Chapter 21
Conservation Status of the Nancy Ma's Owl Monkey (*Aotus nancymaae*, Hershkovitz, 1983) on the Colombian-Peruvian Amazon Border

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Abstract This chapter presents an overview of the general characteristics of the Nancy Ma's owl monkey (*Aotus nancymaae*) and a case study where we explore the genetic composition of wild populations in northern Peru and its genetic representation in an ex situ population. The species has historically been heavily exploited for biomedical research. On the Colombian-Peruvian border of the Amazon, trafficking, post-experimentation releases into the wild, and deforestation present growing threats for this vulnerable species. We provide evidence of the heterogeneous geographical origin of the ex situ population and relatively high diversity in wild populations. Unexpectedly, we found no evidence of the Amazon River as a barrier to dispersal, based on the scarce genetic differentiation among populations on opposite riverbanks. We conclude recommending binational collaboration concerning data sharing, population health assessment, and adherence to recent policies to improve the conservation of the most threatened owl monkey species in the Amazon region.

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21.1 Introduction

The Nancy Ma's owl monkey *(Aotus nancymaae*) is distributed in the Amazonian region of Peru, Brazil, and Colombia where it is mainly found in flooded forests or *varzea* (Aquino and Encarnación 1994; Shanee et al. 2023b this volume). Despite the wide distribution range of this species, its nocturnal habits in the high canopies of the dense Amazonian forest limit the study of wild populations (Fernandez-Duque et al. 2023 this volume) and assessment of their conservation status (Aquino and Encarnación 1986b). Likewise, its social behavior in natural habitats is poorly understood (Wolovich and Evans 2007), even when the species has been extensively used in biomedical research (Collins et al. 2006). However, the existing evidence hints that *A. nancymaae* shares similar ecological and social behavioral traits to those described in other *Aotus* taxa. Two of the most prominent threats faced by this species are habitat loss and the trade for biomedical research. The Nancy Ma's owl monkey has the highest demand, among owl monkey species, for research purposes; this is in part because of its use as an animal model for the study of malaria (Williams et al. 2005).

We provide an overview of the available information about the Nancy Ma's owl monkey. We present first a brief description of its distribution range, population biology and ecology, as well as an explanation of the main threats to the sustainability of wild populations, describing in some detail the trade for biomedical research in the Brazil-Colombia-Peru border region. Following, we present a case study providing preliminary genetic information of the populations in northern Peru; these new data provide support for the distribution of the species in the southern bank of the Amazon River where Peru borders Colombia. We conclude with recommendations for establishing a binational conservation strategy to improve the conservation of this unique and highly threatened owl monkey species.

21.1.1 General Characteristics of Aotus nancymaae

The taxonomic status of the genus *Aotus* has been a matter of academic dispute due to the wide number of different karyotypes reported (Defler and Bueno 2007; Fernandez-Duque et al. 2023a this volume; Rogers et al. 2023 this volume; Ruiz-Herrera et al. 2005). Until 1983, all owl monkeys were considered a monotypic genus with the single species *A. trivirgatus*; then Hershkovitz divided the genus into two groups: the red-necked group distributed mainly south of the Amazon-Solimões River, and the gray-necked species group found north of the Amazon River (Hershkovitz 1983; Hernández-Camacho and Defler 1989). Hershkovitz described

Aotus nancymaae as a red-necked species based on a distinct karyotype ($2n = 54$), its resistance to infection with several strains of *Plasmodium*, and patterns of pelage color. With the advent of molecular tools, the systematic relationships among species were tested with mitochondrial genetic data and show the lack of reciprocal monophyly of the gray and red phenotypes but also demonstrate the monophyly of *A. nancymaae* (Ashley and Vaughn 1995; Babb et al. 2011; Di Fiore et al. 2023 this volume; Plautz et al. 2009; Menezes et al. 2010; Ruiz-García et al. 2011). *A. nancymaae* is characterized by a throat with at least the anterior portion of the sides mostly or completely agouti gray to orange/brown, along with gray to brown agouti of the sides of the body and no interscapular whorl. The upper surface of the portion near the tail is orange with a black stripe, and the ventral surface is mostly light yellow to orange/brown (Defler 2004, 2010; Hernández-Camacho and Defler 1989; Hershkovitz 1983).

21.1.2 Distribution and Ecology

In the Brazilian-Peruvian Amazon, *A. nancymaae*, also known as the Peruvian red-necked owl monkey, occurs from western Brazil and northeastern Peru with a geographical distribution ranging from the Amazon-Solimões River in the northern Peruvian department of Loreto and the Jandiatuba River in Brazil (Shanee et al. 2023b this volume). Its southern limit is in the headwaters of the Jutai River, stretching west in a line to cross the Javari River at the level of the headwaters of the Tapiche River, across the Ucayali basin to the upper Marañon River, reaching an enclave between the Tigre and Pastaza rivers (Aquino and Encarnación 1988, 1994; Shanee et al. 2023b this volume, Hershkovitz 1983; Groves 2005). The western boundary of the species' distribution in Peru is the Andean foothills in San Martin up to ~1000 m above sea level (Shanee et al. 2015). *A. nancymaae* is the only species that straddles the ranges of the gray-necked and red-necked taxa. Sympatric populations of Nancy Ma's owl monkey and Spix's owl monkey (*Aotus vociferans*) from the gray-necked group have been described in Brazil, Colombia, and Peru with cytogenetic evidence of no hybridization between them (Pieczarka et al. 1992). However, the specific location of sympatry events is ambiguous in the case of Isla Amaraza, since the authors state that it is located in Colombia, but it is actually in Brazilian territory. Hybridization between *A. nancymaae* and *Aotus griseimembra* has been observed in captivity (Kumamoto and Houck 2001).

Until recently, the Amazon River at the border between Peru and Colombia was considered to act as a biogeographical barrier between *A. nancymaae* (south) and *A. vociferans* (north) (Defler 2004). In the 1970s, Hershkovitz and Hernández-Camacho examined specimens of *A. nancymaae* in captivity in Leticia, Colombia, as part of the collection of a local primate laboratory (Defler 1994), which were deemed most likely purchased and brought to Colombia from the southern side of the Amazon River in Peru and/or Brazil (Defler 2010). Likewise, a cytogenetic study on Colombian owl monkeys confirming the chromosome number and

taxonomic status of *A. nancymaae* used specimens that came from the same laboratory in Leticia (Torres et al. 1988). Similarly, captive specimens that are held in the Bioterium of the Central National Health Institute whose origin was Leticia also came from the same biomedical facility and were thought to have been imported to Colombia (Defler 1994, 2004).

The distribution of *A. nancymaae* was uncertain until the relatively recent description of a population endemic to Colombia in the Loretoyacu River basin, north of the Amazon River (Bloor et al. 2012; Maldonado and Peck 2014; Ruiz-García et al. 2013), followed by a report in Siete de Agosto and Atacuari and a number of other sites on the border with Peru along the Amazon River (Hernández and Díaz 2011; Roncancio-Duque et al. 2019). Although the species may be present all along the Amazon River, the distribution in Colombia has been confirmed by georeferenced visual inspections within an area of 532 km^2 (Henao-Díaz et al. 2020). Álvarez-Gonçalvez et al. (2015) predicted the geographical distribution of *A. nancymaae* combining three bioclimatic models that showed suitable areas for this species in a large region in northern Peru and west of Brazil, near the tri-border area of Peru-Brazil-Colombia.

The distribution area of *A. nancymaae* in Colombia coincides with those places where the species has been released for over 30 years after being subjected to malaria vaccine trials, suggesting that local populations were established by exotic animals imported from Peru (Maldonado and Peck 2014). However, genetic data have revealed the presence of two highly differentiated mitochondrial lineages in Colombia, presumably originated through the vicariant effect of the Amazon River (Bloor et al. 2012). These data indicate that there could be genetic lineages native to Colombian populations affected by recent hybridization with others south of the Amazon River. Despite the confirmation of *A. nancymaae* in Colombia, further population studies are necessary to reconstruct the phylogeographic history of this species, identify the role of the Amazon River in this process, and measure the impact of traffic on the composition and structure of current populations. Meanwhile, there is lack of agreement in the scientific community about *A. nancymaae* as a primate species native to Colombia (Guzmán-Caro et al. 2018).

Population assessments of the species have been conducted mainly in Peru, and there is a lack of information for Brazil. Aquino and Encarnación (1986a, 1988) have estimated densities of 24 to 46 individuals/km^2 in northeastern Peru. Maldonado and Peck (2014) reported densities for northwestern Peru in the localities of Chineria, Yahuma, and Vista Alegre of 3 to 24 ind/km^2. In southwestern Colombia, Roncancio-Duque et al. (2019) present density estimates of 24 ind/km^2 in Naranjales, while Hernández and Díaz (2011) provide population densities in San Juan de Atacuari and Siete de Agosto Indigenous communities with 10 ind/km^2, where animals have been extracted for malaria research.

Although the Nancy Ma's owl monkeys are found in flooded and unflooded Amazonian forests, higher population densities have been registered in flooded forests possibly due to the high productivity of food resources and availability of tree holes and epiphytes which are their preferred sleeping and refuge sites (Aquino and Encarnación 1994). Lowland swamp, pre-montane forests up to ~1000 m.a.s.l.,

and inundated dense vine tangles are also used by the species (Aquino and Encarnación 1994; Roncancio-Duque et al. 2019). In the Tahuayo River (Peru), 21% of *A. nancymaae* sleeping sites were in the shrub stratum of the understory, 64% in the lower, and 14% in the middle. No sleeping sites were found in the upper story or in emergent trees (Aquino and Encarnación 1986a).

