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RESEARCH PAPER

Mitochondrial and karyotypic evidence reveals a lack of support for the genus *Nasuella* (Procyonidae, Carnivora)

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Abstract. Coatis are traditionally divided into two genera (*Nasua* and *Nasuella*). Coatis from the lowlands of the Neotropics are larger (*Nasua nasua* in South America and *Nasua narica* in Central America) than those from the highlands in the Andean Cordilleras (*Nasuella olivacea* and maybe *Nasuella meridensis*). Some authors have claimed that *Nasuella* should be included in *Nasua* but strong data have not been provided to support this statement. We reported an extensive mitochondrial (mt) DNA analysis with 205 specimens with complete mitogenomes. Some *N. olivacea* were intermixed among haplogroups of *N. nasua*, some haplotypes of *N. narica* were intermediate between *N. nasua* and the most recent haplotypes of the Central American *N. narica*, and *N. narica* from southern Central America and northern Colombia were introgressed with mtDNA from *N. olivacea*. Furthermore, the spatial genetic structure of *N. nasua*, *N. narica*, and *N. olivacea* were practically identical. Additionally, we also show, for first the time, the karyotype of *N. olivacea*. The chromosome morphology of *N. olivacea* was un-differentiable from that of *N. nasua*. These data fail to support the independence of these two genera.

Key words: coatis, karyotypes, mitogenomes, *Nasua*, *Nasuella*

Introduction

Coatis are social carnivores from the Procyonidae distributed in the Neotropics (from Arizona, USA, to northern Argentina and Uruguay). Traditionally, three species of coatis are placed in two different genera (*Nasua* Storr, 1780 (the brown-nosed coati *Nasua nasua* distributed in South America; the white-nosed coati *Nasua narica*, distributed in Central America) and *Nasuella* Hollister, 1915 (the mountain coati *Nasuella olivacea*, distributed in the Andean Cordilleras of Venezuela, Colombia and Ecuador)). Recently, a new species of *Nasuella* was reported (Eastern mountain coati *Nasuella*

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meridensis) in the Venezuelan Andean Cordillera (Helgen et al. 2009) based on craniometrics and sequences of the mitochondrial (mt) Cyt*b* gene. However, Ruiz-García et al. (2020) showed that the specimen used to define this new species was clustered with other specimens of mountain coati from the Eastern Colombian Andean Cordillera using three mt genes (*ND5,* Cyt*b* and control region), leaving open the debate about the validity of this species. Traditionally, the mountain coati has been classified as a different genus from *Nasua* because 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 (Nowak 1999).

Similarly, the body size of *Nasuella* is significantly smaller than that of *Nasua*. The baculum is shorter in *Nasua* than in *Nasuella* (Mondolfi 1987, Decker 1991) although the utility of this diagnostic is ambiguous (Gompper & Decker 1998). Although, the difference in size between these genera are obvious, some authors have noted that *Nasuella* should be included in *Nasua* (Glatston 1994) because of the similarity in many other anatomical characters.

Few molecular studies have been conducted on the coatis. McFadden (2004) and McFadden et al. (2008) concluded that the *Nasua nelsoni* from Cozumel Island is a full species differentiated from *N. narica*. Helgen et al. (2009) concluded that a sample from Venezuela was a different species (*N. meridensis*). Tsuchiya-Jerep (2009) and Neves-Chaves (2011) analysed the genetic structure of some populations of *N. nasua* in Brazil. The same were carried out by Silva et al. (2017) and Nigenda-Morales et al. (2019) for *N. narica* in Central America. Finally, Ruiz-García et al. (2020) analysed the genetic structure of *N. olivacea* in Colombia and Ecuador. However, not one of these studies analysed the possibility that both genera, *Nasuella* and *Nasua*, were un-differentiable. Only Helgen et al. (2009) and Nigenda-Morales et al. (2019), with a limited number of specimens and genes, suggested that all coati taxa should belong to one genus (*Nasua*).

Here we attempt to assess this last possibility through an extensive mitochondrial (mt) gene analysis and examination of karyotypes. We selected mt genes to determine the degree of relationships between *Nasua* and *Nasuella*. The mt genes are appropriate markers for this task because they include a rapid accumulation of mutations, rapid coalescence time, a negligible recombination rate, haploid inheritance and lack introns (Avise et al. 1987). They also have a large number of copies per cell, which makes mitochondrial 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 mtDNA (Galtier et al. 2006, Nabholz et al. 2012). Also, particular substitution patterns and base composition biases exist among sites and strands (Reyes et al. 1998), which are related to different evolutionary pressures affecting this kind of DNA. For all of these reasons, mt gene trees are more precise in reconstructing the divergence history among closely related taxa than other molecular markers (Moore 1995). For these reasons, we sequenced 205 samples (total of 179 haplotypes) of these three species for complete mitogenomes. However, the chance of detecting whether *Nasuella* should be included within *Nasua* depends not only on the results of phylogenetic analysis, since if these three coati taxa are closely related then they should belong to the same genus. In addition, we would expect that their spatial genetic structures – consequences of the evolutionary causes that generated them – should be similar or identical (Ruiz-García et al. 2017). Sokal & Wartenberg (1983), Sokal et al. (1986, 1987, 1989a), and Epperson (1990, 1993) showed that identical correlograms are created by identical spatial evolutionary forces affecting the same genes.

The karyotypes of *N. nasua* and *N. narica* are known. For the former species, the diploid chromosome number is 38, the fundamental number (FN) is 72, including 28 metacentric, submetacentric and subtelocentric autosomes, eight acrocentric autosomes, a submetacentric X, and a subtelocentric Y (Wurster & Benirschke 1968, Hsu & Benirschke 1970). For the latter species, the karyotype is similar. It has 38 diploid chromosomes; FN = 72, including 30 metacentric and submetacentric autosomes, and six acrocentric autosomes. The sex chromosomes include a relatively large submetacentric X and an acrocentric or small submetacentric Y (Hsu & Arrighi 1966, Todd et al. 1966, Hsu & Benirschke 1970). *Nasua nasua* differs from the karyotype of *N. narica*, by having one additional acrocentric pair and one less metacentric pair (Wurster & Benirschke 1968). Verleye et al. (1987) examined a

zoo colony of *N. narica* and *N. nasua* by G-banding, and noted hybridization resulting from complex chromosome rearrangements. However, no karyotype of *N. olivacea* has hitherto been reported. Here we report the first karyotype of a male and a female *N. olivacea* and we compare them with those reported for the two species of *Nasua* and other Procyonidae.

The main objectives of the current work were: 1) to determine the degree of molecular differentiation with mt genes, and the phylogenetic relationships, among a large sample of specimens of *Nasuella* and *Nasua* to assess whether there is support for the distinction of the genera; 2) to compare the spatial genetic structure of these three coati taxa; and 3) to compare the morphology of the karyotype of *Nasuella* with that of species of *Nasua*.

Material and Methods

We analysed mitogenomes of 205 coatis (110 *N. nasua*, 38 *N. olivacea* and 57 *N. narica*), and used two *Bassarycion medius* (Ecuador) as the outgroup (Table S1, Fig. 1). Samples came from individuals hunted in Indian communities as well as from road kill specimens. A minor fraction of the samples (of Colombian origins) were obtained from the museum of the Instituto Alexander von Humboldt (Villa de Leyva) with appropriate permissions. No ethics review was required, as our research work used a combination of museum skins and road kill and previously hunted animals and did not involve any direct manipulation or disturbance to live animals by researchers. For the karyotype analysis, we obtained blood from two specimens of *N. olivacea* (one male and one female) seized by the Secretaria Ambiental del Ambiente (SDA) in Bogotá (Colombia) from Chingaza National Park in the Eastern Colombian Andean Cordillera near Bogotá. The mitochondrial analyses were carried out in the laboratory of molecular population genetics of the Pontificia Universidad Javeriana (Bogotá DC, Colombia) and the karyotype analysis was carried out in the laboratory of genetics and cytogenetics of the National University (Medellín, Colombia).

Mitochondrial molecular procedures

DNA was extracted and isolated from either hair, skin, teeth, or muscle samples using the QIAamp DNA Micro Kit (Qiagen, Inc.) following the manufacturer's protocol. Mitochondrial genomes were sequenced by long-template PCR, which minimizes the chance of amplifying mitochondrial pseudogenes from the nuclear genome (numts) (Thalmann et al. 2004, Raaum et al. 2005). PCR amplification of mitochondrial DNA was carried out using a LongRange PCR Kit (Qiagen, Inc.), with a reaction volume of 25 µl and a reaction mix consisting of 2.5 µl of 10× LongRange PCR Buffer, 500 µM of each dNTP, 0.6 µM of each primer, 1 unit of Long-Range PCR Enzyme, and 50-250 ng of template DNA. Cycling conditions were as follows: 94 °C for 5 min, followed by 45 cycles denaturing at 94 °C for 30 s, primer annealing at 50-57 °C (depending on primer set) for 30 s, and an extension at 72 °C for 8 min, followed by 30 cycles of denaturing at 93 °C for 30 s, annealing at 45-52 °C (depending on primer set) for 30 s, and extension at 72 °C for 5 min, with a final extension at 72 °C for 8 min. Four sets of primers were used to generate overlapping amplicons from 3,687 to 4,051 bp in length, thereby enabling a quality test for genome circularity (Bensasson et al. 2001, Thalmann et al. 2004). Both mt DNA strands were sequenced directly using BigDye Terminator v3.1 (Applied Biosystems, Inc.). Sequencing products were analysed on an ABI 3730 DNA Analyzer system (Applied Biosystems, Inc.). Sequences were then assembled and edited using Sequencher 4.7 software (Gene Codes, Corp., Ann Arbor, MI). Overlapping regions were examined for irregularities such as frameshift mutations and premature stop codons. A lack of such irregularities indicates an absence of contaminating numt sequences.

The alignments with all the genes (16,114 bp) were concatenated after removing problematic regions using Gblocks 0.91 (Talavera & Castresana 2007) under a relaxed approach. This software removes all poorly aligned regions and is particularly effective in phylogenetic studies including highly divergent sequences (Castresana 2000, Talavera & Castresana 2007). The individual alignments were then concatenated by means of the SequenceMatrix v1.7.6 software (Vaidya et al. 2011) to create a master alignment. The GenBank accession numbers of the coati specimens analysed are from MT587713 to MT587788, MW410859 to MW410908, and MW419814 to MW419853.

