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How Many Taxa or Groups are within *Nasua nasua* and *Nasuella olivacea* (Procyonidae, Carnivora)? The Mitochondrial Reconstruction of the Complex Evolutionary History of the Coatis throughout the Neotropics and Some Insights into the Systematics of the Genus *Bassarycion*

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Abstract

The coatis are social carnivores of the family Procyonidae that inhabit the Neotropics (from Arizona, U.S., to northern Argentina and Uruguay). Traditionally, three species of coatis are placed in two different genera: Nasua (*Nasua nasua* distributed in South America; *Nasua narica*, distributed in Central America) and Nasuella (*Nasuella olivacea*, distributed in the Andean Cordilleras of Venezuela, Colombia, and Ecuador). Recently, a supposed new species of Nasuella has been reported (*N. meridensis*) in the Venezuelan Andean Cordillera. However, the systematics and the evolutionary history of the coatis are extremely confusing. Herein, we present an phylogenetic analysis of 345 coati specimens sampled from southern Mexico to Uruguay sequenced at eight mitochondrial genes (*ND5, ND4,Cytb*, *D-loop, COI, COII*, *ATP6*, and *12s rRNA*). We detected 19 main haplogroups (10 haplogroups in *N. nasua*, five haplogroups in *N. olivacea*, and four haplogroups in *N. narica*) and eight to ten sub-haplogroups (five-seven in *N. nasua*, and three in *N. narica*). The oldest and original haplotypes were from Colombian and Ecuadorian Andean Cordillera *N. nasua* specimens, followed by some haplotypes of specimens with the phenotype of *N. olivacea* also in the Colombian and Ecuadorian Andes, but with DNA closer to that of *N. nasua*. Thus, the Northern Andes seems to be the original point where the mitochondrial DNA coati diversification began around 13 millions of years ago (Late Miocene). This is in contrast to the traditional paleontological view, which considers that coati appeared in North America and later migrated into South America during the Pleistocene. The present molecular results more strongly support a single unique genus (*Nasua*) rather than the traditional two genera. If we apply the Phylogenetic Species Concept (PSC), at least 19 species of coatis should be described. However, the evolutionary history of the coatis is complex and as such, we consider that a greater preponderance of evidence supports one to three species. Two (or three) *N. olivacea* haplogroups were more related to some *N. nasua* haplogroups than to other *N. olivacea* haplogroups. This means that *N. olivacea* is polyphyletic or there were some Andean *N. nasua* groups that have evolutionarily converged to similar phenotypes as those of the "true" *N. olivacea*. In fact, we detected the presence of a "true" *N. olivacea* haplogroup distributed within the Cuzco Department in southern Peru. Thus, this is the first study to molecularly detect this taxon in Peru. Furthermore, we detected *N. nasua* specimens with haplotypes very related with those of the original *N. narica* haplogroup. The first *N. narica* haplogroup was detected in the trans-Andean and Pacific Ecuador. This haplogroup later generated the Central American *N. narica*. In Central America, we clearly detected three haplogroups, two of them closely related (northern Costa Rica, Nicaragua, El Salvador, Honduras, Guatemala, and southern Mexico). However, the fourth *N. narica* haplogroup present in southern Costa Rica, Panama, and northern Colombia was mitochondrially introgressed by *N. olivacea*. Therefore, *N. narica* originated in South-America and later migrated to Central America.

Keywords: Coatis • Genetic heterogeneity • Nasua • Nasuella • Miocene • Mitochondrial genes • Phylogenetic analyses • Pleistocene • Pliocene • Speciation

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Introduction

The Carnivora Order encloses 16 families divided into two monophyletic super-families, Caniformia and Feliformia [1-3]. The first super-family encloses the families Canidae, Ursidae, Mephitidae, Ailuridae, Procyonidae, Mustelidae, Otaridae, Odobenidae, and Phocidae, while the second super-family contains Felidae, Nandiniidae, Prionodontidae, Eupleridae, Herpestidae, Hyaenidae, and Viverridae [4].

The biogeographic history of the Procyonidae in the Americas is complex and the evolution of this family has been very important in understanding the history of the Great American Biological Interchange (GABI) [5,6]. The fossil

record shows that Procyonidae originated and dispersed two separate times from North America into South America.

The first dispersion event occurred in the Late Miocene (9-5 Million Years Ago, MYA) with the appearance of the fossils of the genera Cyonasua and Chapalmalania from the Huayqueriense–Montehermosense in South America [7]. This was before the closure of the Isthmus of Panama around 3-2.5 MYA [8-11]. However, the Procyonidae of this first colonization event apparently went extinct by the end of the Middle Pleistocene [6,12]. The second dispersion of Procyonidae from North America into South America is thought to be the one made by the ancestors of the extant genera during the late Pleistocene, 0.126 MYA [11]. Of the current Procyonidae genera, only Nasua and Procyon had fossils dated to the Lujanense period (0.126-0.009 MYA) [6,7,13]. However, Koepfli, et al. (2007) showed, by using nine nuclear and two mitochondrial (mt) genes that the diversification within the extant genera of Procyonidae occurred in the Middle to Late Miocene. This agrees quite well with the first colonization event but not with the second one as supported by the paleontological record.

One of the most known Procyonidae is the coatis. They are diurnal, highly social mesocarnivore (1.3 – 6.0 kg). Coatis forage both arboreally and terrestrially, and their diet includes primarily fruits, invertebrates and occasionally small vertebrates [14,15]. Females and immature males form permanent groups while mature males are solitary, joining the groups only during the mating season [15,16].

Among coatis, three, or five, extant species are currently recognized: the white-nosed coati (*Nasua narica*), the South American or brown-nosed coati (*Nasua nasua*), and the Western Mountain Coati (*Nasuella olivacea*). Recently, there were claims that the Eastern Mountain Coati (*Nasuella meridensis*) in the Venezuelan Andean Cordillera should be a new full species [17,18]. In the same way, McFadden, et al. (2008) designated the white-nosed coati from the Cozumel Island (Caribbean Mexico) as a distinct species (*Nasua nelsoni*) [19]. However, we are reluctant to accept these two species, as we show below.

N. narica is the only coati species distributed in North, Central and South America, from Arizona and New Mexico in the United States to northern Colombia [20]. The brown-nosed coati (*N. nasua*) is distributed in South America, from Colombia and Venezuela to Uruguay and northern Argentina [14,15,21]. It is found primarily in forested habitats, ranging from tropical rainforest and gallery forest to chaco, cerrado and dry scrub environments [21,22]. *N. olivacea* is found in the Andes of Venezuela, Colombia, and Ecuador in cloud forest and paramo at altitudes of 1,300-4,250 meters above sea level.

Koepfli, et al. (2007) were the first to try to determine the phylogenetic relationships among the genera within Procyonidae although they did not include samples of *N. olivacea*. This work was important because it was the first to estimate the temporal splits among and within the extant Procyonidae genera. The study also analyzed the possible phenomena which affected the colonization of this family in Central and South America.

Only a scarce number of molecular studies have covered the coatis. McFadden (2004) and McFadden, et al. (2008) analyzed a small fraction of mtDNA and concluded that the *N. nelsoni* from Cozumel Island is a full species [19,23]. Helgen, et al. only studied four specimens of *Nasuella*, three from the Western Colombian Andes (one specimen) and from the northern Ecuadorian Andes (two specimens), and one specimen from the Venezuelan Merida Cordillera. They analyzed only a small fraction of the mt*Cyt-b* gene (366 base pairs, bp). They reached the conclusion, with these preliminary data, that the unique sample from Venezuela was a different species (*Nasuella meridensis*) from the three samples from the Western Colombian Andean Cordillera and northern Ecuador. Tsuchiya-Jerep (2009) analyzed 90 specimens of *N. nasua* throughout Brazil. MtDNA analyses showed levels of diversity that were up to ten-fold higher for *N. nasua* relative to *Procyon cancrivorus* in Brazil. Five reciprocally monophyletic mtDNA phylogroups were recognized for *N. nasua* in this country, which were also supported as distinct populations by microsatellite analyses [24]. Neves-Chaves (2011) analyzed 215 specimens of *N. nasua* from seven localities from Minas Gerais state in Brazil. The control regions of mtDNA and microsatellite loci were analyzed. One of these populations (Mangabeiras Park) showed low intrapopulation genetic diversity, high coefficient of relatedness between individuals, reduced effective population size, Hardy-Weinberg departure, evidence of recent bottleneck, and moderate isolation from other sampled populations. [25] Silva, et al. (2017) analyzed 60 specimens in five populations of *N. narica* distributed throughout Mexico by means of the mt*Cyt-b* gene and 12 microsatellite loci. They found moderate to high levels of genetic diversity for both genetic markers. There were twenty-two different mt*Cyt-b* haplotypes throughout the five sampled areas and each sampled population harbored unique haplotypes. Only three haplotypes were shared between two different populations that were close geographically. All populations had high haplotype diversity ($H_{d\pm}$=0.968+0.008) but lower levels of nucleotide diversity (π=0.007+0.001) [26]. Niguenda-Morales, et al. (2019) found a high degree of genetic structure and divergence among populations that conform to five evolutionarily significant units of *N. narica* in Central America. They analyzed three mt genes and 11 microsatellite loci in 85 specimens of *N. narica*. The most southerly distributed population (Panama) split much earlier (3.8 MYA) than the northern populations (1.2 MYA). Estimated gene flow among populations was low and mostly northwards and westwards. They described phylogeographic patterns within *N. narica* which were associated with geographic barriers and habitat shifts likely caused by Pliocene-Pleistocene climate oscillations. In addition, they suggested that the dispersal of *N. narica* was south-to-north beginning in the Pliocene, not in the opposite direction during the Pleistocene as suggested by the fossil record [27]. Jaramillo and Ruiz-García (2021) analyzed the genetic structure of 74 specimens of *N. narica* sampled in Central America and in northwestern South America (Colombia and Ecuador) by means of two data sets (three mt genes, and complete mitogenomes). They detected six well-differentiated groups of *N. narica*, one of them, in southern Central America, and another in northern Colombia, were introgressed by mtDNA of *N. olivacea* [28]. The colonization route was south to north, such as Niguenda-Morales, et al. (2019) claimed. Therefore, for *N. narica,* there are more molecular phylogeographic and systematic studies than for *N. nasua* and *N. olivacea*. For this reason, we centered this work especially in *N. nasua* and *N. olivacea*, although we included the sequences of *N. narica* that we obtained because we want to determine the relationships of the diverse *N. narica* haplogroups with those of *N. nasua* and *N. olivacea*.

We selected the study of mt genes as a first step to understand the phylogeography and the possible existence of full species within *Nasua* and *Nasuella* because these markers were those originally chosen to carry out the studies of phylogeography [29,30]. The mitochondrial genes are interesting markers for phylogenetic tasks because they include a rapid accumulation of mutations, rapid coalescence time, lack introns, a negligible recombination rate, and haploid inheritance [31]. They also have a high number of copies per cell which makes mitogenomic data easy to obtain and sequence especially in low quality samples, such as hair, teeth or small pieces of skin [32,33]. Despite representing a single linked locus, selection pressures and evolutionary rates are highly heterogeneous across the mtDNA [34,35]. For all of these reasons, mt genes have a higher discrimination power of genetic groups within species, or very related species, than the nuclear genes do [36-39].