Nancy Ma's owl monkeys live in small groups composed of an adult pair and offspring of different ages. Group size ranges from two individuals in hunting areas (Maldonado and Peck 2014) to three to four individuals in northwestern Peru and southwestern Colombia; solitary animals have also been observed (Aquino and Encarnación 1988; Hernández and Díaz 2011; Roncancio-Duque et al. 2019). They are pair-living and socially monogamous with extensive male care of offspring (Fernandez-Duque et al. 2020; García de la Chica et al. 2023 this volume; Wolovich and Evans 2007). A peak in births has been reported between December and March, during the rainy season, and age of first reproduction is approximately 40 months. Interbirth intervals in captive females are 9–11 months (Aquino et al. 1990; Spence-Aizenberg et al. 2023 this volume). The social behavior of *A. nancymaae* has been the focus of substantial research on captive individuals, including research on communication, pair bonding, and biparental care (Evans and Wolovich 2023 this volume) in captivity, and it has been reported that they possess a unique composite of communicative behaviors (see complete description in Evans and Wolovich 2023 this volume and Spence-Aizenberg et al. 2023 this volume).

21.1.3 Threats

21.1.3.1 Hunting

Hunting for consumption has not been historically a serious threat for the species; locals report that owl monkeys are not widely consumed owing to the strong and disagreeable odor and taste from their subcaudal gland (Aquino et al. 2009; Maldonado and Waters 2020). However, more recently, Indigenous and caboclos communities living in deforested regions are hunting them given the reduction in the abundance of bigger preys. For example, Maldonado and Waters (2020) reported the consumption of 22 individuals in Mocagua and San Martin, Colombia. On the other hand, Nancy Ma's owl monkeys are commonly kept as pets in Indigenous communities.

21.1.3.2 Habitat Loss

The Amazon rainforest is suffering devastating levels of deforestation from ongoing pressures that vary in type and magnitude for each country. The Amazon regions in Colombia, Brazil, and Peru, the range countries of *A. nancymaae*, are threatened by extractive industries, illegal crops, industrial agriculture, and infrastructure projects

(Armenteras et al. 2019; Etter et al. 2020; Fearnside 2017; Vilela et al. 2020). Expansion of agricultural activities and road development undertaken across the region, mainly in Brazil and Peru, are major drivers of habitat loss and fragmentation. Forest fires, overhunting, and overall environmental degradation often create irreversible impacts on the ecosystems inhabited by arboreal mammals, such as owl monkeys, that rely on primary and secondary forests (Armenteras et al. 2013; Laurance et al. 2014). The additive effect of these pressures, which increases the risks of disease emergence and spillover (Everard et al. 2020), could have devastating consequences in Colombia for a habitat-restricted species such as *A. nancymaae*. As mentioned above, with only 532 km^2 of potential range of distribution in the southernmost part of the Amazonas department, the species could be highly susceptible to local extinction (Henao-Díaz et al. 2020). This small region has been classified as Endangered by the latest Threats and Risks Assessment of Colombian Ecosystems due to the increasing threats to its long-term viability (Etter et al. 2020).

21.1.3.3 Nancy Ma's Owl Monkeys as a Model for Biomedical Research

Owl monkey species have been developed as suitable models for biological, immunologic, and chemotherapeutic research (Collins et al. 2006; Spence-Aizenberg et al. 2023 this volume). From early on, research testing potential malaria vaccines and antimalarial drugs sparked a high demand by the biomedical industry (Garcia de la Chica et al. 2021; Herrera et al. 2002; Williams et al. 2005). The Nancy Ma's owl monkey can be productively infected by multiple strains of *Plasmodium falciparum* and *Plasmodium vivax*, the most virulent and widespread species of the human malaria parasites. The availability and susceptibility to infection in intact spleens and splenectomized test subjects have made *A. nancymaae* the model of choice for malaria research (Williams et al. 2005). Among other research developments based on use of this taxon, *A. nancymaae* provides a robust transmission model which has made it a system of choice for the testing of anti-sporozoite and liver stage vaccines against *P. falciparum* (Collins et al. 2006, Moreno-Pérez et al. 2017). Additionally, the species has been proven valuable as their differences, relative to other nonhuman primates and humans, in adjusting the immune parameters in studies testing the efficacy of potential therapeutic agents or vaccine candidates (Nehete et al. 2017). It has also been used in research on viral pathogenesis, vaccine development for dengue and hepatitis, and HIV-1 research (Nehete et al. 2017; Schiavetta et al. 2003; Williams et al. 2005).

Overall, because owl monkeys are susceptible to multiple viral infections that also affect humans and they are smaller, less expensive, and less aggressive than haplorhine primates (e.g., baboons, macaques), they have become the optimal model for a variety of research purposes (Schiavetta et al. 2003; Menezes et al. 2010; Smith 2012). Since the discovery that certain species of owl monkeys make excellent biological models for vaccine development, *A. nancymaae* has been subjected to legal and illegal trade across range countries at a local, regional, and

global scale with minimal considerations for impacts on wild populations (Maldonado et al. 2009; Shanee et al. 2023a this volume). A recent review of trade in owl monkeys as reported in the CITES database for the period 1975–2014 documents exports of live and dead individuals, as well as specimens (e.g., fecal or blood samples) (Svensson et al. 2016). *A. nancymaae* was the most commercial and scientific traded species, representing 40% of the trade. Although national bans in Colombia and Peru and CITES regulations have restricted the export of primates over the last few decades with the biomedical industry largely transitioning to captive-bred primate models, wild-caught *A. nancymaae* are still widely traded for biomedical research in Colombia (Defler 2010; Maldonado et al. 2009; Ruiz-García et al. 2013). An updated assessment of the legal and illegal trade in owl monkeys shows this trade remains a threat to the conservation of this species (Shanee et al. 2023a this volume). Trade estimates are not comprehensive since in-country captures for domestic trade and research purposes are not reported to CITES and national authorities are not informed of confiscations by local authorities (Maldonado 2011; Shanee et al. 2023a this volume).

Beginning in the early 1980s, owl monkeys were continuously harvested from the tri-border region to supply the Fundación Instituto de Inmunología de Colombia (FIDIC), a biomedical research facility in Leticia, Amazonas, Colombia, which was working on the development of a synthetic malaria vaccine. Permits issued by Corpoamazonia (Corporación para el Desarrollo Sostenible del Sur de la Amazonía), the governmental environmental authority in the Colombian Amazon, approved the annual capture of 800 *A. vociferans* in Colombian Indigenous territory, calculating quotas based on FIDIC's requests, rather than on a systematic population assessment (Maldonado and Lafon 2017; Roncancio-Duque et al. 2019). Although these permits only authorized the capture of *A. vociferans*, both *A. nancymaae* and *A. nigriceps* were observed in FIDIC's laboratory and reported in their research publications (Baquero et al. 2006; López et al. 2014; Suárez et al. 2011). Furthermore, legal licenses were issued to registered Indigenous collectors in Colombia to capture *A. vociferans* from their territories, but interviews with local trappers and official lab records showed that other owl monkey species, including *A. nancymaae*, continue to be captured in Peru and Brazil and illegally transported to Leticia (Maldonado et al. 2009; Ruiz-García et al. 2013). This international trafficking transpired under the supervision of Corpoamazonia, even though it contravened permits by trapping an unauthorized species (*A. nancymaae*) and by exceeding its annual extraction quota (Gil-Botero 2013; Maldonado and Lafon 2017). In 2016, Corpoamazonia included the species *A. nancymaae* in FIDIC's permits (Resolution No.0993, 2016), and in April 2020, they released Resolution 0366 allowing the trapping of 1200 owl monkeys (*A. nancymaae* and *A. vociferans*) for a 3-year period (Corpoamazonia 2020). The Ministry of Environment and Sustainable Development of Colombia (MADS) in collaboration with the National University signed Agreement 518 from 2018 and carried out a national assessment using the IUCN Red List criteria for *A. nancymaae* classifying the species as Vulnerable.

21.1.3.4 Impacts of Released Animals

Additional threats to the populations of owl monkeys at the tri-border area come from the release of primates that outlive biomedical experiments. FIDIC released 4041 owl monkeys in Leticia between 2006 and 2012 and at least 815 in 2017 (Maldonado and Lafon 2017; Roncancio-Duque et al. 2019; Ruiz-García et al. 2013). Only 10% of the released animals received a veterinarian checkup (Corpoamazonia 2017); thus, the zoonotic risks on resident populations of releasing animals after experimentation are unknown (Maldonado and Peck 2014). The ecological impacts caused by release of, for example, a colony of 278 animals subjected to malarial research on the resident population have not been assessed by the environmental authorities (Corpoamazonia 2012). As territorial species living in groups of no more than five individuals, there are likely several impacts on competition for food, territory, behavior, and health (Aquino and Encarnación 1994; Fernandez-Duque 2011; Maldonado and Peck 2014).

Animals trafficked across the borders, and laundered through hunting licenses and trapping permits issued for Colombia, are released in Colombia or returned to Peru without the supervision of Peruvian authorities. Unregulated releasing of trafficked animals may have resulted in the introduction of *A. nancymaae* north of the Amazon River (Bloor et al. 2012; Maldonado and Peck 2014), and this is possibly causing the displacement, or hybridization, of resident populations of this and other owl monkey species. Evidence suggesting that *A. nancymaae* harvested from neighboring countries for biological experimentation have been released in Colombia outside their historical distributional area is difficult to prove without genetic testing (Roncancio-Duque et al. 2019).