Phylogenetical analyses to determine the relationships between the genera *Nasua* and *Nasuella* by using mitochondrial sequences. jModeltest v2.0 (Darriba et al. 2012), Kakusan4

Fig. 1. Map with the geographical origins and sample sizes of specimens of three coati species (*Nasua nasua, Nasua narica* and *Nasuella olivacea*) for which mitogenomes were sequenced (n = 205) throughout the Neotropics.

(Tanabe 2011) and MEGA X 10.0.5 software (Kumar et al. 2018) were used to determine the best evolutionary mutation model for the sequences analysed for each individual gene, for different partitions and for all the concatenated sequences. Akaike information criterion (AIC; Akaike 1974, Posada & Buckley 2004) was used to determine the best evolutionary nucleotide model.

Phylogenetic trees with mitogenomes were constructed using two procedures: Maximum Likelihood tree (ML), and Bayesian Inference tree (BI). The ML tree were obtained using the RA × ML v8.2.X software (Stamatakis 2014) implemented in CIPRES Science Gateway (Miller et al. 2010). The GTR + G + I model (General Time Reversible model + gamma distributed rate variation among sites + proportion of invariable sites; Lanave et al. 1984) was used to search for the ML tree because it was the best model for the major part of the mitochondrial genes. We estimated support for nodes using the rapid-bootstrapping algorithm (–f a –x option) for 1,000 non-parametric bootstrap replicates (Stamatakis et al. 2008). The groups of

coatis were considered significant when bootstraps were higher than 70% (lax limit; Hillis & Bull 1993). The BI tree was also performed using a GTR + G + I model for mitogenomes. This tree was completed with the BEAST v2.5.1 program (Drummond et al. 2012, Bouckaert et al. 2014). 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 30 million generations after a burn-in period of six million generations. Evidence of convergence and stationarity of model parameter posterior distributions was assessed based on ESS values > 200 and examination of trace files in Tracer v.1.7 (Rambaut et al. 2018). The burn-in was set at 20% and separate runs were assembled using LOGCOMBINER v.2.5.1 and TREEANNOTATOR v.2.5.1 (Rambaut et al. 2018). A Yule speciation model and a relaxed molecular clock with an uncorrelated log-normal rate of distribution (Drummond et al. 2006) was used. Posterior probability values provide an assessment of the degree of support of each node on the tree. Majority-rule consensus trees were constructed for each Bayesian analysis. Following Erixon et al. (2003), nodes supported by posterior probability (pp) ≥ 0.95 were considered strongly supported. Trees were visualized in the FigTree v1.4 software (Rambaut 2012).

To determine whether *N. olivacea* is nested within *Nasua*, we consider the mitogenome data set but also a data set with only three mt genes (*ND5*, Cyt*b* and *D-loop*) with more specimens analysed (particularly for critical geographic areas) and with a wider geographical range (345 specimens), which unfortunately did not amplify for all the mitogenome. We obtained ten different trees (we show them in a simplified version), with or without different outgroups to determine the relationships between *Nasuella* and *Nasua*. We wanted to see the influence of different outgroups in the relationships of both genera, *Nasua* and *Nasuella*, as well as the presence or absence of outgroups and its influence on the relationships of taxa with relatively recent phylogenetic splits (Ho et al. 2008). We also reconstruct the possible relationships among the haplotypes of *Nasuella* and *Nasua* with a Median Joining Network (MJN) with Network v4.6.0.1 software (Fluxus Technology Ltd.) (Bandelt et al. 1999) with the mitogenome data set.

Genetic distances

The Kimura 2P genetic distance (Kimura 1980) was applied to determine the percentage of genetic differences among the different groups detected in the three species of coatis analysed for the mitogenome data set. The Kimura 2P genetic distance is a standard measurement for barcoding tasks (Hebert et al. 2003, 2004). Kartavtsev (2011) analysed sequences of 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. At 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. Bradley & Baker (2001) and Baker & Bradley (2006) claimed for Cyt*b* 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, we take as an average for mitochondrial genes values above 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 above 16-18% (Kartavtsev 2011).

Spatial genetic analyses

Three Mantel tests (Mantel 1967) were used to detect possible overall relationships between the genetic matrices (Kimura 2P genetic distance) among specimens of each one of the three coati taxa and their respective geographic distance matrices among the specimens analysed for each one of these three taxa. Both genetic distances and geographical distances were log transformed. In this study, Mantel's statistic was normalized according to Smouse et al. (1986). This procedure transforms the statistic into a correlation coefficient.

Three spatial autocorrelation analyses were carried out for each of the three coati species. This analysis utilized the Ay statistic (Miller 2005) for each distance class (DC), where Ay = $\Sigma i = 1, n\ \Sigma j > i, n (Dijwyij)/\Sigma i = 1, n\ \Sigma j > i, n\ wyij$, where n is the number of individuals in the data set, and Dij is the genetic distance between observations i and j. Elements of a binary matrix, wyij, 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

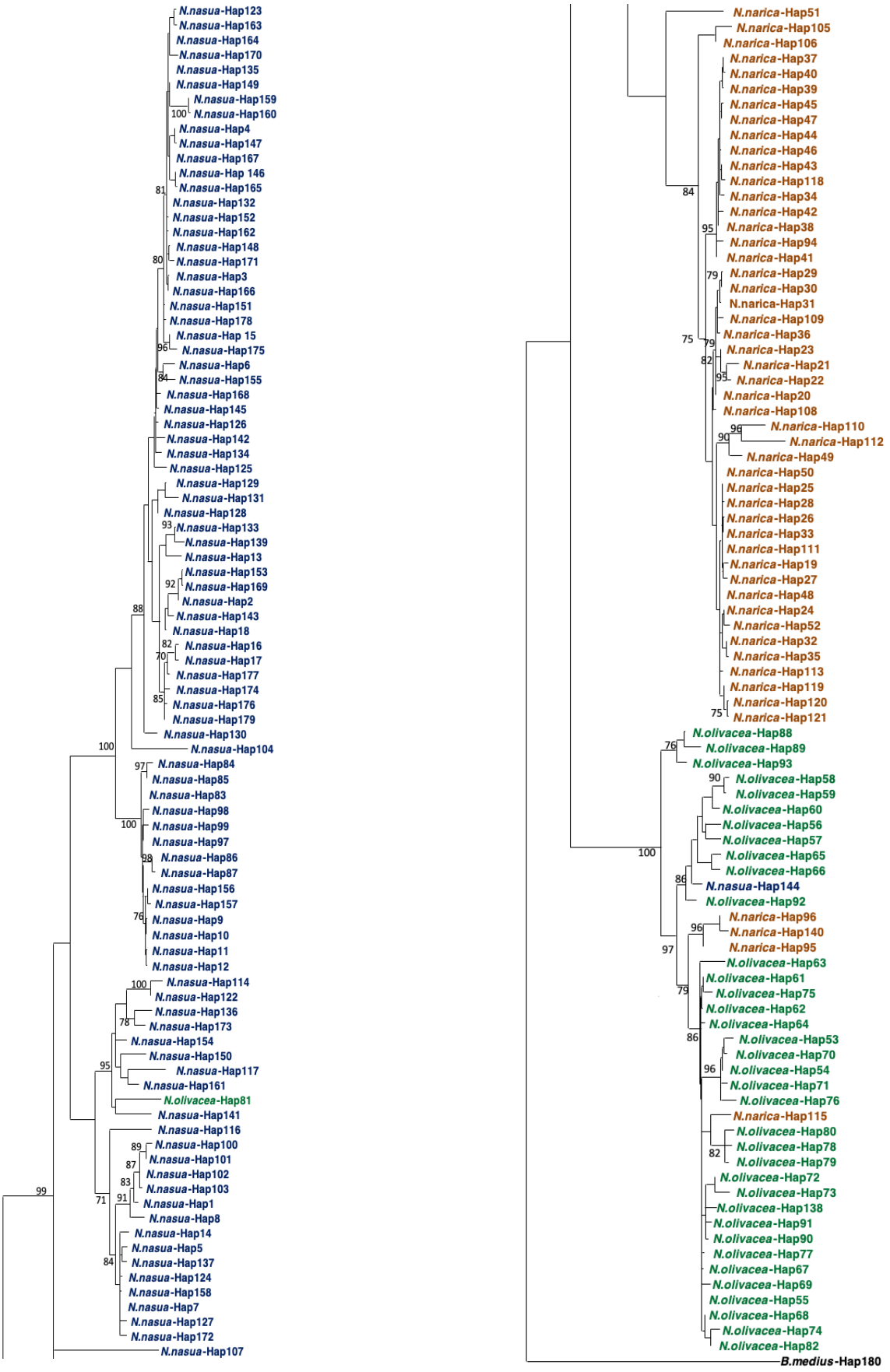


Fig. 2. Maximum Likelihood tree based in complete mitogenomes with 179 haplotypes for three species of coatis (*Nasua nasua, Nasua narica* and *Nasuella olivacea*) sampled in Latin America. Nodes are labelled with bootstrap percentages. H144 corresponded to a specimen "a priori" classified as *N. nasua* that might represent the first confirmed record of *N. olivacea* in Peru (the River Urubamba, Cuzco).

dissimilar. The probability for each DC is obtained using 1,000 randomizations. For this analysis there were ten defined DCs for both *N. nasua* and *N. narica* (*N. nasua*: 0-210 km; 210-368 km; 368-475 km; 475-614 km; 614-774 km; 774-1,001 km; 1,001-1,288 km; 1,288-1,718 km; 1,718-2,256 km; 2,256-3,514 km; *N. narica*: 0-26 km; 26-83 km; 83-183 km; 183-270 km; 270-337 km; 337-381 km; 381-547 km; 547-730 km; 730-1,089 km; 1,089-2,298 km) and six defined DCs for *N. olivacea* (0-46 km; 46-144 km; 144-206 km; 206-268 km; 268-429 km; 429-753 km). These DCs were the best to contain approximately the same number of comparisons in each DC. The size of each DC differs for each of the species analysed because the geographical range sampled for each species is also different, but the number of DCs is relatively similar to compare the shape of the correlograms of each species analysed. This analysis was carried out with AIS software (Miller 2005).