However, the mitochondrial gene poses several problems including heterogeneity in base composition at each codon position, and third-codon position saturation [38,39]. Additionally, extreme care should be taken when using mitochondrial genes for resolving taxonomic problems because gene trees do not necessarily correspond well with species trees. If we use the gene tree to estimate divergence time for the species tree, the species will appear to have diverged more recently than they really did [40]. Furthermore, mitochondrial data show only the evolution of the female lineages, and this could miss hybridization events between close species when males are the gene flow vectors ('mitochondrial capture') [41]. To fully understand the evolutionary biology and number of taxa in the coatis, there first needs to be supporting evidence from nuclear data (microsatellites or SNPs), not just mtDNA data. Conscious of this two-pronged approach, we offer our analysis of mitochondrial DNA as the first step towards resolving this question. In fact, mtDNA have been extremely useful in detecting new and previously unnoticed taxa [42-47]. The mt results should be very important in helping to determine conservation strategies based in the systematic classification of the

differentiated populations below the species level. The uncertainty about these conservation units can lead to confusion in the establishment of management plans and errors in setting priorities [48] (O'Brien, 1994). Therefore, there will be a necessary second step of analyzing nuclear markers to detect possible hybridization or gene flow events among different lineages.

The main aims of the current work were as follows: 1) To determine how many significant different main haplogroups (and sub-haplogroups) were detected in the three traditional coati species with eight mt genes, and their systematic implications especially in *N. nasua* and *N. olivacea*; 2) To determine how many species of coati are really there; 3) To identify which were the oldest and original coati haplotypes, at what species belonged, where, and when they appeared; 4) To determine if there are incomplete lineage sorting, introgression or hybridization among the different traditional species of coatis; 5) To determine if *N. olivacea* is also distributed outside the current known distribution near the Venezuelan, Colombian, and Ecuadorian Andes; 6) To determine if the sequence of the evolution of coatis occurred from Central America to South America (colonization from north to south) or vice versa (colonization from south to north), and 7) To show some new insights into the systematics of the genus *Bassarycion*, which was used as outgroup of *Nasua-Nasuella*.

Materials and Methods

Samples

We analyzed eight mt genes in 345 coati (222 *N. nasua*, 51 *N. olivacea*, and 72 *N. narica*) across 16 Latin American countries. Twenty-four specimens were used as outgroups: 5 *Bassarycion neblina* (Colombia and Ecuador), 5 *Bassarycion medius* (Colombia and Ecuador), and 14 *Bassarycion alleni* (Ecuador, Peru, and Bolivia). For a detailed origin of all the specimens studied, (Table 1 and Figure 1). The samples analyzed were basically coming from specimens hunted in Indian communities as food resources as well as from road kill specimens. A minor fraction of the samples (of Colombian origins) were obtained from the mammologist museum of the Instituto Alexander von Humboldt (Villa de Leyva) with the permissions of their responsibles. No review from the ethics committee was required, as our research work applied on samples of museum skins and killed road and previously hunted animals for human food and did not involve any direct manipulation or disturbance of live animals by the researches.

Table 1. Sources of the coatis collected and analyzed at eight mitochondrial loci (ND5, ND4, Cytb, D-loop, COI, COII, ATP6, and 12s rRNA) (222 *Nasua nasua*, 51 *Nasuella olivacea*, and 72 *Nasua narica*).

Species and Location		Number of samples (*n*)
Nasua nasua n=222		
Colombia *n*=53	Amazonas Department	10
	Antioquia Department	7
	Cauca Department	2
	Caquetá Department	1
	Cauca Department	1
	Cundinamarca Department	2
	Guaviare Department	3
	Huila Department	3
	Meta Department	8
	Nariño Department	1
	Norte de Santander Department	2
	Quindio Department	1
	Risaralda Department	3
	Santander Department	2
	Tolima Department	1
	Valle del Cauca Department	5
	Vichada Department	1
Ecuador *n*=37	Esmeraldas Province	3
	Guayas Province	3
	Imbabura Province	3
	Manabí Province	3
	Napo Province	6
	Orellana Province	3
	Pastaza Province	8
	Pichincha Province	4
	Santo Domingo de Tsáchilas Province	2
	Sucumbios Province	1
	Zamora-Chinchipe Province	1
Peru *n*=69	Apurimac Department	5
	Cuzco Department	6
	Huánuco Department	1
	Junín Department	1
	Loreto Department	18
	Madre de Dios Department	17
	Pasco Department	1
	San Martín Department	6
	Ucayali Department	14
Bolivia *n*=26	Beni Department	16
	Cochabamba Department	5
	La Paz Department	3
	Santa Cruz Department	2
Brazil *n*=23	Amazonas State	7
	Goias State	1
	Paraná State	15
Paraguay *n*=9	Alto Paraná Department	9
Uruguay *n*=4	Tucuarembó Department	3
	Artigas Department	1
Chile *n*=1	Robinson Crusoe Island	1
Nasuella olivacea n=51		
Colombia *n*=42	Antioquia Department	1
	Boyacá Department	5
	Caldas Department	4
	Cauca Department	2
	Chocó Department	1
	Cundinamarca Department	14
	Nariño Department	1
	Norte de Santander Department	9
	Risaralda Department	3
	Tolima Department	1
	Valle del Cauca Department	1
Ecuador *n*=6	Carchi Province	1
	Cotopaxi Province	1
	Morona-Santiago Province	1
	Napo Province	1
	Pichincha Province	2
Peru *n*=2	Apurimac Department (possible *N. olivacea*)	1
	Cuzco Department	1
Bolivia *n*=1	Cochabamba Department (possible *N. olivacea*)	1
Nasua narica n=72		

Mexico *n*=24	Campeche State	10
	Chiapas State	3
	Quintana Roo State	1
	(Cozumel Island)	
	Tabasco State	7
	Yucatan State	3
Guatemala *n*=24	Alta Verapaz Department	3
	Guatemala Department	1
	Izabal Department	2
	Peten Department	18
Belize *n*=3	Rio Bravo Conservation Area	3
Honduras *n*=7	Olancho Department	5
	Roatán Island	2
El Salvador *n*=3	La Libertad Department	3
Nicaragua *n*=1	Nueva Segovia Department	1
Costa Rica *n*=3	Alajuela Province	2
	Puntarenas Province	1
Panama *n*=1	Colón Province	1
Colombia *n*=3	Antioquia Department	1
	Chocó Department	2
Ecuador *n*=3	Guayas Department	2
	Morona-Santiago Department	1

To clasificate the 345 coati specimens analyzed, we took into accont several facts. First, the geographical origins and the previous reported species in the area sampled. For instance, in Central America, there is not any report of *N. nasua* or *N. olivacea*, only *N. narica*. Second, pelage and body characteristics. *N. nasua* is distinguished from *N. narica* by pelage on the muzzle that is brown or gray (white in *N. narica*), and hairs on the nape of the neck that are in a reversed anterior position (different to *N. narica*). *N. narica* has a clear (white) area around the eyes (dark in *N. nasua*) and the paws are very dark or pale in *N. nasua*, meanwhile they are of a chocolate color. *N. nasua* has seven to nine dark bands in the tail, while *N. narica* has less marked bands in the tail. The hair of *N. nasua* has generally two color band (reddish and black), meanwhile the hair of *N. narica* has three color bands (pale yellow, chocolate, and pale yellow). *N. olivacea* has nine dark bands in the tail but sometime less notorious than in Nasua, because these bands are considerably thinner than in *Nasua*. The body color is pale yellow and olivaceus. The hair of *N. olivacea* has four bands (cream, dark coffe, pale yellow, and black). The length of the tail is shorter in Nasuella than in Nasua, and the length of the leg in *N. olivacea* in smaller than 68 mm (the total length of the body is smaller than 600 mm). Third, when it was possible, skull characters. For *N. nasua*, the palate is flat along the midline, rather than concave as in *N. narica*. In the first species, the sides of the nasal bones converge posteriorly rather than being parallel as in the second species. The skull of Nasuella is smaller and more slender than that of Nasua, the middle part of the facial portion is greatly constricted laterally, and the palate extends farther posteriorly [14,49].

Molecular methods

We extracted DNA from skin, muscle and blood samples with a modified phenol-chloroform procedure [50]. DNA from hair/follicles and teeth were extracted using 10-20% Chelex 100 resin (Bio-Rad, USA), with several modifications from Walsh, et al. (1991) [51].

We amplified the eight mt genes with primers from the following papers: 1) 1,140 base pairs (bp) of *Cytb* [52], 2) 657 bp of *COI* [53], 3) 720 bp of *COII* [54], 4) 710 bp of *ND4* [55], 5) 1,800 bp of *ND5* [56], 6) 329 pb of *ATP6* [57], 7) 376 pb of *12s rRNA* [58], and 8) 306 bp of *D-loop* [59]. This equaled a total of 6,038 bp.

PCR conditions for these mt genes were brought to a volume of 25 µl with 13.5 µl of Mili-Q H_2O, 3 µl of $MgCl_2$ 1 mM, 1 µl of dNTPs 0.2 mM, 1 µl of each primer (0.1 µM), 2.5 µl of buffer 10X, and one unity of Taq Polymerase with 50-100 ng of DNA. The PCR temperatures were 95° for 5 minutes followed by 10 cycles of 1 minute at 95°C, 1 minute at 58-64°C and 1.5 minute at 72°C, 25 cycles of 1 minute at 95°C, 1 minute at 52-60°C and 1.5 minute at 72°C and one final extension of 15 minutes at 72°C. All amplifications, including positive and negative controls, were checked in 2% agarose gels. Those samples that amplified were purified using membrane-binding spin columns (Qiagen). The PCR products were sequenced in both directions using the Big Dye™ kit in an ABI 377A automated DNA sequences. A consensus of the forward and reverse sequences was determined using the Sequencher program. The sequences were concatenated using the criteria of Vaidya, et al. (2011) [60].

The GenBank accession numbers of the coati specimens analyzed are from MT587713 to MT587788, MW410859 to MW410908, and MW419814 to MW419853.

Phylogenetic studies

jModeltest v2.0 [61] and Mega 6.05 software [62] were applied to determine the best evolutionary mutation model for the sequences analyzed for each individual gene, for different partitions and for the concatenated sequences. Akaike information criterion (AIC) [63,64] and the Bayesian information criterion (BIC) [65] were used to determine the best evolutionary nucleotide model.

Phylogenetic trees were constructed by using two procedures: Maximum Likelihood tree (ML), and Bayesian Inference tree (BI). ML tree was applied to the specimens of the coati taxa analyzed, whilst BI tree was applied to the haplotypes found in these coati taxa.

The ML tree was obtained using RA × ML v7.2.6 software [66]. We used the partitioning scheme and best-fit models chosen by the PartitionFinder software [67]. This program was used to objectively determine the optimal model of evolution and partitioning scheme simultaneously (for the partitions, codons 1+2 combined, and codon 3, for each gene, including control region and 12s rRNA). Therefore, a total of 16 blocks were analyzed, although the major part of them showed similar results. For this reason, the phylogenetic ML tree showed was using simultaneously all the information. Best-fit models were selected using Bayesian information criteria under a 'greedy' search scheme using a subset of models specific to RA × ML. The GTR+G+I model (General Time Reversible model+gamma distributed rate variation among sites+proportion of invariable sites) [68] was used to search for the ML tree. We estimated support for nodes using the rapid-bootstrapping algorithm (–f a -x option) for 1,000 non-parametric bootstrap replicates [69]. The haplogroups of coatis were considered significant when bootstraps were higher than 70% (lax limit) [70].