21.1.3.5 Conservation Litigation for *A. nancymaae*

In order to protect owl monkey populations and ecosystems, civil society took legal actions against authorities and the biomedical facility. The goal was to prove that the continuous extraction of *Aotus* spp. for malaria research conducted by FIDIC, as well as the negligence of authorities in complying with Colombian legislation, had inflicted environmental damage on owl monkey populations and their habitat. As a result, permits were revoked for 4 years; and authorities were ordered to abide by environmental protection laws, including Law 1333, 2009, which describes procedures for sanctioning activities that violate Colombian environmental regulations (Gil-Botero 2013).

In response to the legal conflict, Corpoamazonia and the MADS conducted a demographic and genetic study in the territories where animals were trapped for biomedical research in collaboration with two CITES scientific authorities in Colombia – the SINCHI Amazonian Scientific Research Institute (Leticia, Amazonas) and the Genetic Institute of the Universidad Nacional de Colombia

(IGUN, Bogotá, Colombia). The study, which covered 4 of the 20 FIDIC's capture and release sites and a 5th control site, where animals had been captured, but not released (Bloor et al. 2012; Roncancio-Duque et al. 2019), confirmed the occurrence of a historical population of *A. nancymaae* in the western part of the Colombian Amazon, and a second population probably established after recent introductions to Colombia.

Aotus vociferans was found solely at the control site, supporting the hypothesis that this species is possibly being displaced by the continuous release of *A. nancymaae* into their native range (Maldonado and Peck 2014). Likewise, studies conducted over the past 13 years have not reported the presence of *A. vociferans* in the southern Colombian Amazon (Hernandez and Díaz 2011). The study recommended that FIDIC cease the trapping and releasing of owl monkeys in the western Amazonian border between Colombia and Peru until further research could determine the genetic origins of this species in Colombia. Nevertheless, FIDIC, under the supervision of Corpoamazonia, continued releasing animals in this area, including the control site, until as recently as 2019 (Corpoamazonia 2019).

Despite upgrading the IUCN (International Union for the Conservation of Nature) Red List global category for *A. nancymaae* from Least Concern to Vulnerable (Maldonado et al. 2017), Corpoamazonia has continued granting trapping permits for this species until 2022 (Corpoamazonia 2020) without prior population assessments to demine extraction quotas, impacts of released animals on wild populations, or zoonotic risks. There are currently in progress penal and disciplinary investigations requested by the Environmental Comptroller to the Attorney General of Colombia against MADS and Corpoamazonia (Shanee et al. 2023a this volume).

21.2 Current Genetic Structure of Wild and Ex Situ Populations

Most research on *Aotus* species have focused on resolving the phylogeny of the genus rather than on the analyses at the status of current populations. Given the history of captures and releases of *A. nancymaae* in the Colombian-Peruvian border for purposes of biomedical research, we used genetic data from the mitochondrial genome to explore the population genetic composition of this species in the northern Peruvian department of Loreto. We also studied the genetic representation of this species in the ex situ population held at the Veterinary Institute for Tropical and Altitude Research – IVITA, Peru.

21.2.1 Methods

21.2.1.1 Study Sites

After consultations and approval by local communities, we carried out the study in the territories of six Indigenous communities in the Ramón Castilla and Yavarí provinces of the Loreto department, Peru (Table 21.1 and Fig. 21.1). We chose to sample from these communities because of their history of involvement in the trapping of owl monkeys for malaria research and their distance from one another along the northern and southern banks of the Amazon River at the border with Colombia.

This region has high levels of anthropogenic habitat fragmentation including deforestation caused by more than 50 years of illicit crop cultivation and large-scale timber extraction through permits granted by the environmental authority (Maldonado 2011). Forest degradation is the highest in Yahuma, Nuevo Oriente, and Isla El Tigre. However, despite evident human influence, many parts of the forest in Vista Alegre and Uranias remain unaltered, displaying an unbroken canopy (25–35 m height) and several layers of understory. The sites selected for the study have an average temperature of 29 °C and an average altitude of 78 m.a.s.l. None of the sites are under government protection, and all are located in seasonal flooded forests. During the peak of the rainy season (February to May), all sites are completely flooded.

Table 21.1 Characteristics of *Aotus nancymaae* groups captured from wild populations in Loreto, Peru

Localities	Geographical coordinates	Number of groups	Group size (mean)	Total individuals	Number captured/ capture rate
Chineria	S4° 10.121’	4	2	8	5
	W70° 02.607’				(62%)
Yahuma	S4° 05.993’	2	3 (3.5)	7	5
	W70° 07.594’				(71%)
Vista Alegre	S3° 52.816’	4	4 (3.8)	15	14
	W 70° 17.420’				(93%)
Nuevo Oriente	S3° 52.359	3	4	12	9
	W70° 37.894				(75%)
Uranias	S3° 48.073	4	2	8	7
	W70° 42.877				(88%)
Isla El Tigre	S3° 50.743	1	2	2	1
	W70° 37.830				(50%)

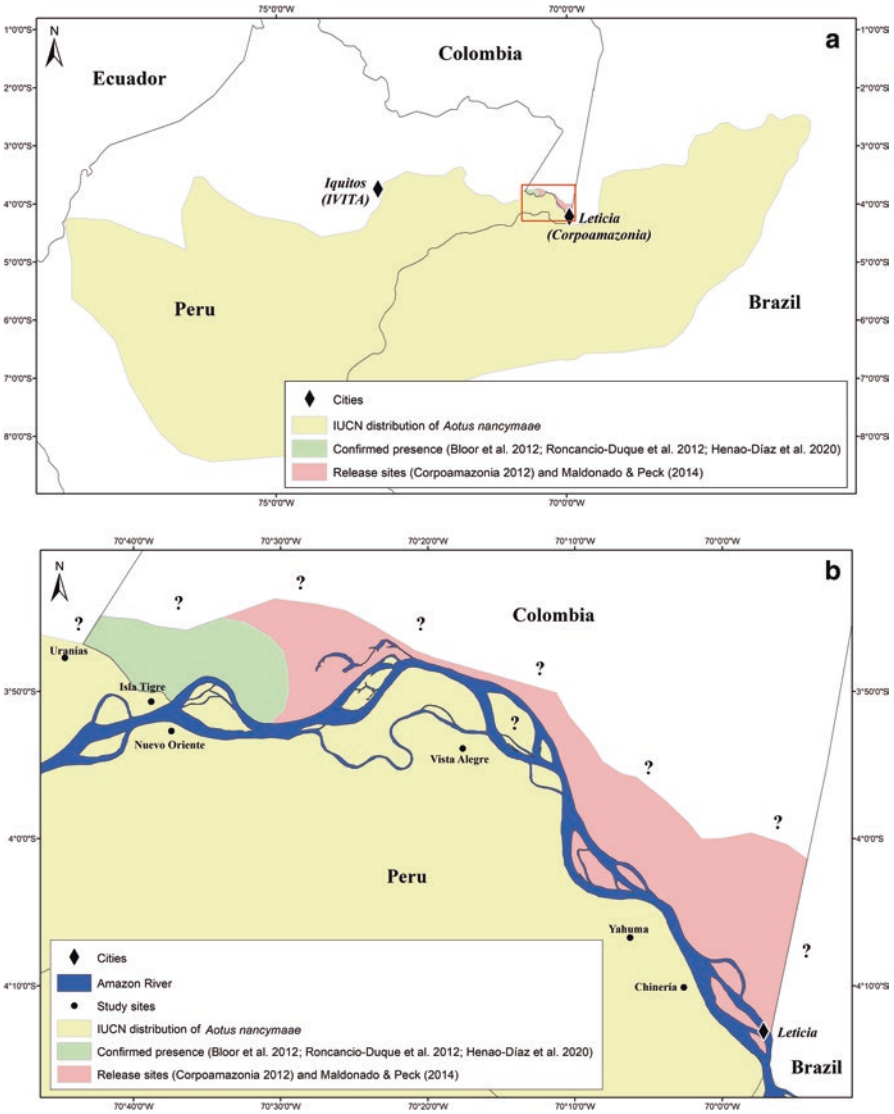

Fig. 21.1 Geographic distribution of *Aotus nancymaae* based on research available to date and study site location. The first map shows the distribution recognized by the International Union for the Conservation of Nature (IUCN); the distribution of *A. nancymaae* on the northern riverbank in Colombia confirmed by Bloor et al. (2012), Roncancio (2012), and Henao-Díaz et al. (2020); and the predicted distribution where *A. nancymaae* have been released post-experimentation by FIDIC according to Corpoamazonia (2012) and Maldonado and Peck (2014). Diamonds denote the cities where samples for the study were taken, from captive primates held at the Veterinary Institute for Tropical and Altitude Research (IVITA) in Iquitos, Peru, and from wild populations near Leticia, Colombia. The rectangle indicates the location of the study site. The second map shows a closer look at the study site including the six localities indicated by the dots along the Amazon River where wild *Aotus nancymaae* were captured: Chineria, Yahuma, Vista Alegre, Nuevo Oriente, Uranias, and Isla del Tigre. This figure also shows the range of confirmed presence of *A. nancymaae* in Colombia and the estimated area where owl monkeys have been translocated. The true distribution may extend beyond this area, but information is currently lacking as indicated by the question marks

21.2.2 Capture Methods

This research is part of a larger study aimed at analyzing the health (e.g., viral agents) and genetic characteristics of wild *A. nancymaae* populations. Thus, we were interested in the collection of blood, fecal, and saliva samples. Even though a noninvasive sampling of feces is possible and advised to minimize stress, live captures were necessary for genetic and parasitological screenings of blood and fecal samples. These methods were also necessary to ensure that collected samples correspond to different individuals, which is not entirely feasible with opportunistic fecal sampling, and enable a reliable estimation of genetic parameters at the group and population levels.

