



The phylogeographic structure of the mountain coati (*Nasuella olivacea*; Procyonidae, Carnivora), and its phylogenetic relationships with other coati species (*Nasua nasua* and *Nasua narica*) as inferred by mitochondrial DNA

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Abstract

Carnivores are important elements of Neotropical biomes that are in need of conservation efforts. However, successful conservation methods rely on the identification of accurate evolutionary taxa. Unfortunately, in the case of Procyonidae systematics, there has been little knowledge in some genera. Two of these genera are *Nasuella* and *Nasua*, also known as the coatis. Herein, we analyzed a dataset obtained in South America and Central America, containing sequences of three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) collected from 42 mountain coati (*Nasuella olivacea*) specimens, plus 50 white-nosed coati (*Nasua narica*) and 51 ring-tailed coati (*Nasua nasua*) (total sample of 143). Our results support four main findings. (1) We detected four significantly different groups of *N. olivacea*. There were two small groups, one distributed in the Central Colombian Andean Cordillera and Western Ecuadorian Andean Cordillera, and another in the Western Colombian and Ecuadorian Andean Cordilleras. The specimens of these small groups were phenotypically un-differentiable from *N. olivacea*, but their mtDNA were more related to that of *N. nasua* than to the mtDNA of the other *N. olivacea*. The other two groups of *N. olivacea* contained the major part of the specimens analyzed. One is in the Eastern Colombian Andean Cordillera and is molecularly un-differentiable from the proposed “new” endemic Venezuelan species, *Nasuella meridiensis*. The ancestor of this group gave origin to another expanded group in the Western and Central Colombian and Ecuadorian Andean Cordilleras. (2) Different analyses (network, temporal splits, genetic diversity analyses) showed that the mitochondrial haplotypes of *N. nasua* were the first to appear (temporal diversification during the Late Miocene, and Pliocene), followed by the haplotypes of the current groups of *Nasuella* (temporal diversification during the Pliocene and beginning of the Pleistocene), and then the haplotypes that of the Central American *N. narica* (temporal diversification during the Pleistocene). Within *N. nasua*, we detected, at least, four highly differentiated groups that contain cryptic species or highly differentiated subspecies. (3) All of the taxa we analyzed showed high levels of mitochondrial genetic diversity, but *N. nasua* showed the highest levels, whereas *N. narica* showed the lowest levels. (4) Some groups of *N. olivacea*, and *N. narica* showed Pleistocene population expansions, but all the taxa showed a very strong signal of population declination in the last 20,000 years ago (YA), which could be correlated with the drastic climatic changes in that epoch.

Keywords Central America · Coatis · Mitochondrial genes · *Nasua narica* · *Nasua nasua* · *Nasuella meridiensis* · *Nasuella olivacea* · Phylogeography · Population expansions · Spatial genetic structure · South America

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Introduction

The Carnivora Order encloses 16 families divided into two monophyletic super-families, Caniformia and Feliformia (Eisenberg 1989; Wozencraft 2005; Wyss and Flynn 1993). 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 (Wilson and Mittermeier 2009).

The majority of molecular studies including those of Procyonidae were undertaken to clarify the phylogenetic relationships among Carnivores (Yu et al. 2004). Koepfli et al. (2007) were the first to try to resolve the phylogenetic relationships among the genera within Procyonidae using nuclear and mitochondrial genes. However, they did not include sequences of the mountain coati (*Nasuella olivacea*). This work was important because it was the first effort to estimate the temporal splits among and within the Procyonidae genera as well as to analyze possible phenomena which affected the colonization of this family in Central and South America.

Only a small number of molecular studies have focused at the level of species for Procyonidae in the Neotropics (McFadden 2004 for *Nasua* and *Procyon*; Helgen et al. 2009, 2013 for *Nasuella* and *Bassarycion*; Tsuchiya-Jerep (2009) for *Nasua*; Neves-Chaves (2011) for *Nasua*; Ruiz-García et al. 2013, 2019 for *Potos*; Nascimento et al. 2017 for *Potos*; Silva Caballero et al. 2017 for *Nasua*; and Nigenda-Morales et al. 2019 for *Nasua*).

There are only two publications (Helgen et al. 2009; Nigenda-Morales et al. 2019) including *N. olivacea*, the species covered in the current study. The first study only included four specimens of *Nasuella*, one from the Western Colombian Andes (Cauca Department), two from the northern Ecuadorian Andes, and one from the Venezuelan Merida Cordillera. Additionally, they studied two *N. nasua* (Bolivia and Brazil) and two *Nasua narica* (Panama and southern USA). They sequenced and analyzed only a small fraction of the mitochondrial (mt) *Cyt-b* gene (366 base pairs, bp). However, even with these scarce data, they reached the conclusion, that the unique sample from Venezuela was a different species (*Nasuella meridensis*). It was differentiated from the specimens from the Western Colombian Andean Cordillera and northern Ecuador, which belonged to *N. olivacea* (100%, bootstrap percentage, and 84%, Bayesian posterior probability). Overall, this work was too preliminary to completely elucidate the genetic structure and phylogeography of the mountain coati.

The second work, with three mtDNA genes and with 11 microsatellites, found a high degree of genetic structure

and divergence in *N. narica* that conformed to five evolutionarily significant units. The phylogeographic patterns found within *N. narica* were associated with geographic barriers and habitat shifts caused by Pliocene–Pleistocene climate oscillations. Additionally, their findings suggested the dispersal of *N. narica* was from southern Central America to north beginning in the Pliocene, and not from north to south direction during the Pleistocene as suggested by the fossil record.

In the current work, we analyzed three mitochondrial genes (mt genes) of 42 mountain coatis and 101 *Nasua* specimens (total 143 specimens). We elected to focus on mt genes as a first step to understand the phylogeography and the possible existence of full species within *N. olivacea*. Mitochondrial genes are interesting markers for phylogenetic tasks because they include a rapid accumulation of mutations, rapid coalescence time, lack introns, have a negligible recombination rate and haploid inheritance (Avise et al. 1987). They also have a high number of copies per cell which makes mitogenome data easy to obtain and sequence especially in low-quality samples, such as hair, teeth or small pieces of skin (Mason et al. 2011; Guschanski et al. 2013). Despite representing a single linked locus, selection pressures and evolutionary rates are highly heterogeneous across the mtDNA (Galtier et al. 2006; Nabholz et al. 2012). For all of these reasons, mitochondrial gene trees are more precise in reconstructing the divergence history within species, or among very related species, than other molecular markers (Moore 1995).

Extreme care should be taken when using mitochondrial genes for resolving taxonomic problems because gene trees do not necessarily correspond well with species trees. Species can diverge simultaneously with a pair of mitochondrial haplotypes or they can diverge after a pair of haplotypes diverge. However, it is possible for a new haplotype to develop some time after a population divides. A migrant could carry the new haplotype to another population and, both it and the ancestral haplotypes could eventually become lost from their respective populations. Therefore, if we use the gene tree to estimate genetic heterogeneity and the divergence time for the species tree, the species will appear to have diverged more recently than they really did (Freeman and Herron 1998). Furthermore, mitochondrial data only show the evolution of the female lineages, and this could miss hybridization events between close species when males are the gene flow vectors ('mitochondrial capture'; Burrell et al. 2009). To fully resolve the issue about the complete understanding of the evolutionary biology and number of taxa in *N. olivacea*, there 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 (see Derenko et al. 2012; Krause et al. 2010; Sawyer et al. 2015). The mtDNA results should be instrumental 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 (O'Brien 1994). An essential second step in the future will be to analyze nuclear markers to detect possible hybridization or gene flow events among different lineages.

Thus, the main aims of the current work were: (1) To determine the number of significantly different groups within *N. olivacea* and to determine if any are full species; (2) To determine if the presumed “new” endemic Venezuelan *N. meridiensis* is a “real” full species; (3) To determine the kind of phylogenetic relationship of *N. olivacea* with *N. nasua* and *N. narica*; (4) To determine possible significant groups within *N. nasua* and *N. narica*; (5) To estimate the levels of genetic diversity in *Nasuella* and *Nasua*; (6) To analyze possible demographic changes in these taxa; and (7) To determine the possible spatial genetic structure in *N. olivacea*.

Materials and methods

We analyzed a set of 143 individuals, all analyzed at the mt genes *ND5*, *Cyt-b*, and *D-loop*. Forty-two were *N. olivacea*, 50 were *N. narica*, and 51 were *N. nasua*. Additionally, as outgroups, we included three specimens of *Bassarycion medius* (Ecuador), two specimens of *Bassarycion neblina* (Colombia), and 11 specimens of *Bassarycion alleni* (three from Ecuador, four from Peru, and four from Bolivia) (see Table 1 and Fig. 1).

DNA was obtained from hair, teeth, muscle and blood from living and dead animals in diverse Indian, colono, or mestizo communities. We requested permission to collect biological materials from either carcasses or live animals that were already present in the community. We sampled small pieces of muscle, blood drops, or teeth from hunted animals that were discarded during the cooking process, or hairs with bulbs plucked from live pets. Communities were visited only once, all sample donations were voluntary, and no financial or other inducement was offered for supplying specimens for analysis. During the sampling process (pets and hunted animals), we interviewed the indigenous hunters who claimed that the hunted and captured specimens came from within 10–15 km of their respective communities. For more information about sample permissions, see the Acknowledgment section. All the samples used were directly obtained by the authors for the current work.

Table 1 Sources of the coatis collected and analyzed at three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) (42 *Nasuella olivacea*, 50 *Nasua narica*, and 51 *Nasua nasua*)

Species and location	Number of samples
<i>Nasuella olivacea (n=42)</i>	
<i>Colombia (n=37)</i>	
<i>Boyacá Department</i>	
(Chita, Cocuy, Iguaque, Paipa, Villa de Leyva)	5
<i>Caldas Department</i>	
(Guasca, Ubaté, Chingaza NP)	2
<i>Cauca Department (Versalles, Puracé)</i>	
<i>Chocó Department</i>	
(Alto Galápagos, San José del Palmar)	2
<i>Cundinamarca Department</i>	
(Cumbal)	1
<i>Nariño Department</i>	
(Támará NP)	6
<i>Risaralda Department</i>	
(Lago Otún, Santa Rosa, Santa Cecilia)	4
<i>Seized in Bogotá</i>	
<i>Tolima Department</i>	
(Los Nevados NP)	1
<i>Ecuador (n=5)</i>	
<i>Carchi Province</i>	
(Tulcán)	1
<i>Cotopaxi Province</i>	
(Llanganates NP)	1
<i>Morona-Santiago Province</i>	
(Sangay NP)	1
<i>Pichincha Province</i>	
(Chiriboya, Guajacito)	2
<i>Nasua narica (n=50)</i>	
<i>Mexico (n=19)</i>	
<i>Campeche State</i>	
(San Cristobal de las Casas)	9
<i>Chiapas State</i>	
(Cozumel Island)	2
<i>Quintana Roo State</i>	
(Livingstone, Puerto Barrios)	1
<i>Tabasco State</i>	
(Tikal NP, Uaxactun)	7
<i>Guatemala (n=13)</i>	
<i>Alta Verapaz Department</i>	
(Cobán)	1
<i>Izabal Department</i>	
(Rio Bravo Conservation Area)	2
<i>Belize (n=3)</i>	
<i>Honduras (n=7)</i>	
<i>Olancho Department</i>	
(Tegucigalpa)	3

Table 1 (continued)

Species and location	Number of samples
(Juticalpa)	5
Roatán Island	2
El Salvador (n=3)	
La Libertad Department	
(Jayaque)	3
Nicaragua (n=1)	
Nueva Segovia Department	
(Ocotal)	1
Costa Rica (n=3)	
Alajuela Province	
(El Arenal)	2
Puntaarenas Province	
(Golfo Dulce RF)	1
Panama (n=1)	
Colón Province	
(Nombre de Dios)	1
Nasua nasua (n=51)	
Colombia (n=10)	
Amazonas Department	
(Leticia, Macedonia, Amacayacu NP)	5
Caquetá Department	
(Cuemani River)	1
Guaviare Department	
(El Raudal)	2
Meta Department	
(La Macarena, Pinacita)	2
Ecuador (n=2)	
Napo Province	
(Tena)	1
Sucumbíos Province	
(Lago Agrio)	1
Peru (n=13)	
Cuzco Department	
(Quincemil, Manu NP)	5
Loreto Department	
(Nanay River, Napo River)	3
Madre de Dios Department	
(Hermosa Grande)	2
Ucayali Department	
(Yarinacocha)	3
Bolivia (n=5)	
Beni Department	
(Moxos, Mamoré River)	3
La Paz Department	1
Santa Cruz Department	1
Brazil (n =12)	
Amazonas State	
(Tabatinga, Javarí River, Manaus)	5
Goias State	

Table 1 (continued)

Species and location	Number of samples
(Isla do Banal)	1
Parana State	
(Iguazú)	6
Paraguay (n=5)	
Alto Paraná Department	
(Pubio, Hernandarias)	5
Uruguay (n=4)	
Tucuarembó Department	
(Laureles)	3
Artigas Department	1

The number of specimens analyzed at three mitochondrial genes in each country is in bold

Molecular methods

We extracted DNA from the skin, muscle and blood samples with a modified phenol–chloroform procedure (Sambrook et al. 1989). 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).

We amplified three mitochondrial genes with primers from the following papers: (1) 407 base pairs (bp) of mtCyt-b (Irwin et al. 1991), (2) 1,800 bp of mtND5 (Trigo et al. 2008), and (3) 306 bp of mtD-loop (Hoelzel et al. 1994). The total length was 2513 bp.

We used a PCR reaction with 2 µl of MgCl₂ 1 mM, 1 µl of dNTPs 0.2 mM, 1 µl of 0.1 µM of each primer, 1 unit of Taq Polymerase, 100–200 ng of DNA (2–4 µl of DNA), 2 µl of Buffer 10X and 13.5 µl of H₂O. The PCR temperatures were 95° for 5 min followed by 40 cycles of 1 min at 94 °C, 1 min at 52–56 °C (depending on the primers used) and 1 min at 72 °C, and one final extension of 10 min 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 (QIAquick PCR Purification Kit; Qiagen). The PCR products were sequenced in both directions using the Big Dye™ kit in an ABI 377A automated DNA sequencer. A consensus of the forward and reverse sequences was determined using the Sequencher software (Gene Codes Corporation).