The BI tree was performed also using a GTR+G+I model. BI trees were completed with the BEAST v1.8.1 program [71]. Four independent iterations were run using three data partitions (codon 1, codon 2, codon 3) with six Markov Chain Monte Carlo (MCMC) chains sampled every 1,000 generations for 20 million generations after a burn-in period of two million generations. We checked for convergence using Tracer v1.6 [72]. We plotted the likelihood versus generation and estimated the effective sample size (ESS>200) of all parameters across the four independent analyses. The results from different runs were combined using LogCombiner v1.8.0 software [73] and TreeAnnotator v1.8.0 software [74]. A Birth-Death speciation model and a relaxed molecular clock with an uncorrelated log-normal rate of distribution [75] were used. Posterior probability values provide an assessment of the degree of support of each node on the tree. Values higher than 0.90 were considered strong support for monophyletic haplogroups [76,77]. Trees were visualized in the FigTree v1.4 software [78]. The BEAST v1.8.1 program was run to estimate the time to most recent common ancestor (TMRCA) for different nodes of the BI trees. We used a prior of 12.5+1 MYA (97.5% confidence interval: 10.54-14.46 MYA) for the split of the common ancestor of *Bassarycion* and Nasua+Nasuella, and a prior of 2.7+0.2 MYA (97.5% confidence interval: 2.31-3.09 MYA) for the split of the common ancestor of *Bassarycion medius* and *Bassarycion alleni*. These priors were obtained from Koepfli, et al. (2007).

To reconstruct the possible relationships among the haplogroups of the three "a priori" coati species analyzed and to estimate possible divergence

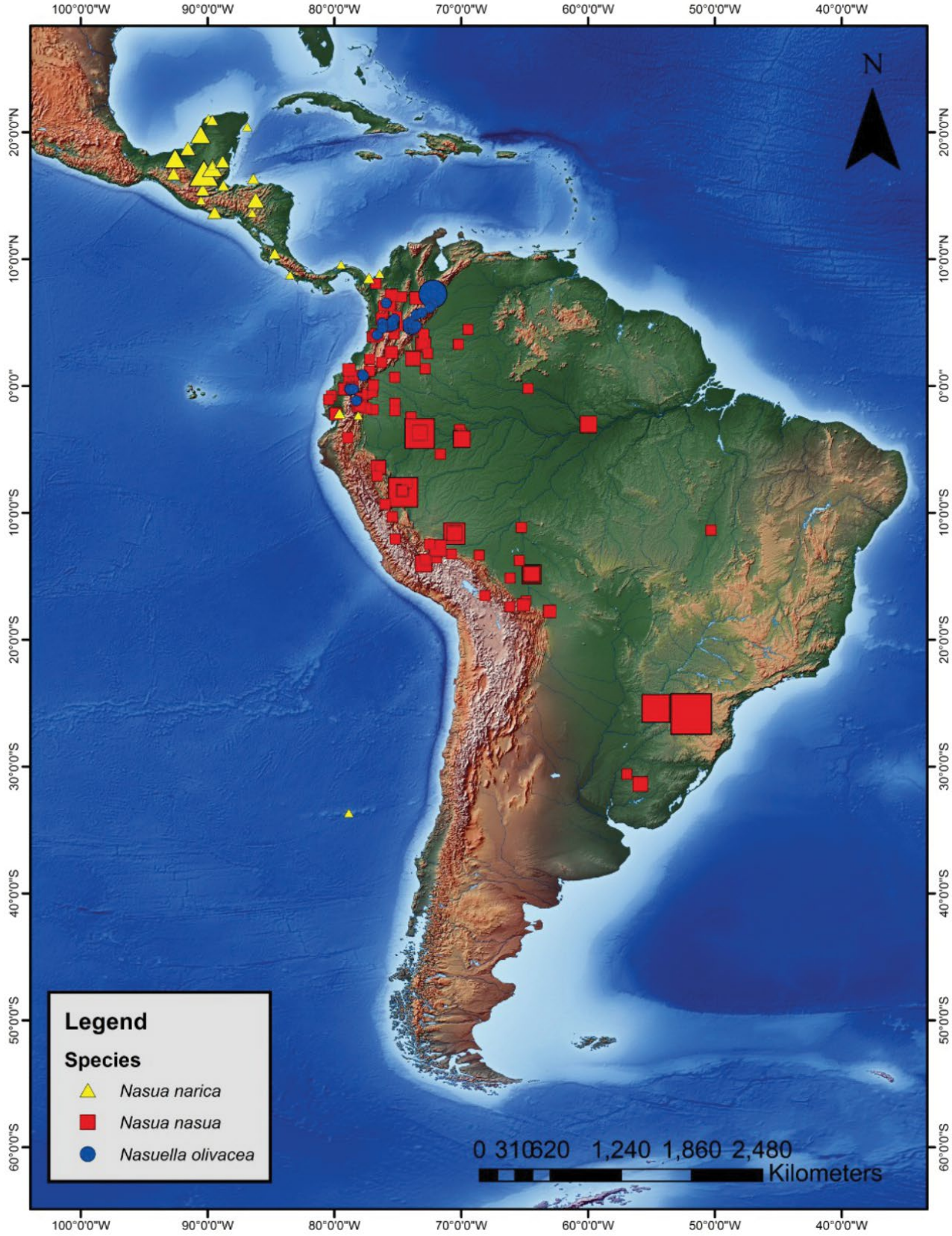

Figure 1. Map with the geographical origins and sample sizes of the specimens of three coati species (*Nasua nasua, Nasua narica*, and *Nasuella olivacea*) for which we sequenced eight mitochondrial loci (ND5, ND4, Cytb, D-loop, COI, COII, ATP6, and 12s rRNA) (*n*=345) throughout the Neotropics.

times among these haplogroups, we constructed a Median Joining Network (MJN) using Network v4.6.0.1 software (Fluxus Technology Ltd) [79]. Additionally, the ρ statistic [80] and its standard deviation [81] were estimated and transformed into years. The ρ statistic is unbiased and highly independent of past demographic events. This approach is named "borrowed molecular clocks" and uses direct nucleotide substitution rates inferred from other taxa [82]. We used an evolutionary rate of 1.3-1.7%per one MY [83] (values for Canidae), which represented one mutation each 12,740-9,742 years for the 6,038 bp analyzed. Network analyses could be more useful to reconstruct the evolutionary history within species or among much related species than bifurcating trees as is the case of the coatis.

The Kimura 2P genetic distance [84] was applied to determine the percentage of genetic differences among the different haplogroups detected in the three species of coatis analyzed. The Kimura 2P genetic distance is a

standard measurement for barcoding tasks [85,86]. In this case, the Kimura 2P distance practically offered identical values to the p-distance. Kartavtsev (2011) analyzed sequences of mt*COI* from 20,731 vertebrate and invertebrate animal species and obtained 0.89% ± 0.16% for populations within species, 3.78% ± 1.18% for subspecies or semi-species, and 11.06% ± 0.53% for species within a genus [87]. At mt*COII*, Ascunce, et al. (2003), and Ruiz-García, et al. (2014) reported an average genetic distance of around 8% among species within a genus, and around 2–5% for subspecies [38,88]. Bradley & Baker (2001) and Baker & Bradley (2006) claimed, for mt*Cytb*, that values<2% would equal intra-specific variation, values between 2% and 11% would merit additional study, and values>11-13% would be indicative of specific recognition [89,90]. Therefore, we take as an average for mitochondrial genes, above 4-10% for possible subspecies, and values higher than 12-13% for different species of the same genus. For species of different genera, this value should be above 16-18% [87].

Results

Phylogenetics of the coatis

The best nucleotide substitution models for the concatenated sequences were TN93+G (20,268.155) and GTR+G+I (13,234.043) for BIC and AIC, respectively.

The ML tree is shown in Figure 2. The first to diverge were the sequences of *Bassarycion* (100% bootstrap percentage). Within *Bassarycion*, the situation is confusing. There were three strongly supported clades. The most divergent one consisted of three *B. neblina* from Ecuador (92%), although, curiously, a specimen of *B. alleni* from Cochabamba Department (Bolivia) was associated with it. The second robust clade contained two specimens "a priori" also classified as *B. neblina* (but both from Colombia in this case; 99%), whilst the thrid robust clade was contained two specimens "a priori" classified as *B. medius* (Valle del Cauca, Colombia, and Santo Domingo de Tsáchilas, Ecuador; 93%). Later, we found various intermixed specimens from "a priori" different species (*B. medius* and *B. alleni*). One clade only had specimens of *B. alleni* (Ecuador, Peru, and Bolivia) but had a low bootstrap (58%). Thus, the systematics of *Bassarycion* is confusing and in need of a thorough molecular analysis.

This ML tree detected a stronger relationship between *N. nasua* and *N. olivacea* than either of these had with *N. narica*. This suggests that the genus Nasuella should be enclosed in the genus Nasua, as indicated by Ruiz-García, et al. (2020) and Ruiz-García, et al. (2021) [91,92].

The ML tree detected 10 significant clades within *N. nasua* with geographic significance. Haplogroup 1 (H1-NAS; 80%) was comprised by three sub-haplogroups. The first sub-haplogroup (H1-sh1-NAS; 71%) contained specimens from Bolivia (the major part of the Bolivian specimens analyzed were within this group), and southern Peru (mainly from the Madre de Dios River). The second sub-haplogroup (H1-sh2-NAS; 81%) was integrated by some specimens distributed throughout the Peruvian Amazon (north and south) and in the central Brazilian Amazon (Negro River). The third sub-haplogroup (H1-sh3-NAS; 61%), with a low bootstrap support contained Bolivian specimens together with some specimens distributed throughout all the Peruvian Amazon (north and south).

The second haplogroup (H2-NAS; 87%) was integrated by specimens distributed across all the Peruvian Amazon (north and south) and Peruvian mountain zones in the Departments of Cuzco, Pasco, and San Martin.

The third haplogroup (H3-NAS; 90%) was mainly expanded across a large fraction of Peru, both in the Amazon (north, central, south) and within the Andean zones (Apurimac, Cuzco, and San Martín Departments). But this haplogroup was also expanded in part of the Colombian (Leticia) and western Brazilian Amazon (Tabatinga) up to the Ecuadorian Pacific coast. Within this haplogroup, we detected some small clusters of specimens mixed of different geographical areas where this haplogroup was established (65%, 84%, and 71%, respectively). Thus, some sub-haplogroups should be defined within it.

The fourth haplogroup (H4-NAS; 89%) was established at the southern area of South America (southern Brazil, Paraguay, and Uruguay). It is possible that this haplogroup can arrive to more northern Brazilian areas because one specimen from Araguaia (Goias State) was included at this haplogroup.

The fifth haplogroup (H5-NAS; 91%) was a small one integrated by specimens of the northern Peruvian Amazon and it reached the trans-Andean area of Ecuador (Santo Domingo de Tsáchilas Province).

The sixth haplogroup (H6-NAS; 80%) had specimens distributed within the Andean Cordillera areas of Colombia and Ecuador. In Colombia, these specimens were found in the Norte de Santander, Santander, Antioquia, Caldas, Risaralda, Quindío, Tolima, Huila, Cauca, and Nariño Departments, covering practically the entire Colombian Andes. In Ecuador, these specimens were found in the Imbabura, Pichincha, and Esmeraldas Provinces. As we will comment below, a group of Nasuella was associated with this *N. nasua*'s haplogroup.

The seventh haplogroup (H7-NAS; 76%) was basically distributed within the trans-Andean Pacific area of Ecuador, but included interesting specimens. One of them was a specimen of "a priori" classified as *N. narica* from the Robinson Crusoe Island (Chile), where the coatis were introduced by a German couple in 1950 to combat rodent plagues. Additionally, we found one specimen sampled in an Andean locality of the Peruvian Apurimac Department in this haplogroup. It was classified by its morphology as *N. olivacea* (although this species is not reported in Peru) and one specimen from another Andean locality at the Jatun River in the Bolivian Cochabamba Department and also with a morphotype similar to *N. olivacea* (a species which has not been reported in Bolivia).