Local trappers stated that owl monkeys are not a species that could be captured with traps, while tamarins and squirrel monkeys are easily caught in this way. This strategy has been proven unsuccessful and time-consuming also elsewhere due to difficulties in attracting owl monkeys to baited traps (Fernandez-Duque and Rotundo 2003; Fernandez-Duque et al. 2023 this volume). From 2009 to 2010, we placed 18 traps in different nesting trees with daily monitoring. In agreement with previous experience of locals and other researchers, our previous attempts to use baited traps had failed. In addition, our local coinvestigators expressed that, considering the illegal crops that are grown in the area, using rifles might create security problems for our team. Thus, we captured free-ranging owl monkeys by adapting local methods common to the area, similar to those described in Aquino and Encarnación (1988), Maldonado (2011), and Roncancio-Duque et al. (2019).

We carried out captures during 63 days of fieldwork throughout the rainy season (February 10 to March 7) and during the dry season (August 14 to September 19) of 2018. The fieldwork team was composed of two Peruvian wildlife veterinarians, two biologists, and five to seven Indigenous trappers with over 20 years of trapping experience.

To locate a group, collectors went to the forest at dawn (6.00–7.00) and dusk (18.00–19.00) to scan for owl monkey activity. The following day, the team returned to the trapping site to record characteristics of the nest, nest tree species, and geographic information of the field site including GPS coordinates and altitude. At each trapping site, we set up a waterproof field laboratory (3.5 × 3.5 m and 4 m height), with mosquito netting and plastic roof where the veterinarians could take samples from the captured individuals and monitor their recovery from anesthesia.

Depending on the location of the nest in the tree, collectors used one of two trapping techniques. If the nest was in a hollowed tree trunk, a collector climbed the tree to block the opening with branches, or with a synthetic sack, to prevent monkeys from using it as an exit. If there were no other holes in the trunk, then a second one was made with a machete. Collectors shouted and banged on the tree trunk to startle the monkeys, forcing them to pass through the unblocked hole to escape, where a collector waited to catch them.

The second technique was employed if the nest was made in the tree branches and tangling vines of the understory (Aquino and Encarnación 1994). These captures

required that approximately 10 m^2 of trees be cleared around the nesting tree, leaving only a bridge of trees leading away from the nest as a means of escape. This technique was employed in Vista Alegre on two occasions, and alternative nests used by the two groups were located to release animals after sampling. Local coinvestigators and veterinarians reforested the cleared areas, but follow-up on the saplings was only performed in Vista Alegre and Chineria, where we work on a monthly basis. Field team members recorded the local vernacular names of the felled tree species, and they measured the diameter at breast height (DBH). They also measured the size of the cleared area along two perpendicular diameters. A double nylon fishing net approximately 50 m long was placed around the perimeter of the deforested area. Collectors were provided with harnesses and security ropes. Captures were carried out during the daylight hours (8.30–16.00 h). Captured monkeys were placed in a cloth drawstring sack and transported to the nearby makeshift laboratory where animals were then transferred to individual kennels to await sampling collection.

21.2.3 Animal Handling and Sampling

Out of 41 animals captured, we anesthetized 35 juveniles, subadults, and adults using ketamine hydrochloride administered by the veterinarian team (10 mg/kg). Age categories were determined by the amount and size of wear on the canine teeth and pigmentation of the externa genitalia (Fernandez-Duque and Rotundo 2003), as well as average body mass and size as reported by Encarnación and Aquino (1986b) (adults: 530–774 mm, 550–950 gr; subadults: 574–651 mm, 425–825 gr; juveniles: 490–609 mm, 450–575 gr; infants: 298–525 mm, 100–375 gr). Prior to sample collection, we performed a full clinical assessment, including a physical evaluation and measurement of physiological parameters; and we searched for clinical signs or visible lesions (Fernandez-Duque and Rotundo 2003). The life history characterization of individuals included age class, sex, and reproductive status (Aquino and Encarnación 1994; Setchell and Curtis 2003). Following Aquino and Encarnación (1986b), age and sex qualitative data were complemented by the description, measurements, and photographs of reproductive characters including vulva/testes, external scent glands, nipples, canines, teeth and gums, and dental casts. Additional full-body photographs and close-ups were taken for phenotypic species identification (Ancrenaz et al. 2003; Setchell and Curtis 2003).

A maximum amount of 0.5 ml of blood was collected from the femoral vein and stored in vials with absolute ethanol at ambient temperature. Similarly, around 20 root hairs were plucked from the animal's dorsoventral flank and stored in dry paper envelopes and Ziploc® bags at ambient temperature. After anesthesia and processing, each individual was placed in a kennel and allowed to recover for a minimum of 1 hour or until the sampling process was complete for all group members before release at the capture site. To prevent recapturing the same animals, we cut hair in the end of the tails to mark them. Kennels were covered all the time

with a blanket to decrease stress and reduce visual stimulation during recovery (Fernandez-Duque and Rotundo 2003).

In addition to the captures of wild individuals, we collected blood and hair samples from 34 captive-born individuals at the IVITA Center for Reproduction and Conservation of Nonhuman Primates (Iquitos, capital of Loreto department, Peru), following the same procedures described above.

21.2.4 DNA Extraction and Sequencing

Genomic DNA was extracted from the blood and hair using the DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands) following the standard protocol. We amplified two overlapped fragments (780 and 1230 bp) of the cytochrome oxidase I (cox1) gene for a total of 1320 bp and 690 bp of the D-loop using primers shown in Table 21.2. Each PCR reaction contained 1X buffer, 0.2 μM dNTPs, 2.0–2.5 mM $MgCl_2$, 0.2 μM of each primer, 0.5 U *Taq* polymerase (Qiagen, Venlo, Netherlands), and 1 μL DNA in a final volume of 15 μl. The amplification protocol consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60–63 °C for 45 s, and 72 °C for 1 min, and a final extension step of 72 °C for 10 min. Amplification was checked by 2% agarose gel electrophoresis and then purified with a mix of 0.5 U exonuclease I and 0.25 U alkaline phosphatase (New England Biolabs, Ipswich, MA, USA) in a final volume of 30 μL incubated at 37 °C for 30 min. The PCR products were then sequenced through a standard Sanger method in an ABI 3730xl system outsourcing a commercial laboratory.

The sequences were read, edited, and aligned with Geneious software v.6.1.6 (Biomatters Ltd., Auckland, New Zealand). These sequences were also aligned with the reference mitochondrial genomes of *Aotus lemurinus*, *A. vociferans*, *A. nancymaae*, *A. azarae azarae*, *A. azarae boliviensis*, *A. infulatus* (2), *A. griseimembra*, *A. trivirgatus*, and *A. nigriceps* (GenBank accession codes FJ785421, HQ005482, JN161100, JN161099, HQ005472, KC592390, HQ005476, HQ005484, AY250707, HQ005478).

Table 21.2 Primers used to amplify and sequence two mitochondrial regions (D-loop and Cytochrome Oxidase-I)

Primer name	Locus	Primer sequence (5′ – 3′)
Aot-15375F	D-loop	CCATCAACACCCAAAGCTGAAA
Aot-1668R		CCGGGTTTGGTCGAGCTA
Aot-5370F	CO-I	CAGCCATTTTACCTCTCSTACCTA
Aot-6140R		TCCAAAGCCCGGTARAATAAGGAT
Aot-5780F		TGAACAGTYTATCCACCCTTAGCA
Aot-6990R		GTCATAGGGTCTATGGAATTGGCT

21.2.5 Genetic Analyses

Nucleotide and haplotype diversity were calculated in DnaSP v6 (Librado and Rozas 2009). We constructed a median joining haplotype network for the mitochondrial D-loop and the cox-1 gene using PopArt (Bandelt et al. 1999). Pairwise genetic distances K3-P (Kimura 3-parameters) were calculated in MEGA7 using bootstrapping with 1000 replications (Kumar et al. 2016).

Ethical Note

This research complies with Peruvian legislation and was authorized by the National Forest and Wildlife Service (SERFOR) through General Directorate Resolutions No. 318-2015-SERFOR-DGGSPFFS and No. 050-2018-MINAGRI-SERFOR-DGGSPFFS. This study also adhered to the International Primatological Society International Guidelines for the Acquisition Care and Breeding of Nonhuman Primates (Cann et al. 2007). All animal procedures at IVITA followed the institutional guidelines of Universidad Nacional Mayor de San Marcos on animal care and use (Gozalo et al. 1994).

21.3 Results

We captured 41 free-ranging owl monkeys in 6 localities (Table 21.1). More than half of them were classified as adults (59%, $n = 24$), followed by infants (17%, $n = 7$), subadults (15%, $n = 6$), and juveniles (9.8%, $n = 4$). Captured individuals belonged to 18 groups, whose size ranged from 1 to 5 individuals with an average of 3 (SD ± 1.10) individuals. We registered two solitary males; one of these males was captured in Chineria and had a tattoo on its left inner thigh with number A280 as marked by FIDIC (Fig. 21.2).

The number of polymorphic sites and haplotypes and the nucleotide and haplotype diversity were identical in the two mitochondrial loci (Table 21.3). However, there were higher diversity levels in the IVITA specimens compared to the wild ones. Genetic distances between localities ranged between 0.002 and 0.006. IVITA exhibited the highest pairwise distances (Table 21.4), which were comparable to the distance between Uranias and Chineria, the two farthest locations in this study.