We translated the mtCyt-b and mtND5 gene sequences into amino acids to exclude the possibility of nuclear mitochondrial DNA segments (NUMTs) in the dataset analyzed (Lopez et al. 1994). We note that all amino acid translations of the obtained sequences showed initial start and terminal stop codons and the absence of premature stop codons. All the mutations we observed were synonymous changes. This agrees quite well with the fact that there were no NUMTs in our sequence data. Moreover, one

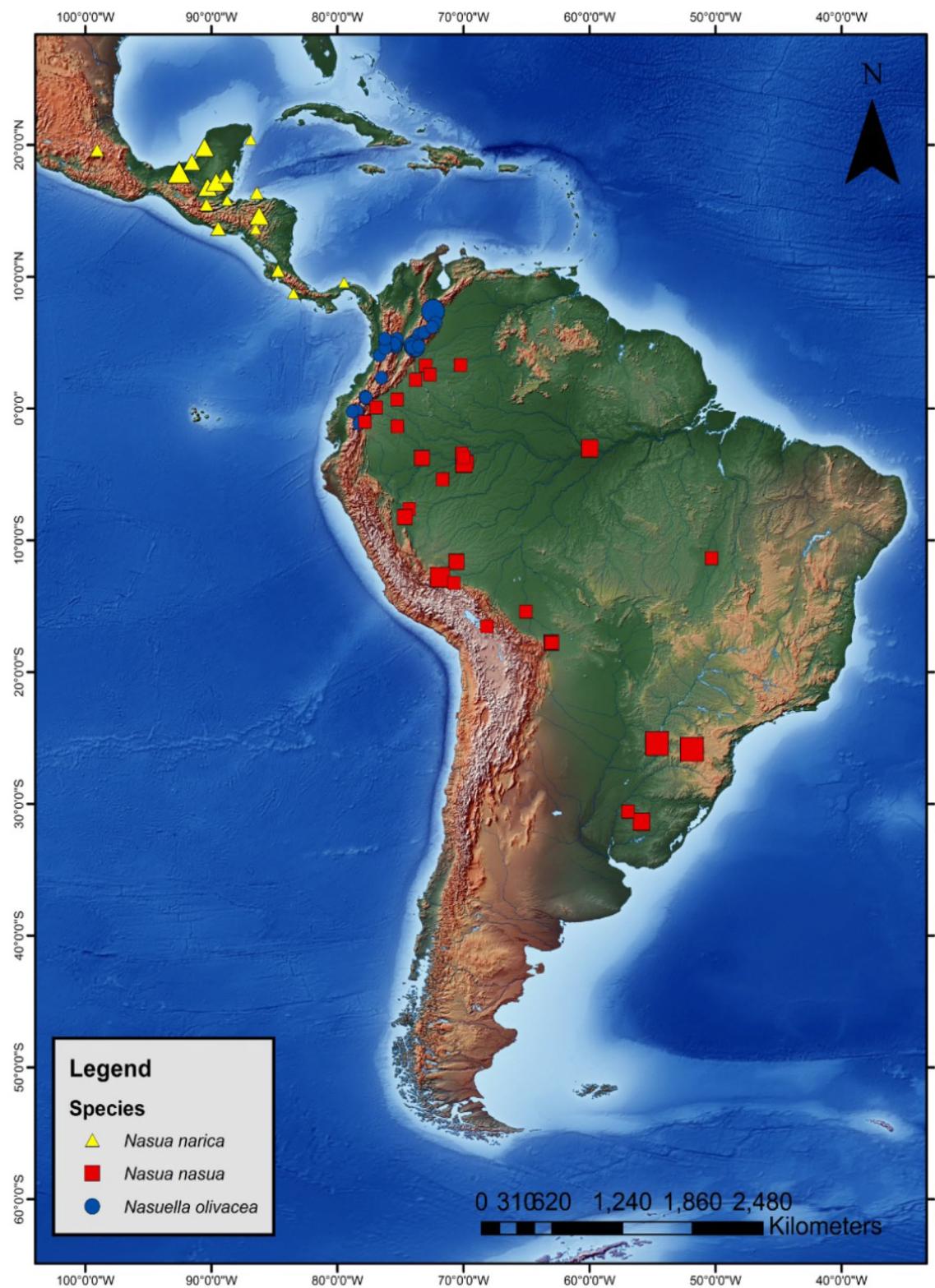


Fig. 1 Map of Latin America where specimens of *Nasuella olivacea*, *Nasua nasua*, and *Nasua narica* were sampled. These specimens were sequenced for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*). A total of 42 *Nasuella olivacea*, 50 *Nasua narica*, and 51 *Nasua nasua* were sampled

would expect to see a signal relating to NUMTs in the DNA chromatogram. When the ratio of nuclear to mitochondrial genomes per cell is around 1/1000 (Takamatsu et al. 2002), the proportion of NUMTs and real mitochondrial sequences is expected to be amplified. If the potential amplified NUMTs were highly differentiated from the real mitochondrial gene or included some insertions or deletions, many double peaks are expected in the chromatogram. We did not observe this in our chromatograms.

The GenBank accession numbers of the coati specimens analyzed are from MT587713 to MT587855.

Phylogenetic studies

MrModeltest v2.3 (Nylander 2004) and MEGA 6.05 software (Tamura et al. 2013) were applied to determine the best evolutionary mutation model for the sequences analyzed for each individual gene, for different partitions and for all the concatenated sequences. Akaike information criterion (AIC; Akaike 1974; Posada and Buckley 2004) and the Bayesian information criterion (BIC; Schwarz 1978) were used to determine the best evolutionary nucleotide model.

We constructed a Maximum Likelihood phylogenetic tree (MLT) using RAxML v.7.2.6 software (Stamatakis 2006). To select the best fitting model, 50 independent iterations were run using three data partitions (codons 1, 2, and 3). For each analysis, the GTR + G + I model (General Time Reversible model, Tavaré 1986, + gamma-distributed rate variation among sites + proportion of invariable sites, Yang 1994) was used to search for the MLT and topological support was estimated with 500 bootstrap replicates (Stamatakis 2006).

To estimate possible divergence times among the haplotypes found in *N. olivacea*, *N. nasua*, and *N. narica*, we relied on a Median Joining Network (MJN) (Bandelt et al. 1999) using Network 4.6.0.1 software (Fluxus Technology Ltd). Additionally, the ρ statistic (Morral et al. 1994) and its standard deviation (Saillard et al. 2000) 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 (Pennington and Dick 2010). We used an evolutionary rate of 1.75% per one MY, which represented one mutation every 22,742 years for the 2513 bp analyzed for the dataset analyzed. One advantage of the MJN procedures compared to traditional trees is that they explicitly allow for the co-existence of ancestral and descendant haplotypes, whereas traditional trees treat all sequences as terminal taxa (Posada and Crandall 2001). This allows us to observe which current haplotypes began to evolve first and also to identify the more recently derived haplotypes.

Genetic heterogeneity

The statistical procedures H_{ST} , K_{ST} , K_{ST^*} , γ_{ST} , N_{ST} and F_{ST} (Hudson et al. 1992) were applied to determine the overall genetic heterogeneity among the different taxa of *Nasuella* and *Nasua* detected in the phylogenetic analyses. We obtained indirect gene flow estimates for all the taxa, assuming an infinite island model (Wright 1965). Significance was estimated with permutation tests using 10,000 replicates. These analyses were applied to the three coati species, as well to the four groups detected within *Nasuella olivacea*. In addition, genetic heterogeneity and gene flow statistics were calculated by taxa pairs. We used F_{ST} tests with Markov chains, 10,000 dememorizations parameters, 20 batches, and 5,000 iterations per batch. All of these statistics were calculated with DNAsp 5.1 (Librado and Rozas 2009) and Arlequin 3.5.1.2 programs (Excoffier and Lischer 2010).

The Kimura 2P genetic distance (Kimura 1980) was applied to determine the percentage of genetic differences among the different groups detected in *Nasuella* and *Nasua*. The Kimura 2P genetic distance is a standard measurement for barcoding tasks (Hebert et al. 2003, 2004). Kartavtsev (2011) analyzed sequences of mtCOI from 20,731 vertebrate and invertebrate animal species and obtained $0.89\% \pm 0.16\%$ for populations within species, $3.78\% \pm 1.18\%$ for subspecies or semispecies, and $11.06\% \pm 0.53\%$ for species within a genus. At mtCOII, Collins and Dubach (2000), 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. Bradley and Baker (2001) claimed, for mtCytb, that values < 2% would equal intra-specific variation, values between 2 and 13% would merit additional study, and values > 13% would be indicative of specific recognition. Therefore, for the three mt genes employed, we consider values around 3–5% for possible subspecies and values around 12–13% for different species of the same genus. For species of different genera, this value should be around 16–18% or higher (Kartavtsev 2011).

Genetic diversity and demographic changes

We used the following statistics to determine the genetic diversity for the overall sample of *N. olivacea*, and for the two main haplogroups of *N. olivacea*, and for *N. nasua*, and *N. narica*: number of haplotypes, haplotype diversity (H_d), nucleotide diversity (π), and θ statistic by sequence. These genetic diversity statistics were calculated with DNAsp 5.1 software (Librado and Rozas 2009).

We relied on three procedures to detect possible historical population changes in the overall sample of *N. olivacea*, the two main haplogroups of *N. olivacea*, *N. nasua*, and *N. narica*. (1) We used the Fu and Li D^* and F^* tests (Fu and Li 1993), the Fu F_S statistic (Fu 1997), the Tajima

D test (Tajima 1989) and the R_2 statistic (Ramos-Onsins and Rozas 2002). A 95% confidence interval and probabilities were obtained with 10,000 coalescence permutations. (2) The mismatch distribution (pairwise sequence differences) was obtained following the method of Rogers and Harpending (1992) and Rogers et al. (1996). We used the raggedness rg statistic to determine the similarity between the observed and the theoretical curves. All these previous demographic analyses were carried out with the DNAsp 5.1 (Librado and Rozas 2009) and Arlequin 3.5.1.2 programs (Excoffier and Lischer 2010). (3) A Bayesian skyline plot (BSP) was obtained by means of the BEAST v. 1.8.1 (Drummond et al. 2012) and Tracer v1.6 (Rambaut et al. 2013) software. The Coalescent-Bayesian skyline option in the tree priors was selected with four steps and a piecewise-constant skyline model with 30,000,000 generations (the first 3 million discarded as burn-in), kappa with log Normal [1, 1.25] and Skyline population size with uniform [0, infinite; initial value 80]. Marginal densities of temporal splits were analyzed and the Bayesian Skyline reconstruction option was selected for the trees log file using Tracer v1.6. We selected a stepwise (constant) Bayesian skyline variant with maximum time as the upper 95% high posterior density (HPD) and the trace of the root height as the treeModel.rootHeight. We considered the last few million years as part of this analysis. Nevertheless, it is important to recall that all of these demographic procedures have several caveats. Selection has effects on effective population sizes reducing the effective numbers for a time and increasing the coalescence rate later (Schridler et al. 2016). The same occurs with little changes in the mutation rates (μ), which can greatly affect the effective numbers and which in turn affect estimated divergence times (Sheehan et al. 2013).

Spatial genetic structure in *N. olivacea*

For all the spatial analyses carried out, we used the latitude and longitude recorded for each specimen analyzed. No populations or groups of individuals were employed for these analyses.

A Mantel's test (Mantel 1967) was used to detect possible overall relationships between a genetic matrix among specimens of *N. olivacea* (Kimura 2P genetic distance) and the geographic distance matrix among the specimens analyzed. In this study, Mantel's statistic was normalized according to Smouse et al. (1986). This procedure transforms the statistic into a correlation coefficient.

The spatial autocorrelation analysis utilized the Ay statistic (Miller 2005) for each distance class (DC), where $Ay = \sum_{i=1}^n \sum_{j>i}^n (D_{ij}w_{ij}) / \sum_{i=1}^n \sum_{j>i}^n w_{ij}$, where n is the number of individuals in the data set, and D_{ij} is the genetic distance between observations i and j . Elements of a binary matrix, W_{ij} , take on values of 1 if the geographical

distance between observation i and j fall within the boundaries specified for a specified DC and are 0 otherwise. Ay can be interpreted as the average genetic distance between pairs of individuals that fall within a specified DC. Ay takes on a value of 0 when all individuals within a DC are genetically identical and takes on a value of 1 when all individuals within a DC are completely dissimilar. The probability for each DC are obtained using 1,000 randomizations. For this analysis, there were six defined DCs for the three-gene datasets (0–50 km; 50–150 km; 150–210 km; 210–270 km; 270–440 km; 440–800 km) each of different size but with an equal number of individual comparisons. This analysis was carried out with AIS software (Miller 2005).

Another spatial analysis was carried out with Monmonier's Algorithm (Monmonier 1973; MMDA) using AIS software (Miller 2005). This geographical regionalization procedure is used to detect the locations of putative barriers to gene flow by iteratively identifying sets of contiguous, large genetic distances along with connectivity networks (Doupanloup et al. 2002; Manel et al. 2003; Manni et al. 2004). A Delaunay triangulation (Watson 1992; Brouns et al. 2003) was used to generate the connectivity network among sampling points. A graphical representation of putative "barriers" inferred by the algorithm is superimposed over the connectivity network to detect rapid identification of important geographical features reflected by the genetic dataset. In this case, we used this procedure to detect the five most important geographical barriers contained in the dataset for *N. olivacea*.

Results

Phylogenetic analyses

The nucleotide substitution models which were the best for the three mt genes taken together were TN93 + G for BIC (20,268.155) and GTR + G + I for AIC (13,234.043), respectively.