Karyotype procedures

Cultures were carried out following the method described by Moorhead et al. (1960). Heparinized blood was sowed (1 mL) in a supplemented culture RPMI 1640 (SIGMA) with 10% bovine foetal serum (GIBCO) and with 1% antibiotic (streptomycin 100 µg/mL, and penicillin 100 UI) with a final volume of 10 mL. The mitogene phytohemagglutinin (SIGMA) was added (100 µL). The culture was incubated at 37.5 °C for 72 hours. To obtain R-replicative bands (RGB), 100 µL of 5-bromodeoxyrudine (BrdU) with a concentration of 2 mg/mL was added at 66 hours to obtain the metaphase chromosomes. After 71 hours, 100 micro litres of colcemid with a concentration of 10 µg/mL was added. After culturing, cultures were transferred to 15 mL centrifuge tubes and centrifuged at 1,000 rpm for seven minutes. The supernatant was discarded and the lymphocyte pellet was resuspended. KCl (0.075 M) was added to achieve a volume of 7 mL and the sample was incubated at 37 °C for seven minutes. It was again centrifuged, the supernatant as discarded, and the remaining pellet was resuspended. A methanol-acetic acid (3:1) fixing solution was vigorously added to reach a volume of 7 mL. The sample was again centrifuged and the supernatant discarded and a fixation solution added. This procedure was repeated until a translucent supernatant was obtained (Spowart 1994). Samples were dripped into clean plates with alcohol and then cooled. Alcohol was added (tincture) to the plates to reveal the RGB bands (Camargo & Cervenka 1982, López & Márquez 2002). The extended chromosomes were evaluated at 100x magnification using a ZEISS optical microscope. We analysed fifty cells undergoing mitosis for each of the cases. Chromosome size and centromere ubication were considered in preparation of the karyotype.

Results

Mitochondrial phylogenetic procedures and their consequences on the systematics of the coatis

The most probable nucleotide substitution model considering the complete mitogenomes (all concatenated sequences; 16,114 bp, n = 205) was GTR + G + I (–Ln = 150, 195, 765, AIC). The mitogenome data set indicated a total of 179 coati haplotypes. The ML tree (Fig. 2) did not recover the three "a priori" species as monophyletic. In the clade of *N. nasua*, one haplotype of *N. olivacea* (H81) appeared from San José del Palmar (Chocó, Colombia). Between the clades of *N. nasua* and *N. narica*, one haplotype of *N. nasua* (H107) appeared from the PN Tamá (Norte de Santander, Colombia). Within the clade of *N. olivacea*, we found H144, which corresponded to a specimen "a priori" classified as *N. nasua* by its geographical origin (it was a road kill specimen and, therefore, its phenotype was not clearly recognized, although some traits seemed to be of *N. olivacea*) but, it could be the first real register of *N. olivacea* in Peru (the River Urubamba, Cuzco), and four haplotypes of *N. narica* (H95, H96, H140 and H115), which belonged (three of them) to southern Costa Rica, Nombre de Dios (Colón, Panama), and Arboletes (Antioquia, Colombia), and the other to western Ecuador (San José Cruz, Pichincha). These were undoubtedly specimens with *N. narica's* phenotype (they were alive) and in a geographical area where only *N. narica* lives but with mitogenomes of *N. olivacea*. The BI tree (Fig. S1) yielded the same inconsistences as the previous tree with two additions: the presence of the H51 (one specimen of *N. narica* from Costa Rica) within the clade of *N. nasua* and the H107 (*N. nasua*) within the *N. narica* clade and not in an intermediate position between *N. nasua* and *N. narica* as in the previous tree. Therefore, it is clear that no reciprocal monophyly existed among the three traditional species of coatis, nor between the two genera considered (*Nasua* and *Nasuella*) when mitogenomes were used. Note that most of the specimens that indicate monophyly were living or museum samples and thus there was no ambiguity regarding their identification.

Ten different phylogenetic trees (with different procedures and different outgroups) based on

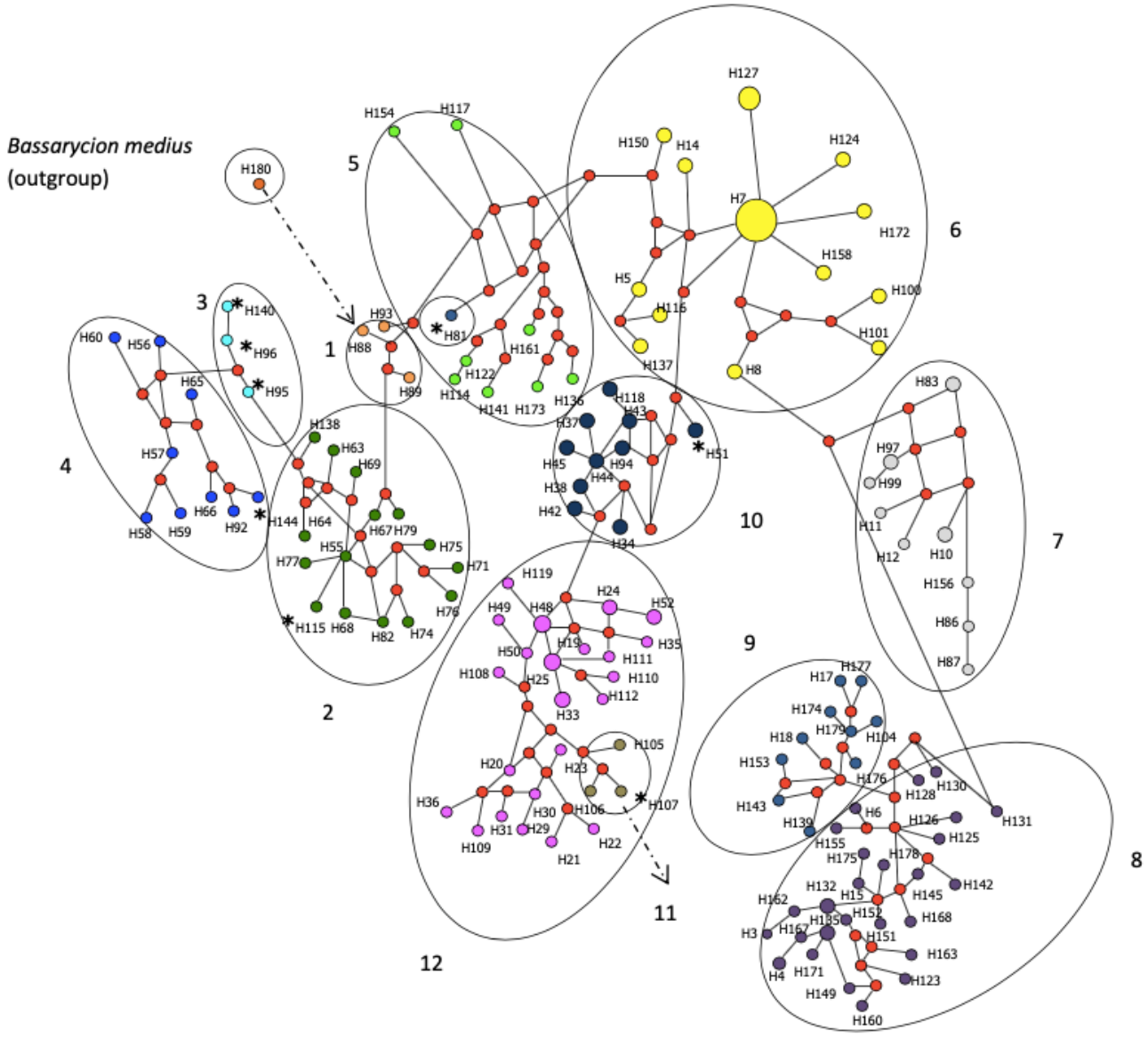


Fig. 3. Median Joining Network on mitogenomes of 205 specimens of three species of coatis (*Nasua nasua, Nasua narica* and *Nasuella olivacea*) sampled in Latin America. Haplogroups are shown with different colours; *Bassarycion neblina* (outgroup); 1) orange circles = *N. olivacea*; "transition-intermediate" haplotypes in the Colombian (Cauca, and Nariño Departments) and Ecuadorian (Carchi Province) Andean Cordilleras; 2) dark green circles = *N. olivacea* from the Eastern Colombian Andean Cordillera, including Norte de Santander, Boyacá, and Cundinamarca Departments; 3) light green circles = *N. narica* from southern Costa Rica, Panama and northern Colombia (Antioquia Department) introgressed with mtDNA from *N. olivacea*; 4) navy blue circles = *N. olivacea* from Western and Central Colombian and Ecuadorian Andean Cordilleras; Colombian Caldas, Risaralda, Chocó, and Tolima Departments, and Ecuadorian Pichincha, and Cotopaxi Provinces; 5) green circles = *N. nasua* from the Colombian and Ecuadorian Andean Cordilleras (one *N. olivacea*, H81, was included in this group); 6) yellow circles = *N. nasua* from the Ecuadorian and Colombian Amazon, Colombian Cundinamarca, Meta and Valle del Cauca Departments, and trans-Andean and Pacific Ecuador; 7) grey circles = *N. nasua* from southern Brazil, Paraguay, and Uruguay; 8) greenish-blue circles = *N. nasua* from different areas of the Peruvian Andes and Amazon, Colombian and western Brazilian Amazon); 9) lilac circles = *N. nasua* from southern Peru and Bolivia, different areas of the Peruvian Amazon and central Brazilian Amazon; 10) dark blue circles = *N. narica* from northern Costa Rica, Nicaragua, El Salvador, Honduras and Guatemala; 11) greenish-brown circles = *N. narica* from trans-Andean and cis-Andean Ecuador; 12) fuchsia-pink circles = *N. narica* from Guatemala, Belize, southern Mexico and Yucatan. Red circles indicate missing intermediate haplotypes. Some haplotypes have an asterisk (*). These are the cases of H40, 51, 81, 95, 96, 107, 115 and 144. All belonged to specimens with the morphotype of a determined species but with mitogenomes belonging to a different species. H144 corresponded to a specimen "a priori" classified as *N. nasua* that might represent the first confirmed record of *N. olivacea* in Peru (the River Urubamba, Cuzco).

the three mt gene data set are shown in Fig. S2. Sixty percent of these trees (ML tree with only *Bassarycion neblina* as the outgroup, ML tree with all of the *Bassarycion* species as the outgroup, ML tree without an outgroup, NJ (neighbour-joining, Saitou & Nei 1987) tree with only *B. neblina* as