The topology and relationships between lineages in the median joining networks were similar in the two mitochondrial loci (Figs. 21.3 and 21.4). Both loci showed 27 haplotypes combining wild and captive animals and the reference sequence for *A. nancymaae* (cox1: Hap 13; D-loop: Hap 1). All the sequences for *A. nancymaae* showed a clear differentiation from all other species. Both networks showed five haplotypes shared by two or more localities, including a central modal haplotype (Hap_3 in cox-1 and Hap_9 in D-loop. IVITA specimens exhibited a larger number of different haplotypes (14) but also exclusive haplotypes (11) in both mitochondrial loci compared to the wild populations. Six of these haplotypes, exclusively found in IVITA specimens, were also highly differentiated from the remaining haplotypes, in

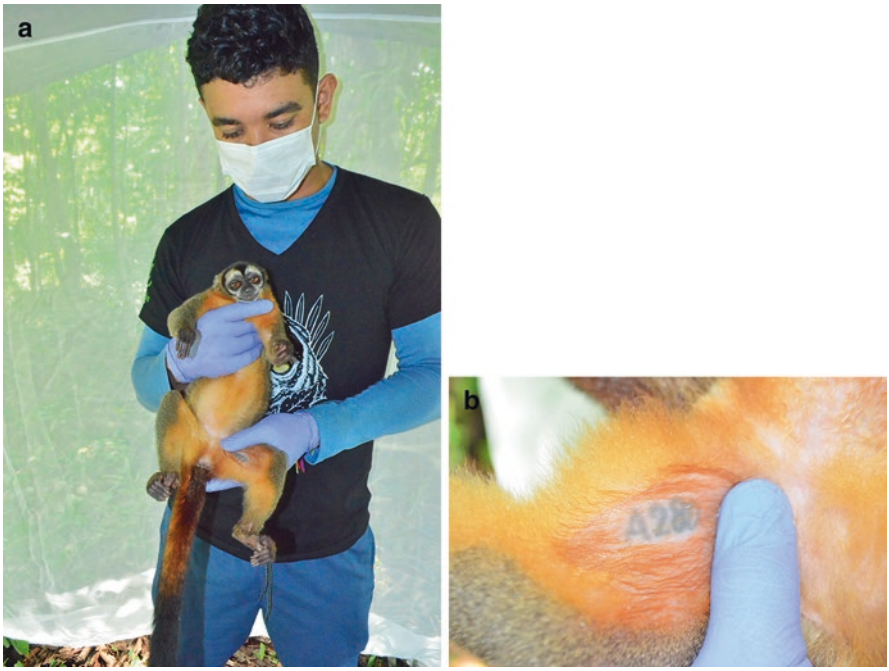

Fig. 21.2 Solitary adult male *Aotus nancymaae* captured in Chineria with tattoo A280

Table 21.3 Levels of genetic diversity for D-loop and Cytochrome Oxidase I in wild (33 individuals) and captive specimens (31 individuals) of *Aotus nancymaae*, for a total of 64 individuals

	D-loop		Cytochrome Oxidase I	
	Wild	Captive	Wild	Captive
Nucleotide diversity	0.00751	0.01106	0.00455	0.00605
Number of polymorphic sites	23	44	23	44
Haplotype diversity	0.960	0.930	0.902	0.920
Number of haplotypes	14	17	14	17

both cox1 and D-loop. Both haplotype networks failed to show differentiation between localities, suggesting high gene flow between social groups and localities.

21.4 Discussion

Our mitochondrial data revealed high levels of genetic diversity in both the wild and IVITA populations with no clear pattern of differentiation in the haplotype composition among the six localities in northeastern Loreto, indicating high levels of gene flow in this area. The fact that only three widely distributed haplotypes are

Table 21.4 Estimates of evolutionary divergence over sequence pairs between groups of *Aotus nancymaae*

	IVITA	Chineria	Yahuma	Vista Alegre	Nuevo Oriente	Uranias	Isla El Tigre
IVITA		0.002	0.001	0.001	0.001	0.001	0.001
Chineria	0.006		0.003	0.004	0.004	0.001	0.004
Yahuma	0.005	0.001		0.003	0.001	0.001	0.001
Vista Alegre	0.004	0.001	0.001		0.001	0.001	0.001
Nuevo Oriente	0.004	0.001	0.003	0.002		0.001	0.001
Uranias	0.005	0.005	0.003	0.003	0.003		0.002
Isla El Tigre	0.004	0.002	0.003	0.002	0.002	0.001	

The number of base substitutions per site from averaging over all sequence pairs between groups is shown. Standard error estimates are shown above the diagonal

shared between IVITA and natural populations, and that diversity levels are higher in IVITA, suggests that the captive population was established from multiple sources, including populations outside the area covered in this study. Similarly, preliminary genomic analyses have also shown elevated levels of polymorphism and heterozygosity in single nucleotide polymorphisms (SNPs) of a captive population of *A. nancymaae* in the USA, which has been explained by the mixed origin of the population founders (Rogers et al. 2023 this volume). A similar pattern was previously identified in Colombia, where the genetic diversity of captive animals present at FIDIC exceeds that of natural populations included in their study (Bloor et al. 2012). Even though this can be interpreted as potential proof that animals from foreign populations have been introduced to FIDIC, it could be a consequence of limited geographic sampling of natural populations, which will require future genetic studies and a better understanding of the genetic variation of wild populations. The haplotype diversity and the number of haplotypes in this study were similar to what was previously reported for Colombia (Bloor et al. 2012). Unfortunately, their sequences have not been published or made public; and therefore, it is not possible to make specific comparisons. However, we also found two sets of highly differentiated haplotypes, including one exclusively present in IVITA. Whether or not the two sets of differentiated lineages identified in Peru correspond to those reported in Colombia remains to be studied. But if that were the case, it would mean that populations occurring on opposite banks of the Amazon River are not as genetically isolated as previously thought. Future studies will be needed to contrast populations from opposite riverbanks to determine the potential role of natural and human-induced connectivity.

The area of interest for this study is on the edge of the distributional area of *A. nancymaae* and near the parapatric *A. vociferans* across the Amazon River in Colombia. Despite this fact, all the haplotypes present in the captive and wild populations and the reference sequence for *Aotus nancymaae* form a single and well-supported cluster in both haplotype networks, consistent with lack of recent interspecies hybridization in *A. nancymaae*. This may be due to an absence of international traffic of *A. vociferans* from the north bank of the Amazon River in

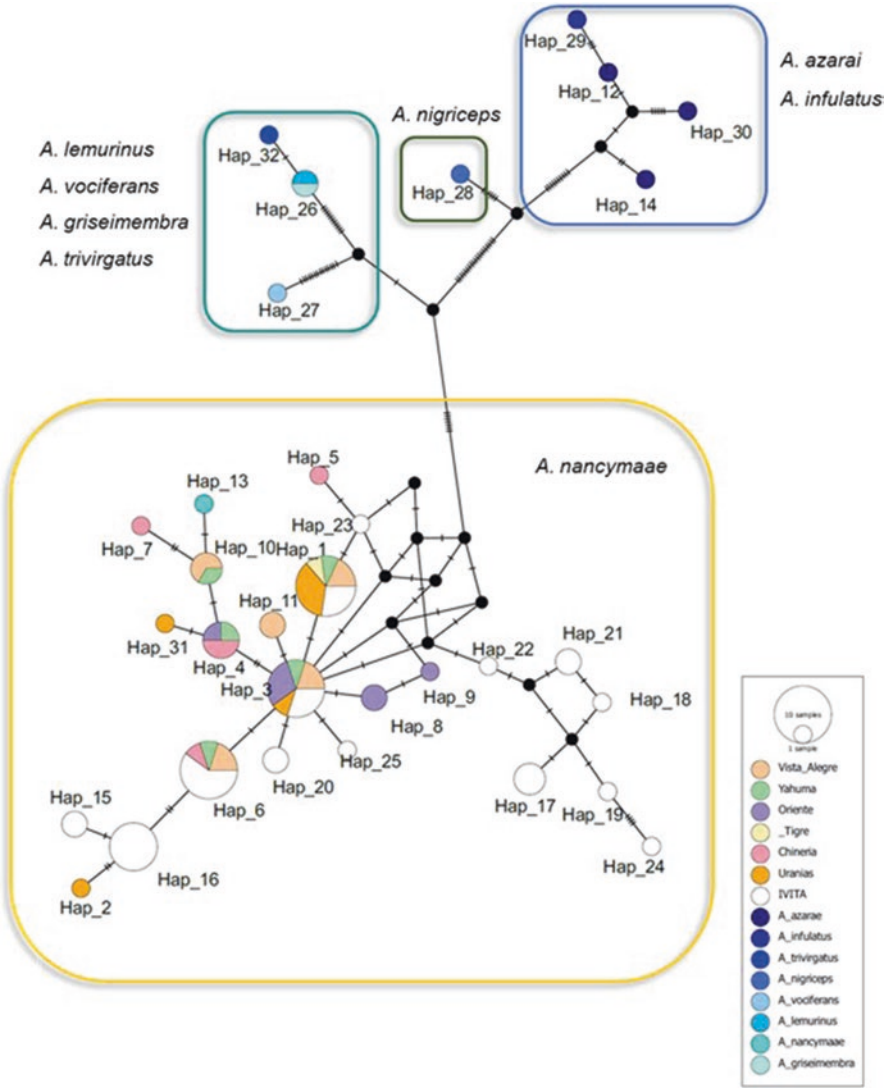

Fig. 21.3 Haplotype network for the mitochondrial Cytochrome Oxidase-I gene of *Aotus nancymaae* from Loreto and IVITA-Peru and reference sequences for other *Aotus* species

Colombia or the action of reproductive barriers that prevent hybridization between *A. nancymaae* and *A. vociferans*.