The MLT (Fig. 2) showed that the first *Nasua-Nasuella* clade to diverge (76%) consisted of two specimens of *N. olivacea* (Risaralda-Colombia, and Pichincha-Ecuador; we named this group FGO-N) and 14 specimens of *N. nasua* from the Colombian, Ecuadorian and northern Peruvian Amazon, Colombian Eastern Llanos and one specimen from Santa Cruz-Bolivia (bootstrap support, BS = 97%). The second clade to diverge had four specimens of *N. olivacea* (BS = 82%; we named this group SGO-N), all from the trans-Andean area of Colombia (Chocó, Cauca, and Nariño Departments) and Ecuador (Pichincha Province). The third clade to diverge was completely integrated by 37 specimens of *N. nasua*. Within this clade, there were two specimens from the western-central Brazilian Amazon, which presented

Fig. 2 Maximum likelihood tree (ML) for 42 *Nasuella olivacea*, 50 *Nasua narica*, and 51 *Nasua nasua* specimens sampled throughout the Neotropics sequenced at three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*). Nodes are labelled with bootstrap percentages

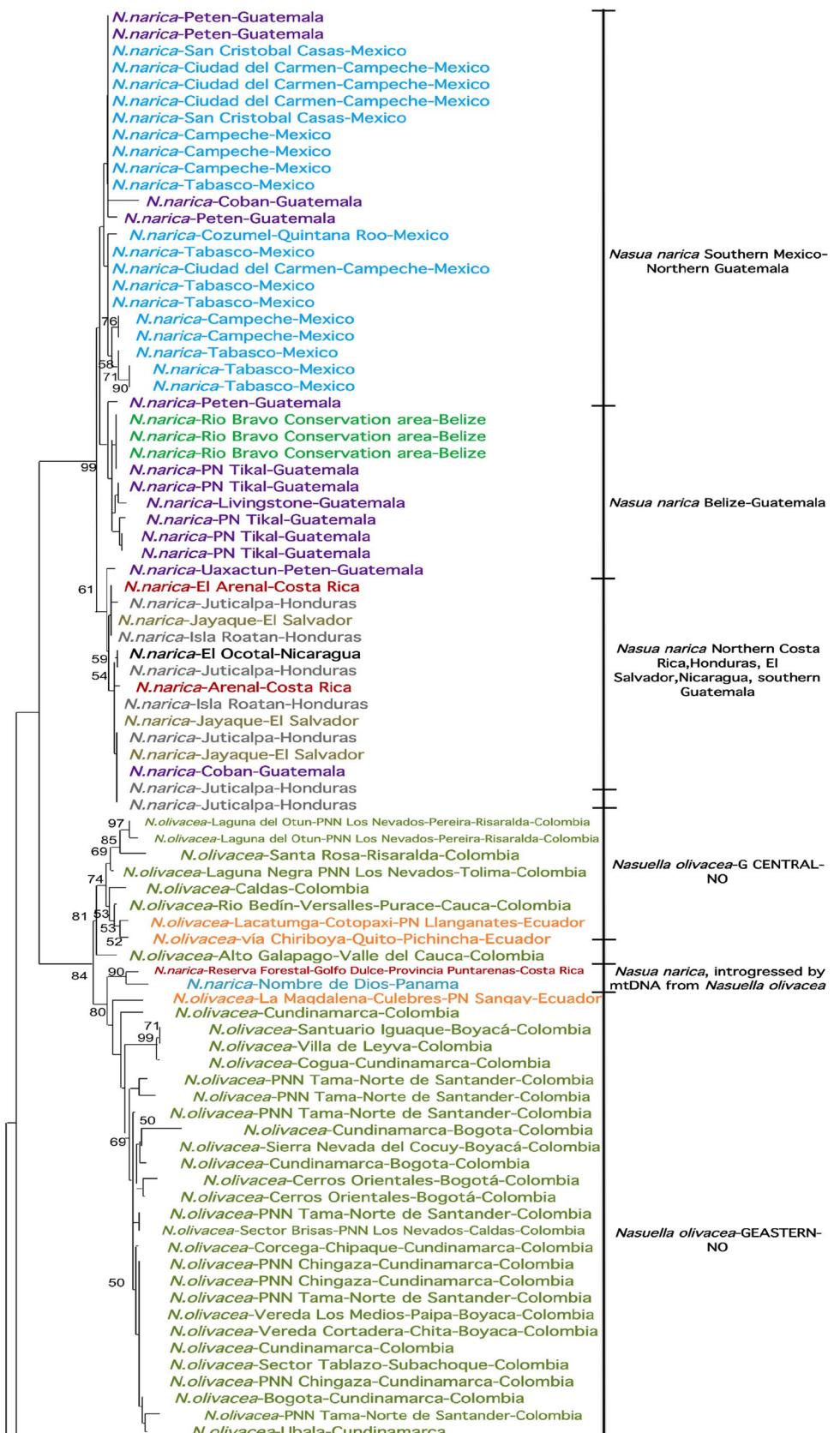
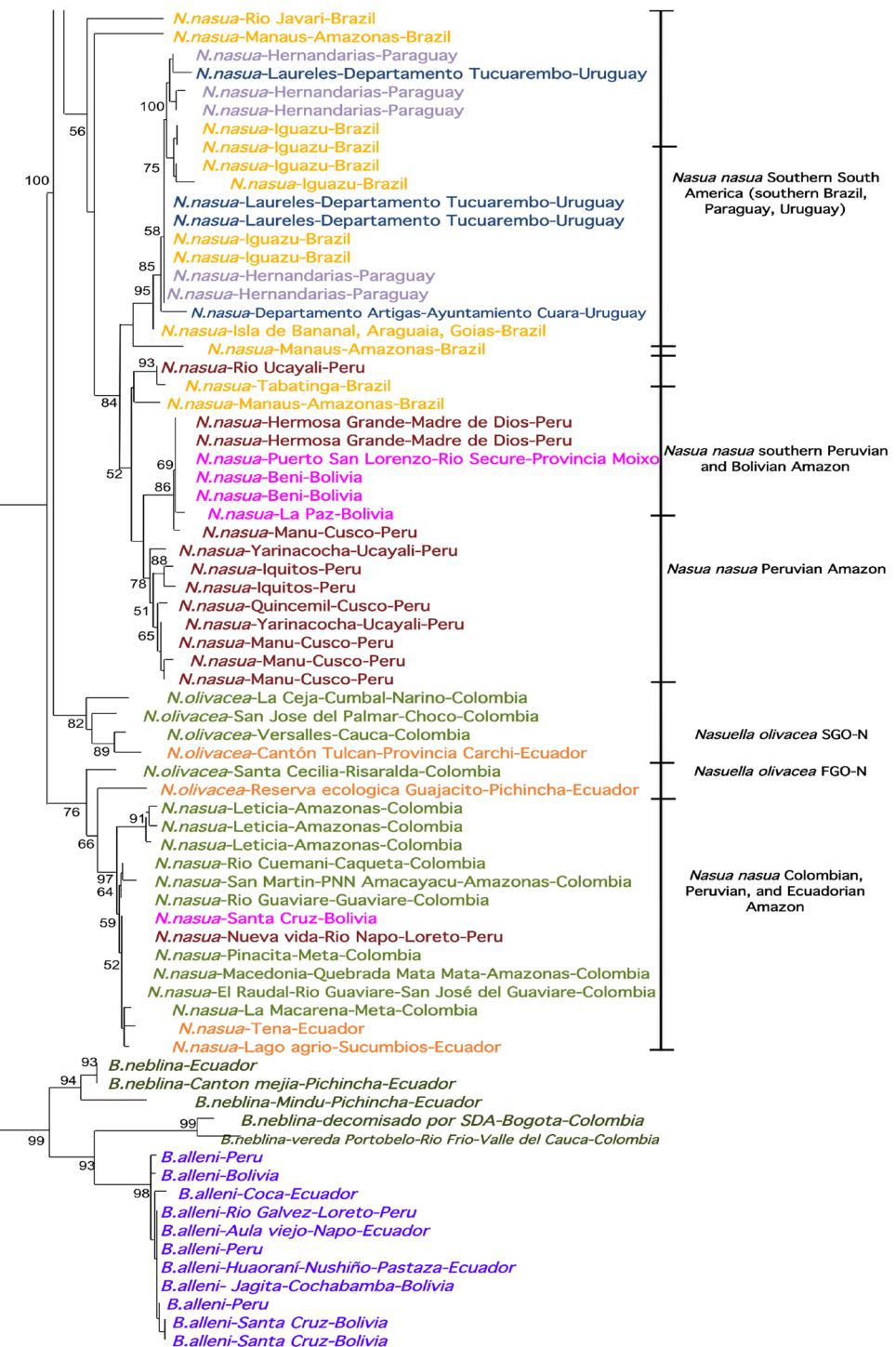


Fig. 2 (continued)



a low bootstrap. However, the remaining specimens of this clade yielded an elevated bootstrap ($BS=84\%$). This clade was characterized by three large sub-clades from precise geographical areas: throughout the entire Peruvian Amazon (Loreto, Ucayali, and Manu National Park, Cusco; $BS=78\%$), part of the southern Peruvian Amazon and Bolivia ($BS=86\%$), and southern South America and the Brazilian state of Goias (Paraguay, Uruguay, and Iguazú

National Park in southern Brazil; $BS=95\%$). A few specimens from the western and central Brazilian Amazon were present among these three geographical sub-clades. The fourth large clade to diverge consisted of the major fraction of specimens studied of *N. olivacea* ($BS=84\%$). Within this clade, we detected two significant sub-clades. One composed of nine specimens from the Central Colombian Andean Cordillera, and a few from the Western Colombian

Andean Cordillera, plus some specimens from the trans-Andean northern area of Ecuador ($BS = 81\%$; we named this group GCENTRAL-NO). Another contained 27 specimens ($BS = 80\%$; we named this group GEASTERNO-NO), all from the Eastern Colombian Andean Cordillera (Boyacá, Cundinamarca, and Norte de Santander Department), and curiously, also a specimen from the Ecuadorian Sangay National Park in Eastern Ecuador. There was also a mysterious small sub-clade consisting of two *N. narica* from Panama and southern Costa Rica, but with mitochondrial DNA from *N. olivacea* ($BS = 90\%$). The fifth and last large clade to diverge was composed of all the *N. narica*, except the two from southern Central America with the DNA of *N. olivacea* ($BS = 99\%$). There were no sub-clades with high bootstrap percentages within this clade. However, the specimens sampled in southern Mexico and few of northern Guatemala tend to clump together, whereas the specimens from central and northern Guatemala and Belize tend to create another cluster, and the specimens from southern Guatemala, Honduras, El Salvador, Nicaragua, and northern Costa Rica form another cluster. One specimen from Cozumel Island (*N. narica nelsoni* or *N. nelsoni* for some authors) had mitochondrial sequences that were undifferentiated from the other Mexican coati specimens.

Therefore, the MLT showed the existence of four groups of specimens with the typical morphology and within the geographical distribution of *N. olivacea*. Two small groups were integrated by a few specimens more related with mtDNA of *N. nasua* (these specimens are always from Central and Western Colombian Andean Cordilleras and trans-Andean Ecuador) and the main group of *N. olivacea*. This, in turn, showed two differentiated groups, one in the Eastern Colombian Andean Cordillera and another in Central and Western Colombian Andean Cordillera and northern trans-Andean Ecuador. Two specimens with the phenotype of *N. narica* and within the southern Central American range of this species had mitochondrial DNA related with the group of *N. olivacea* from the Eastern Colombian Andean Cordillera. It is rather remarkable that the main group of *N. olivacea* was more related with *N. narica* than this last with *N. nasua*. This provides evidence against *Nasuella* as a different genus from *Nasua*.

The MJN (Fig. 3) showed the following picture. If we assumed that the oldest haplotypes are closest to the out-groups (three species of *Bassarycion*), then haplotypes 56 and 79 (two specimens of *Nasuella*, FGO-N), and haplotype 3 (and related) of *N. nasua*, the last one widely distributed in the Amazon and Eastern Colombian Llanos, were the first to appear within *Nasua-Nasuella*. Next to diverge (from haplotypes 56 and 79), was a group of haplotypes from specimens phenotypically similar to *N. olivacea* (SGO-N). These haplotypes are a “bridge” between the first (and oldest) haplotypes of *Nasuella* and *N. nasua* and the remaining haplotypes of

N. olivacea. GEASTERNO-NO was the first to appear within the main body of haplotypes of *N. olivacea*. GCENTRAL-NO derived from this first group. Within GEASTERNO-NO, two haplotypes (81 and 82) belonged to specimens of *N. narica* (Panama and southern Costa Rica), similar to what we identified with the MLT. Finally, the haplotypes of all the Central American coatis, *N. narica*, derived from GCENTRAL-NO. Based on these data, haplotypes of *N. narica* were the youngest to appear.

There were striking temporal splits between and within the different taxa and groups detected in *Nasua-Nasuella*. (1) We estimated a temporal split between the haplotypes of *Bassarycion. neblina-medius* and the most original haplotype of *N. nasua* (H3) of around 17.7 ± 0.4 MYA; (2). The temporal splits between the GEASTERNO-NO and *N. nasua* was estimated to have occurred 2.835 ± 0.051 MYA, whereas the temporal divergence between the GCENTRAL-NO and *N. nasua* was dated to around 3.763 ± 0.051 MYA. Between both main groups of *N. olivacea*, the temporal splits were dated around 3.612 ± 0.785 MYA. The temporal splits between *N. nasua* and *N. narica* were estimated to have occurred around 5.415 ± 0.236 MYA. (3) The internal temporal split in *N. nasua* (6.145 ± 1.293 MYA) and in *N. narica* (1.089 ± 0.230 MYA) were other time splits. For additional time splits, see Table 2.

Taking into account the diversification within each taxon, it seems that the oldest haplotypes were that of *N. nasua*, followed by the haplotypes of both main groups of *N. olivacea*, with the haplotypes of *N. narica* as the youngest ones.