Table 1. Kimura (1980) 2P genetic distances among the main groups of *Nasua nasua* (five groups), *Nasua narica* (four groups), and *Nasuella olivacea* (three groups), together with *Bassarycion medius* as out-group, using the mitogenome data set. 1) *N. nasua* haplogroup from Colombian and Ecuadorian Amazon and Eastern Colombian Llanos; 2) *N. nasua* haplogroup from the Colombian and Peruvian Amazon; 3) *N. nasua* haplogroup from southern Peru and Bolivia; 4) *N. nasua* haplogroup from Colombian and Ecuadorian Andes; 5) *N. nasua* haplogroup from southern Brazil, Paraguay, and Uruguay; 6) *N. narica* haplogroup from southern Central America (southern Costa Rica and Panama) and northern Colombia introgressed by mtDNA of *N. olivacea*; 7) *N. narica* haplogroup from southern Mexico and part of Guatemala; 8) *N. narica* haplogroup from part of Guatemala and Belize; 9) *N. narica* haplogroup from part of Guatemala, Honduras, El Salvador, Nicaragua, and Costa Rica; 10) *N. olivacea* haplogroup from Colombian and Ecuadorian Andean Cordilleras more related to *N. nasua*; 11) *N. olivacea* haplogroup from Western-Central Colombian and Ecuadorian Andean Cordilleras; 12) *N. olivacea* haplogroup from Eastern Colombian Cordillera; 13) *B. medius*. Standard deviations are not shown because they were practically 0. Genetic distances in %.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13
1	-												
2	7.9	-											
3	8.6	1.6	-										
4	3.5	8.3	8.9	-									
5	7.7	4.0	4.8	8.4	-								
6	13.8	15.4	15.1	13.1	15.4	-							
7	10.1	11.5	11.8	9.4	10.5	11.9	-						
8	10.3	11.5	11.7	9.4	10.7	12.6	0.9	-					
9	10.2	11.8	12.0	9.5	11.4	11.7	1.2	1.7	-				
10	6.6	10.3	11.1	2.9	10.7	14.6	11.3	10.7	11.3	-			
11	13.4	14.8	14.8	12.3	14.4	1.8	10.5	10.9	10.4	13.6	-		
12	13.9	15.3	15.3	12.9	15.3	2.0	11.9	12.3	11.9	13.1	2.3	-	
13	19.4	20.1	20.7	19.4	19.0	17.7	19.2	19.4	19.0	20.8	18.4	19.2	-

the outgroup, NJ tree with all of the *Bassarycion* species as the outgroup, and NJ tree without an outgroup) yielded *N. olivacea* + *N. narica* with *N. nasua* as the most differentiated taxon. In contrast, 40% of the trees indicated *N. nasua* + *N. narica* and *N. olivacea* as the most differentiated taxon (ML tree with *Procyon cancrivorus* as outgroup, ML tree with *P. cancrivorus* + all the species of *Bassarycion* as the outgroup, NJ tree with *P. cancrivorus* as the outgroup, and NJ tree with *P. cancrivorus* + all the species of *Bassarycion* as the outgroup) but without reciprocal monophyly among these putative species.

The MJN for mitogenomes is shown in Fig. 3. The results obtained for this analysis were insensitive to the outgroup employed. Some haplotypes of *N. olivacea* were the first to appear (H88, H89, H93). From these, two pathways developed. The first gave rise to the remaining haplotypes of *N. olivacea* (with the exception of one haplotype). These were the first to derive from the Eastern Colombian Andean Cordillera (H67, H79, and related haplotypes) and, later, the Western and Central Colombian and Ecuadorian Andean Cordilleras (H56, H60, and related haplotypes). One group of *N. narica* in southern Central America (H95, H96, H140) was introgressed by mtDNA from *N. olivacea*. This haplogroup is an intermediate group between both main groups of *N. nasuella*. The second pathway first gave rise to the majority of the Andean Colombian and Ecuadorian *N. nasua* haplotypes (H154 and related haplotypes) together with a haplotype of a specimen of *N. olivacea* (Chocó Department, Colombia; H81). This group showed high internal heterogeneity. These Andean coati haplotypes (both *N. nasua* and *N. olivacea*) are the origin of the group of *N. nasua* distributed mainly within the Colombian and Ecuadorian Amazon and some Colombian Andean Departments. This genetic result confirmed the existence of sympatry in the Andes of *N. nasua* and *N. olivacea*, as was demonstrated by ecological analyses by González-Maya et al. (2015). This Colombian and Ecuadorian group of *N. nasua* is the origin of *N. narica* and, as well as all the other differentiated groups of *N. nasua*. These differentiated groups of *N. nasua* are: 1) southern South America (southern Brazil, Paraguay, and Uruguay; H83, H97, and related haplotypes), 2) the Peruvian Amazon and Peruvian Andes (H18, H176, and related haplotypes), and 3) the Andean Peru, Peruvian Amazon, including the Madre de Dios River basin (southern Peru), Bolivia, and central Brazilian Amazon (the River Negro)

(H131, H132, H135, and related haplotypes). The first haplogroup to appear on the branch of *N. narica* was from the middle area of Central America (northern Costa Rica, Nicaragua, El Salvador, Honduras and certain areas of Guatemala; H51, H118, and related haplotypes). From this, the most northern haplotypes in Guatemala, Belize and southern Mexico (H25, H48, and related haplotypes) originated. The Ecuadorian specimens of *N. narica* were a derived haplogroup from this last Central American group. With the MJN analysis, a group of *N. narica* from the Yucatan Peninsula (Mexico) was not clearly discriminated from the northern Central American *N. narica* group, whereas in the phylogenetic trees, the Yucatan group was differentiated (H110 and H112). Therefore, we observed ancestral haplotypes of *N. olivacea* more related to those of the Andean haplogroup of *N. nasua* compared to most derived *N. olivacea*. Furthermore, the most southern Central America and northern Colombian *N. narica* showed mtDNA from *N. olivacea* because there was introgression of mtDNA of this last species in the first around 0.9-0.7 millions of year ago (Ruiz-García et al. 2020). Thus, the haplotypes of *Nasuella* and *Nasua* were not completely isolated and, in many cases, were mixed in some evolutionary trajectories. Thus, no reciprocal monophyly existed between *Nasuella* and *Nasua*.

Genetic distances among coati taxa

Kimura (1980) 2P genetic distances among the main groups of *N. nasua* (five groups), *N. narica* (four groups), and *N. olivacea* (three groups), together with *B. medius*, were estimated using the mitogenome data set (Table 1). The genetic distance among the coati group and *B. medius* was around 20%, which is within the range obtained by Kartavtsev (2011) (higher than 16-18% for species of different genera). The highest genetic distance values obtained among coati groups were around 15.4% (*N. nasua* from Colombian and Peruvian Amazon *vs*. *N. narica* introgressed by *N. olivacea*, and *N. nasua* from southern Brazil, Paraguay, and Uruguay *vs*. *N. narica* introgressed by *N. olivacea*). The two main groups of *N. olivacea vs*. the five groups of *N. nasua* showed genetic distances ranging from 12.3% to 15.3%, whilst the same two groups of *N. olivacea vs*. the three groups of *N. narica* (excluded the group of *N. narica* introgressed by *N. olivacea*) varied from 10.4% to 12.3%. The genetic distances among the five groups of *N. nasua* and these three groups of *N. narica* were of the same magnitude, ranging from 9.4% to 12%. All of these values were clearly lower than 16-18% identified by Kartavtsev (2011) as a threshold for species of different genera. Moreover, the smaller group of *N. olivacea* yielded lower genetic distances with reference to the five groups of *N. nasua* (2.9-11.1%) than with the two main groups of *N. olivacea* being "a priori" the same species (13.1-13.6%). In fact, the genetic distances of the smaller group of *N. olivacea* with reference to the group of *N. nasua* from the Colombian and Ecuadorian Andes (2.9%), or the group of *N. nasua* from the Colombian and Ecuadorian Amazon and Colombian Eastern Llanos (6.6%), were lower than some genetic distances among the five groups of *N. nasua*, which ranged from 1.6% to 8.9%. For instance, the genetic distances among the group of *N. nasua* from the Colombian and Ecuadorian Amazon and Colombian Eastern Llanos *vs*. the group of *N. nasua* from the southern Peru and Bolivia or the group of *N. nasua* from southern Brazil, Paraguay, and Uruguay were 8.6% and 7.7%, respectively. The smaller genetic distances were found among the groups of *N. narica* (excluding the group introgressed by *N. olivacea*), ranging from 0.9% to 1.7%.

Thus, the magnitude of the genetic distances between the groups of *Nasuella* and *Nasua* were lower than that expected for species of different genera based on Kartavtsev (2011) using two mt genes. Additionally, the genetic distances of the groups of *N. narica vs*. the groups of *N. nasua* and *vs*. the groups of *N. olivacea* were of the same magnitude, with one group of *N. olivacea* showing lower genetic distances with some groups of *N. nasua* than the genetic distances among many groups of *N. nasua.* These results question the designation of *Nasuella* as a different genera from *Nasua*.

Comparative spatial structure among *N. nasua*, *N. narica* and *N. olivacea*

The three taxa showed similar results with Mantel tests (Fig. 4). In the case of *N. nasua*, the relationship between the geographical distances and the genetic distances was significant ($r = 0.44$, $P < 0.001$). Geographic distance explains about 19.7% of the genetic distance. Similarly, for *N. narica*, the relationship between the geographical distances and the genetic distances was significant ($r = 0.44$, $P < 0.001$). About 19.6% of the genetic distance is explained by geographical distance. For *N. olivacea*, the relationship between the geographical distances and the genetic distances was also significant ($r = 0.46$, $P < 0.001$) with the geographical distance

