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Investigaciones Mastozoológicas

Volume 2

Primatology in Argentina

La primatología en Argentina

Martín M. Kowalewski and **Luciana I. Oklander**, Editors

The Argentinean Society for the Study of Mammals (Sociedad Argentina para el Estudio de los Mamíferos – SAREM) was created in 1983, and currently has about 300 members from several countries. SAREM is an interdisciplinary society of natural sciences professionals whose main goals are the promotion of scientific and technical research, the consolidation of national collections and research centers, and the publication and diffusion of research on living and/or extinct mammals. SAREM has organized scientific meetings for mammal researchers since 1994, publishes the journal *Mastozoología Neotropical* and has edited books on the systematics, distribution and conservation of the mammals of southern South America, including *Libro Rojo de los mamíferos amenazados de la Argentina* (first ed. 2000, second ed. 2012) and *Mamíferos de Argentina. Sistemática y distribución (2006),* as well as contributing to the *Libro Rojo de los mamíferos y aves amenazados de la Argentina* (currently out of print).

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PRIMATOLOGY IN ARGENTINA

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Cover photograph: *Alouatta caraya*
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In 2013, during the Annual Assembly of SAREM in the city of Mar del Plata, Dr. Mariano Merino, then President of the Society, together with the rest of the Directive Committee, announced the launch of a new editorial project: **SAREM Series A: Mammalogical Research (Investigaciones Mastozoológicas)**. The goal of this publication was to be the dissemination of scientific works on Neotropical mammals from wide and varied perspectives (evolutionary history, systematics, paleontology, biogeography, morphology, ecology, physiology, ethology, conservation, genetics, etc.) aimed at a public formed by the mammalogy research community, graduates, students and other interested readers, at both national and international levels. Each volume of this series is dedicated to a specific subject, be it a particular taxon (*e.g.*, taxonomy of caviomorphs, marsupials, carnivores, primates, etc.) or discipline (*e.g.*, ecology of small mammals, conservation, etc.). The series allows publication of unpublished works and revisions resulting from scientific meetings, symposia or workshops, so that they may achieve wide distribution in the international scientific community.

With this volume, *Primatology in Argentina* (*La primatología en Argentina*), SAREM once again sponsors the publication of novel works of a different nature compared to those already published in the journal *Mastozoología Neotropical*. The second volume of the series presents a series of topics developed by active groups of Argentinian and foreign primatologists, covering different fields of primatology developed in the country, such as behavior, ecology, evolution and zoonoses.

It is our hope that this new series becomes a tool for further development of the study of mammals, one that can be used by the mammalogical community with the unwavering purpose of promoting the knowledge and dissemination of mammalogy in South America.

Dra. Emma Carolina Vieytes
Editor-in-Chief, SAREM Series A

Dr. David Alfredo Flores
President, SAREM

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Martín Kowalewski dedicates this volume to his parents,
María A. Rebollo and Raúl García, who supported
him since the early years of his career; and especially
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Luciana Oklander dedicates this volume to her daughters
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interesting primates she could ever observe; and to her
husband Julián, because through his support she can
continue working on what she loves: primate biology.

FOREWORD

It is coincidentally fitting that I have the chance to say a few words at the start of this first volume of collected works of Argentinian primatologists since systematic study of Argentinian primates started in the 1980's. I first visited Argentina as a teenager, spending a full year between the austral winters of 1970 and 1971 in Buenos Aires at the Centro Panamericano de Zoonosis, as assistant to the public health ecologist Dr. Rexford Lord. Although I had the chance to visit many parts of Argentina (mostly in pursuit of vampire bats, *Desmodus rotundus*), including Misiones province, I was not then focused on primates. Despite political and financial uncertainties that pervaded that time, I felt welcome and made many good friends. One of my friends, at the farewell party when I left to return to the U.S. in 1971 said, prophetically, “You know you will return, don't you?”