Genetic heterogeneity and genetic distances

The overall genetic heterogeneity tests of the three species revealed very significant differences (Table 3). All the gene flow statistics were considerably lower than 1 ($Nm_{\gamma ST} = 0.34$; $Nm_{NST} = 0.22$; $Nm_{FST} = 0.23$), which supports that these taxa are three different species. However, we also analyzed the overall genetic heterogeneity of the four groups of *N. olivacea* that were detected in the phylogenetic analyses. In all the cases, the genetic heterogeneity was highly significant, even more than in the previous case where the different groups of *N. olivacea* were not considered. This means that these four groups of *N. olivacea*—detected with the phylogenetic procedures—are genetically, highly differentiated (Table 4). Again, the gene flow estimates were considerably lower than 1 (three-gene dataset only for *N. olivacea*: $Nm_{\gamma ST} = 0.30$; $Nm_{NST} = 0.20$; $Nm_{FST} = 0.22$), which not only ratifies the distinctness of the species but also the differentiation of the four groups detected within *N. olivacea*.

The genetic heterogeneity analysis by taxa pairs (the four groups of *N. olivacea*, *N. nasua*, and *N. narica*) was estimated by the F_{ST} statistic (Table 5a). This statistic showed that all the taxa pair comparisons were significant (with the

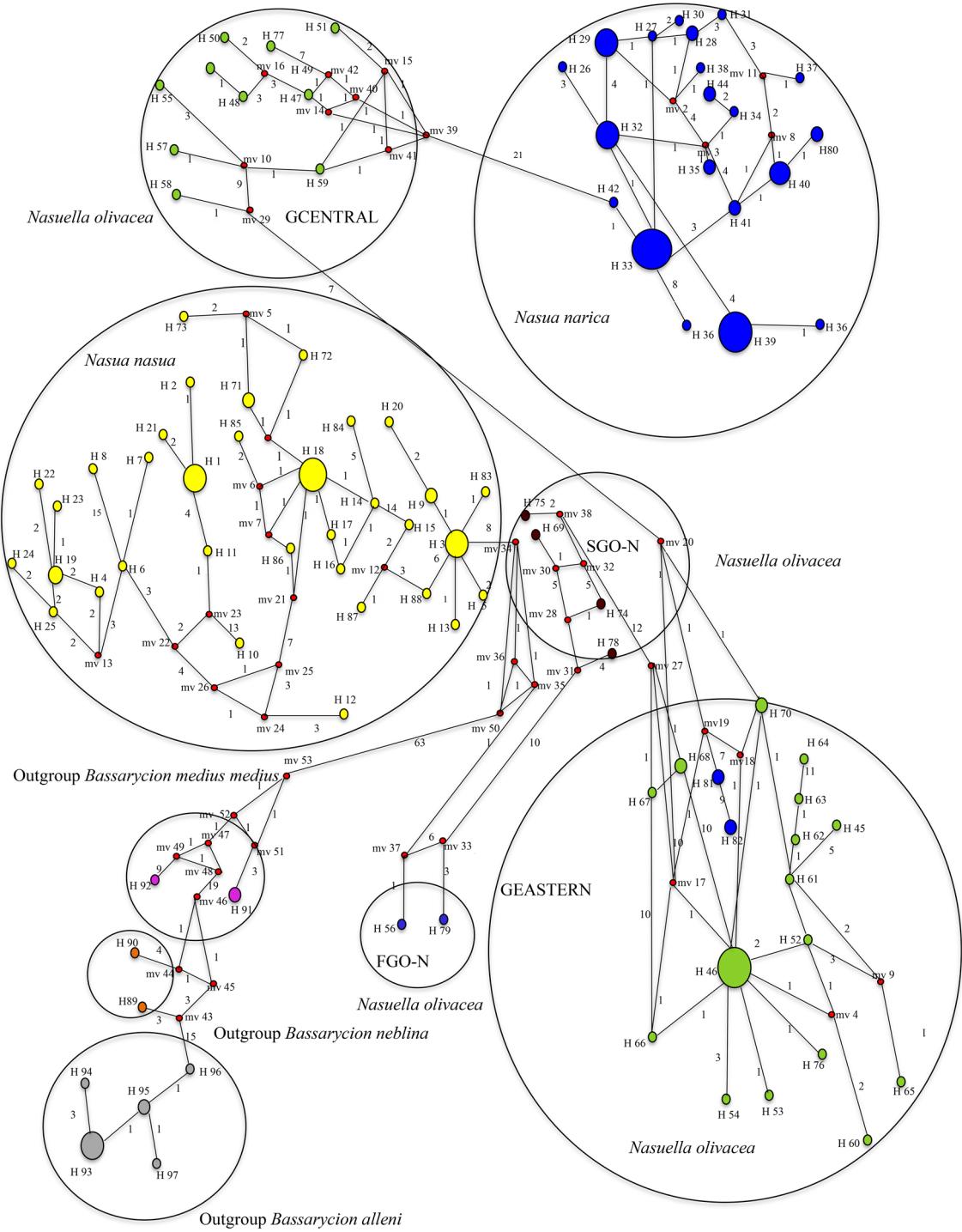


Fig. 3 Median Joining Network on three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) of 42 *Nasuella olivacea*, 50 *Nasua narica*, and 51 *Nasua nasua* specimens from the Neotropics. Taxa analyzed are showed with different colors for their haplotypes: grey circles = *Bassarycion alleni* (outgroup); orange circles = *Bassarycion neblina* (outgroup); pink circles = *Bassarycion medius medius* (outgroup); lilac circles = some specimens phenotypically *Nasuella olivacea* (Risaralda Department, Colombia; Carchi Province, Ecuador; FGO-

N); brown circles = some specimens phenotypically *Nasuella olivacea* (Chocó, Cauca, Nariño Departments, Colombia; Pichincha Province, Ecuador; SGO-N); yellow circles = *Nasua nasua*; green circles = *Nasuella olivacea*; blue circles = *Nasua narica*. Red circles (with mv) indicate missing intermediate haplotypes. Numbers between haplotypes = number of mutations that differentiated the haplotypes

Table 2 Some estimated temporal splits among and within the two species of *Nasua* (*N. nasua* and *N. narica*) and *Nasuella olivacea* (four groups: FGO-N, SGO-N, GEASTERN-NO, and GCENTRAL-NO; for geographical origins of these four groups, see text), and *Bassaryc*

Species and groups	Estimated temporal splits among and within species and groups \pm SD in MYA
Between <i>N. nasua</i> and <i>N. olivacea</i> (FGO-N)	0.618 \pm 0.053
Between <i>N. nasua</i> and <i>N. olivacea</i> (SGO-N)	1.598 \pm 0.103
Between <i>N. narica</i> and <i>N. olivacea</i> (GEASTERN-NO)	4.227 \pm 0.472
Between <i>N. narica</i> and <i>N. olivacea</i> (GCENTRAL-NO)	3.557 \pm 0.708
Within <i>N. olivacea</i> (GEASTERN-NO)	3.331 \pm 0.843
Within <i>N. olivacea</i> (Gcentral-NO)	1.900 \pm 0.459
Within <i>Bassaryc allenii</i>	0.253 \pm 0.128

SD=Standard deviation; MYA=Millions of years ago

Table 3 Genetic heterogeneity and gene flow statistics across the specimens of *Nasuella olivacea*, *Nasua narica*, and *Nasua nasua* analyzed in this study for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*); ** $p < 0.01$, df=degrees of freedom

Genetic heterogeneity and gene flow statistics	Values	Probabilities
χ^2	272.000 df=160	0.00001**
H_{ST}	0.0039	0.00001**
K_{ST}	0.5908	0.00001**
K_{ST}^*	0.3057	0.00001**
Z_S	1594.771	0.00001**
Z_S^*	7.0623	0.00001**
S_{nn}	1.0000	0.00001**
γ_{ST}	0.5969	0.00001**
N_{ST}	0.6980	0.00001**
F_{ST}	0.6814	0.00001**
Nm^1	0.34	
Nm^2	0.22	
Nm^3	0.23	

Nm gene flow statistics, Nm^1 Gene flow estimated from γ_{ST} , Nm^2 Gene flow estimated from N_{ST} , Nm^3 Gene flow estimated from F_{ST}

Bonferroni's correction $\alpha=0.0033$), with the exception of FGO-N with SGO-N ($p=0.074$), and FGO-N with GEASTERN-NO ($p=0.018$). The gene flow estimates for the taxa pair comparisons (Table 5b) showed low levels of gene flow (lower than 1) for all the comparisons. The value nearest to 1 was that between *N. nasua* and FGO-N ($Nm=0.94$).

The Kimura 2P genetic distances (Table 6) showed the highest range of values among the different taxa of *Nasua*-*Nasuella* and the outgroups (three species of *Bassaryc*; between *N. narica* and *Bassaryc neblina*, 47.5%, and FGO-N and *Bassaryc neblina*, 34.9%). These genetic distances are in the range of well differentiated genera. The major part of the genetic distances among the seven *Nasua*-*Nasuella* groups considered were above the value considered

saryc allenii analyzed at three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*), by means of the ρ statistic applied on the median joining network (assuming 1 mutation every 22,742 years)

Table 4 The genetic heterogeneity and gene flow statistics across the specimens for the four groups of *Nasuella olivacea* detected in the phylogenetic analyses at three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) * $p < 0.05$; ** $p < 0.01$, df=degrees of freedom

Genetic heterogeneity and gene flow statistics	Values	Probabilities
χ^2	126.000 df=93	0.01291*
H_{ST}	0.0371	0.0035**
K_{ST}	0.5798	0.00001**
K_{ST}^*	0.3031	0.00001**
Z_S	216.0235	0.00001**
Z_S^*	5.1281	0.00001**
S_{nn}	1.0000	0.00001**
γ_{ST}	0.6223	0.00001**
N_{ST}	0.7114	0.00001**
F_{ST}	0.6928	0.00001**
Nm^1	0.30	
Nm^2	0.20	
Nm^3	0.22	

Nm gene flow statistics, Nm^1 Gene flow estimated from γ_{ST} , Nm^2 Gene flow estimated from N_{ST} , Nm^3 Gene flow estimated from F_{ST}

for different species of the same genus (11–12%; see introduction). The lowest values, below this limit were the cases of *N. nasua* vs. SGO-N (9.9%), FGO-N vs. SGO-N (9.5%), GEASTERN-NO vs. GCENTRAL-NO (8.9%), GCENTRAL-NO vs. the two *N. narica* with mtDNA of *N. olivacea* (6.8%), and GEASTERN-NO vs. the two *N. narica* with mtDNA of *N. olivacea* (6.5%). Henceforth, values less than the average genetic distance values of full species were between *N. nasua* and one of the small and differentiated groups of *N. olivacea*. In addition, they were among some of the groups of *N. olivacea*, and among the two main groups of *N. olivacea* and the two *N. narica* with mtDNA of *N. olivacea*. Additionally, the magnitude of genetic differentiation between *N. narica* and the groups of *N. olivacea* was

Table 5 A F_{ST} pair statistics (below main diagonal; significance upper main diagonal) and B gene flow estimates among six groups of coatis, four groups of *Nasuella olivacea* (FGO-N, SGO-N, GEAST-

ERN-NO, GCENTRAL-NO), *Nasua narica*, and *Nasua nasua* analyzed by means of three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*)

A F_{ST} pair statistics

Taxa	<i>N. olivacea</i> FGO-N	<i>N. olivacea</i> SGO-N	<i>N. olivacea</i> GEASTERN-NO	<i>N. olivacea</i> GCENTRAL-NO	<i>N. narica</i>	<i>N. nasua</i>
<i>N. olivacea</i> FGO-N			*		*	*
<i>N. olivacea</i> SGO-N	0.479		*	*	*	*
<i>N. olivacea</i> GEASTERN-NO	0.856	0.787		*	*	*
<i>N. olivacea</i> GCENTRAL-NO	0.785	0.740	0.696		*	*
<i>N. narica</i>	0.855	0.836	0.846	0.799		*
<i>N. nasua</i>	0.346	0.526	0.708	0.657	0.735	

B gene flow

Taxa	<i>N. olivacea</i> FGO-N	<i>N. olivacea</i> SGO-N	<i>N. olivacea</i> GEASTERN-NO	<i>N. olivacea</i> GCENTRAL-NO	<i>N. narica</i>	<i>N. nasua</i>
<i>N. olivacea</i> FGO-N						
<i>N. olivacea</i> SGO-N	0.544					
<i>N. olivacea</i> GEASTERN-NO	0.084	0.135				
<i>N. olivacea</i> GCENTRAL-NO	0.138	0.175	0.219			
<i>N. narica</i>	0.085	0.098	0.091	0.125		
<i>N. nasua</i>	0.943	0.450	0.206	0.261	0.180	

* $P < 0.0033$ (Bonferroni's correction)

Table 6 Kimura 2P genetic distance (Kimura 1980) in percentages (%) among different taxa of *Nasuella olivacea* (four groups; 1=FGO-N, 2=SFGO-N, 3=GEASTERN-NO, 4=GCENTRAL-NO), 5=*Nasua narica*, 6=southern Central American *Nasua narica* sharing mtDNA of *N. olivacea*, 7=*Nasua nasua*, 8=*Bassarycion*

neblina, 9=*Bassarycion medius*, 10=*Bassarycion alleni* (below main diagonal) and standard deviations in percentages (%) (above main diagonal) estimated by means of three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*)

Taxa	1	2	3	4	5	6	7	8	9	10
1		1.5	2.0	2.1	1.9	2.3	1.9	4.8	3.8	4.6
2	9.5		2.7	2.4	2.1	2.6	1.3	4.8	4.2	4.7
3	13.6	17.4		1.4	2.2	1.3	2.5	5.1	4.4	4.8
4	15.6	16.8	8.9		2.0	1.2	2.4	5.0	3.9	4.4
5	14.5	14.6	13.8	11.3		2.6	2.3	5.5	4.2	5.0
6	16.0	17.6	6.5	6.8	12.8		2.5	4.9	4.0	4.7
7	14.1	9.9	18.1	18.0	17.2	18		4.8	4.4	5.0
8	43.3	42.6	43.8	47.3	47.5	44.6	43.0		2.6	2.0
9	36.4	34.9	39.0	39.0	36.9	38.6	37.9	15.6		3.0
10	39.6	40.0	40.2	41.0	43.2	41.7	41.7	11.9	19.1	

similar, or even lower, than the values between *N. narica* and *N. nasua*.