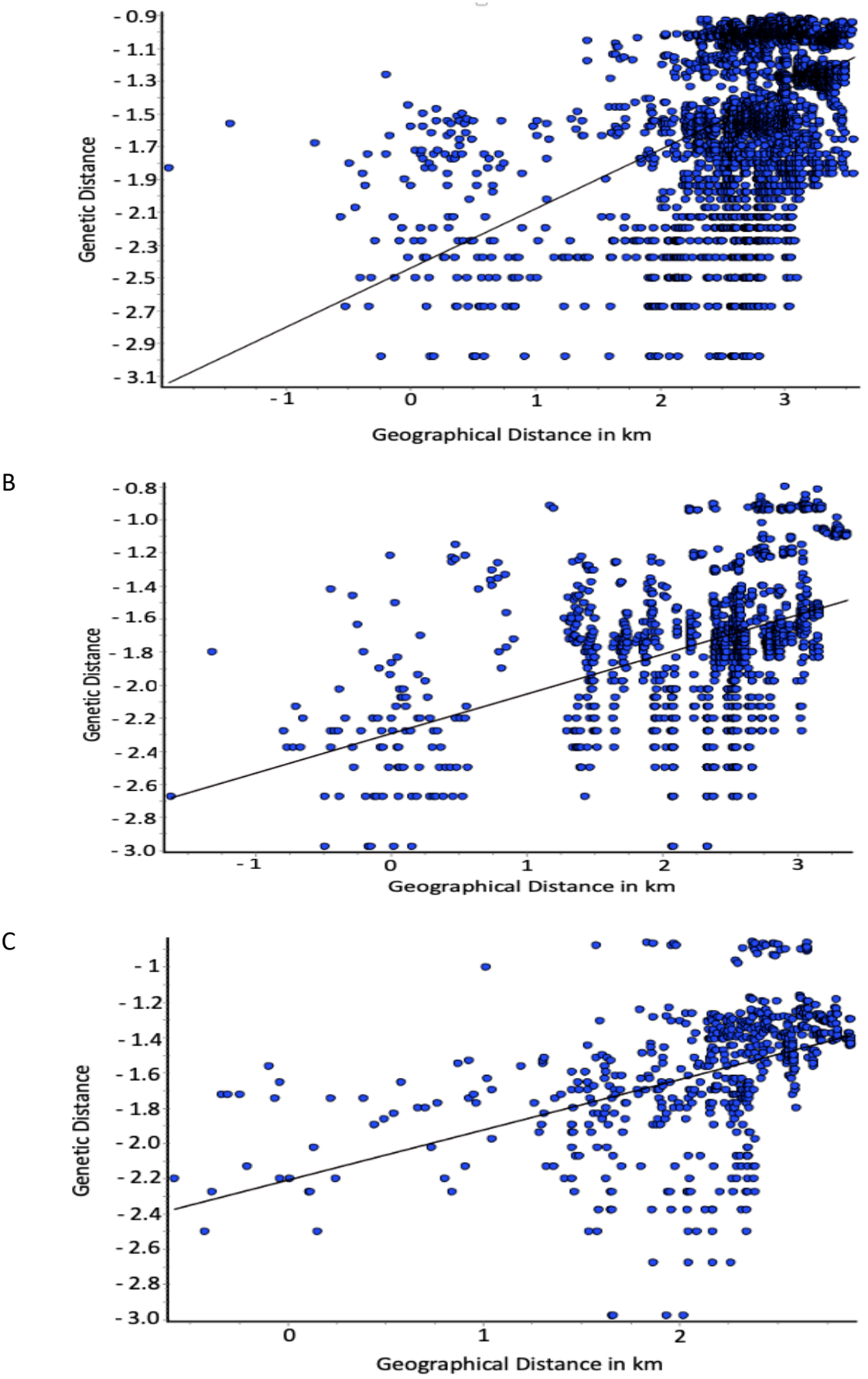
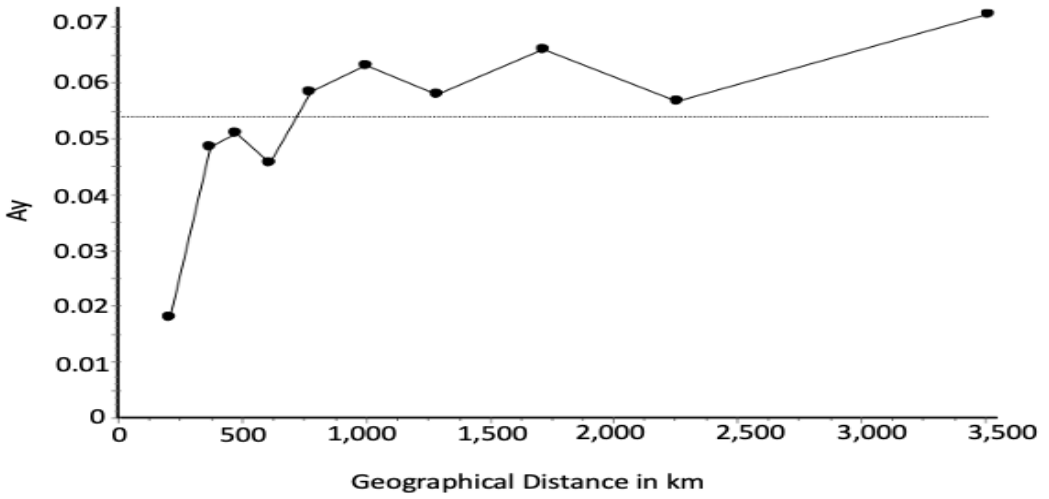
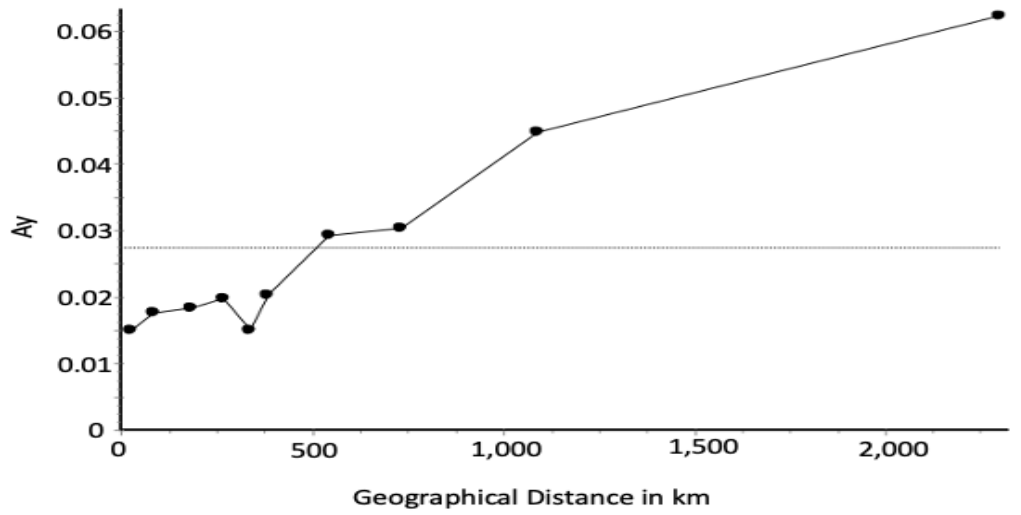


Fig. 4. Mantel test (log transformed) between the geographic and genetic distances for the entire mitogenomes of the specimens of *Nasua nasua*, *Nasua narica* and *Nasuella olivacea* studied. A) *N. nasua*; B) *N. narica*; C) *N. olivacea*.

A



B



C

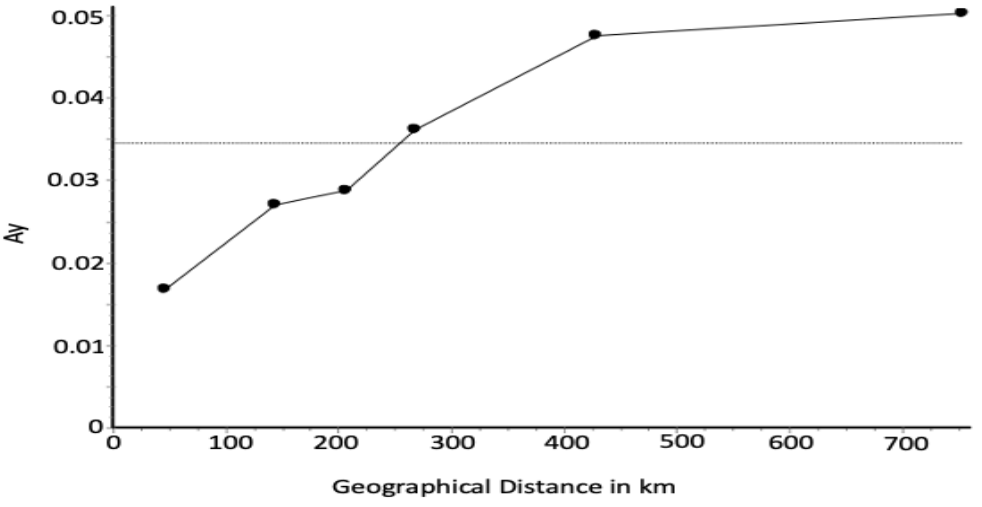


Fig. 5. Spatial autocorrelation analyses for specimens of *Nasua nasua*, *Nasua narica* and *Nasuella olivacea* with their entire mitogenomes sequenced. A) *N. nasua* with ten Distance Classes (DC); B) *N. narica* with ten Distance Classes (DC); C) *N. olivacea* with six Distance Classes (DC).

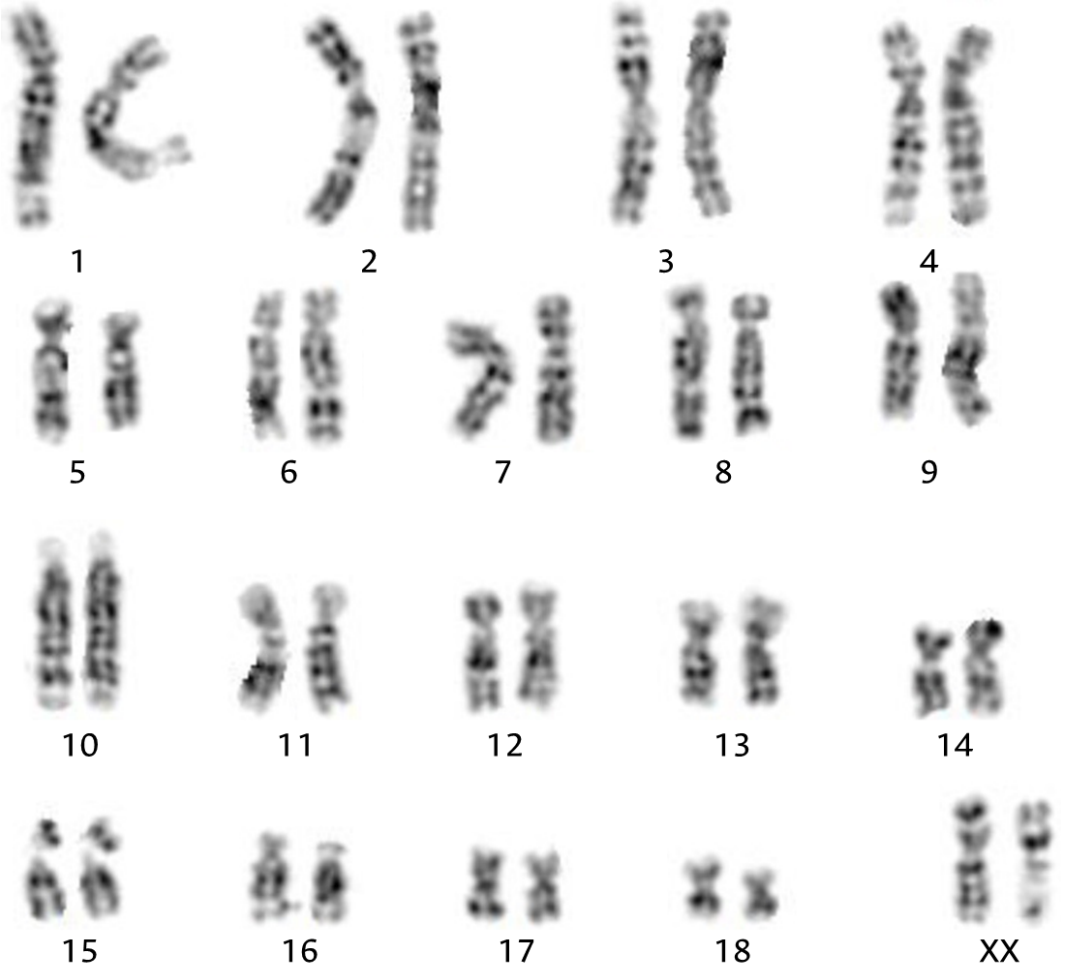


Fig. 6. Karyotype of a female (XX) of *Nasuella olivacea* with RBG bands.