Jump forward 17 years to 1988, when I was in Brasilia at the XII Congress of the International Primatological Society. I was looking for a long-term study site where I might study *Cebus* (now *Sapajus*) species under ecological conditions far different from the tropical moist forests of Peru that I used for my dissertation work. Ideally, a new site would allow me to use experimental methods to gain more solid insights into the causes of capuchin social ecology. I had visited two sites in Brasil (Manaus, and Fazenda Montes Claros in Minas Gerais), but neither was ideal for my needs. I ran into Alejandro Brown at the Congress, and he at once set out to convince me that Iguazú National Park might be exactly the site for my needs, and that I could contribute an important part as well to helping to develop primatology in Argentina. I was particularly intrigued by his (and Gabriel Zunino's) data that the *caí* (capuchins) of Iguazú lived in both larger and apparently less stable societies than I had been used to seeing in Peru. Alejandro's enthusiasm and energy were contagious, and I soon found myself spending a short field season in Iguazú, doing some basic census work and getting a feel for the area and what it was like to work with the Argentinian Administration of National Parks. In just a couple of weeks, I was able to confirm Alejandro's conjecture that the groups at least sometimes broke apart in the austral winter, an intriguing pattern documented at that time in very few other primates. That observation plus the ease of access of the national park and the sympathetic attitude of the park authorities toward ecological research convinced me to try to find the funding to set up a long-term study site there. Within two years, I had found funds and Alejandro had found a small group of young and enthusiastic field assistants to collaborate in setting up the study site. These assistants were a great group, some experienced (Sandra Arditi and Guillermo Placci had conducted field studies of *Aotus* in Formosa), others almost completely new to the field, but we shared in the adventure of discovering the biology of a site that was new to all of us. Several of these early field assistants are now well-established biologists (Mario Di Bitetti, Sandra Chediak, Inés Horovitz), despite the very tough work of establishing the field site; this involved not only cutting and marking a series of narrow trails along which we could move and observe the *caí,* but tagging and identifying hundreds of trees to study food availability, battling frequent power outages, and spending considerable time improving the physical structure of the laboratory building that the National Park authorities had graciously allowed us to use. Throughout this first year (1991), Alejandro Brown was a frequent visitor and collaborator, and it is certainly true that he is as (or more) responsible as I was for making the Centro de Investigaciones Ecológicas Subtropicales (CIES) a reality and a durable haven for ecological studies of the area.

The CIES has become the site with the longest continuous data set on identified primate groups in Argentina, under the broad umbrella of the Proyecto Caí. These long-term studies have allowed the demography of individuals and groups to be documented in detail, supported excellent basic research on many aspects of capuchin biology (vocalizations, agonism, mating systems, feeding ecology, cognition, parasitism), and offered a training ground for dozens of field researchers from both Argentina and other counties nearby and far away. Within a decade of the establishment of the CIES, two other long-term Argentinian study sites were established (EBCo in Corrientes, and Proyecto Mirikiná in Formosa), and have offered similarly beneficial long-term study sites for howler monkeys (*Alouatta*) and night monkeys (*Aotus*) respectively, some of the results of which are reported in this volume. The scientific and management value of these long-term study sites cannot be overstated!

When I started work at the CIES in 1991, there were few opportunities for advanced training in primate biology, especially for field studies. I was lucky to have two of the project's early field assistants (Mario Di Bitetti, Inés Horovitz) come to Stony Brook to pursue (and succeed) in doctoral research. Mario worked with me, and quickly showed his extraordinary intuition for biology in his dissertation research (some of which appears in this book) as well as in his later work both on primates and other mammals in the province of Misiones. I quickly came to appreciate him as a colleague and as a friend. It has been especially gratifying to see him bring in and mentor a diverse set of students from Argentina and other countries, many of whom are now established and interesting scientists and contributors to this volume. I note with pride and interest a recent article in the prestigious journal *Nature* by one of these “second-generation” students, Ilaria Agostini, and her colleagues, showing experimentally for the first time in the wild that capuchin monkeys that are fed more have lower parasite loads.

Speaking of experiments, if there is one special way that the Iguazú study site was and remains a pioneer and international example, it is in the consistent use of experimental approaches to separate the often-entangled web of causes and effects that combine to make the observed patterns of primate ecology and behavior. There are simply no other primate research sites in the world where experiments manipulating ecological aspects of the environment (food and predation risk) have been used so often and so successfully. To accomplish this, I have to single out the collaboration of the Argentinian Administration of National Parks, and especially its local scientific office (Delegación Regional Nordeste Argentina). They showed unusual trust and willingness to allow the Proyecto Caí to use experimental manipulations of food abundance and location in the wild to promote the best science that addresses both basic and applied questions. It is my hope that my own few contributions to understanding primate biology will be long outlived by the legacy of my students and the experimental approach that I encouraged all my students to adopt.