Genetic diversity levels and demographic trajectories

The genetic diversity analyses are shown in Table 7 (total sample of *N. olivacea*; GEASTERN-NO, GCENTRAL-NO, *N. nasua*, and *N. narica*). In general, the data showed that

the levels of genetic diversity were very high for all the lineages studied. The number of haplotypes and the H_d were elevated and similar in all five lineages. These statistics are influenced by the sample sizes and therefore a comparative discussion is not very explanatory. In contrast, the comparison of the statistics π and θ per sequence is more interesting because they are not affected by sample size. The *N. nasua* sample presented the highest levels of genetic diversity ($\pi = 0.0647 \pm 0.012$ and $\theta = 16.447 \pm 4.846$), followed

Table 7 Genetic diversity statistics (and \pm standard deviation) in the total sample of *Nasuella olivacea*, in the *N. olivacea* from Eastern Colombian Andean Cordillera (GEASTERN-NO), in the *N. olivacea*

Taxa	<i>N</i>	H_d	π	θ
Overall sample of <i>N. olivacea</i>	27	0.943 ± 0.032	0.0503 ± 0.007	17.008 ± 5.319
GEASTERN-NO	17	0.892 ± 0.055	0.0230 ± 0.0049	11.156 ± 3.842
GCENTRAL-NO	9	1.000 ± 0.052	0.0339 ± 0.0045	9.566 ± 4.312
<i>N. narica</i>	20	0.916 ± 0.024	0.0195 ± 0.0016	6.985 ± 2.274
<i>N. nasua</i>	34	0.969 ± 0.012	0.0647 ± 0.0033	16.447 ± 4.846

N Number of haplotypes, H_d Haplotype diversity; π Nucleotide diversity; θ $N_e\mu$, N_e effective female population size, μ mutation rate per generation

by the total sample of *N. olivacea* ($\pi=0.0503 \pm 0.007$ and $\theta=17.008 \pm 5.319$), GCENTRAL-NO ($\pi=0.0339 \pm 0.0045$ and $\theta=9.566 \pm 4.312$) and GEASTERN-NO ($\pi=0.0230 \pm 0.0049$ and $\theta=11.156 \pm 3.842$). The taxa with the lowest genetic diversity analyses was *N. narica* ($\pi=0.0195 \pm 0.0016$ and $\pi=6.985 \pm 2.274$). These results agree quite well with the phylogenetic tree and MJN procedures. The haplotypes of *N. nasua* seems to be the oldest with the highest degree of differentiation among populations classified within this species. The haplotypes of *N. olivacea* appeared later and then *N. narica*, with the youngest of all the haplotypes analyzed.

The demographic analyses showed the following picture. For the analyses with diverse statistics and a mismatch distribution, the overall sample of *N. olivacea* only showed one out of six analyses as significant ($F_S=-6.713$, $p=0.025$), thus indicating population expansion. This was a rather weak support of a possible population expansion for this species. For GCENTRAL-NO, there was little evidence of demographic changes. Only one statistic was significantly in agreement with a population expansion ($F_S=-3.089$, $p=0.034$). In contrast, GEASTERN-NO yielded strong evidence of population expansion. The five statistics were significant ($D=-1.888$, $p=0.014$; $D^*=-2.308$, $p=0.029$; $F^*=-2.558$, $p=0.026$; $F_S=-4.963$, $p=0.024$; $R2=0.074$, $p=0.042$) as well as for the mismatch distribution ($rg=0.0173$, $p=0.048$). *N. narica* also presented strong evidence of population expansion ($D^*=-2.666$, $p=0.019$; $F^*=-2.449$, $p=0.023$; $F_S=-5.014$, $p=0.044$). No demographic changes were detected for the last species, *N. nasua*.

For the BSP analyses (Fig. 4), the overall sample of *N. olivacea* showed a considerable female population increase starting 500,000 YA and a very strong population decrease in the last 20,000 Y. The first temporal estimate was relatively similar to that obtained with the mismatch distribution, assuming a generation time for the coatis of 1–2 years ($\tau=17.129$; 251,100–502,200 YA). For GCENTRAL-NO, the demographic size remained constant during the last 0.9 MYA until the last 20,000 Y when there was a very strong population decrease. For GEASTERN-NO, there was a

from Western and Central Colombian and Ecuadorian Andean Cordilleras (GCENTRAL-NO), in *Nasua narica*, and *Nasua nasua* analyzed at three mitochondrial (mt) genes (*ND5*, *Cyt-b*, and *D-loop*)

strong population expansion in the last 250,000 Y and a strong population decrease in the last 20,000 Y. With the mismatch distribution, the initial expansion was estimated to have occurred 70,800–141,600 YA ($\tau=4.829$). For *N. nasua*, there was very little clear evidence of population increase. Maybe, there was a very slight increase from 70,000 YA to 20,000 YA, but there was a clear decrease in the last 20,000 Y. Finally, *N. narica* presented evidence of population expansion in the last 120,000 Y, with a strong population decrease in the last 20,000 Y. With the mismatch distribution, the initial expansion was very similar to the BSP estimate. It was dated to 63,400–126,800 YA ($\tau=4.322$). Therefore, there was suggestive population expansion for the overall sample of *N. olivacea*, for GEASTERN-NO, and for *N. narica*. However, there was no clear evidence of population increase for GCENTRAL-NO or for *N. nasua*. Based on the BSP analysis of all five taxa, there was an extremely similar strong population decrease in the last 20,000 Y.

Spatial genetic structure of *Nasuella olivacea*

The application of Mantel's test offered very similar results in the detection of global spatial structure. The Mantel's test with the three-gene dataset (Fig. 5) showed a significant relationship between geographical and genetic distances ($r=0.3713$; $p=0.000099$). This means that the geographic distance significantly explained around 13.79% of the genetic distances.

The spatial autocorrelation analysis using 6 DCs showed a significant overall correlogram ($V=0.0237$; $p=0.0001$) (Fig. 6). The first two DCs showed significant positive autocorrelation (1 DC: 0–50 km, $p=0.000001$; 2 DC: 50–150 km, $p=0.0320$). The third DC presented negative autocorrelation without reaching statistical significance. The fourth DC was also significantly positive (4 DC: 210–270 km, $p=0.0001$). The two last DCs were significantly negative (5 DC: 270–440 km, $p=0.000001$; 6 DC: 440–800 km, $p=0.0131$). Henceforth, the overall correlogram showed a significant spatial pattern with regional

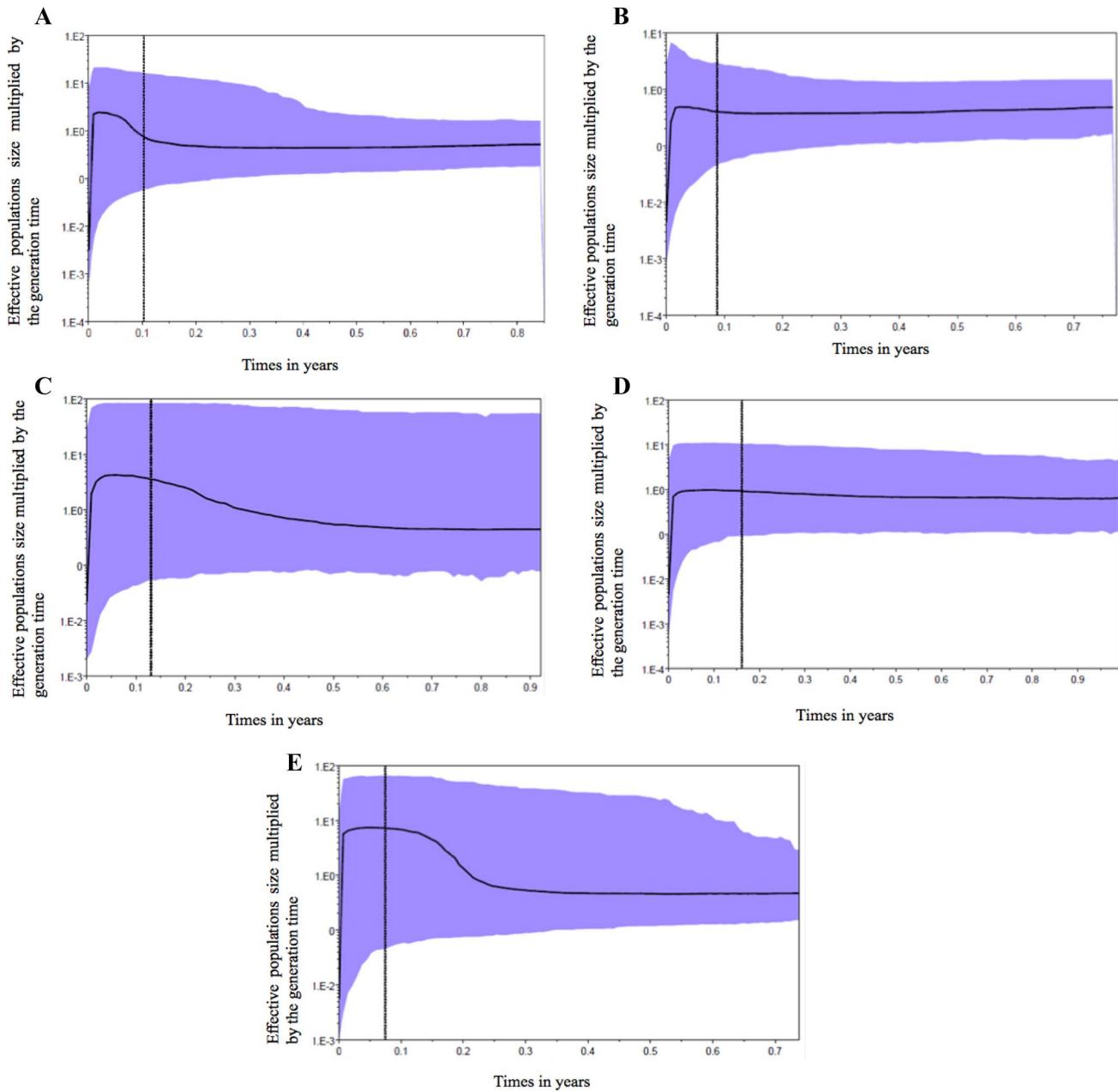


Fig. 4 Bayesian skyline plot analyses (BSP) to determine possible demographic changes across the natural history of different taxa of *Nasuella* and *Nasua* for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) in the last 0.7–1 million of years. **a** *Nasua narica*; **b** *Nasua*

nasua; **c** Overall sample of *Nasuella olivacea*; **d** Sample of *Nasuella olivacea* from Western and Central Andean Colombian and Ecuadorian Cordilleras (GCENTRAL-NO); **e** Sample of *Nasuella olivacea* from Eastern Andean Cordillera (GEASTERN-NO)

patches in the first 200 km and isolation by distance from around 200 to 800 km.

The MMDA procedure for the three-gene dataset is shown in Fig. 7. Five possible geographical barriers were analyzed. The first barrier (blue in Figure) delimited the geographical area from the Risaralda Department (Colombian Central Andean Cordillera) to the Pichincha Province (northern Ecuadorian Western Andean Cordillera). This area agrees quite well with the FGO-N detected in the

MLT and in the MJN. The second barrier (green) discriminated a geographical area from the Chocó Department (Colombian Western Andean Cordillera) to the Carchi and Pichincha Provinces (northern Ecuadorian Western Andean Cordillera). This coincides with the SGO-N and the major part of the specimens from GCENTRAL-NO detected in the MLT and in the MJN. The third barrier (green bluish) was located in a geographical area within Ecuador (Pichincha and Cotopaxi Provinces), which

Fig. 5 Mantel test between the geographic and genetic distances for specimens of *Nasuella olivacea* sequenced for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*)