The eighth haplogroup (H8-NAS; 81%) was a small one distributed across the Colombian Amazon and the Colombian Eastern Llanos, although we detected within this group a specimen from the trans-Andean Pacific Ecuador.

The ninth haplogroup (H9-NAS; 85%) was composed of two sub-haplogroups. The first (H9-sh1-NAS; 75%) was integrated by specimens of trans-Andean and Andean areas of Colombia (Risaralda, Chocó, and Valle de Cauca Departments) and Ecuador (Esmeraldas Province). The second (H9-sh2-NAS; 88%) was composed of specimens of the Colombian Eastern Llanos. Associated to this haplogroup, we found a *Nasuella* haplogroup as we will comment below.

The tenth haplogroup (H10-NAS; 92%) was composed of specimens mainly distributed by the Colombian and Ecuadorian Amazon (it should be a sub-haplogroup, although its bootstrap percentage was low), but with specimens which were also distributed near the Andean Colombian Departments of Antioquia, Cundinamarca, Valle del Cauca as well as near the Colombian Eastern Llanos, and by the trans-Andean and Pacific areas of Ecuador (Pichincha, Santo Domingo de Tsáchilas, and Guayas Province) (it should also be another sub-haplogroup with a low bootstrap percentage).

In the case of *N. olivacea*, we detected four different haplogroups, and maybe a fifth haplogroup, as we mentioned earlier. There were some Andean specimens of *N. nasua*, which were a transition, or "bridge" toward the main group of *N. olivacea*. These Andean specimens were from the Antioquia Department (Colombia), Imbabura Province (Ecuador), and from the Junín Department (Peru). This last specimen should be indirect proof of the possible existence in the present, or in the past, of *N. olivacea* in Peru.

The first haplogroup (H1-OLI; 82%) was clustered with H6-NAS, which agrees quite well with that reported by Ruiz-García, et al. (2020) that specimens with the typical morphotypes of *N. olivacea* shared mitochondrial DNA more related to that of *N. nasua* than to that of the major fraction of *N. olivacea*. However, they were also significantly differentiated from the more related mitochondrial DNAs of *N. nasua*, which invalidates that these *N. olivacea* are simply specimens of Andean coatis recently introgressed by mitochondrial DNA from *N. nasua*. The specimens of this haplogroup were extended to the Colombian (Norte de Santander, Antioquia, Caldas, Chocó, Cauca, and Nariño Departments) and Ecuadorian (Carchi Province) Andean Cordilleras.

The second haplogroup (H2-OLI; 88%) was associated, as in the previous

Figure 2. Maximum-likelihood tree (ML) based on eight mitochondrial loci (ND5, ND4, Cytb, D-loop, COI, COII, ATP6, and 12s rRNA) of 345 specimens of three species of coatis (*Nasua nasua, Nasua narica*, and *Nasuella olivacea*) sampled in Latin America. Nodes are labeled with bootstrap percentages, but only significant haplogroups based on bootstrap percentages were marked in the figure to avoid number saturation. The numbers in bold are those corresponding to that we consider significant haplogroups or subhaplogroups. The specimens sampled in the same localities were indicated with a number into parentheses to reduce the size of the tree.

case, to another haplogroup of *N. nasua* (H9-NAS). This could be explained as in the previous case. The specimens of this haplogroup were also distributed in the Colombian (Caldas Department) and Ecuadorian (Pichincha Department) Andean Cordilleras.

The third haplogroup (H3-OLI; 95%) contained specimens at the Western and Central Colombian (Caldas, Chocó, Tolima, and Cauca Departments) and Ecuadorian (Pichincha, and Cotopaxi Provinces) Andean Cordilleras. Within this haplogroup, there was a very interesting specimen. This specimen was from Alba (Urubamba River; Cuzco Department, Peru). This is the first clear report of *N. olivacea* in Peru. Unfortunately, this specimen was hunted by a peasant and we sampled a little sample of skin. A picture of this dry skin was taken and it is available from the first author.

A haplogroup of *N. narica* was present and associated with this haplogroup of *N. olivacea*, as we will comment below.

The fourth haplogroup (H4-OLI; 90%) was mainly composed by specimens from the Eastern Colombian (Norte de Santander, Boyacá, and Cundinamarca Departments) Andean Cordillera. However, two specimens outside this geographical area were included in this haplogroup. One was from the Los Nevados NP (Caldas Department) in the Central Colombian Andes Cordillera, and another was from the Sangay NP in the Ecuadorian Province of Morona-Santiago.

Potentially, a fifth haplogroup of *N. olivacea* (H5-OLI; 62%) should be associated with H7-NAS as we aforementioned. If so, the geographical distribution of this haplogroup, in the case that they were Andean coatis, extends from the Apurimac Department in southern Andean Peru to the Cochabamba area in Bolivia. However, we have not complete evidence if this haplogroup is composed by two specimens of real *N. olivacea* or they are specimens of *N. nasua* adapted to live in Andean conditions.

In the case of *N. narica,* we detected four haplogroups, one of them integrated by three sub-haplogroups.

The first haplogroup (H1-NARI; 86%), probably the most ancestral for this species, contained three specimens from Ecuador. Two of them were from the Guayas Province at the trans-Andean and Pacific area of Ecuador. The third specimen was sampled in Fatima at the cis-Andean Amazon area of Ecuador. This haplogroup is extremely interesting because it seems to be the origin of the mainly Central-American *N. narica*.

The second haplogroup (H2-NARI; 82%) had a couple of specimens from the Yucatan Peninsula in Mexico.

The third haplogroup (H3-NARI; 87%) had a wide geographical distribution in Central America with three well differentiated sub-haplogroups. The first sub-haplogroup (H3-sh1-NARI; 79%) consisted of specimens from Guatemala, Honduras, El Salvador, Nicaragua, and northcentral Costa Rica. The second sub-haplogroup (H3-sh2-NARI; 88%) included specimens from Guatemala and Belize, while the three sub-haplogroup (H3-sh3-NARI; 98%) was distributed near northern Guatemala, and southern Mexico. This sub-haplogroup contained one specimen from the Cozumel Island.

The fourth haplogroup (H4-NARI; 94%) was associated, as we aforementioned, to the H3-OLI. This haplogroup was recorded at the southern area of Central America (Puntaarenas Province, southern Costa Rica; Nombre de Dios, Colon Province, Panama), but also in Colombian lands (Chocó and Antioquia Departments) close to the border with Panama. These specimens had undoubtedly morphotypes of *N. narica* (see Material and Methods) but with mitochondrial DNA of *N. olivacea*.

Henceforth, with 345 coati specimens and with eight mt genes, we detected 19 main haplogroups (10 *N. nasua*, five *N. olivacea*, and four *N. narica*) and 8-10 sub-haplogroups (five-seven *N. nasua*, and three *N. narica*).

The BI tree (Figure 3) was constructed by using haplotypes (not specimens as in the previous tree). It showed a similar perspective in reference to the different haplogroups found within the three species in the ML tree. The major fraction of the haplogroups detected in that analysis was found with the BI tree: H1-NAS (p=0.82), H3-NAS (p=0.88), H4-NAS (p=0.9), H6-NAS (p=0.91) and H1-OLI (p=0.76) both associated, H7-NAS (p=1), H9-sh1-NAS (p=0.98) and H2-OLI (p=0.78) both associated, H10-NAS (p=0.80), H3-OLI (p=0.70), H4-OLI (p=0.88), H2-NARI (p=1), H3-NARI (p=0.93), including H3-sh1-NARI (p=0.87), H3-sh2-NARI (p=0.83), H3-sh3-NARI (p=0.96), and H4-NARI (p=1). Nonetheless, there were some differences with regard to the ML tree. For instance, H2-NAS and H7-NAS disappeared as a significant clades and H5-NAS (p=0.98) was, in this case, integrated within H10-NAS. In the ML tree, the introgressed *N. narica*'s haplogroup (H4-NARI) was associated with H3-OLI. In this case, it was associated with H4-OLI. The H1-NARI was the most divergent haplogroup of *N. narica* in the ML tree. However, in the BI tree, H1-NARI (p=0.73) was included in one sub-haplogroup of the Central American *N. narica* (H3-sh1-NARI). Last, in the BI tree, two haplotypes (two specimens) of *N. olivacea* appeared as the most divergent of all the coatis analyzed and potentially their ancestors as the original one (Colombian and Ecuadorian Andes: Risaralda Department, Colombia, and Carchi Province, Ecuador). However, the major difference between both trees was the relationship of Nasuella regards to Nasua. In the ML tree, Nasuella was more related to *N. nasua*, meanwhile, in the BI tree, Nasuella was more related to *N. narica* than to *N. nasua*. In whatever case, Nasuella seems to be a taxon which should be enclosed into Nasua.

If we took the origin of the evolution of the coati's haplotypes as those less differentiated in reference to the common ancestor with the outgroup (*Bassarycion*), the first haplotypes which appeared in the MJN for the eight mt genes were H90, H91, and H27 (Figure 4). These haplotypes corresponded to diverse specimens of *N. nasua* from the trans-Andean Pacific Ecuador, and Western and Central Colombian Andean Cordilleras (Esmeraldas Province, Ecuador, and Risaralda, Chocó, and Valle del Cauca Departments, Colombia). Based on a morphological point of view, the first haplotypes of *N. olivacea* (H140 and H146; Caldas Department, Colombia, and Pichincha Province, Ecuador, also in the northern Andes) appeared from these. Two routes appeared. The first originated a major fraction of the Andean *N. nasua* throughout Colombia and Ecuador (H88 and related haplotypes). This in turn, gave origin to some haplotypes of *N. olivacea* (H181, H159, and associated haplotypes). The specimens with these haplotypes had, undoubtedly, phenotypes of *N. olivacea*, although these haplotypes were more related to those of the Colombian and Ecuadorian Andean *N. nasua* than to the next derived group of *N. olivacea*. These "transition" haplotypes gave origin to a group of *N. olivacea* mainly (but not exclusively) distributed near the Eastern Colombian Andean Cordillera (H132 and related haplotypes). In the past, haplotypes of this group intruded into a fraction of *N. narica* distributed in the southern area of Central America (southern Costa Rica, and Panama) and northern Colombian frontier with Panama. Eventually, this group of *N. olivacea* from the Eastern Colombian Andean Cordillera gave place to another group of *N. olivacea* in the Western and Central Colombian and Ecuadorian Andes Cordilleras. Thus, this last group of *N. olivacea* was the most recent one for this species. The second way was initiated with haplotypes from the Colombian Eastern Llanos (H49 and H50). Two additional pathways originated from these. The first one gave rise to some transition haplotypes of *N. nasua* (H130; Buenaventura, Valle del Cauca Department, Colombia), which finally gave origin to the first haplotypes of *N. narica* in the Ecuadorian Pacific area, although later some of these *N. narica*'s haplotypes crossed the Andean Cordillera until they at least reached the Ecuadorian Amazon (H86, H98, and H38). The evolution of the haplogroups of *N. narica* was discussed in Jaramillo and Ruiz-García (2021) and it is not here discussed [28]. The second one gave origin to all the detected groups of *N. nasua*. One was composed of haplotypes mainly distributed in the Colombian and Ecuadorian Amazon as well as near the Pacific Ecuador and some Andean areas of Colombia (H3, H31, and related haplotypes). Another group was mainly composed of haplotypes of *N. nasua* placed from trans-Andean and Pacific Ecuador, but some haplotypes were from more distant locations. One was found in a coati from Robison Crussoe Island (Chile), but two others were found in Peru and Bolivia and these specimens had phenotypes very similar to *N. olivacea* (H22, H94, H150). There were single and dispersed haplotypes (H51, H62) within these groups of *N. nasua*, distributed within the Eastern Colombian Llanos (Meta Department) and the Huila Department, both in Colombia. They seem to play a very important role in the origin and generation