Future comparative analyses of genetic data from Colombian and Peruvian populations of *A. nancymaae* will be necessary to evaluate the hypothesis of international traffic of this species and its potential effect on populations. However, we have confirmed the presence of released animals in Peruvian territory. This situation raises more questions about the potential genetic, ecological, and epidemiological

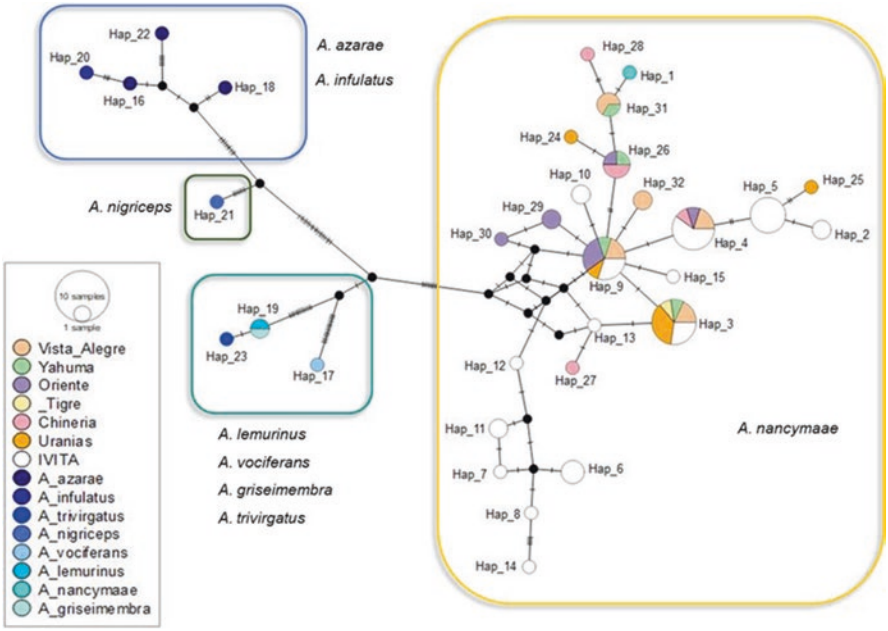

Fig. 21.4 Haplotype network for the D-loop mitochondrial marker of *Aotus nancymaae* from Loreto and IVITA-Peru and reference sequences for other *Aotus* species

impacts of animal translocations carried out by local experimental facilities. Many infectious agents circulate naturally in wild populations at the tri-border area; but the lack of health assessments makes it impossible to determine the effect of endemic diseases on the survival of released animals, as well as the impact of these translocations on the stability of receptive populations whether by introducing human-associated pathogens or disrupting natural disease cycles (Leendertz et al. 2006; Nichols et al. 2017).

21.5 Recommendations

Binational data sharing and actions (a) Make the DNA sequences of *Aotus* spp. held at the National University of Colombia open to the public, allowing comparative analyses using data from other studies in order to expand our knowledge of the genetic diversity of *Aotus* in the Colombian-Peruvian border. Such knowledge is needed to refine strategic actions for the management of this genus. (b) Conduct a comprehensive population health assessment including the surveillance of infectious agents. Monitoring of free-ranging populations provides opportunities for data collection through invasive (i.e., clinical evaluations, invasive sample collection) and noninvasive methods (i.e., observation of wounds and lesions indicative of aggression and healing capacity, noninvasive sample collection) (Leendertz et al.

2006; Gilardi et al. 2015). (c) Implement collaboration between CITES and environmental authorities SERFOR-Peru and MADS-Colombia to simplify procedures for the acquisition and transport of captive-bred specimens from IVITA (Peru) to FIDIC (Colombia), to promote adherence to international protocols for the use of primates in biomedical research, decreasing the impact of wild populations. (d) Include the species in the Colombian List of Endangered Species, expected to be released in 2021, following the global and national IUCN assessments for *A. nancymaae* classified as Vulnerable. (e) Adopt and apply recent binational conservation policies such as the "Leticia Pact for the Amazon" and the "Lima Declaration on Illegal Wildlife Trade" from 2019. These legal agreements/treaties require the signing countries to recognize illegal wildlife trade as a serious crime that has adverse consequences not only for the species and ecosystems of the region but for the economy, security, and well-being of its Indigenous and local populations. Thus, a binational plan for socioeconomic investment in the Amazon border region is urgently needed to accomplish these treaties as described in the "Leticia Pact for the Amazon," numeral 10:

> Strengthen the mechanisms that support and promote the sustainable use of the forest, sustainable production systems, responsible production and consumption patterns and that promote value chains and other sustainable production approaches, including those based on biodiversity.

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References

Álvarez-Gonçalvez CV, Mudry MD, Nieves M (2015) Accuracy assessment of datasets on the geographic distribution of *Aotus* spp. using a new Georeferencing Reliability Index. Ann Zool Fenn 52(3):177–185

Ancrenaz M, Setchell J, Curtis D (2003) Handling, anesthesia, health evaluation and biological sampling. In: Setchell J, Curtis D (eds) Field and laboratory methods in primatology: a practical guide. Cambridge University Press, Cambridge, UK, pp 122–139

Aquino R, Encarnación F (1986a) Characteristics and use of sleeping sites in *Aotus* (Cebidae: Primates) in the Amazon lowlands of Peru. Am J Primatol 11(4):319–331

Aquino R, Encarnación F (1986b) Population structure of *Aotus nancymai* (Cebidae, Primates) in Peruvian Amazon lowland forest. Am J Primatol 11:1–7

Aquino R, Encarnación F (1988) Population densities and geographic distribution of night monkeys (*Aotus nancymai* and *Aotus vociferans*) (Cebidae: Primates) in northeastern Peru. Am J Primatol 14(4):375–381

Aquino R, Encarnación F (1994) Owl monkey populations in Latin America: field work and conservation. In: Baer JF, Weller RE, Kakoma I (eds) *Aotus*: the owl monkey, San Diego, pp 59–95

Aquino R, Puertas PE, Encarnación F (1990) Supplemental notes on population parameters of northeastern Peruvian night monkeys, genus *Aotus* (Cebidae). Am J Primatol 21(3):215–221. https://doi.org/10.1002/ajp.1350210305

Aquino R, Terrones W, Navarro R, Terrones C, Cornejo FM (2009) Caza y estado de conservación de primates en la cuenca del río Itaya, Loreto, Perú. Rev Peru Biol 15(2):33–39

Armenteras D, Cabrera E, Rodríguez N, Retana J (2013) National and regional determinants of tropical deforestation in Colombia. Reg Environ Chang 13(6):1181–1193. https://doi.org/10.1007/s10113-013-0433-7

Armenteras D, Negret P, Melgarejo LF, Lakes TM, Londoño MC, García J, Krueger T, Baumann M, Davalos LM (2019) Curb land grabbing to save the Amazon. Nat Ecol Evol 3(11):1497. https://doi.org/10.1038/s41559-019-1020-1

Ashley MV, Vaughn JL (1995) Owl monkeys (*Aotus*) are highly divergent in mitochondrial Cytochromec Oxidase (COII) sequences. Int J Primatol 16(5):793–806. https://doi.org/10.1007/BF02735720

Babb PL, Fernandez-Duque E, Baiduc CA, Gagneux P, Evans S, Schurr TG (2011) mtDNA diversity in Azara's owl monkeys (*Aotus azarai azarai*) of the Argentinean Chaco. Am J Phys Anthropol 146(2):209–224. https://doi.org/10.1002/ajpa.21567

Bandelt H-J, Peter Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16(1):37–48

Baquero J, Miranda S, Murillo O, Mateus H, Trujillo E, Suarez C, Patarroyo M, Parra-López C (2006) Reference strand conformational analysis (RSCA) is a valuable tool in identifying MHC-DRB sequences in three species of *Aotus* monkeys. Immunogenetics 58(7):590–597. https://doi.org/10.1007/s00251-006-0101-x

Bloor P, Ibáñez C, Arciniegas S, Hoyos M, Hernández S, Pedraza L (2012) Informe Final del Convenio de Cooperación Científica y Tecnológica No. 10F Fondo Nacional Ambiental – FONAM; la Universidad Nacional de Colombia – Instituto de Genética – IGUN; el Instituto Amazónico de Investigaciones Científicas – SINCHI y la Corporación para el Desarrollo Sostenible del Sur de la Amazonia – CORPOAMAZONIA. IGUN - Instituto de Genética Universidad Nacional de Colombia, Colombia

Cann C, Buchanan-Smith H, Farmer KH, Fitch-Snyder H, Jones-Engel L, Prescott MJ, Taylor S (2007) IPS international guidelines for the acquisition, care and breeding of non-human primates, 2nd edn, p 35

Collins W, Sullivan J, Williams A, Nace D, Williams T, Galland G, Barnwell J (2006) *Aotus nancymaae* as a potential model for the testing of anti-sporozoite and liver stage vaccines against *Plasmodium falciparum*. Am J Trop Med Hyg 74(3):422–424