explaining about 20.9% of the genetic distances. Thus, the three taxa showed a similar overall genetic structure, which is symptomatic of species closely related phylogenetically and, probably, not from different genera.

For the spatial autocorrelation analyses (Fig. 5), the situation is also similar for the three taxa. Using 10 DCs, for *N. nasua*, the overall correlogram is significant (V = 0.014, $P < 0.001$). The first DC (0-210 km, $P < 0.001$), second DC (210-368 km, $P = 0.014$), and fourth DC (475-614 km, $P < 0.001$) were all significantly positive. In contrast, the fifth (614-774 km, $P = 0.0159$), sixth (774-1,001 km, $P < 0.001$), eighth (1,288-1,718 km, $P < 0.001$) and tenth DCs (2,256-3,513 km, $P < 0.001$) were all significantly negative. Therefore, for *N. nasua*, we found a patch diameter of around 600 km and later a structure of isolation by distance at around 3,500 km.

For *N. narica*, the overall correlogram of the 10 DCs was also significant (V = 0.015, $P < 0.001$). The first (0-26 km, $P < 0.001$), second (26-83 km, $P = 0.026$), third (83-183 km, $P = 0.018$), fourth (183-270 km, $P = 0.034$), and fifth DCs (270-337 km, $P < 0.001$) were all significantly positive. In contrast, the ninth (730-1,089 km, $P = 0.007$) and tenth DCs (1,089-2,298 km, $P = 0.002$) were significantly negative. Therefore, for *N. narica*, we found a patch diameter of around 340 km and later a structure of isolation by distance, or clinal pattern, of around 2,300 km.

Finally, the overall correlogram representing six DCs for *N. olivacea* was positive (V = 0.012, $P < 0.001$). The first (0-46 km, $P < 0.001$), second (46-144 km, $P = 0.023$), and third DCs (144-206 km, $P = 0.039$) were all significantly positive. The fifth (268-429 km, $P < 0.001$), and sixth DCs (429-753 km, $P = 0.003$) were significantly negative. Therefore, for *N. olivacea*, we found a patch diameter of around 200 km and later a structure of isolation by distance, or clinal pattern, of around 750 km.

Although the geographical range distribution for the three taxa are unequal, their geographical structures are similar (patches and later clinal

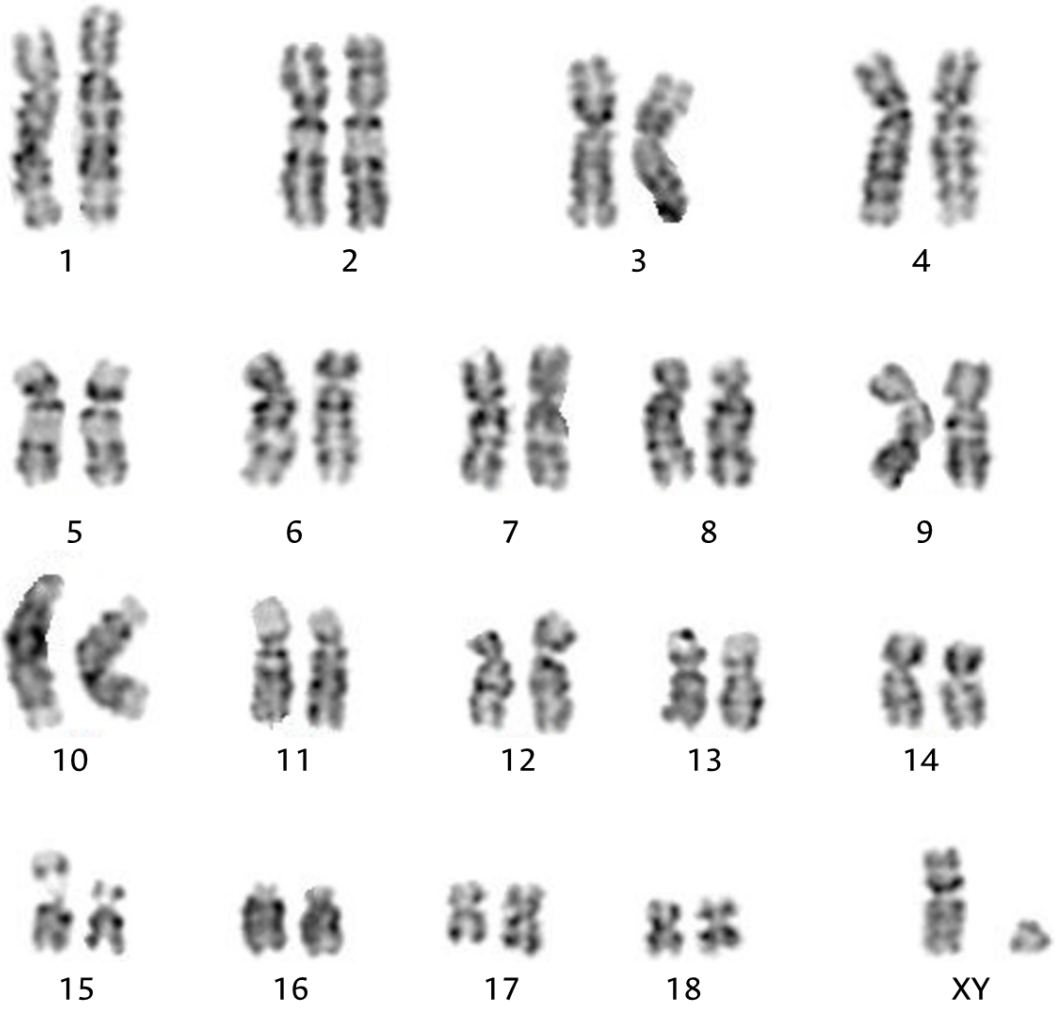


Fig. 7. Karyotype of a male (XY) of *Nasuella olivacea* with RBG bands.

pattern), which means that different evolutionary forces were acting upon these taxa in a similar fashion. In turn, this revealed strong phylogenetic relationships among the three taxa, which contradicts with the species belonging to different genera. As we will show in brief, other procyonids more differentiated phylogenetically from the coatis, also has more differentiated spatial genetic patterns.

Karyotype

We found 2n = 38, the FN = 72 (Figs. 6 and 7), and there were two metacentric, ten submetacentric, four acrocentric, and two subtelocentric autosomic chromosome pairs. The X chromosome was submetacentric and the Y chromosome was subtelocentric. As such, the morphology of the chromosomes of *N. olivacea* was indistinguishable from that of *N. nasua* (Wurster & Benirschke 1968). Additionally, the morphology of chromosome 15 was identical for both *N. olivacea* and *N. nasua.* However, our banding pattern was not comparable with other studies, because we obtained RGB and the banding patterns previously reported for *N. nasua* and *N. narica* were G-banded.

The relative length of the 19 chromosome pairs expressed as an average ± standard deviation with the Centromeric Index are shown in Table 2. Based on Fig. 6 and 7, and the correlation of the CI, several chromosomes in the two studies specimens had polymorphisms. Chromosome pairs 5 and 11 presented chromosome polymorphisms in the p arm, and chromosome 15 showed a polymorphism in the centromere of the p arm. This last one should be a marker chromosome due to the differential behaviour between males and females. This hypothesis requires confirmation with the analysis of additional specimens.

The sex chromosomes were easily identified with the RBG procedure. The X chromosome represented about 5% of the total genome length, a characteristic in mammalian genomes. The Y chromosome showed subtelocentric morphology with a high heterochromatin content.

Table 2. Relative length of the chromosomes found in the karyotype of *Nasuella olivacea*. RL = Relative Length; CI = Centromeric Index. SD = Standard Deviation.

Chromosome	Mean RL	SD RL	Mean CI	SD CI
1	9.00	0.32	29.35	0.52
2	8.37	0.34	37.77	2.00
3	7.62	0.64	42.37	2.17
4	7.82	0.12	32.58	2.52
5	5.02	0.46	27.92	4.79
6	5.71	0.14	24.96	3.08
7	5.59	0.32	34.68	2.31
8	5.54	0.27	23.11	1.37
9	5.57	0.28	35.08	2.26
10	6.17	0.39	2.17	0.93
11	5.14	0.25	30.44	4.46
12	4.64	0.47	29.73	2.39
13	4.04	0.13	37.96	1.78
14	3.57	0.40	39.15	3.66
15	3.84	0.51	37.16	6.50
16	2.88	0.58	8.79	0.71
17	2.70	0.11	43.01	2.38
18	2.19	0.26	46.00	4.66
X	4.58	0.09	45.24	0.76
Y	1.06	0.08	4.9	1.21

Discussion

The main aim of the current study is to obtain data to clarify whether the genus *Nasuella* Hollister, 1915 should be integrated within the genus *Nasua* Storr, 1780. Other topics, such as the number of significant groups within each species, the systematics of these subspecies in each species, temporal origins of these groups, geological and climatic events which generated these splits, etc. were exhaustively treated in Ruiz-García et al. (2020, 2021) and Ruiz-García & Jaramillo (2021) and therefore, are not included here.