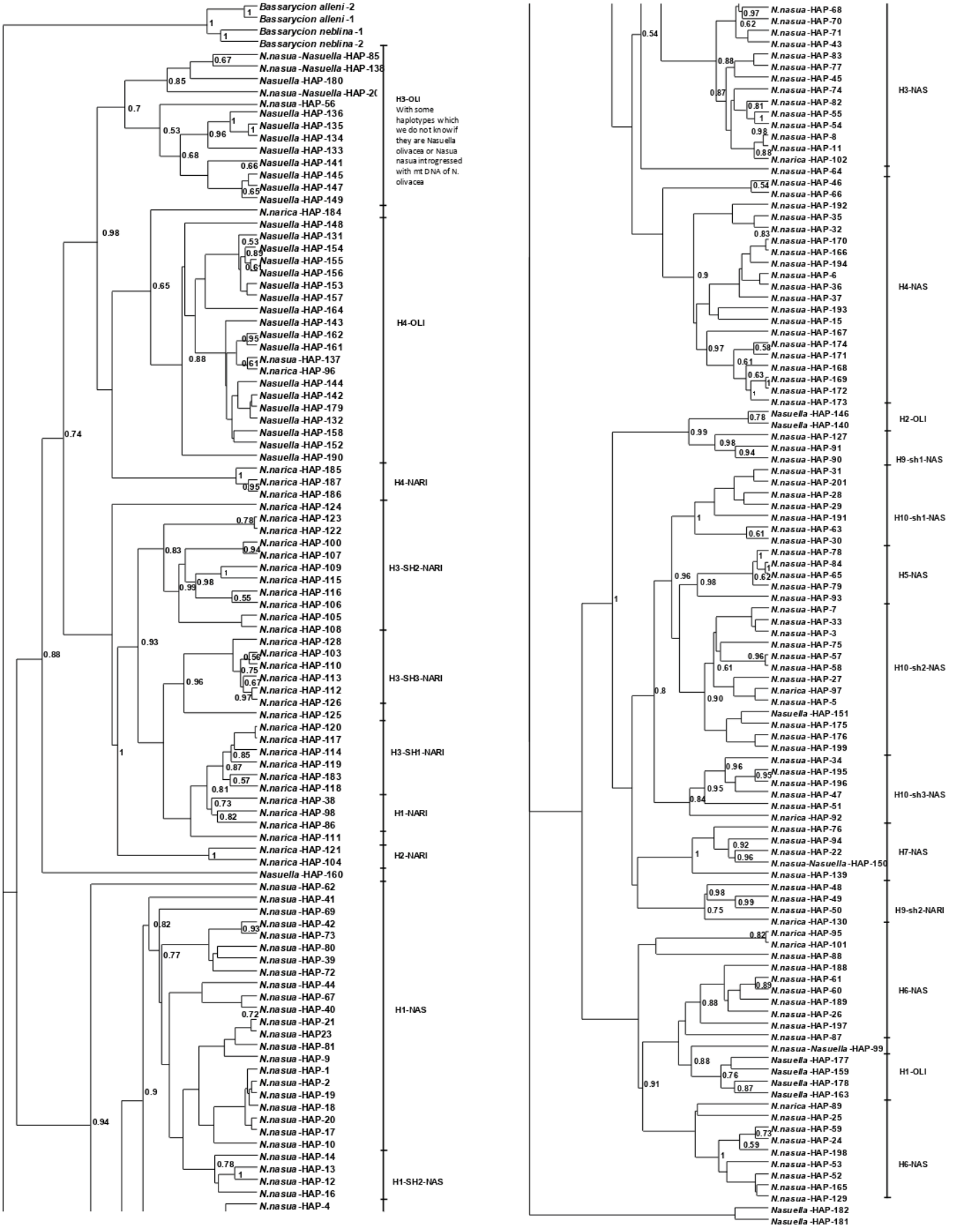

Figure 3. Bayesian Inference tree (BI) based on eight mitochondrial loci (ND5, ND4, Cytb, D-loop, COI, COII, ATP6, and 12s rRNA) of 201 haplotypes of three species of coatis (*Nasua nasua, Nasua narica*, and *Nasuella olivacea*) sampled in Latin America. Significant haplogroups based on posterior probabilities were marked. The numbers in the nodes correspond to those with posterior probabilities higher than 0.5 to avoid number saturation.

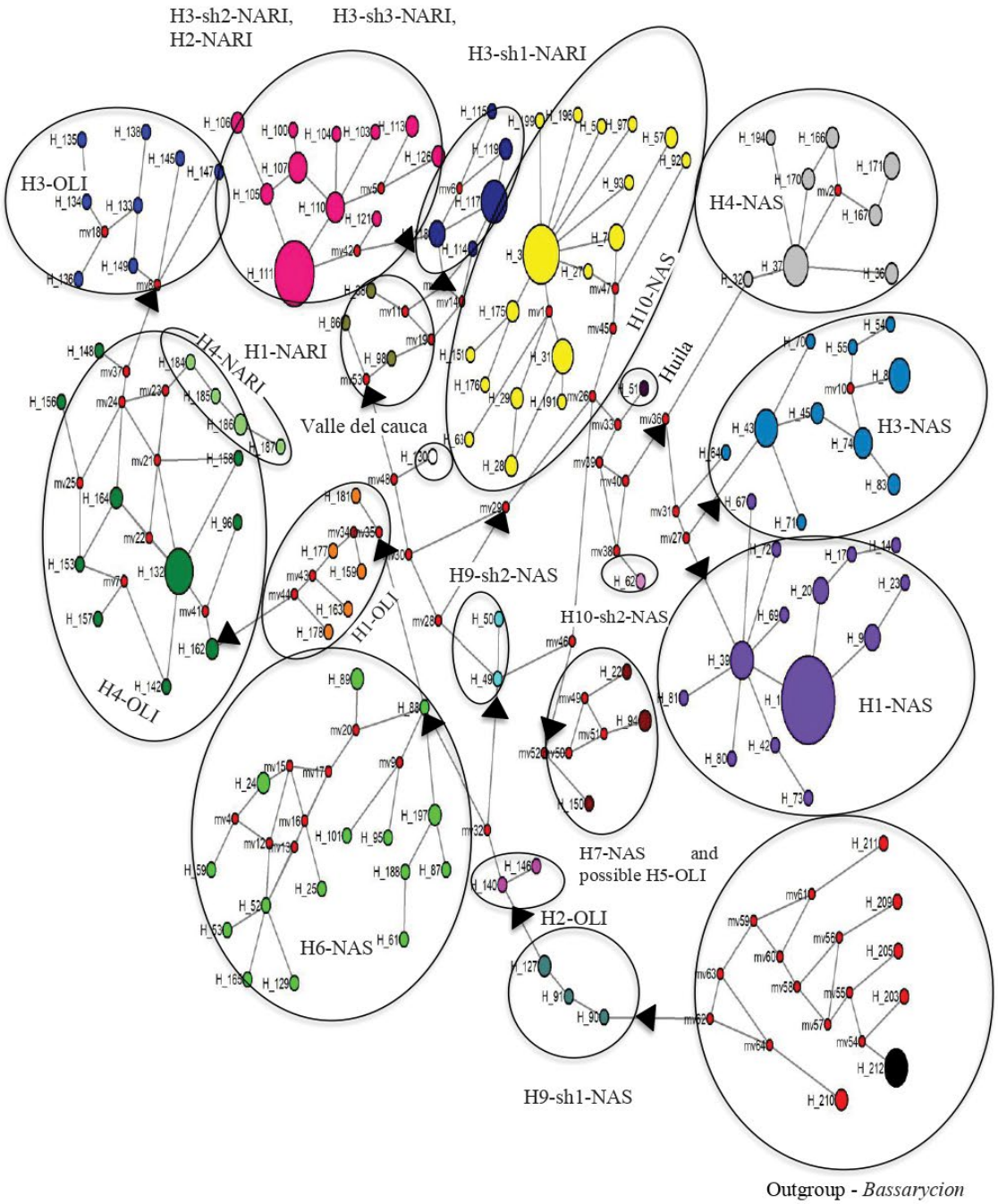

Figure 4. Median Joining Network based on eight mitochondrial genes *(ND5, ND4, Cytb, D-loop, COI, COII, ATP6, and 12s rRNA)* of 345 specimens of three species of coatis (*Nasua nasua, Nasua narica*, and *Nasuella olivacea*) sampled in Latin America. Haplogroups are showed with different colors; black circles=*Bassarycion alleni* (outgroup); red circles with H_number=*Bassarycion neblina* and *Bassarycion medius* (outgroup); bluish green circles=H9-sh1-NAS (*N. nasua* from the Colombian Chocó, Risaralda, and Valle del Cauca Departments, and the Ecuadorian Esmeralda Province); pink circles=H2-OLI (*N. olivacea* from the Colombian Caldas Department, and the Ecuadorian Pichincha Province); very light blue circles=H9-sh2-NAS (*N. nasua* from Eastern Colombian Llanos); dark lilac and very light pink circles=one independent haplotype and H10-sh2-NAS (*N. nasua* from Eastern Colombian Llanos); brown circles=H7-NAS and possible H5-OLI (*N. nasua* from trans-Andean and Pacific Ecuador and one specimen transported to the Robinson Crusoe Island-Chile-, but also two specimens with phenotypes similar to *N. olivacea* from southern Peru and Bolivia); grey circles=H4-NAS (*N. nasua* from southern Brazil, Paraguay, and Uruguay); greenish blue circles=H3-NAS (*N. nasua* from different areas of the Peruvian Andes and Amazon, Colombian and western Brazilian Amazon, and Pacific Ecuador); lilac circles=H1-NAS (*N. nasua* from southern Peru and Bolivia, different areas of the Peruvian Amazon, and central Brazilian Amazon); yellow circles=H10-NAS (*N. nasua* from the Ecuadorian and Colombian Amazon, Colombian Antioquia, Cundinamarca, Meta, and Valle del Cauca Departments, and trans-Andean and Pacific Ecuador-Pichincha, Santo Domingo de Tsáchilas, and Guayas Provinces); green circles=H6-NAS (*N. nasua* from the Colombian and Ecuadorian Andean Cordilleras); orange circles=H1-OLI (*N. olivacea*; "transition" haplotypes in the Colombian (Norte de Santander, Antioquia, Caldas, Chocó, Cauca, and Nariño Departments) and Ecuadorian (Carchi Province) Andean Cordilleras); dark green circles=H4-OLI (*N. olivacea* mainly from the Eastern Colombian Andean Cordillera, including Norte de Santander, Boyacá, and Cundinamarca Departments, but also some specimens in the Central Colombian Andean Cordillera-Caldas Department-, and Sangay NP in Ecuador); very light green circles=H4-NARI (*N. narica* from southern Costa Rica, Panama, and northern Colombia (Antioquia and Chocó Departments) introgressed with mtDNA from the *N. olivacea* of H4-OLI); navy blue circles=H3-OLI (*N. olivacea* from Western and Central Colombian and Ecuadorian Andean Cordilleras; Colombian Caldas, Risaralda, Chocó, Tolima, and Cauca Departments, and Ecuadorian Pichincha, Napo, Cotopaxi Provinces); white circle=an individual haplotype (*N. nasua* from Valle del Cauca Department in Colombia. This specimen has a haplotype of "transition" between *N. nasua* and *N. narica*); greenish brown circles=H1-NARI (*N. narica* from trans-Andean and cis-Andean Ecuador); very dark blue circles=H3-sh1-NARI (*N. narica* from northern Costa Rica, Nicaragua, El Salvador, Honduras, and Guatemala); and fuchsia pink circles=H3-sh2-NARI, H3-sh3-NARI, and H2-NARI (*N. narica* from Guatemala, Belize, southern Mexico, and Yucatan). Red circles (with mv) indicate missing intermediate haplotypes. With arrows, the evolutionary direction of the original haplotypes given place to the derived haplotypes is shown in the Median Joining Network.

of three other large groups of *N. nasua*. One group was situated in southern South America (southern Brazil, but also distributed to more northern areas of Brazil, Paraguay, and Uruguay; H32, H37, and related haplotypes). Another group was mainly in the Peruvian Amazon and Peruvian Andes but also the Colombian and western Brazilian Amazon and even the Ecuadorian Pacific coast (H64, H43, and related haplotypes). Another group (H1, H39, and other related haplotypes) had some sub-groups. The first sub-group was distributed near Andean Peru and some areas of the Peruvian Amazon, which, in turn, originated the majority of the haplotypes of the Madre de Dios River basin (southern Peru) and Bolivia. In turn, these haplotypes generated haplotypes of *N. nasua* in the central Brazilian Amazon (Negro River).