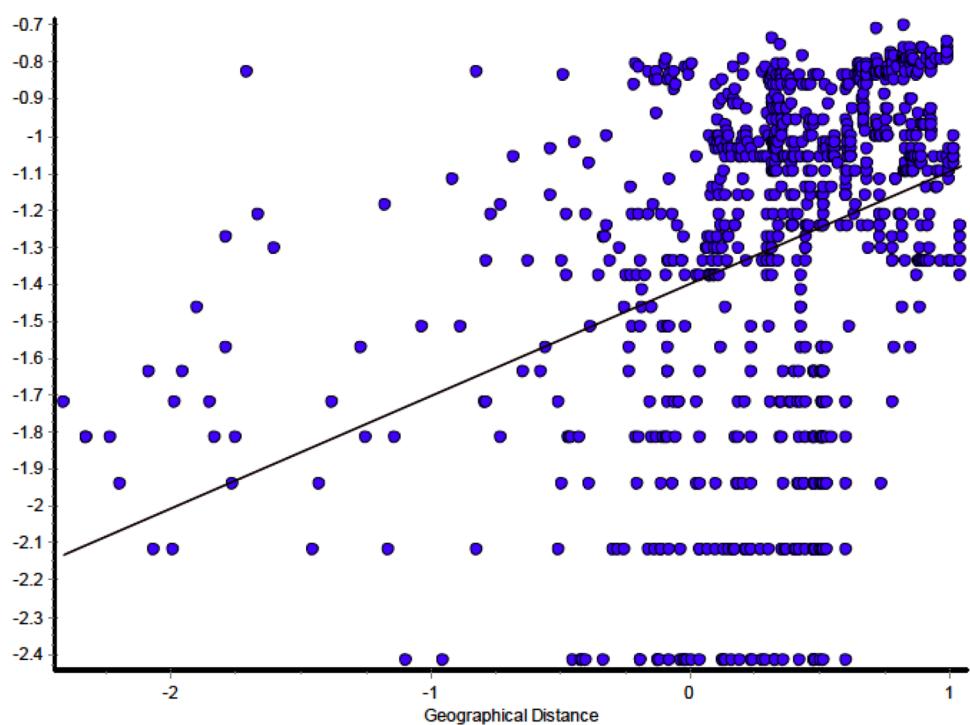
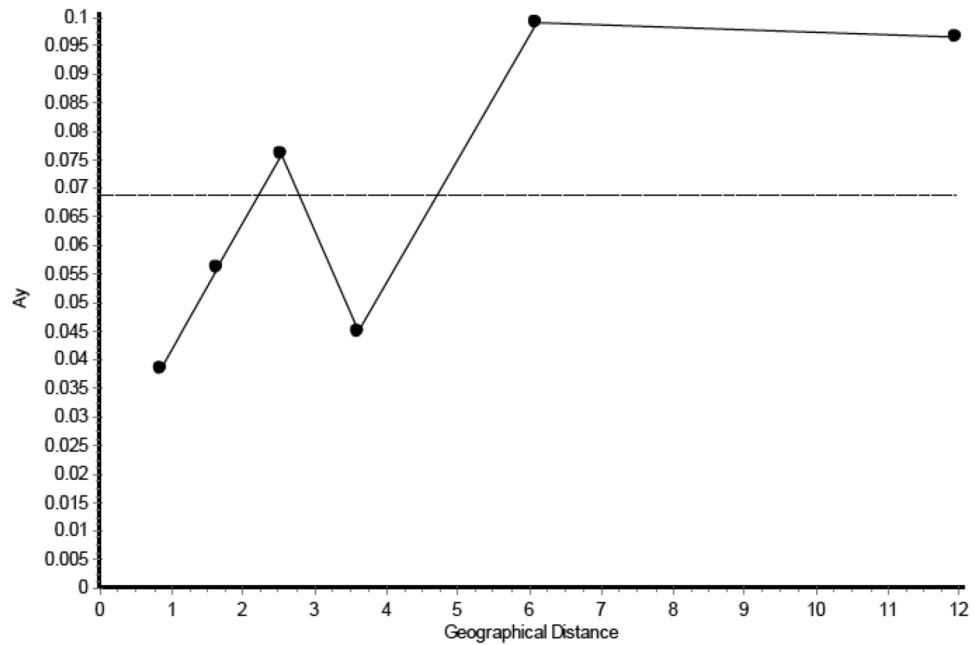


Fig. 6 Correlograms with the A_y statistic and six distance classes from a spatial autocorrelation analysis for specimens of *Nasuella olivacea* sequenced for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*)



agreed quite well with an Ecuadorian sub-group detected within GCENTRAL-NO in the MLT. The fourth and fifth barriers (brown and lilac, respectively) delimited two geographical areas which corresponded to GEASTERN-NO. The first one contained specimens sampled in the Departments of Cundinamarca and a fraction of Boyacá, whereas the second one delimited another fraction of the Boyacá Department as well as the Norte de Santander

Department. Therefore, there was good correspondence between the MMDA and the MLT. Maybe the unique differences were that the MMDA differentiated less SGO-N from GCENTRAL-NO than did the MLT. However, it more remarkably differentiated the Ecuadorian sub-group within GCENTRAL-NO and distinguished more sharply two sub-groups within GEASTERN-NO.

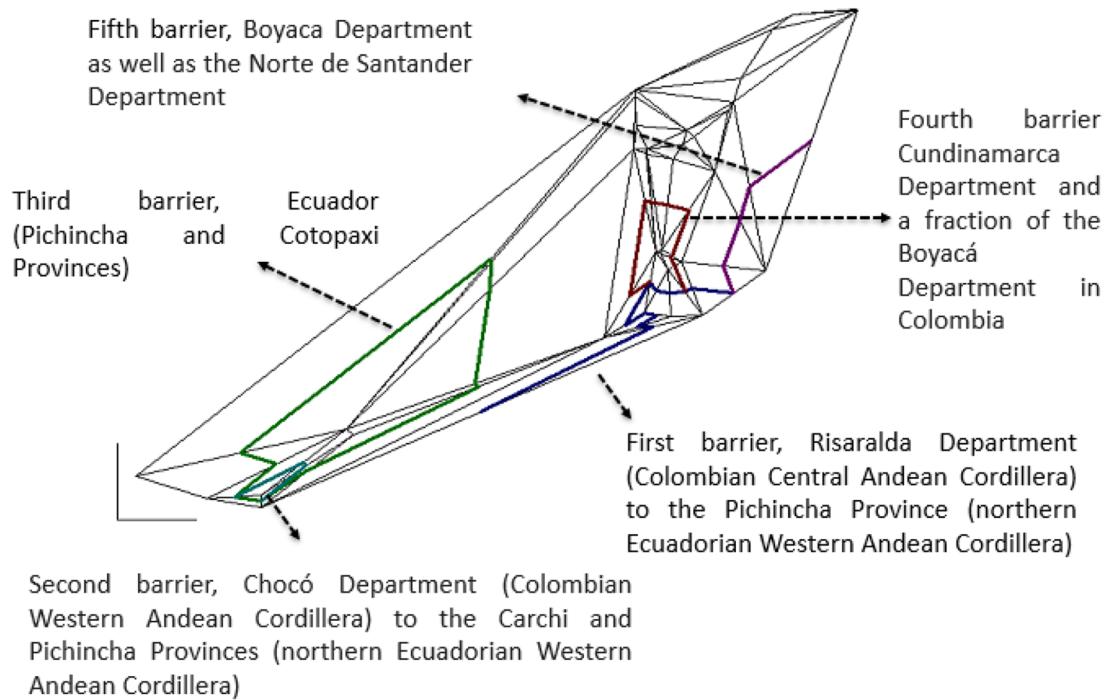
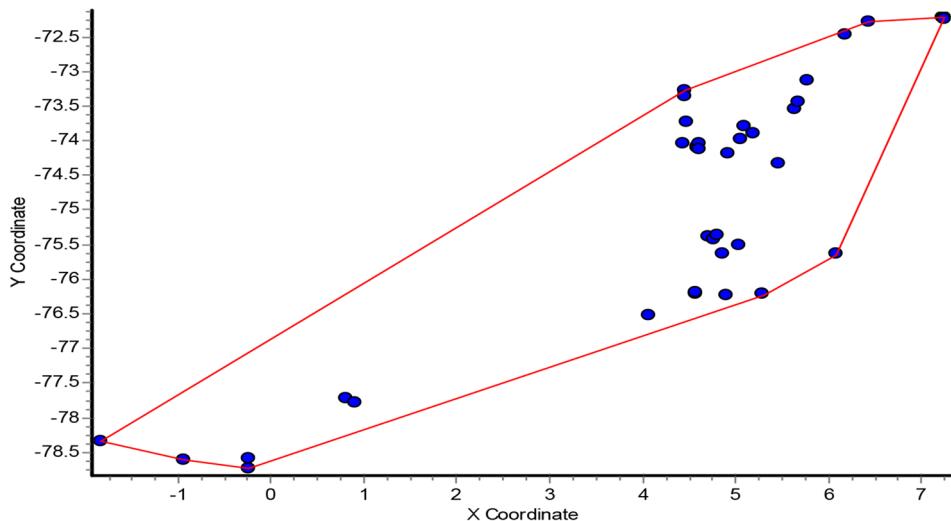


Fig. 7 Monmonier's algorithm analysis (MMAA) to detect the five most important geographical barriers for the specimens of *Nasuella olivacea* sampled in Colombia and Ecuador for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*). First barrier (blue)=Geographical area from the Risaralda Department (Colombian Central Andean Cordillera) to the Pichincha Province (northern Ecuadorian Western Andean Cordillera); second barrier (green point)=Geographical area from the Chocó Department (Colombian Western Andean Cordillera)

to the Carchi and Pichincha Provinces (northern Ecuadorian Western Andean Cordillera); third barrier (green-bluish)=Geographical area within Ecuador (Pichincha and Cotopaxi Provinces); fourth barrier (brown)=Geographical area, which enclosed the Cundinamarca Department and a fraction of the Boyacá Department in Colombia; and fifth barrier (lilac)=Geographical area, which enclosed a fraction of the Boyacá Department as well as the Norte de Santander Department

Discussion

The systematics of *Nasuella*

This work shows the most extensive molecular analysis to

date for *N. olivacea* trying to establish how many differentiated taxa are within the mountain coati. Aforementioned, Helgen et al. (2009) only studied a small fragment of mt*Cyt-b* from an extremely small sample and still determined that the unique sample of the mountain coati from the Merida

Cordillera in Venezuela was a “new” species, *N. meridensis*. Since then, the majority of authors consider the existence of two mountain coati species: the Eastern mountain coati (*N. meridensis*, Thomas 1901) and the Western mountain coati (*N. olivacea*, Gray 1865). The type locality for the first was Monte de Culata, Merida State, Venezuela (Cabrera 1958), while the type locality for the second was Santa Fé de Bogotá, Colombia (“Bogotá, what should be interpreted as the mountains near the capital” claimed Cabrera 1958). Allen (1913) had defined another taxa, *N. lagunetae*, also with the type locality in Bogotá and which is usually considered as a synonym of *N. olivacea*. Additionally, Helgen et al. (2009) considered that within *N. olivacea*, two subspecies should be proposed, although they did not show any molecular result in favor of this: *N. o. olivacea* (Gray 1865), distributed throughout the Colombian Andes with a paler pelage (brown) and dark tail rings, and *N. o. quitensis* (Lönnberg 1913; type locality—the southern slope of the Pichincha volcano), distributed in the Ecuadorian Andes and slightly smaller than the previous one with a darker pelage (more blackish) and tail rings less visible than in the previous subspecies (darker tail).

However, our results from the analysis of three mt genes from 42 specimens of the traditional *N. olivacea* (37 from the three Colombian Andean Cordilleras, and five from the two Ecuadorian Andean Cordilleras) showed a very different perspective. We detected four differentiated molecular groups within *N. olivacea*. The first group of specimens with the morphology and distribution of *N. olivacea* were composed by two specimens that originated in the middle of the Central Colombian Andean Cordillera (Risaralda Department) and in the Ecuadorian Pichincha Province (Western Ecuadorian Andes). The haplotypes of these specimens are more related to the haplotypes of the oldest haplogroup of *N. nasua* we detected (Western Amazon Basin and Colombian Eastern Llanos) than to the majority of the haplotypes detected in the remainder *N. olivacea* specimens. The same occurred with a second small group of *N. olivacea* composed of four specimens distributed in the Western Colombian Andean Cordillera (Chocó, Cauca, and Nariño Departments) as well as in the Western Ecuadorian Andes (Pichincha Province). The group’s haplotypes were more related to those of *N. nasua* than to the majority of *N. olivacea* studied. The MJN analysis showed that the haplotypes of these two small groups were “a bridge” between the haplotypes of *N. nasua* and those from main groups of *N. olivacea*, although more related to *N. nasua*, such as it was found with the MLT. The haplotypes of these two small groups, although more related to *N. nasua* than to the remainder haplotypes of *N. olivacea* (in the MLT, not in the genetic heterogeneity analyses), are sufficiently different from those of *N. nasua* to form highly distinctive branches that are significantly different in many analyses (both phylogenetic and genetic heterogeneity ones).

We discard the notion of very recent or current hybridization between *N. nasua* and specimens of *N. olivacea* as an explanation of these results because their haplotypes were significantly differentiable from the current haplotypes of the most related *N. nasua*. However, we offer three hypotheses as an explanation. (a) These two small groups were the product of two independent events of old introgression of *N. nasua* (Western Amazon) into *N. olivacea*, which came about 0.6–1.6 MYA, respectively. (b) These two small groups represent transitional forms from *N. nasua* haplotypes to the most derived haplotypes of *N. olivacea* from the GEASTERN-NO and GCENTRAL-NO haplogroups. Figure 3 and genetic heterogeneity analyses (see Table 5) agree quite well with this hypothesis. It is clear that the haplotypes of FGO-N were derived from haplotypes of *N. nasua* and, in turn, a haplotype of FGO-N gave origin to the haplotypes of SGO-N, which, also in turn, gave origin to the first haplotype of GEASTERN-NO. This hypothesis, which we consider the most probable, however, is very problematic from a systematic point of view. Transitional mtDNA, in some analyses more related to *N. nasua* (example, MLT) but with bodies fully of *N. olivacea*. If so, we should consider these two groups as the most ancient *N. olivacea*. The results of Ruiz-García et al. (2020b), with mitogenomes, reinforced this hypothesis. For this reason, we have enclosed these two small groups inside of all the analysis of *N. olivacea* that we carried out. (c) The FGO-N and SGO-N haplotypes would represent *N. nasua* haplotypes but the specimens with these haplotypes morphologically evolved by convergent adaption to very similar morphotype to that shown by *N. olivacea* by living in the same Andean biome, but they are really *N. nasua*. Only an analysis of nuclear DNA markers should help to test the three hypotheses.

The other two groups of *N. olivacea*’s haplotypes were considerably more represented in the specimens analyzed. The first to appear seemed to be composed by all the specimens sampled in the Eastern Colombian Andean Cordillera from the Cundinamarca, Boyacá, and Norte de Santander Departments. Although this group was basically found in this cited area, we also detected within it, one specimen from the Caldas Department (Central Colombian Andean Cordillera) and one specimen from Eastern Ecuador (Sangay NP). Therefore, after this group formed, some migrations could have occurred relatively recently from the Eastern Colombian Andean cordillera towards the West and South because the haplotypes of these two specimens are very similar. Within this group, we found two specimens of *N. narica* from southern Central America (Panama, and southern Costa Rica). Their morphotypes, as well as their geographic origins, were undoubtedly specimens of *N. narica*. Something similar was found by Nigenda-Morales et al. (2019) as we will discuss in brief. Such as in the previous case, they cannot represent events of recent hybridization between *N.*

olivacea and *N. narica*. Although the differences between the haplotypes of southern Central American *N. narica* and GEASTERN-NO were not high, as we observed between the haplotypes of the two first small groups of *N. olivacea* and those from *N. nasua*, they were significantly different. This could be interpreted as a case of genetic introgression of the *N. olivacea* from GEASTERN-NO into the southern Central American distribution of *N. narica*. However, this introgression event occurred more recently than the two possible introgression events of *N. nasua* in *N. olivacea* that we previously described.