How many genera of coatis are there by studying mitochondrial genes?

All of the phylogenetic trees that we generated did not show reciprocal monophyly between *Nasua* and *Nasuella*. The results obtained showed that within the clades with haplotype characteristics of *N. nasua*, there were haplotypes of specimens with typical phenotypes (and also typical geographical distributions) of *N. narica* (H51, Fig. S1) and *N. olivacea* (H81; Fig. 2, Fig. S2). Furthermore, within the clades with haplotype characteristics of *N. narica*, some specimens appeared with the full characteristics of *N. nasua* (H107, Fig. S1), and within of the main clade of *N. olivacea*, there were specimens with full phenotypes of *N. narica* (H95, H96, H140 and H115). In fact, specimens of *N. narica* within the main clade of *N. olivacea* represent two different introgression or hybridization events. The first is a clear old introgression event from *N. olivacea* into *N. narica* (H95, H96, H140; for time splits, see Ruiz-García et al. 2020), which affected all the specimens of *N. narica* distributed in southern Central America (southern Costa Rica and Panama) and the northern Colombian frontier with Panama. This was the case for all specimens of *N. narica* studied in this area by Nigenda-Morales et al. (2019), as well as all specimens herein studied from this geographical area. Additionally, these three haplotypes comprised a homogeneous haplogroup within the *N. olivacea* clade but clearly differentiated from other haplogroups of *N. olivacea* (Figs. 2, 3; Fig. S1), which agrees well with the fact that this introgression event was enough old to differentiate the mtDNA of this introgressed *N. narica* haplogroup from other haplotypes and haplogroups of *N. olivacea*. However, the specimen of *N. narica* from north-western Ecuador (H115) with a haplotype of *N. olivacea* seems to be a case of recent hybridization because its haplotype is similar to the current *N. olivacea* haplotypes from Ecuador and its morphology, although nearest to *N. narica*, has some traits similar to *N. olivacea*. These results were typical of taxa that have relatively small genetic differences among them and that have a typical reticulated evolution, with introgression or hybridization at different times (Ruiz-García et al. 2018, 2019b). Therefore, the interchange of genes among specimens of these three putative taxa is not consistent with species belonging to different genera. One alternative hypothesis is that these specimens were misclassified when the samples were obtained. Nevertheless, this seems unlikely because, at least for the specimens of *N. narica*, all of them were alive when they were sampled and they had the unmistakable physical characteristics of *N. narica*. Additionally, *N. olivacea* is not distributed in the frontier between Colombia and Panama, nor in Panama and southern Costa Rica, where only *N. narica* occurs. In the case of *N. nasua-N. olivacea*, it should be possible that the specimen of "a priori" *N. olivacea* with mtDNA of *N. nasua* (H81) would represent one specimen of *N. nasua* which morphologically evolved by convergent

adaption to a similar morphotype to that shown by *N. olivacea* through occupation of the same Andean biome. However, the skull, mandible, and teeth of this exemplar were typically of *N. olivacea*. Furthermore, with the three mt gene data set, as well as in Ruiz-García et al. (2021), more specimens of *N. olivacea* were nested inside *N. nasua* and they conformed to homogeneous haplogroups within this last species but clearly differentiated from other haplogroups of *N. nasua*. Additionally, the skulls, mandibles, and teeth of these specimens were typically of *N. olivacea*. Introgression, recent hybridization, and intermediate haplotypes among the three species of coatis seem more likely than misclassifications or morphological convergent adaption (possible case of *N. nasua* from the Andean mountains of Colombia and Ecuador) and, therefore, this correlates well with there being no genetic differences among the three species of coatis. Furthermore, the haplogroup of *N. narica* introgressed with mtDNA of *N. olivacea* showed lower genetic distances with the main haplogroups of *N. olivacea* relative to haplogroups of its own species. The existence of introgression indicates no reproductive barriers between the ancestors of the current *N. narica* and *N. olivacea.* In fact, it correlated well with a possible scenario based on biogeographic grounds (Toews & Brelsford 2012) and the introgressed descendent expanded through northern Colombia, Panama, and southern Costa Rica. Henceforth, these introgressed specimens were highly successful showing no genetic incompatibilities between *N. olivacea* and *N. narica*. This is an improbable outcome for specimens of fully differentiated genera.

Another relevant result obtained here is in relation to the haplotype H144 found within *N. olivacea*. This specimen was "a priori" classified as *N. nasua* (Fig. 2) because in the geographical area where it was sampled there was no record of the presence of *N. olivacea*. However, the mitogenome obtained is typical of *N. olivacea* and a detailed analysis of the morphotype of the individual revealed that it probably corresponded with a "true" *N. olivacea*. Therefore, it could be the first real register of *N. olivacea* in Peru (the River Urubamba, Cuzco).

With the mitogenome tree analyses, we only discovered one relationship: ((*N. nasua* + *N. narica*) + *N. olivacea*) with the percentages of bootstraps considerably higher than those obtained with the three mt genes, potentially supporting the maintenance of two traditional genera of coatis. We, however, think that a unique genus (*Nasua*) should be considered as the preferential option because with mitogenomes no reciprocal monophyly was observed among the three coati taxa, which was consistent with the karyotype analysis with no relevant differences between *Nasuella* and *Nasua*. Also, for the three mt gene data set, the major part of the trees obtained with different outgroup species showed a major relationship between *N. narica* and *N. olivacea* that was greater than either of these two taxa with *N. nasua*, as Helgen et al. (2009) found for mtCyt*b*. It is interesting to note that when the sequence of *P. cancrivorus* was present as an outgroup, with or without the other outgroup species, *N. olivacea* was differentiated from *N. nasua* + *N. narica*. In contrast, if *P. cancrivorus* was excluded (whether or not other species of out-groups were included), then, the relationship was *N. olivacea* + *N. narica*, with *N. nasua* more differentiated. It is curious that with the three mt genes, *Nasua* + *Nasuella* yielded a stronger relationship with *P. cancrivorus* than with *Bassarycion*. This finding agrees well with morphological studies (Baskin 2004), but contradicts the molecular relationships recovered by Koepfli et al. (2007), who showed that the sister species of *Nasua* + *Nasuella* was *Bassarycion*.

The absence of differentiation of *Nasuella* from *Nasua* is likely because the evolutionary trajectory for the coatis is a continuous process and not a discrete one. This is more apparent in the MJN than in phylogenetic trees. We consider this true for intra-specific relationships, or for closely related species (such as in this case). A MJN better reflects the evolution of taxa than do traditional phylogenetic bifurcating trees (PBT) for four reasons (Freeland et al. 2011). 1) Population genealogies are frequently multifurcated. In our case, MJN allowed multifurcated events, whereas PBT did not. 2) Within species, or among closely related species, genetic similarity can be generally high, or very high. Whilst MJN can reconstruct genealogies with restricted genetic variability, PBT requires more differentiated characters to discriminate among the taxa analysed. 3) At an intra-specific level, or among closely related species, ancestral and derived haplotypes can coexist within populations or closely related taxa. MJN allows for both original and descendant haplotypes, whereas PBT assumes that ancestral haplotypes no longer exist. 4) At the intra-specific or closely related species level, hybridization and recombination can occur often and be important.

MJN can easily reveal hybridization and with some procedures, recombination (nuclear genes) as well. This is much more limited for PBT.

The MJN carried out here showed that one haplogroup of *N. olivacea* followed some Andean *N. nasua* haplotypes that were basal. In fact, some *N. nasua*, living at the Colombian and Ecuadorian Andean Cordilleras, were more related with one haplogroup of *Nasuella* than with other haplogroups of *N. nasua.* However, Ruiz-García et al. (2020, 2021) showed the most basal haplogroup to be coatis of the Colombian and Ecuadorian Andean *N. nasua* haplogroup, followed by one haplogroup of *N. olivacea*. Therefore, the mitogenome data set (with few specimens studied, but longer sequences) and the three mt data set (with a greater sample size and more diversified geographical origins, but shorter sequences) did not offer the same conclusion about which of the current coati haplotypes are basal. More Andean coatis (both *N. nasua* and *N. olivacea*) should be analysed with both mitochondrial and nuclear genes to resolve this question. Nevertheless, both mt data sets showed that the origin of the current coatis seem to have originated in the Andean cordilleras from north-western South America (current Colombia and Ecuador). This process could have begun around 13-10 MYA, during the Miocene (Ruiz-García et al. 2021) and from here the ancestors of the current coatis expanded to southern South America and Central America. Support in favour of the origin of the current coatis in north-western South America is the fact that the majority of introgression and hybridization cases were in the territory of Colombia and Ecuador. This result agrees well with the findings of Nigenda-Morales et al. (2019). The two S-DIVA and BBM biogeographic analyses conducted by these authors identified South America as an area of distribution for the most recent common ancestor of *Nasua* and *Bassaricyon*. They estimated the split between the ancestors of *N. nasua* and *N. narica* to have occurred around 6 MYA, which is compatible with that reported by Ruiz-García et al. (2021) and with the results shown here.

We detected intermediate haplotypes between *N. nasua* and *N. narica.* For instance, the genetic distance between a haplogroup of *N. nasua*, in the Colombian and Ecuadorian Amazon and Eastern Colombian Llanos, and the most basal haplogroup of *N. narica* was 7.7% for the mitogenome data set. This value is lower than the genetic distances of different haplogroups of *N. nasua* (for instance, 8.6% between this Colombian and Ecuadorian Amazon and Eastern Colombian Llanos one, and one haplogroup from southern Peru and Bolivia, or 8.9% between this last haplogroup and one haplogroup from the Colombian and Ecuadorian Andes). This result is consistent with colonization from northern South America into Central America. Indeed, Nigenda-Morales et al. (2019) detected asymmetric patterns of colonization, with migration from Panama into northern Central-American populations to be greater than in the opposite direction, which is the reverse of the traditional paleontological viewpoint (Soibelzon & Prevosti 2013).

Finally, the genetic distances of the most differentiated haplogroups of *N. olivacea* in relationship with *N. nasua* and *N. narica* were 12.3-15.3% and 10.4-12.3%, respectively. However, these values were not of the order of 16-18% or higher (Kartavtsev 2011), which is expected among species of well differentiated genera. As such, we suggest there are sufficient reasons to consider that all of the coatis are part of a single genus.

