 (nuc)	934	163 (17.4)	162 (17.3)	977	535 (54.8)	393 (40.2)
GAPDH (nuc)	430	96 (22.3)	93 (21.6)	543	338 (62.2)	266 (49.0)
HMGN2 (nuc)	678	165 (24.3)	164 (24.2)	767	518 (67.5)	428 (55.8)
RHOD (nuc)	999	131 (13.1)	131 (13.1)	1059	650 (61.2)	489 (41.2)
TGFb2 (nuc)	591	87 (14.7)	80 (13.5)	714	376 (52.7)	252 (35.3)
FGB (nuc)	616	83 (13.4)	83 (13.4)	--	--	--
MB (nuc)	722	64 (8.8)	63 (8.7)	--	--	--
PEPCK (nuc)	696	85 (12.2)	80 (11.5)	--	--	--
VIM (nuc)	544	84 (15.4)	81 (14.9)	--	--	--
All (nuc)	6210	958 (15.4)	937 (15.1)	4060	2417 (59.5)	1828 (45.0)
All (nuc/mt)	8239	1682 (20.4)	1433 (17.4)	6225	3578 (57.5)	2855 (45.9)