Temporal divergence between and within different haplogroups of coatis

We mainly showed here the temporal splits estimated throughout the MJN procedure as well as for the BI estimates for the eight mt genes. Generally, the BI estimates, were higher than the MJN estimate especially in the oldest time estimates and lower in the most recent temporal estimates. However, the geological epochs of the main temporal splits were more or less the same. The most ancestral haplotype (H90) found for whatever coati studied belonged to H9-sh1-NAS (*N. nasua*) (see Figure 4). The temporal differentiation between this haplotype and the most derived haplotype in H3-OLI (*N. olivacea*) (13.09 ±0.15 MYA for a substitution rate of 1.3%/MYA; 9.99 ±0.11 MYA for a substitution rate of 1.7%/MYA, respectively), in H3-sh3-NAR (*N. narica*) (12.97 ± 0.34 MYA; 9.91 ± 0.26 MYA), and in H1-NAS (*N. nasua*) (8.42 ± 0.29 MYA; 6.44 ± 0.22 MYA). The BI estimation for the initial mitochondrial diversification in the coatis was 11.92 MYA (6.54-13.45 MYA). Therefore, the initial mitochondrial diversification for the coatis was during the Miocene (10-13 MYA) "in situ" in South America. The first haplotypes of H9-sh1-NAS (Colombian and Ecuadorian Andes), that we detected, generated the first haplotypes of *N. olivacea* (H2-OLI; Colombian and Ecuadorian Andes) around 0.19 ± 0.14/0.15 ± 0.10 MY later. H6-NAS (Colombian and Ecuadorian Andes) appeared next around 2.47 ± 0.32/1.89 ± 0.25 MY after the emergence of H9-sh1-NAS. H9-sh2-NAS (Central Andean Colombia, and Eastern Colombian Llanos) appeared next around 2.61 ± 0.36/1.99 ± 0.27 MY later. The intermediate haplogroup of *N. olivacea* (H1-OLI; Colombian and Ecuadorian Andes) appeared 4.50 ± 0.32/3.44 ± 0.25 MY later. Approximately, 7.26 ± 0.41/5.54 ± 0.31 MY later, the first haplotypes of *N. narica* (H1-NARI; trans-Andean Pacific Ecuador) appeared. The last temporal splits support that the evolution of coatis in South America occurred before the complete closure of the Isthmus of Panama around 3 MYA. We previously observed that the H9-sh2-NAS (Central Andean Colombia, and Eastern Colombian Llanos) played an important role in the origin of all the other haplogroups of *N. nasua* in South America. After the emergence of H9-sh2-NAS, H10-NAS appeared (1.31 ± 0.21/1-00 ± 0.16 MY later), then H7-NAS (3.05 ± 0.32/2.33 ± 0.25 MY later), H4-NAS and H3-NAS (3.48 ± 0.29/2.66 ± 0.22 MY later), and H1-NAS (7.71 ± 0.50/5.90 ± 0.38 MY later).

For *N. nasua*, within H10-NAS, the mitochondrial diversification began around 0.95 ± 0.31/0.72 ± 0.24 MYA (the BI estimation: 2.04 MYA, 0.92-3.11 MYA), within H4-NAS around 0.61 ± 0.31/0.47 ± 0.23 MYA (the BI estimation: 2.95 MYA, 1.73-5.88 MYA), within H3-NAS around 2.88 ± 0.80/2.20 ± 0.62 MYA (the BI estimation: 2.18 MYA, 1.19-4.57 MYA), within H1-NAS around 0.62 ± 0.31/0.47 ± 0.24 MYA (the BI estimation: 3.09 MYA, 1.70-4.85 MYA), and within H6-NAS around 1.16 ± 0.27/0.89 ±0.20 MYA (the BI estimation: 2.92 MYA, 0.6-5.56 MYA).

For *N. olivacea*, the time split between H1-OLI and H4-OLI (mainly Eastern Colombian Andean Cordillera) was estimated to have occurred around 4.64 ± 0.70/3.55 ± 0.53 MYA, between H4-OLI and the introgressed haplogroup of *N. narica* (N4-NARI) around 0.87 ± 0.26/0.67 ± 0.19 MYA, and between H4-OLI and H3-OLI around 1.45 ± 0.14/1.11 ± 0.11 MYA. Within H1-OLI, the mitochondrial diversification began around 2.49 ± 0.47/1.91 ± 0.36 MYA (the BI estimation: 1.72 MYA, 0.70-3.79 MYA), within H4-OLI around 3.05 ± 0.82/2.33 ± 0.63 MYA (the BI estimation: 4.41 MYA, 1.34-5.08 MYA), within H4-NARI around 2.09 ± 0.68/1.60 ± 0.52 MYA, and within H3-OLI around 1.67± 0.42/1.28 ± 0.32 MYA (the BI estimation: 5.34 MYA, 1.53-6.12 MYA).

For *N. narica*, the time split between H1-NARI (Pacific Ecuador) and H3-NARI (central and northern Central America) was estimated to have occurred around 4.18 ± 0.28/3.56 ± 0.26 MYA, between H3-sh1-NARI and H3-sh3-NARI around 1.16 ± 0.27/0.89 ± 0.21 MYA, within H3-sh1-NARI around 0.46 ± 0.29/0.36 ± 0.22 MYA (the BI estimation: 1.52 MYA, 0.92-3.95 MYA), and within H3-sh3-NARI around 0.74 ± 0.17/0.56 ± 0.13 MYA (the BI estimation: 2.46 MYA, 0.98-3.99 MYA).

Discussion

Some comments on the taxonomy of the out-group: The case of *Bassarycion*

To date the study of Helgen, et al. (2013) [93] of the *Bassarycion* systematics is the most complete one. Analyzing the genes *CHRNA1* and mt*Cyt-b*, they showed that the most divergent branch was that composed by *B. neblina neblina* (from western slopes of the western Ecuadorian Andes) and *B. neblina osborni* (from the eastern slopes of the western Colombian Andes and western slopes of the central Colombian Andes). Our results agree well with the fact that the most divergent *Bassarycion* branch in our tree was composed by three exemplars of *B. n. neblina* from Ecuador. However, a specimen of *B. alleni* from Cochabamba Department (Bolivia) was included in this clade. Other difference in our analysis is that the two specimens classified "a priori" as *B. neblina* from Colombia did not clustered with the Ecuadorian specimens. Helgen, et al. (2013) defined three possible *B. neblina* subspecies in Colombia. One *B. n. osborni* was aforementioned. The other two were *B. neblina hershkovitzi* (from eastern slopes of central Colombian Andes) and *B. neblina ruber* (from Urrao, Antioquia Department; western slope of northern western Colombia Andes). However, the two Colombian specimens we analyzed were not of the same areas where these subspecies were described. One is from the Nariño Department in southern Colombia and the other was seized in Bogotá and the real origin is unknown but it is possible that it is from the eastern Colombian Andes. Therefore, it is possible that they are part of undescribed taxa very similar phenotypically to *B. neblina*. The following branch to diverge was that of *B. gabbi* from Costa Rica [93]. We did not enclose any specimen from this taxon in our analysis. Finally, the most recent split gave origin to the current *B. medius* and *B.alleni*. We also found that the major part of the specimens of *B. medius* and *B. alleni* were more related among them than with reference to the other *Bassarycion* taxa. However, we did not found strictly monophyletic clades for these two species. In fact, we found different polyphyletic clades of *B. medius*. Even we found some haplotypes of *B. alleni* (from Ecuador) intermixed between the two *B. neblina* clades. This could be explained using two hypotheses. (a) Really there are more *Bassarycion* taxa that those recognized until today and a very exhaustive sampling is urgently needed and/or (b) there are cases of mtDNA introgression between different *Bassarycion* taxa. Therefore, it is urgently a proof molecular analysis of this genus.

How many species of coatis are within the genus *Nasua?*

We consider genetic distances above 4-10% for possible subspecies, values around 12-13% for different species of the same genus, and values above 16-18% for species of different genera [87-90]. One problem in distinguishing species of coatis within the genus Nasua is that genetic distance values among multiple haplogroups can conform a continuous range of values. Here, we acknowledge one example of *N. nasua*.

We begin with genetic distances between H1-sh1-NAS (*N. nasua* from Bolivia and southern Peru) and other haplogroups. The genetic distance with H1-sh3-NAS was 0.8% (population differentiation), with H4-NAS, it was 5.9% (subspecies differentiation), with H10-sh1-NAS, it was 9.8% (subspecies differentiation near to the limit of full species), with H7-NAS, it was 12% (limit of full species), with H1-NARI (first *N. narica* from Ecuador), it was 12.8% (limit of full species), with H1-OLI and H2-OLI (the two first haplogroups of *N. olivacea*), the values were 10.2% and 11.5%, respectively (limit between subspecies and species). If we consider these *N. nasua* taxa as subspecies (H1-sh1-NAS vs. H10-sh1-NAS, or H7-NAS), then the original Ecuadorian *N. narica* haplogroup and two original *N. olivacea* haplogroups should be considered as subspecies

of *N. nasua* too. In contrast, if we consider these *N. narica* and *N. olivacea* as different species from the *N. nasua* taxon, then different haplogroups of *N. nasua* should be considered different species. This shows the difficulty in distinguishing different taxa of *N. nasua* as different species and the oldest groups of *N. narica* and *N. olivacea*. This is further evidence favouring the existence of one unique genus for the coatis (Nasua). However, the genetic differentiations between H1-sh1-NAS and the most derived *N. narica* taxa (H2-NARI, H3-sh1-NARI, H3-sh3-NARI), and the most derived *N. olivacea* (H3-OLI, H4-OLI) were in the range of well-differentiated species (17.3%, 15.8%, 15.9%, 15.7%, and 17%, respectively).

These results put forward that the most derived haplogroups of *N. nasua* and the most derived haplogroups of *N. narica* and *N. olivacea* are within the genetic distance range of well differentiated species. However, a key question remains about the relationships of the most original haplogroups of *N. nasua* with the original groups of *N. narica* and *N. olivacea*, which were typically of subspecies.

The question with *N. olivacea* is complex. The genetic distances among the "intermediate" (haplotypes between the ancestral haplotypes of *N. nasua* and the most derived haplotypes of *N. olivacea*, with morphological characters and geographical distributions typical of *N. olivacea*) H1-OLI, with the remainder haplogroups of *N. olivacea* (H2-OLI, H3-OLI, H4-OLI) were 5.7%, 10.9%, and 9.4%, respectively, and between H3-OLI and H4-OLI was 5.4%. These values were in the range of subspecies. Nonetheless, the genetic distances among H2-OLI versus H3-OLI and H4-OLI were 15.4% and 16.4%, respectively, which are clearly in the range of full species. The genetic distances agree quite well with that observed in the phylogenetic trees and in the MJN procedure: the polyphylia of *N. olivacea* or the existence of groups of Andean *N. nasua*, which have independently evolved towards a morphotype very similar to that of the real *N. olivacea* (H3-OLI and H4-OLI).