Corpoamazonia (2012) A-DTA-001-Acta de seguimiento al proceso de liberación y reintroducción al medio natural de 278 ejemplares de *Aotus* de la Estación Experiental-FIDIC (enero 21 y 23 de 2012). Corpoamazonia, Leticia, Amazonas

Corpoamazonia (2017) A-009-2017. Comité de ética Estación de Primates FIDIC-CEEPA. Visita general por parte del comité a las instalaciones y valoración de 120 especímenes de *Aotus vociferans* y *Aotus nancymaae* candidatos a reubicarse en medio natural. Marzo 7. Corpoamazonia, Leticia, Amazonas

Corpoamazonia (2019) A-030. Seguimiento al proceso de reubicación al medio natural de 27 especímenes de *Aotus vociferans* y *Aotus nancymaae* de la Estación de Primates-FIDIC del 4 de abril. Corpoamazonia, Leticia, Amazonas

Corpoamazonia (2020) Resolución 0366. Por medio del cual se otorga permiso individual de recolección de especímenes de especies silvestres de la diversidad biológica con fines no comerciales a la Fundación Instituto de Inmunología de Colombia -FIDIC, identificada con NIT:830084143–6, representada legalmente por el señor Manuel Elkin Patarroyo, identificado con cédula de ciudadanía No. 3.046.783 de Girardot, para el "proyecto recolección no comercial de especímenes silvestres de monos del género *Aotus* de las especies *A. vociferans* y *A. nancymaae* presentes en el área del Trapecio Amazónico colombiano, entre los ríos Amazonas y río Putumayo, sus afluentes y sub afluentes, para fines de investigación biomédica". Expediente PE-06-91-001-X-009-032-19

Defler TR (1994) La conservación de los primates colombianos. Trianea (Acta científica y tecnológica Inderena) 4:255–287

Defler TR (2004) Primates of Colombia. Tropical field guide series. Conservation International, Bogotá

Defler TR (2010) Historia natural de los primates de Colombia, 2nd edn. Universidad Nacional de Colombia. Facultad de Ciencias. Departamento de Biología, Bogotá

Defler TR, Bueno ML (2007) *Aotus* diversity and the species problem. Primate Conserv 22(1):55–70. https://doi.org/10.1896/052.022.0104

Di Fiore A, Valencia LM, Martins A (2023, this volume) Phylogenetics and biogeography of Owl Monkeys (*Aotus*): the molecular evidence. In: Fernandez-Duque E (ed) Owl Monkeys. Biology, Adaptive Radiation, and Behavioral Ecology of the Only Nocturnal Primate in the Americas. Springer, Cham

Etter A, Andrade A, Saavedra K, Amaya P, Cortés J, Arévalo P (2020) Colombian ecosystems: threats and risks. Application of the red list of ecosystems to the continental terrestrial ecosystems. Pontificia Universidad Javeriana and Conservación Internacional-Colombia, Bogotá

Evans S, Wolovich C (2023, this volume) The owl monkeys of the DuMond conservancy in Miami, USA: a scientific, educational and community asset. In: Fernandez-Duque E (ed) Owl monkeys - biology, adaptive radiation and behavioral ecology of the only nocturnal primate in the Americas. Springer, Cham

Everard M, Johnston P, Santillo D, Staddon C (2020) The role of ecosystems in mitigation and management of Covid-19 and other zoonoses. Environ Sci Pol 111:7–17. https://doi.org/10.1016/j.envsci.2020.05.017

Fearnside P (2017) Deforestation of the Brazilian Amazon. In: Shugart H (ed) Oxford research encyclopedia of environmental science. Oxford University Press, New York. https://doi.org/10.1093/acrefore/9780199389414.013.102

Fernandez-Duque E (2011) Rensch's rule, Bergmann's effect and adult sexual dimorphism in wild monogamous owl monkeys (*Aotus azarai*) of Argentina. Am J Phys Anthropol 146(1):38–48. https://doi.org/10.1002/ajpa.21541

Fernandez-Duque E, Rotundo M (2003) Field methods for capturing and marking *Azarai* night monkeys. Int J Primatol 24:1113–1120

Fernandez-Duque E, Huck M, Van Belle S, Di Fiore A (2020) The evolution of pair-living, sexual monogamy, and cooperative infant care: insights from research on wild owl monkeys, titi monkeys, sakis, and tamarins. Yearb Phys Anthropol:1–56. https://doi.org/10.1111/j.1365-2958.2003.03935.x

Fernandez-Duque E, Juárez CP, Defler T (2023a, this volume) Morphology, systematics and taxonomy of owl monkeys. In: Fernandez-Duque E (ed) Owl monkeys - biology, adaptive radiation and behavioral ecology of the only nocturnal primate in the Americas. Springer, Cham

Fernandez-Duque E, Rotundo M, Seltzer J, Di Fiore A, Link A (2023) Past, present and future use of technology for field studies of owl monkeys. In: Fernandez-Duque E (ed) Owl Monkeys. Biology, Adaptive Radiation, and Behavioral Ecology of the Only Nocturnal Primate in the Americas. Springer, Cham

García de la Chica A, Spence-Aizenberg A, Wolovich C, Evans S, Fernandez-Duque E (2023, this volume) The social life of owl monkeys. In: Fernandez-Duque E (ed) Owl monkeys - biology, adaptive radiation and behavioral ecology of the only nocturnal primate in the Americas. Springer, Cham

García de la Chica A, Fernandez-Duque E, Williams LE (2021) Behavioral biology of Owl Monkeys. In: Coleman K, Shapiro S (eds) Behavioral biology of laboratory animals, 1st edn. CRC Press, UK

Gilardi KV, Gillespie TR, Leendertz FH, Macfie EJ, Travis DA, Whittier CA, Williamson EA (2015) Best practice guidelines for health monitoring and disease control in great ape populations. IUCN SSC Primate Specialist Group, Gland, Switzerland

Gil-Botero E (2013) Acción popular - apelación sentencia en acción popular. Consejo de Estado, Sala de lo Contencioso Administrativo, Sección Tercera, Subsección C, Bogotá, Colombia

Gozalo A, King N, Montoya E (1994) Possible spontaneous mycobacteriosis in an owl monkey. J Med Primatol 23(1):58. https://doi.org/10.1111/j.1600-0684.1994.tb00098.x

Groves CP (2005) Order Primates. In: Wilson DE, Reeder DA (eds) Mammal species of the world: a taxonomic and geographic reference, 3rd edn. Johns Hopkins University Press, Baltimore, pp 151–177

Guzmán-Caro D, Vargas S, Cárdenas S, Castro J, Stevenson P (2018) Estudio y conservación de primates en Colombia: Avances, retos y el papel de parques nacionales naturales. In: Urbani B, Kowalewski M, Teixeira da Cunha R, de la Torre S, Cortés-Ortiz L (eds) La primatología en Latinoamérica 2 - A primatologia na America Latina 2. Tomo 1 Argentina-Colombia. Ediciones IVIC. Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela, pp 283–294

Henao-Díaz F, Stevenson P, Carretero-Pinzón X, Castillo-Ayala C, Pacheco J, Defler T, García-Villalba J, Caro D, Link A, Moreno M, Palacios E, Duque N, Soto-Calderón I, Soto L, Velásquez-Tibata J, Olaya-Rodríguez M, Noguera-Urbano E, Valencia L (2020) Atlas de la biodiversidad de Colombia. Primates. Mejores modelos con el apoyo de expertos. Atlas de la Biodiversidad de Colombia. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá D. C., Colombia

Hernández AF, Díaz AS (2011) Estado preliminar poblacional del mono nocturno (*Aotus* sp. Humboldt 1812) en las comunidades indígenas Siete de Agosto y San Juan de Atacuari - Puerto Nariño, departamento de Amazonas, Colombia. Universidad del Tolima, Ibagué, Tolima

Hernández-Camacho J, Defler TR (1989) Algunos aspectos de la conservación de primates no-humanos en Colombia. In: Saavedra CJ, Mittermeier RA, Bastos Santos I (eds) La primatología en Latinoamérica. WWF-U.S, Washington, D.C, pp 67–100

Herrera S, Perlaza BL, Bonelo A, Arévalo-Herrera M (2002) *Aotus* monkeys: their great value for anti-malaria vaccines and drug testing. Int J Parasitol 32(13):1625–1635. https://doi.org/10.1016/s0020-7519(02)00191-1

Hershkovitz P (1983) Two new species of night monkeys, genus *Aotus* (Cebidae, Platyrrhini): a preliminary report on *Aotus* taxonomy. Am J Primatol 4(3):209–243. https://doi.org/10.1002/ajp.1350040302

Kumamoto AT, Houck ML (2001) Cytogenetic identification of a hybrid owl monkey, *Aotus nancymaae* x *Aotus lemurinus griseimembra*. J Zoo Wildl Med 32(1):130–133. https://doi.org/10.1638/1042-7260(2001)032[0130:cioaho]2.0.co;2

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874. https://doi.org/10.1093/molbev/msw054

Laurance WF, Clements GR, Sloan S, O'Connell CS, Mueller ND, Goosem M, Venter O, Edwards DP, Phalan B, Balmford A, Van Der Ree R, Arrea IB (2014) A global strategy for road building. Nature 513(7517):229–232. https://doi.org/10.1038/nature13717

Leendertz F, Pauli G, Maetz-Rensing K, Boardman W, Nunn C, Ellerbrok H, Jensen S, Junglen S, Christophe B (2006) Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biol Conserv 131(2):325–337. https://doi.org/10.1016/j.biocon.2006.05.002

Librado PJR, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11):1451–1452. https://doi.org/10.1093/bioinformatics/btp187