Total agreement in the spatial genetic structure of *N. nasua*, *N. narica* and *N. olivacea*

The results for the Mantel tests and those of the spatial autocorrelation showed similar structures for the three coati taxa studied although the geographical extent of each species was different (for instance, for *N. nasua*, we sampled specimens over a distance of more than 3,500 km, whereas, this distance was around 750 km for *N. olivacea*) as well as the geographical barriers and biomes where the three coati taxa occur are different.

Generally speaking the few spatial genetic studies carried out with Procyonids have detected significant spatial structure. Cullingham et al. (2008b), with *Procyon lotor*, detected that some geographical barriers could enhance significant genetic differences between populations of raccoons in North America. In the Niagara region, two genetically different raccoon populations were identified corresponding to either side of the River Niagara. However, for the St. Lawrence region, spatially congruent clusters were not identified, despite the presence of the intervening St. Lawrence River. Cullingham et al. (2008a) sequenced, for the mt control region, specimens from four putative morphological subspecies of *P. lotor* that occur along the eastern seaboard of North

America through to the central United States. They showed three distinct lineages. One of them was found primarily in Florida, one along the eastern seaboard, and the third predominantly to the west of the River Mississippi. A SAMOVA analysis indicated that different barriers contributed to differentiate these three lineages (river-mountains at the east of the studied area, river-mountains at the west of the studied area, and by regions). However, there was considerable lineage mixing across the eastern seaboard and to the west of the River Mississippi. Rioux Paquette et al. (2014) analysed several microsatellites for raccoons in southern Quebec and they detected that the genetic distance among the raccoon males was strictly a function of geographic distance, while dispersal in raccoon females was significantly reduced by the presence of agricultural fields. Thus, females were more affected by barriers than males, which could agree with that reported here with coatis based on mtDNA. Biedrzycka et al. (2014) examined the microsatellite and mitochondrial diversity of raccoon populations recently introduced in Central Europe (Germany, Poland and Czech Republic). They detected two genetically different groups with isolation-by-distance showing a significant but weak positive relationship between geographic and genetic distance. Thus, procyonids seems to easily develop spatial structuring like we showed here for coatis. Nevertheless, the spatial genetic structures observed in *P. lotor* could not be compared with those shown here for the three coati species because correlograms with the A_y distance were not employed in these studies. The unique result obtained for a Procyonid with the same procedure employed here was the case of the kinkajou, *Potos flavus* (Ruiz-García et al. 2019a).

Diverse microevolutionary processes can differentially affect genomes if they are in some degree different and therefore develop different spatial structuring. In contrast, if genomes are similar they can respond to geography in a similar way (Sokal & Jacquez 1991). Sokal & Wartenberg (1983) and Sokal et al. (1989b) showed, in metasimulations, that stochastic generating processes produced genetic surfaces with characteristics that were a function of parameters such as parent vagility and neighbourhood size. Different simulations with identical parameters generated identical, or very similar, spatial correlograms, including different kinds of migration or selection. We wish to show that the spatial correlogram of *N. olivacea* is significantly more similar to those of *N. nasua* and *N. narica* than to the correlogram of other procyonids of other genera, such as *P. flavus*.

To demonstrate this, we generated a correlogram with the same number of DCs (ten) for the four Procyonid taxa with the size of each DC being as similar as possible. The correlograms were later compared by computing average Manhattan distances (Sneath & Sokal 1973) between pairs of correlograms over the ten DCs constructed. Sokal et al. (1986, 1987, 1989a) demonstrated that spatial correlograms generated by the same microevolutionary forces affecting identical genomes showed Manhattan distances among their correlograms of 0.1-0.2. The Manhattan distances between the correlogram of *P. flavus* and those of *N. nasua*, *N. narica* and *N. olivacea* were 0.354, 0.619 and 0.488, respectively (significantly different to 0.2; Fisher exact test, $P < 0.001$; Everitt 1992). *Potos flavus* also has a significant spatial structure like the three species of coatis, but its spatial structure was higher than that detected in the coatis and its correlogram was significantly different to the correlograms of the three coati taxa. Thus, the microevolutionary processes that affected kinkajous were different to those that affected the coatis. In contrast, the Manhattan distances between *N. olivacea vs*. *N. nasua* and *N. narica* were 0.147 and 0.183, respectively, and they did not differ from 0.2 (Fisher exact test, $P > 0.6$). Hence the three coatis have mitogenomes similar enough to be affected by the same microevolutionary processes in an identical way. In fact, the correlograms of *N. olivacea* and *N. nasua* were more similar (affected more similarly by identical evolutionary processes, 0.147) than the correlograms between *N. nasua* and *N. narica* (affected by less similar evolutionary processes, 0.265). This finding suggests that the reproductive and the migratory behaviours of the coatis are more relevant for this spatial structure than the geographical features in the distribution range of each taxon. Coatis are highly gregarious, forming social groups of up to 20-40 females and associated juveniles. Males are typically solitary and disperse once they reach sexual maturity, with brief contact with the female groups only during the mating period. Females are highly philopatric and their home ranges generally include their birth area (Gompper 1995, 1997, Gompper et al. 1997, 1998, Valenzuela & Ceballos 2000, Hass 2002). This means that female capacity to migrate is low and the high levels of philopatry may lead to pronounced fine-scale genetic structuring

(Ruiz-García 1998, 1999). Although there are some reports that males have moved more than 20 km between years (Lanning 1976), our spatial genetic results suggest that the three coati taxa have limited capacity for dispersion and, therefore, their behaviours are strongly similar because they are not very differentiated taxa, which is more indirect evidence to include *Nasuella* within *Nasua*.

The karyotype of *N. olivacea*

This is the first time that the karyotype of *N. olivacea* (one male, and one female) is reported. Although our banding pattern for *N. olivacea* was not comparable with the banding patterns obtained for the two species of *Nasua*, the chromosome morphology is comparable. As mentioned, the chromosome morphology of the karyotype of *N. olivacea* was un-differentiable from that reported by Wurster & Benirschke (1968) for *N. nasua*. It is composed of 28 metacentric, submetacentric, and subtelocentric autosomic chromosomes, eight acrocentric autosomic chromosomes, one submetacentric X chromosome, and one subtelocentric Y chromosome. In fact, the chromosome morphology of *N. narica*, although highly similar to that of the other coatis, showed some minor differences to that described for *N. olivacea* and *N. nasua.* It has one additional pair of metacentric and submetacentric autosomic chromosomes and one less pair of the acrocentric autosomic chromosomes, as well as a different acrocentric or small submetacentric Y chromosome.

All other Procyonidae genera also have 2n = 38. However, these karyotypes show some differences to the karyotype of the coatis. For example, *Bassaricyon gabii* has an autosomal complement of 28 meta- and submetacentric chromosomes and eight acrocentric chromosomes. One pair of small acrocentric chromosomes in this species has satellites on its short arms. The X chromosome is a medium-sized submetacentric chromosome, similar to that found in *Nasua* and *Nasuella*, but the Y chromosome is a small subacrocentric chromosome different to that of *Nasua* and *Nasuella* (Wurster & Bernirsche 1967, 1968). The North American raccoon (*P. lotor*) has 30 metacentric, submetacentric or subtelocentric chromosomes and six acrocentric or telocentric chromosomes (different numbers than the coatis). The X chromosome is submetacentric and the Y chromosome is submetacentric or subtelocentric. A pair of small subtelocentrics (pair 14) possesses a distinctive satellite on each short arm, similar to the E1 pair of domestic cat (Benirschke et al. 1966, Hsu & Arrighi 1966, Todd et al. 1966), which is not present in the coatis. Finally, the karyotype of *Bassariscus astutus* (ring-tailed cat) has a FN = 68, which is different from the FN = 72 of *Nasua* and *Nasuella*. The autosome chromosomes were 36 submetacentrics and subtelocentrics. Additionally, the karyotype of this species includes a large submetacentric X chromosome and a small acrocentric Y, which is not present in the coatis (Hsu & Arrighi 1966, Wurster-Hill & Gray 1975). Although the karyotype of the Procyonidae is conservative, the differences observed among the different genera and the close similarity between *N. nasua* and *N. olivacea* support one unique coati genus, *Nasua*, rather than two well-differentiated genera. One of the *Nasua* species (*N. nasua*) was more related to *Nasuella* than to the other species of *Nasua* (*N. narica*).

Taking into consideration the mitochondrial and the karyotype results presented here, it seems clear that all coatis belong to a unique genus: *Nasua*. Nuclear genes, immunological, reproductive, and ethological studies should be conducted to further investigate the status of *Nasuella* as a "true" genus.

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Supplementary online material

Table S1. Haplotypes, number of samples by species and geographical localities of 205 coatis (*Nasua nasua, Nasua narica* and *Nasuella olivacea*) sequenced for their mitogenomes. IVM = Mammal Museum of the Instituto von Humboldt (https://www.ivb.cz/wp-content/uploads/JVB-vol.-71-2022-Ruiz-Garcia-et-al.-Table-S1.pdf).

Fig. S1. Bayesian Inference tree based in complete mitogenomes with 179 haplotypes found from three species of coatis (*Nasua nasua*, *Nasua narica* and *Nasuella olivacea*) sampled in Latin America. Nodes are labelled with "a posteriori" probabilities. H144 corresponded to a specimen "a priori" classified as *N. nasua* that might represent the first confirmed record of *N. olivacea* in Peru (the River Urubamba, Cuzco) (https://www.ivb.cz/wp-content/uploads/JVB-vol.-71-2022-Ruiz-Garcia-et-al.-Fig.-S1.pdf).

Fig. S2. Ten different phylogenetic trees obtained with three mitochondrial genes (*ND5*, Cyt*b*, and *D-loop*) to analyse the influence of outgroups on the relationships among *Nasuella olivacea*, *Nasua nasua* and *Nasua narica*. ML = Maximum Likelihood; NJ = Neighbour-Joining. A) ML tree with only *Bassarycion neblina* as the outgroup; B) ML tree with all the *Bassarycion* species analysed as the outgroup; C) ML tree with *Procyon cancrivorus* as the outgroup; D) ML tree with *P. cancrivorus* + all the species of *Bassarycion* analysed as the outgroup; E) ML tree without an outgroup; F) NJ tree with only *B. neblina* as the outgroup; G) NJ tree with all the *Bassarycion* species analysed as an outgroup; H) NJ tree with *P. cancrivorus* as the outgroup; I) NJ tree with *P. cancrivorus* + all the species of *Bassarycion* analysed as an outgroup; J) NJ tree without an outgroup (https://www.ivb.cz/wp-content/uploads/JVB-vol.-71-2022-Ruiz-Garcia-et-al.-Fig.-S2.pdf).