Therefore, how many species we can discriminate within the genus Nasua? We propose three hypotheses:

1) Each one of the significant and monophyletic haplogroups should be a differentiated species using extreme versions of Phylogenetic Species Concept 1 (PSC) [94] and Phylogenetic Species Concept 2 [95]. If we adopted this strategy, then, we should define, at least, 19 different species of coatis versus the three traditional species that is if we only take into account the main haplogroups detected. However, we do not want to make the same mistake as previous authors and indiscriminately apply the PSC without first understanding the reproductive, ethological, or immunological incompatibilities among the groups of coatis or karyological studies. The application of PSC without the knowledge of these previous items can easily spawn many unrealistic new species that are clearly not sustained by a critical analysis. This was named by Isaac, et al. (2004) as "taxonomic inflation" [96]. Zachos, et al. (2013) suggests that some of the proposed new mammal species are completely unjustifiable [97]. We agree with Zachos (2016) who stated that species are such fundamental units that they should not be introduced carelessly [98]. Descriptions of species and their splitting should not be based on simple morphometric differences (even significant ones) or phylogenetic relationships derived from very limited molecular datasets. These datasets may serve to support conclusions derived from larger and more complete datasets, but are not enough on their own.

2) Another possibly more conservative option is that the three traditional species should be conserved in one unique genus (*Nasua nasua, Nasua olivacea*, and *Nasua narica*). Ruiz-García, et al. (2021) discussed the reasons why Nasuella [99] should be enclosed within Nasua [100]. For instance, for the BI tree, *N. narica* was more related to *N. olivacea* than to *N. nasua*. However, for this to be accepted, data must indicate that several groups of *N. olivacea* are really specimens of *N. nasua*. They would have convergently evolved to have phenotypes undistinguishable from *N. olivacea*. Someone could argue that these *N. olivacea* were more related with the *N. nasua* than with the most derived *N. olivacea*. Beyond this last questionable assertion (which means that there was no reproductive isolation between two "a priori" species of two different genera), they are not the product of recent hybridization because the specimens have no intermediate phenotype (they are clearly by size and color specimens of *N. olivacea*). Furthermore, the haplotypes of these specimens formed well defined groups and spread across a wide geographical area (not in specific points which could be expected in occasional recent hybridization events). Additionally, if these specimens were the product of recent hybridization events, their haplotypes should be un-differentiable from the maternal lineage and the temporal split between these haplotypes should be zero or very small. Contrarily, the temporal splits found should be considerable. Thus, these specimens could not be considered product of recent and punctual events. Taking this into consideration, the only "true" *N. olivacea* should be H3-OLI and H4-OLI. All of the other specimens with a phenotype of *N. olivacea* outside of these two molecular groups should be classified as *N. nasua.* Obviously, it would be a challenge to correctly classify specimens of *N. olivacea* in the field without having a molecular analysis. Likely, some coatis that have intermediate haplotypes between *N. nasua* and the Central America *N. narica* should be classified as *N. nasua*. This should be the case of the original Ecuadorian *N. nasua*, as well as of one specimen that we classified as *N. nasua* from the Tamá NP (Norte de Santander Department, Colombia). This last one did show a strong relationship to the origin of *N. narica* (the same occurred with one specimen from Buenaventura, Valle del Cauca, and Colombia). Then, the unique "true" *N. narica* should be the Central American one. However, even in this case, there was another problem: the group of *N. narica* from southern Central America and northern Colombia were introgressed by *N. olivacea.* These particular specimens had phenotypes of *N. narica* although their mitochondrial DNA more closely resembled that of *N. olivacea* than the mitochondrial DNA of the remaining *N. narica* of Central America. They were clearly not the product of recent and occasional hybridization events, because the temporal split between H4-OLI and the introgressed haplogroup of *N. narica* (N4-NARI; southern Central America) was around 0.87 ± 0.26/0.67 ± 0.19 MYA.

3) A third hypothesis is that all the coatis belonged to a unique species or super-species. This hypothesis accommodates the polyphylia of *N. olivacea,* the existence of the "intermediate" haplogroups or haplotypes between *N. nasua* and *N. olivacea*, or between *N. nasua* and *N. narica*, or the introgression of *N. olivacea* within a fraction of *N. narica*. Importantly, this hypothesis supports a broad genetic diversity within species and a continuous evolutionary process especially between much related taxa as it is the case of the coatis. The hypothesis addresses a problem stemming from the fact that the Linnaeus classificatory scheme is Aristotelian, scholastic and the scheme was created to classify static, and discrete, organisms that people thought would never evolve because they were created by God. Thus, in many cases (and the coati should be one of them, as proposed with *Ateles*; [101]), there would be conflict between this typological philosophy and the evolutionary process which is continuous, especially between very related taxa as it is the case of the coatis.

Molecular intra-generic systematics of *Nasua nasua* and *Nasuella olivacea*

We agree more with the second or third hypotheses than with the first one. Indeed, although we believe that future molecular, karyological, ethological, and reproductive data may offer results in favor of a unique species, we adopt the most traditional strategy of three species.

We begin the taxonomic review with *N. nasua*. It shows the northern and western slopes of the Andes to the Pacific coast of Colombia and Ecuador as the original areas for the initial mitochondrial diversification of the coatis. The first were some Andean *N. nasua* (Colombian and Ecuador) which originated (polyphyletically) the first ancestral haplogroups of *N. olivacea*, but also the most derived haplotypes of *N. olivacea* (which we name the "true" *N. olivacea*). These *N. nasua* were also the origin of *N. narica*. Additionally, the Andean area of central Colombia (Huila Department, for instance) and surrounding areas (Eastern Colombian Llanos) were significant to the origin of all the other haplogroups of *N. nasua* across South America.

We traditionally consider ten subspecies for *N. nasua* [21]. Only a small fraction of the haplogroups we found for *N. nasua*, support the traditional morphological subspecies. Just two cases seem to be absolutely in agreement. These were the cases of H4-NAS with *N. n. spadicea* (type

locality: Paraguay; [102]) and H1-sh1-NAS with *N. n boliviensis* (type locality: Chaparé, Cochabamba, Bolivia; [103]). One additional case should be the specimen of Goias (Brazil) that we analyzed. This specimen was the most divergent within H4-NAS. If we could have analyzed additional specimens from this Brazilian area (or relatively neighbor zones), we very well might have had a new haplogroup or sub-haplogroup which could agree with *N. n. nasua* (type locality: Pernanbuco, Brazil; [104]). In fact, Tsuchiya-Jerep (2009) detected five haplogroups (four in Brazil), which could be related to the morphology of the *N. nasua* subspecies in Brazil. One of these haplogroups was detected in northeastern Brazil (Caatinga and northern Brazilian Atlantic forest). It corresponded to *N. n. nasua* (our sample probably belongs to this subspecies). These authors detected another one in the central Brazilian Atlantic forest, corresponded to *N. n. solitaria* (type locality: Bahia, Brazil; [105]). Therefore, we detected two clear cases of correspondence between haplogroups and morphological subspecies, and two other probable cases of correspondence between haplogroups and morfphological subspecies following our result of the specimen of Goias and the data of Tsuchiya-Jerep (2009).

Any possible remaining similarities among haplogroups of *N. nasua* and traditional subspecies are more complex and, even, dubious. *N. n. candace* (type locality: Medellín, Antioquia, Colombia; [106]) and *N. n. judex* (type locality: Bogotá, Cundinamarca, Colombia; [107]) have been defined in Colombia. However, the latter one is considered a synonymous to the former one [21]. Our results showed that, at least, two different haplogroups of *N. nasua* were present in the Antioquia Department (H6-NAS, and H10-NAS). Therefore, we do not know which of the two (either of them) correlated with *N. n. candace*. On the other hand, we detected one specimen from Cundinamarca in the H10-NAS. This specimen should be the same as *N. n. judex*, but then *N. n. candace* and *N. n. judex* could not be synonymous. In fact, only in Colombia, did we detect five different haplogroups (and four sub-haplogroups within of two of these haplogroups) for *N. nasua* although only two subspecies were defined (and one was later synonymized with the senior name) in this country. This demonstrates that the molecular results showed a more complex reality than that described by the putative morphological subspecies. From a conservation point of view, this also introduces a technical complication because many of these haplogroups overlapped geographically.

The same occurred in Peru with *N. n. montana* (type locality: Umanpuqio, Ceja Region, Peru; [108]). Two different haplogroups of *N. nasua* were detected throughout the Peruvian Andes (H2-NAS and H3-NAS). Thus, we ignore which of them coincides with that morphological subspecies. In fact, another subspecies has been described in Peru, *N. n. dorsalis* (type locality: Peru and Ecuador; [109]). However, we detected, at least, seven haplogroups and sub-haplogroups of *N. nasua* in Peru and many of them are geographically overlapped. This introduces considerable complications for a traditional systematic nomenclature approach.

Ecuador is an even more complex case. Three morphological subspecies have been defined in this country: *N. n. manium* (type locality: Balzar, Guayaquil, Ecuador; [106]), *N. n. quichua* (type locality: Jima, Azuay Province, Ecuador; [110]), and the quoted *N. n. dorsalis*. Only for the Andean, and trans-Andean-Pacific Ecuador, we detected seven different haplogroups (H3-NAS, H5-NAS, H6-NAS, H7-NAS, H8-NAS, H9-NAS, and H10-NAS) and one additional haplogroup in the Ecuadorian Amazon (H10-NAS). This did not consider the different groups found in *N. olivacea* and *N. narica* which are highly related to *N. nasua* and which are also located in the Ecuadorian territory.

Therefore, the taxonomic and conservation strategies could be more complex for *N. nasua* in Ecuador, Colombia, and Peru. This puts forward that the northern Andes were transcendental in the origin and diversification of the coatis.

The situation in Bolivia was easier to understand. The majority of the specimens of *N. nasua* in this country were part of H1-NAS. Only three Bolivian specimens were outside of this group. One was from Ibiato (Beni Department), which did not have clear clusters of haplogroups. Another specimen sampled in the Santa Cruz Department, was clustered in the H10-NAS. The third was from Jatun River (Cochabamba Department). The last two specimens are interesting. The first is part of a haplogroup that originated in northern Amazonia (current Colombian and Ecuador), and dispersed to the Bolivian Amazon. The second specimen was even more interesting. It was sampled in an Andean area from the Cochabamba Department and it was closely related with another specimen from the Andean area of the Apurimac Department (southern Peru) and both were included within H7-NAS. Both specimens showed a morphology similar to that of *N. olivacea*, or, at least, intermediate between *N. nasua* and *N. olivacea*, although they are related to an *N. nasua* haplogroup originating in the trans-Andean and Pacific Ecuador. Three hypotheses arose from this finding: 1) It is one additional polyphyletic group of *N. olivacea* distributed in Peru and Bolivia, where this species has not yet been reported; 2) These specimens represent cases of convergent adaptative morphology evolution of *N. nasua* to the phenotype of *N. olivacea* which is adapted to live in high altitudes; or 3) Because these haplotypes were very similar to those of the *N. nasua* haplogroup where they were enclosed, it could be a case of relatively recent hybridization or introgression between *N. nasua* and *N. olivacea*. However, as *N. olivacea* has not been described in these areas of Peru and Bolivia, this could be a proof in favor of the existence of *N. olivacea* in some Andean areas of Peru and Bolivia.