GEASTERN-NO gave origin to another large haplogroup of *N. olivacea*, GCENTRAL-NO. Therefore, there was considerably more genetic heterogeneity among the specimens of *N. olivacea* sampled within the Western and Central Colombian Andean Cordilleras (where we found specimens belonging to four different groups, FGO-N, SGO-N, GCENTRAL-NO, and one specimen from Caldas classified in GEASTERN-NO), and within Ecuador (where we also found specimens belonging to these four groups), than in the Eastern Colombian Andean Cordillera, where only one gene pool was discovered.

These results radically change the systematics of *Nasuella* proposed by Helgen et al. (2009). First, these authors did not include samples of *Nasuella* from the Eastern Colombian Andean in their analyses. We compared the unique sequence they studied of the proposed new *N. meridensis* with some mtCyt-b gene sequences of the *Nasua* and *Nasuella* we studied with a maximum likelihood tree and it was un-differentiable from the sequences of the *Nasuella* that we analyzed from the Eastern Colombian Andean Cordillera (see Fig. 8). This tree is less robust than that we show in Fig. 2, because only a fraction of the specimens were analyzed (110 specimens vs. 143 specimens) and the length of the mtDNA sequence was of 366 bp vs. 2513 bp. This introduces certain inconsistencies in this tree with only a small fraction of the mtCyt-b gen in reference to the three of Fig. 2. This also shows the importance to obtain large sample sizes representing the broader geographical distribution as possible of a species given. However, this tree only wants to show as the Venezuelan specimen considered a “new species” by Helgen et al. (2009) is undifferentiated from the *N. olivacea* from the Eastern Colombian Andean Cordillera. Therefore, from a genetic point of view, *N. olivacea olivacea* (Gray 1865) and *N. meridiensis* (Thomas 1901) are synonyms. This means that the mountain coatis from Eastern Colombian Andean Cordillera and those from the Venezuelan Merida Cordillera should be named as *N. o. olivacea* (Gray 1865). As with the other two Colombian Cordilleras and in Ecuador, at least three other haplogroups were detected. If we consider that the two small groups were caused by genetic introgression from *N. nasua* from the Western Amazon (hypothesis 1), perhaps they should have no systematic value and should be

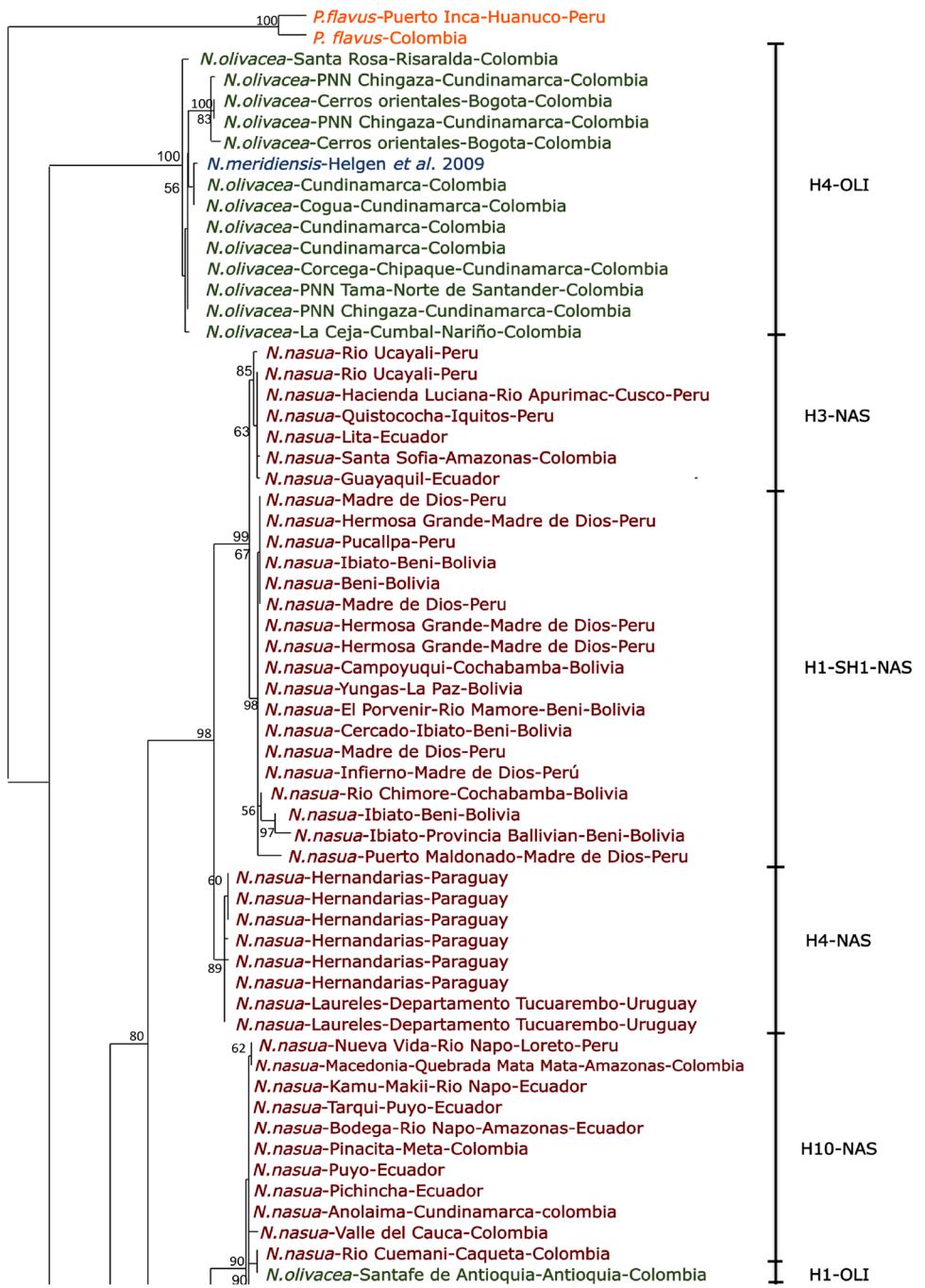
related to GCENTRAL-NO. If so, all of these specimens of *N. olivacea* from Western and Central Colombian Andean Cordilleras and those from the Western and Central Ecuadorian Andean Cordilleras should be named as *N. o. quitensis* (Lönnberg 1913). In contrast, if the two small groups were the first *N. olivacea* derived from *N. nasua*, which, in turn, gave origin to the two main groups of *N. olivacea* (hypothesis 2, which we believe the most probable), they should be subspecifically named. Thus, three subspecific names are required, and only one is available (*N. o. quitensis*), but we have no idea which of the three groups is correlated with *N. o. quitensis*. Confirmation would require a DNA analysis of the holotype of *N. o. quitensis*. In whatever case, two new subspecific names should be required for two of these three groups of *N. olivacea* detected in the Western and Central Colombian Andean Cordilleras and those from the Western and Central Ecuadorian Andean Cordilleras. We consider that the two main *Nasuella* groups to be subspecies more than full species as Helgen et al. (2009) considered. If we take the break for full species values of genetic distances of 11–12%, the relationships of these two groups of *N. olivacea* were below this amount. For example, GEASTERN-NO vs. GCENTRAL-NO showed 8.9%. Until there is extensive work of possible reproductive isolation among these taxa or important karyotypic differences (which appear to have no existence, or they are minimal; Jaramillo et al. 2020), we prefer to treat those taxa as subspecies of *N. olivacea*. Finally, if these two small groups were *N. nasua*, which evolved by convergent adaption to the Andean biome with a similar body of *N. olivacea*, they have not any significance for the systematics of *N. olivacea*.

The phylogenetic relationships between *Nasuella olivacea* and the two species of *Nasua* and some systematic notes for *N. nasua* and *N. narica*

Helgen et al. (2009) concluded that *Nasuella* (Hollister 1915) and *Nasua* (Storr 1780) should be unique genera because *Nasuella* was more related to *N. narica* than this last taxon with *N. nasua*. In fact, Gray (1843, 1865) described the mountain coati as *Nasua olivacea*. Later, Hollister (1915) proposed the genus *Nasuella* for the mountain coati, and this was retained by Goodwin (1953) and is still used today.

Our results agree quite well with Helgen et al. (2009) because we also detected a close relationship between the two main haplogroups of *N. olivacea* and *N. narica* more so than between *N. narica* and *N. nasua*. This was true for all of the analyses we carried out. For instance, the genetic distances between the two main groups of *N. olivacea* (GEASTERN-NO, and GCENTRAL-NO) vs. *N. narica* were 13.8% and 11.3%, respectively, whilst *N. nasua* and *N. narica* offered a value of 17.2%. Nigenda-Morales et al.

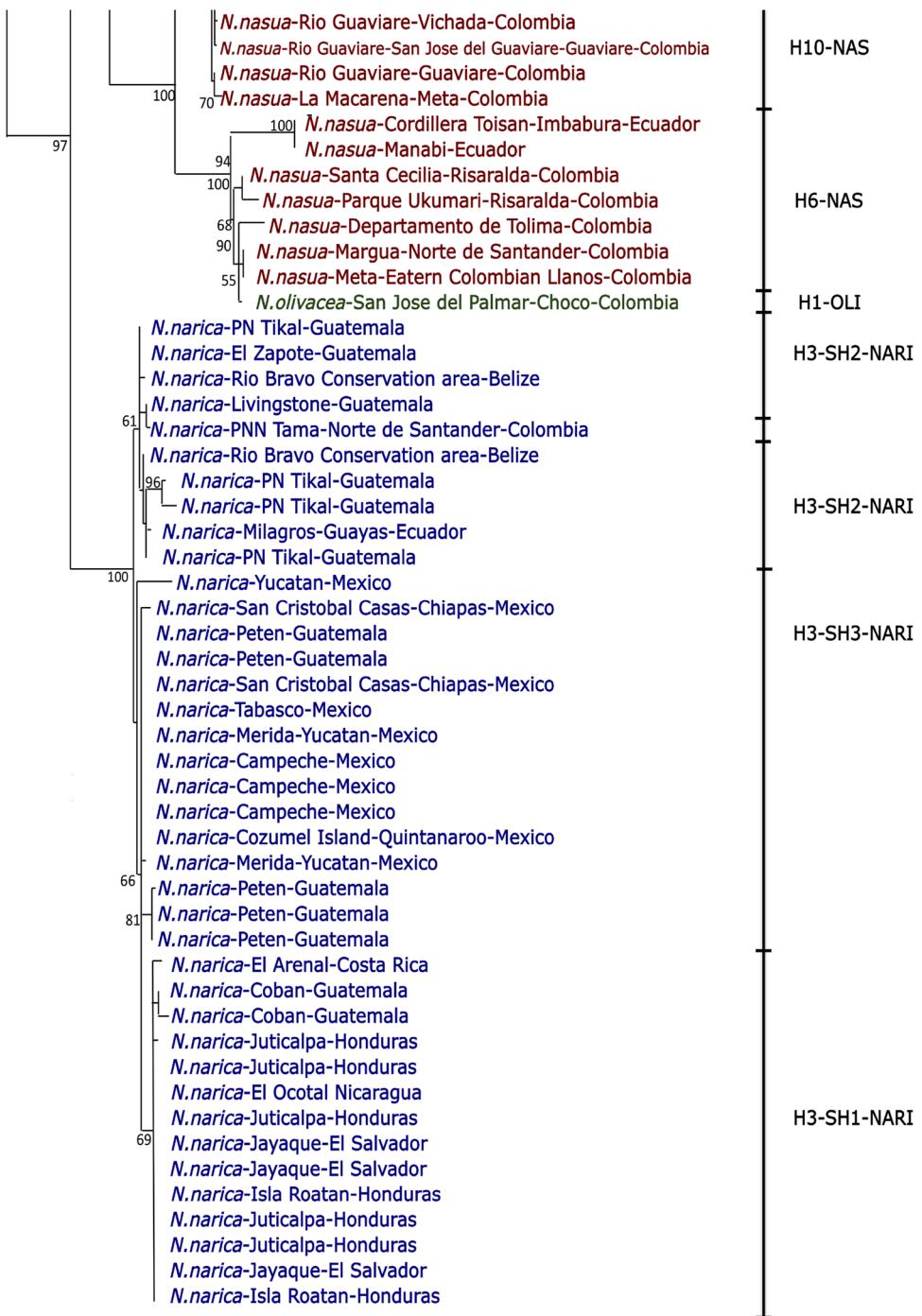
Fig. 8 Maximum likelihood tree (ML) for 1 *Nasuella meridensis*, 15 *Nasuella olivacea*, 41 *Nasua narica*, and 53 *Nasua nasua* specimens sampled throughout the Neotropics sequenced for the mtCyt-*b* gene. *Potos flavus* was employed as out-group. Nodes are labelled with bootstrap percentages



(2019) found three haplotypes from 13 samples derived from several locations in Panama, which constituted the earliest branching lineage within the mitochondrial gene tree they obtained; these haplotypes were highly divergent from the remaining haplotypes of *N. narica* that they found in other areas of Central and North America (9.92–10.78%). Now, thanks to the current work and Ruiz-García et al. (2020b), we know that the Panamanian *N. narica* specimens were introgressed with the mtDNA from *N. olivacea* and that they are not a possible new species. This is the motif why,

both Nigenda-Morales et al. (2019) and the present work, found high genetic distances (very similar in both works) between the Panamanian *N. narica* specimens and the remainder *N. narica* specimens. Nigenda-Morales et al. (2019), throughout the ML and BI analyses that incorporated mtCyt-*b* sequences of two *N. olivacea* specimens, obtained trees showing that these two specimens were placed inside the *N. narica* clade. Now, we know that the Panamanian *N. narica* (as well as some specimens from southern Costa Rica and northern Colombia; Ruiz-García et al. 2020b)

Fig. 8 (continued)



were nested inside one of the haplogroups of *N. olivacea* (GEASTERN-NO).