López C, Suárez CF, Cadavid LF, Patarroyo ME, Patarroyo MA (2014) Characterising a microsatellite for DRB typing in *Aotus vociferans* and *Aotus nancymaae* (Platyrrhini). PLoS One 9(5):e96973. https://doi.org/10.1371/journal.pone.0096973

Maldonado AM (2011) Tráfico de monos nocturnos *Aotus* spp. en la frontera entre Colombia, Perú y Brasil: Efectos sobre sus poblaciones silvestres y violación de las regulaciones internacionales de comercio de fauna estipuladas por CITES. Revista Acad Colomb Ci Exact 35(135):237–254

Maldonado A, Lafon T (2017) Biomedical research vs. biodiversity conservation in the Colombian-Peruvian Amazon: searching for law enforcement where there is lack of accountability. In: Rodríguez GD, Mol H, Brisman A, South N (eds) Environmental crime in Latin America: the theft of nature and the poisoning of the land. Palgrave Macmillan, London, pp 261–294

Maldonado AM, Peck MR (2014) Research and *in situ* conservation of Owl monkeys enhances environmental law enforcement at the Colombian-Peruvian border. Am J Primatol 76:658–669. https://doi.org/10.1002/ajp.22260

Maldonado AM, Waters S (2020) Ethnoprimatology of the Tikuna in the Southern Colombian Amazon. In: Urbani B, Lizarralde M (eds) Neotropical ethnoprimatology: indigenous peoples' perceptions of and interactions with nonhuman primates. Springer Nature Switzerland, pp 89–107

Maldonado AM, Nijman V, Bearder SK (2009) Trade in night monkeys *Aotus* spp. in the Brazil-Colombia-Peru tri-border area: international wildlife trade regulations are ineffectively enforced. Endanger Species Res 9(2):143–149. https://doi.org/10.3354/esr00209

Maldonado AM, Guzmán-Caro D, Shanee S, Defler TR, Roncancio NJ (2017) Aotus nancymaae. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T41540A121725532.en. Accessed 18 March 2020

Menezes AN, Bonvicino CR, Seuánez HN (2010) Identification, classification and evolution of Owl Monkeys (*Aotus*, Illiger 1811). BMC Evol Biol 10(1):248. https://doi.org/10.1186/1471-2148-10-248

Moreno-Pérez D, García-Valiente R, Ibarrola N, Muro A, Patarroyo M (2017) The *Aotus nancymaae* erythrocyte proteome and its importance for biomedical research. J Proteome 152:131–137. https://doi.org/10.1016/j.jprot.2016.10.018

Nehete PM, Nehete BP, Chitta S, Williams LE, Abee C (2017) Phenotypic and functional characterization of peripheral blood lymphocytes from various age- and sex-specific groups of owl monkeys (*Aotus nancymaae*). Comp Med 67(1):67–78

Nichols JD, Hollmen TE, Grand JB (2017) Monitoring for the management of disease risk in animal translocation programmes. EcoHealth 14(1):156–166. https://doi.org/10.1007/s10393-015-1094-4

Pieczarka J, de Souza BR, Nagamachi C, Rodrigues R, Espinel A (1992) Aotus vociferans × Aotus nancymai: sympatry without chromosomal hybridation. Primates 33(2):239–245

Plautz H, Gonçalves EC, Ferrari SF, Schneider MPC, Silva A (2009) Evolutionary inferences on the diversity of the genus *Aotus* (Platyrrhini, Cebidae) from mitochondrial cytochrome c oxidase subunit II gene sequences. Mol Phylogen Evol 51(2):382

Rogers J, Harris RA, Raveendran M, Williams L, Worley KC (2023, this volume) A reference genome assembly and initial polymorphism data for *Aotus nancymaae*. In: Fernandez-Duque E (ed) Owl Monkeys. Biology, Adaptive Radiation, and Behavioral Ecology of the Only Nocturnal Primate in the Americas. Springer, Cham

Roncancio NJ (2012) Revisión de los protocolos de reincorporación de ejemplares del género *Aotus* a la vida silvestre de la FIDIC con recomendaciones. SINCHI – Instituto Amazónico de Investigaciones Científicas, Bogotá, Colombia

Roncancio-Duque N, Osorno M, Calderón-Espitia LM, Acosta-Castañeda A, García-Loaiza LM, Gómez-Melendro N, Henao BE (2019) Densidad poblacional y composición de grupos de *Aotus nancymaae* en áreas de aprovechamiento de la especie para experimentación biomédica en el Trapecio Amazónico colombiano. Neotrop Primates 25(1):30–37

Ruiz-García M, Vásquez C, Camargo E, Leguizamón N, Gálvez H, Vallejo A, Pinedo M, Castellanos L, Shostell J, Alvarez D (2011) Molecular phylogenetic of *Aotus* (Platyrrhini, Cebidae). Int J Primatol 32(5):1218–1241

Ruiz-García M, Vásquez C, Camargo E, Castellanos A, Gálvez H, Leguizamón N, Shostell JM (2013) Molecular genetics analysis of mtDNA COII gene sequences shows illegal traffic of night monkeys (*Aotus*, Platyrrhini, Primates) in Colombia. J Primatol 2(107):2–9. https://doi.org/10.4172/2167-6801.1000107

Ruiz-Herrera A, García F, Aguilera M, García M, Ponsa Fontanals M (2005) Comparative chromosome painting in *Aotus* reveals a highly derived evolution. Am J Primatol 65:73–85

Schiavetta AM, Harre JG, Wagner E, Simmons M, Raviprakash K (2003) Variable susceptibility of the owl monkey (*Aotus nancymaae*) to four serotypes of dengue virus. Contemp Top Lab Anim Sci 42(5):12–20

Setchell J, Curtis D (2003) Field and laboratory methods in primatology: a practical guide. Cambridge University Press, Cambridge, UK

Shanee S, Allgas N, Shanee N, Campbell N (2015) Distribution, ecological niche modelling and conservation assessment of the Peruvian Night Monkey (Mammalia: Primates: Aotidae: *Aotus miconax* Thomas, 1927) in northeastern Peru, with notes on the distributions of *Aotus* spp. J Threat Taxa 7(3):6947–6964. https://doi.org/10.11609/JoTT.o4184.6947-64

Shanee S, Mendoza AP, Maldonado AM, Fernández-Hidalgo AM, Svensson MS (2023a, this volume) Traffic and trade in owl monkeys. In: Fernandez-Duque E (ed) Owl monkeys - biology, adaptive radiation and behavioral ecology of the only nocturnal primate in the Americas. Springer, Cham

Shanee S, Tirira D, Aquino R, Carretero-Pinzon X, Link A, Maldonado AM, Mendez P, Urbani B, Fernandez-Duque E (2023b, this volume) Geographic distribution of owl monkeys. In: Fernandez-Duque E (ed) Owl Monkeys. Biology, Adaptive Radiation, and Behavioral Ecology of the Only Nocturnal Primate in the Americas. Cham: Springer

Smith DG (2012) Taxonomy of nonhuman primates used in biomedical research. In: Abee CR, Mansfield K, Tardif S, Morris T (eds) Nonhuman primates in biomedical research: biology and management, vol 2nd, 2nd edn. Academic Press, Boston, pp 57–85. https://doi.org/10.1016/B978-0-12-381365-7.00003-0

Spence-Aizenberg A, Evans S, Wolovich C, Larson S, Fernandez-Duque E, Williams L (2023) *Aotus*: a model to synthesize studies of captive and wild primates. In: Fernandez-Duque E (ed) Owl Monkeys. Biology, Adaptive Radiation, and Behavioral Ecology of the Only Nocturnal Primate in the Americas. Springer, Cham

Spence-Aizenberg A, García de la Chica A, Evans S, Fernandez-Duque E (2023, this volume) Communication among owl monkeys. In: Fernandez-Duque E (ed) Owl monkeys - biology, adaptive radiation and behavioral ecology of the only nocturnal primate in the Americas. Springer, Cham

Suárez CF, Patarroyo MA, Patarroyo ME (2011) Characterisation and comparative analysis of MHC-DPA1 exon 2 in the owl monkey (*Aotus nancymaae*). Gene 470(1–2):37–45

Svensson MS, Shanee S, Shanee N, Bannister FB, Cervera L, Donati G, Huck M, Jerusalinsky L, Juarez CP, Maldonado AM, Martinez Mollinedo J, Méndez-Carvajal PG, Molina Argandoña MA, Mollo Vino AD, Nekaris KA, Peck MR, Rey-Goyeneche J, Spaan D, Nijman V (2016) Disappearing in the night: an overview on trade and legislation of night monkeys in South and Central America. Folia Primatol 87(5):332–348. https://doi.org/10.1159/000454803

Torres OM, Enciso S, Ruiz F, Silva E, Yunis I (1988) Chromosome diversity of the genus *Aotus* from Colombia. Am J Primatol 44(4):255–275

Vilela T, Malky A, Bruner A, Laísa V, Arruda S, Ribeiro V, Auxiliadora A, Alencar C, Grandez A, Rojas A, Laina A, Botero R (2020) A better Amazon road network for people and the environment. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.1910853117

Williams A, Barefield S, Carter E, Collins W, Sullivan J, Tate M (2005) Adaptation of *Plasmodium vivax* to growth in owl monkeys (*Aotus nancymai*). Comp Med 55(6):528–532

Wolovich CK, Evans S (2007) Sociosexual behavior and chemical communication of *Aotus nancymaae*. Int J Primatol 28:1299–1313. https://doi.org/10.1007/s10764-007-9228-3