There only seems to be one group of *N. nasua* in Paraguay and Uruguay as well as for southern Brazil. It is these southern areas of South America where these groups of *N. nasua* arrived more recently and therefore, the genetic situation is simpler.

Our analyses indicated that the coati introduced to the Juan Fernández Archipielago (Robinson Crusoe Island) in Chile was *N. nasua* (H7-NAS). It was previously thought that the trans-Andean Pacific Ecuadorian coati was *N. narica*, and the coatis in the Juan Fernández Archipielago probably were taken from the Pacific Ecuador as our molecular results demonstrated, but also our findings demonstrated that the majority (but not all) of coatis of this Ecuadorian area are *N. nasua*.

As we did not analyze specimens from Venezuela, Guianas, and northern Brazil, we cannot determine if any haplogroups is related to the morphological subspecies *N. n. vittata* (type locality: Mt. Roraime, Venezuela; [108]).

One really unique way to determine the similarity between the haplogroups and the morphological subspecies would be to sequence the holotypes used to define the different subspecies of *N. nasua*.

We analyzed the systematics of *N. olivacea* in Ruiz-García, et al. (2020). Here, we will very briefly discuss the issue. Helgen, et al. (2009) determined that the population of the mountain coati in Merida Cordillera in Venezuela was an endemic species that they named *Nasuella meridensis*. However, Ruiz-García, et al., (2020, 2021) showed that the Venezuelan *N. meridensis* was undifferentiated from the H4-OLI that we mainly detected in the Eastern Colombian Andean Cordillera. Therefore, *N. olivacea olivacea* [111] and *N. meridiensis* [110] are synonyms. This means that H4-OLI agrees quite well with *N. o. olivacea* [111]. H3-OLI should correlate with *N. o. quitensis* [112] because it is the most common haplogroup of the mountain coati in the Andes region of Ecuador. However, H1-OLI and H2-OLI were also in the northern Ecuadorian Andes. Henceforth, only the molecular analysis of the holotype of *N. o. quitensis* will completely resolve this issue.

Nevertheless, this work brings forth new information on *N. olivacea*: 1) we augmented the number of mountain coatis and we detected one specimen from Tamá NP (Norte de Santander Department, Colombia) belonging to H1-OLI. All of the specimens from Tamá NP were previously analyzed by Ruiz-García, et al. (2020) and belonged to H4-OLI. Thus, we did detect one specimen with other haplogroups of *N. olivacea* on the Eastern Colombian Andean Cordillera. Thus, we detect more cases of geographic sympatry between different groups as we increase the sampling size. 2) The H1-OLI was a haplogroup of phenotypic specimens similar to *N. olivacea* but with the mitochondrial DNA more related to the Colombian and Ecuadorian Andean *N. nasua* than to the *N. olivacea* H3-OLI and H4-OLI. We named this haplogroup as "intermediate" between *N. nasua* (H6-NAS) and the most derived haplogroups of *N. olivacea* (H3-OLI and H4-OLI). This shows the strong relationships between different groups of Andean *N. nasua* and different groups of *N. olivacea*. Again this supports that the mountain coatis are really polyphyletic. 3) Additionally, in this work, we detected an opposing case. Specimen's phenotypically *N. nasua* presented

mitochondrial DNA more related to that of the most derived *N. olivacea* (H3-OLI and H4-OLI) than to any other haplogroups of *N. nasua*. Any specimens that were phenotypically *N. nasua* but had similar (but differentiated) mitochondrial DNA to the derived haplogroups of *N. olivacea* (rather than to the other *N. nasua*' DNAs) were Andean specimens from Antioquia (Colombia), Imbabura and Pichincha Provinces in Ecuador, and Junin Department in central Peruvian Andes. Thus, we detected not only "intermediate" haplotypes of phenotypic specimens of *N. olivacea* from *N. nasua* towards the most derived *N. olivacea*, but we also detected "intermediate" haplotypes of specimens phenotypically *N. nasua* towards the most derived *N. olivacea* haplogroups, which is highly related with reticulate evolution among very related taxa without genetic isolation when these taxa are living in sympatry. For this reason, it would be better to consider all of the coatis as a unique species. 4) But, possibly, the most relevant finding was the clear detection of a specimen of *N. olivacea* from H3-OLI in southern Peru at the Urubamba River (Cuzco Department). This specimen had a morphotype similar to the *N. olivacea* from Colombia and Ecuador and, also, an mtDNA of an undisputable *N. olivacea* from H3-OLI. Therefore, this is the first indubitable proof in favour of the presence of *N. olivacea* in Peru. Until now, there was no clear evidence of mountain coati in Peru. So, we provide the first irrefutable proof of the presence of a "true" *N. olivacea* in Peru (photos of the dry skin of this specimen could be provided by the first author)

Temporal splits in the evolution of the coatis

Traditionally, from a paleontological point of view, the earliest fossils of Nasua were found in North America and dated to the late Hemphillian (6.7-4.7 MYA) to early Irvingtonian (1.6-1.0 MYA) [113-115]. Based on these paleontological records and that the South American fossil record of Nasua was dated to the Pleistocene, the current coatis derived from North American lineages that migrated into South America during the Pleistocene following the emergence of the Panamanian isthmus [113,116,117]. In fact, the entry in South America (Argentina) of the first Holartic mammals during the Huayquerian (Late Miocene; 9-6 MYA) were procyonids of the endemic genera Cyonasua and Chapalmalania [118,119] dated, at least, around 7.3 MYA. Paleontologist considered, however, that these procyonids disappeared and there were not linked with the current Nasua. They considered that the current Nasua in South America is the descendent of a second wave of procyonids that entered into South America during the GABI 4 (Great American Biotic Interchange, phase 4; [11]) developed during the Lujanian and Platan ages (beginning at 0.125 MYA; Late Pleistocene).

Nevertheless, our molecular data completely disagree with this view. The molecular evolution of the coati seems to appear around 13-10 MYA "in situ" in South America and therefore they are correlated with the first procyonid wave, represented by Cyonasua, arrived to South America during the Miocene. With the MJN procdure, we estimated around 13 MY (for the nucleotide substitution rate of 1.3%/MY), or 9.9 MY (for the rate of 1.7%/MY) passed between the first and oldest *N. nasua* haplotype and the most derived haplotype of *N. narica* The Bayesian analyses indicated that the original mitochondrial diversification process in the coatis began around 11.9 MYA. In agreement with our own results, Nigenda-Morales, et al. (2019) determined that the initial evolution of the coatis occurred from South America to Central America. They estimated around the beginning of the evolution of coati in South America to have begun 7-6 MYA. They only studied five specimens of *N. nasua* and therefore they did not detect the oldest haplotypes of the Andean *N. nasua*. Therefore, they did not detect the oldest mitochondrial diversification events that we detected in the current study. Be it as it may be, the initial lineage diversification in the coatis began in South America (and not in North or Central America) in the Late Miocene.

Koepfli, et al. (2007) showed that the ancestors of Bassaricyon and *Nasua* and the ancestors of Bassariscus and Procyon diverged contemporaneously near the end of the Middle Miocene around 12–13 MYA. Additionally, the ancestors of the extant species *Bassariscus astutus* and *B. sumichrasti* diverged around 9.1–10.6 MYA in the Late Miocene. Recently, it was determined the initial diversification process of *Potos flavus* occurred around 9.6 MYA [120].

These correlated patterns of divergence between independent procyonid lineages agreed with the influence of major regional environmental processes on radiation of the Procyonidae [121]. This period coincides with a process of major global cooling, along with formation of the Antarctic ice sheet and a short-term but important drop in global sea level [122-124]. In addition, the Neotropical forests reduced and transformed into savanna-like environments that expanded [125]. These climatic events played an outstanding role in differentiation of other mammalian species [121,126].

The time elapsed from the first and original *N. nasua* haplotype to the most derived haplotypes of *N. nasua* (H1-NAS; Bolivia and southern Peru) was around 8.4-6.4 MY. We detected that the initial Ecuadorian *N. narica* haplotypes originated around 4.2-3.6 MY, whilst Nigenda-Morales, et al. (2019) detected the initial evolution of the southern Central American *N. narica* around 3.8 MYA. All these diversification processes were during the Pliocene.

Two processes were important during the Pliocene, which could help to understand the important diversification of coati during this period. The first was climatic. The cold and dry climate during the Pliocene coincided with the onset of high-latitude glacial cycles, causing an explosive expansion of low-biomass vegetation, in many places of the world. These changes were correlated with the diversification of prey species that exploited new habitats, which in turn could provide new niches for many carnivores as the coatis. Additionally, it caused fragmentation of some Neotropical rain forests. The second was geologic. Strong geological changes occurred in the Northern Andes, which had a noteworthy repercussion in the Andean Cordilleras and in the rivers related with these Cordilleras. It was very important the formation of the Fitzcarrald Arch (4 MYA). It is demonstrated that the Nazca Ridge subduction imprint had a significant influence on the eastern side of the Andes by means of the Fitzcarrald Arch [127,128]. This uplift is responsible for the atypical three-dimensional shape of the Amazonian foreland basin. Related to Nazca Ridge subduction, arc volcanism in the Peruvian Andes ceased around 4 MYA [129]. These geological changes in the Northern Andes were fundamental because many of the oldest *N. nasua* and *N. olivacea* groups appeared in that area of the Northern Andes and, even, the oldest Ecuadorian *N. narica* haplotypes were also generated at this time.

Finally, the diversification among the youngest haplotypes of *N. narica* or within the youngest haplogroups of *N. nasua* and *N. olivacea* were basically during the Pleistocene (2.5-0.01 MYA). We dated the diversification of *N. narica* from middle Central America to northern Guatemala and southern Mexico to around 1.1 MYA. This estimate is very similar to the 1.2 MYA estimate made by Nigenda-Morales, et al. (2019). The Pleistocene Refugia Hypothesis (PRH) [130-132] could be extremely important in helping to explain why in some areas, especially in Colombia, Ecuador, and Peru, many haplogroups of *N. nasua* and *N. olivacea* were found in sympatry. The PRH could be also being extended to the Pliocene (until 2.8 MYA) [131]. This hypothesis claims that alternative dry-wet cycles caused by the Milankovitch cycles [133] affected the forest of the Neotropics. It was provided evidence about the climatic dryness that affected the Amazon and humid tropical environments of the Neotropics [134]. Large inactive dune fields, typical of dry environments, extended several thousand km^2 between the Negro and Branco rivers (Pantanal do Norte, northern Brazilian Amazon) [135]. Today, this area is covered by the caatinga (open vegetation), which suggests that this area was much dryer in the past compared to today. Correlated with this, numerous smaller Aeolian sand fields exist further north around Boa Vista [136]. Some rainforests, 250 kilometers north of Manaus, are underlain by layers of coarse and extremely poorly sorted sediments which must have been deposited under dry climatic conditions of the recent geological past when dense rainforests were absent at this geographical area [137]. Henceforth, extreme sympatry of many groups of *N. nasua* and *N. olivacea*, rather than *N. narica* originated by contraction (isolation and apparition of the haplogroups found) and posterior expansion of these haplogroups which meet in some identical geographical areas (both in high and low altitudes) today.

Conclusion

Our experience shows us that in order to determine how many groups or taxa are within a species, or taxon, we should initially conduct an analysis of mitochondrial DNA. In the second step, we analyze nuclear markers. This allows us to determine the real number of groups or ESUs and if genetic interchange (s) occurred amongst these groups. The second step also helps to determine when the genetic interchanges occurred, and if gene flow is sexually biased. Therefore, this mitochondrial analysis of the coatis is a first step in completely reconstructing the evolutionary history of these amazing species.

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