The ancestors of GEASTERN-NO, and GCENTRAL-NO diverged from the ancestor of *N. narica* around 3.6–4.2 MYA (and the split between these two haplogroups was dated around 3.6 MYA; Late Pliocene). Nigenda-Morales et al. (2019), employing two different analytical schemes that differed in the calibration priors employed, estimated the temporal divergence between the haplotypes of the

Panamanian *N. narica* (introgressed with mtDNA of *N. olivacea*) and the remainder *N. narica* haplotypes around 4 MYA (95% highest posterior density, HPD = 2.0–6.7 MYA and 2.6–5.1 MYA for the two different analytical schemes employed, respectively). Therefore, these temporal divergence estimates were very similar in both works.

All the phylogenetic analyses showed that the haplotypes of *N. narica* were the youngest. This means that the evolution of the current mitochondrial haplotypes of the coatis

began in South America (around 6.1 MYA in *N. nasua*; Late Miocene; and split between the ancestors of *N. nasua* and *N. narica*, around 5.4 MYA). This result totally concords with that found by Nigenda-Morales et al. (2019). These authors estimated divergence times with the two different analytical schemes they employed and they estimated a split between the ancestors of *N. nasua* and *N. narica*, around 6 MYA, very similar to the estimates herein reported. Ruiz-García et al. (2020b), in an extensive work with *N. nasua*, estimated that the original haplotypes of the ancestor of the current coatis were dated around 13 MYA. This agrees quite well with the model M2 of the three DEC models employed by Nigenda-Morales et al. (2019), which suggested that dispersion of the ancestor of *Nasua* across the Isthmus of Panama likely occurred before 9.5 MYA.

More recently, *N. narica* derived from South America and became distributed in Central America (1.1 MYA; Pleistocene). Nigenda-Morales et al. (2019) estimated the divergence time for the four clades of *N. narica* they found, excluding the Panamanian group, in around 1.3 MYA (95% HPD=0.59–2.1 MYA). Thus, both works also showed similar temporal divergence splits.

The traditional paleontological view maintained that the procyonids dispersed from North America into South America two separate times, being one of the first groups of North American mammals to colonize South America. The first dispersion event took place around 7.3–5 MYA (Late Miocene) and the fossil genus *Cyonasua* in South America is an evidence, long before the closure of the Isthmus of Panama and the GABI, which approximately occurred around 2.4 to 2.8 MYA according to Marshall et al. (1979, 1982), Webb (2006), Woodburne (2010). All descendants from that first colonization apparently went extinct by the end of the Middle Pleistocene (Marshall 1985; Soibelzon and Prevosti 2013). The second dispersion of procyonids into South America was, following this hypothesis, the one made by the ancestors of the extant genera in the last 0.125 MYA (Woodburne 2010) in the Late Pleistocene. Therefore, the current procyonid genera are not considered to be descendants of the procyonids that originally invaded South America (Baskin 2004; Soibelzon and Prevosti 2013; Forasiepi et al. 2014). This traditional view is based on that the complete emergence of the Isthmus of Panama was around 3.0–3.5 MYA in the Middle Pliocene, resulting in the closing of the Central American Seaway, CAS (Coates and Obando 1996; O'Dea et al. 2016). After this event, the fossil record indicates that the mammalian lineages predominantly migrated from North America to South America around 2.4–2.8 MYA (Simpson 1980; Woodburne 2010).

More recently, a second possible hypothesis has been established. This hypothesis affirms that the appearance of a land bridge and the closure of the CAS was around 13–15 MYA, during the Middle Miocene (Farris et al. 2011;

Montes et al. 2012, 2015; Carrillo et al. 2015). Indeed, the Isthmus of Panama formation began earlier and seems to be associated with the northern Andean uplift, around 24 MYA (Farris et al. 2011). Some recent studies proposed that the most significant periods of migration of mammals occurred during 20 and 6 MYA, with similar migration rates between North and South America, and that asymmetric migration emerged after 6 MYA, with higher migration from South to North America (Bacon et al. 2015; Marko et al. 2015). In fact, Carrillo et al. (2015) showed that many faunal colonization events associated with GABI began around 10 MYA. Meanwhile, in the first hypothesis, geological events might be responsible for preventing, or not, fauna colonization events, in this second hypothesis, environmental processes are the causes of promoting, or not, the faunal dispersion (Bacon et al. 2015; Montes et al. 2015). For example, moist and warm climate occurring in northern South America and Central America before 3.5 MYA favored tropical environments preventing faunal interchange of some North American mammal species that do not thrive in densely forested environments (Molnar 2008; Leigh et al. 2014). Nevertheless, *N. narica* is well adapted to forested habitats (Gompper 1995) and likely would have easily colonized different areas through tropical forests before dry savanna-like habitats evolved in the Middle Pliocene (3.0–3.5 MYA; Webb 2006; Molnar 2008).

Our results with the coatis, as well as the results of Nigenda-Morales et al. (2019), agree better with the second hypothesis. Our results also agree with the other results with molecular data found that the diversification within some extant procyonid genera, as *Procyon* and *Potos*, occurred in the Middle to Late Miocene, temporally coincident with the diversification of the extinct genera in South America (Koepfli et al. 2007; Ruiz-García et al. 2019). Additionally, the results of Nigenda-Morales et al. (2019), and the current ones, correlated well with the fact that the diversification of the South American extinct species *Cyonasua* spp. and *Chapalmalania* spp. and of extant *Nasua*, *Potos*, and *Procyon* species, 13–5 MYA, may have been part of a temporally concordant diversification event predating the GABI (Koepfli et al. 2007; Eizirik et al. 2010; Forasiepi et al. 2014; Carrillo et al. 2015; Ruiz-García et al. 2019). Moreover, *Nasua* and *Procyon* fossils dated around 1.5–3 MYA from Venezuela, showed the presence of these genera in South America during the time of the full emergence of the Panamanian isthmus (Ruiz-Ramoni et al. 2018).

This hypothesis aligns well with the origin of the current coatis “*in situ*” in South America. It is correlated with the results of the evolutionary histories of other Neotropical mammals. The ancestors of some today's South American mammalian species colonized South America before the complete closure of the Panamanian land bridge. Examples of this, for example, come from data on *Cebus capucinus*

(Ruiz-García et al. 2012a), *Tapirus bairdii* (Ruiz-García et al. 2012b, 2016), *Eira barbara* (Ruiz-García et al. 2017), and *Potos flavus* (Ruiz-García et al. 2019). The mitochondrial diversification of the *Nasua-Nasuella* complex within South America during the Late Miocene-Pliocene aligns with the fossils of some mammalian species collected from the Panama Canal region and their close similarities to taxa in North America. This suggests a broad connection between Central and North America during the Miocene as it was advanced by Whitmore and Stewart (1965). Indeed, some current mammal species, which traditionally were thought to have been radiated from North America during the GABI and which they colonized South America in the Pleistocene, we now know that the original radiation of the current genetic lineages of these species occurred “in situ” in South America. They were the cases of the jaguar (*Panthera onca*; Ruiz-García et al. 2013), the puma (*Puma concolor*; Culver et al. 2000), or the Andean bear (*Tremarctos ornatus*; Ruiz-García et al. 2020a). Some of the results of Nigenda-Morales et al. (2019) were related to this fact. They found that the numbers of migrants between the Panamanian *N. narica* population and all other populations were consistently asymmetric, with migration from Panama into northern populations usually greater than in the opposite direction (migration north to south). The S-DIVA and BBM biogeographic analyses, carried out by Nigenda-Morales et al. (2019), identified South America as an area of distribution for the most recent common ancestor of *Nasua* and *Bassaricyon*.

This work together with that of Nigenda-Morales et al. (2019) showed that the initial diversification of the *Nasua* species may have been in the northern Andes (current Colombian and Ecuadorian Andes). The rapid uplift of the northern Andes during the last 5–10 MYA (Hoorn et al. 2010; Mora et al. 2010) coincides with the temporal coati splits found.

In contrast to the slight genetic differences among the three sub-groups of *N. narica* found, the genetic differences among the four detected groups within *N. nasua* were very elevated. In fact, the mitochondrial divergence process within *N. nasua* was estimated to begin around 6 MYA (Late Miocene). The genetic distances among these four groups were also noteworthy. These groups were placed in (1) the Western Amazon (Colombia, Ecuador, and northern Peruvian Amazon) and Eastern Colombian Llanos, (2) throughout a large area of the Peruvian Amazon, (3) southern Peru and Bolivia, and in (4) Paraguay, Uruguay, and southern Brazil frontier with Argentina. Traditionally, 10 subspecies of *N. nasua* have been established (Cabrera 1958; Hershkovitz 1959; Decker 1991; Gompper and Decker 1998). The four groups we detected could be in agreement with four of these putative subspecies: (1) *N. n. dorsalis* (Western Amazon and Eastern Colombian Llanos); (2) *N. n. montana* (in

the majority of the Peruvian Amazon); (3) *N. n. boliviensis* (southern Peru and majority of Bolivia); and (4) *N. n. spadicea* (Paraguay, Uruguay, and southern Brazil). The genetic distances among these *N. nasua* groups (shown elsewhere) are very high and the temporal splits among them were considerable (between *dorsalis* and *spadicea*: 4.6 MYA; between *spadicea* and *boliviensis*: 2.9 MYA; *dorsalis* and *boliviensis*: 6.4 MYA). Many of these temporal splits were similar, or even higher, to the time splits between *N. nasua* and *N. narica*, or between *N. nasua* and different groups of *N. olivacea*. Henceforth, these groups should be cryptic species affected by morphological stasis (Eldredge et al. 2005; Gould 2007). This was also discovered in another procyonid, *Potos flavus* (Ruiz-García et al. 2019). Coatis are extremely well adapted to many different environments and it could be responsible for morphological stasis and cryptic morphological species in *N. nasua*. There is considerable molecular differentiation among these cryptic species. Obviously, nuclear DNA, karyotypic, ecological, and reproductive behavioral studies are needed to confirm that these *N. nasua* taxon groups are completely different species. If so, these four groups should be named as *N. dorsalis*, *N. montana*, *N. boliviensis*, and *N. spadicea*, respectively.

Genetic diversity, demographic changes, and spatial structure

The levels of genetic diversity (especially nucleotide diversity) were highly correlated with the time splits within each one of the taxa analyzed. *N. nasua* showed the highest levels of genetic diversity, followed by *N. olivacea* from the Eastern Colombian Andes Cordillera, and then *N. olivacea* from the Western and Central Colombian Andes Cordillera. Finally, *N. narica* is the last and least genetically diverse taxon, which also supports it as the youngest.

N. nasua did not show any evidence of population expansion. This could be related to two events. First, if *N. nasua* evolved the first and oldest haplotypes within coatis, it would also be more challenging to detect possible demographic changes. Second, within *N. nasua*, we found robust genetic heterogeneity, to such an extent that maybe each group should be considered as a cryptic species. This strong genetic heterogeneity can make it difficult to detect possible demographic changes in *N. nasua*. However, the overall sample of *N. olivacea* showed a population expansion which began in the last 0.5 MY, although this sample contained several different groups, but with a lower genetic heterogeneity than found among the groups of *N. nasua*. GEASTERN-NO also showed clear evidence of population expansion in the last 0.2 MY. Nevertheless, the other main group of *N. olivacea*, GCENTRAL-NO, did not show clear evidences of demographic changes. This was probably due to the relatively small sample size of this

group. In contrast, *N. narica* showed a clear population expansion in the last 0.12 MY, nicely correlating with it as the youngest taxon. One common feature among the four taxa is that they all had strong population declines in the last 20,000 years. This aligns with the Upper Pleniglacial period, around 22,000–14,000 YA, concomitant with major cold and dry periods (20,000–18,000 YA) of the fourth large Pleistocene glaciation (Climap 1976; Brown 1982; Haffer 1997, 2008; Van der Hammen et al. 1991). Clark (2002) showed that the rain level and the humidity in the Amazon basin at that time were extremely low. Even the Atlantic Ocean along the Brazilian coast had its temperature lowered to almost 6 °C. Metivier (1998) showed that the central and northern Andes had an ice surface area totaling around 371,306 km², 18,000 YA. This means an ice cover nearly 100 times greater than today. The last glacial advance in the Andes occurred during that epoch (Younger Dryas or III Dryas; Clapperton 1993). This last glacial advance is reported to have happened in different parts of the Andean cordilleras (Wright 1983), such as the Manachaque Valley (Cordillera Blanca), the Upismayo-Jalacocha (Cordillera de Vilcanota), and Puna de Junin in Peru, Choque-Yapu mountain in Bolivia and the Chimborazo volcano in Ecuador. Indeed, Rodbell and Seltzer (2000) showed that the glacial limits in the Cordillera Blanca (San Martin Department in Peru), around 12,000 YA, were around 3,170 and 3,827 m above sea level (masl). Compare this to today's limit of around 4,600 masl. Maslin and Burns (2000) showed that the Amazon River reached its maximum dry peak around 16,000–15,000 YA and this situation continued until 12,000 YA. The analysis with O₁₈ isotopes revealed that the mouth of the Amazon River only had 40% of the water compared to today. This climate change might have produced the last massive extinction event, which eliminated around 80% of the large vertebrates of North America. For example, this eliminated the pumas in North America (Culver et al. 2000) as well as 40 other species including *Smilodon*, lions, cheetahs, as well as the giant ground sloths and glyptodonts (Lessa et al. 1997; Lyons et al. 2004).

The existence of a very significant spatial structure for *N. olivacea* is not surprising due to the significant differences within the Western and Central Colombian and Ecuadorian Andean Cordilleras and, especially, by the differences between these last cordilleras and the Eastern Colombian Andean Cordillera.

The current mitochondrial genetic analysis is only a first step to understand the molecular evolution of the coatis. It should be complemented, in the future, with nuclear DNA markers, especially to analyze gene flow among the four groups of *N. olivacea* and determine whether there are subspecies within *N. olivacea* or full species. It would also be helpful to analyze nuclear DNA to determine the possible

level of historical introgression or current hybridization among *N. olivacea*, *N. nasua*, and *N. narica*.

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