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Part I

INTRODUCTION

1 FORTY YEARS OF PRIMATOLOGY IN ARGENTINA, THE VERY FIRST VOLUME

CUARENTA AÑOS DE PRIMATOLOGÍA EN ARGENTINA, EL PRIMER VOLUMEN

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Introduction

For those outside of the primatological community, it may seem surprising that this is the first volume about primatology in Argentina in almost 40 years. In Argentina we found five species of extant primates that inhabit different types of forests in the northern part of the country, especially in the northeastern region. These monkeys have differing conservation status. *Alouatta caraya* (black and gold howler monkeys), *Alouatta guariba clamitans* (brown howler monkeys), *Aotus azarae* (owl monkeys), *Sapajus nigritus*—taxonomically synonymous with *Cebus nigritus*—(black horned capuchins), and *Sapajus cay*—taxonomically synonymous with *Cebus paraguayanus* and *Sapajus paraguayanus*—(brown-capped capuchins) (Tab. 1).

Table 1. Non-human primate species distribution in Argentina, conservation status at the regional level (IUCN) and in Argentina (SAREM 2012), and main field site with long term wild population under study.

Species	Provinces/states found in Argentina	IUCN status	Argentinian Status (SAREM 2012)
Alouatta caraya	Formosa, Corrientes, Misiones, Chaco, Santa Fe	Least Concern	Vulnerable
Alouatta guariba clamitans	Misiones	Least Concern	Critically Endangered
Sapajus (Cebus) nigritus	Misiones	Near Threatened	Near Threatened
Sapajus cay (Cebus paraguayanus)	Salta, Jujuy	Least Concern	Insufficient data
Aotus azarae	Formosa, Chaco	Least Concern	Least Concern

Despite the low number of non-human primate species in comparison to some other countries and despite our economic and political crisis in the 1990s that did not allow an exponential growth of the scientific research through the years, Argentina has a long history of studies both in laboratory and field environments and rich scientific production in primatology. Studies have been, or are being, carried out by researchers at all levels in academia, and perhaps most importantly there has been an increase in the number of students carrying out their Ph.D. research at different Argentinian universities. However, we still lack a national strategy to conserve primates (see Oklander *et al.*, this volume) and the means to process certain type of samples. For this reason, we still depend on foreign labs to process biological samples. There are two sides to this: on one hand advanced students and researchers are able to travel to labs in different countries strengthening collaborative works, but also we still depend on this collaborative work to run and finish basic science projects. There are few long term sites in Argentina, and it is necessary to maintain them and consider opening new ones (Kowalewski *et al.* 2015, Oklander *et al.*, this volume).

In bringing together the various chapters in this volume, we had several goals in mind. First and foremost we wanted to provide the first compilation of primatological research carried out in Argentina, where young professionals and students will be able to identify research groups and research lines to begin studies and research. Second, we wanted to showcase the expansion in research being carried out in the discipline in recent years. The authors who have contributed to this volume are numerous; however, the list does not include many who have contributed greatly to the development of primatology in Argentina, either because they stopped researching or changed study subjects. Additionally absent are many graduate students currently gathering data and whose work will almost certainly broaden our knowledge even further.

The chapters of this volume demonstrate the diversity of studies in Argentina. The first and second parts of this book deal with the history of primatology in Argentina, as well as the evolution, phylogeny and paleogeography of non-human primates here. Mudry and Zunino put together the academic and professional historical development of primatology as a discipline in Argentina since the late 1970s suggesting that the discipline went through critical periods of time where it almost disappeared especially in 1990s, due to economic problems and a neoliberal political program during which scientific research and public education was strongly discouraged by the state.

Tejedor and Novo present an update on platyrrhine evolutionary history and paleobiogeographic scenarios in **Chapter 3**. The detailed review of different fossil forms strongly suggests the evolutionary continuity between Patagonian and Neotropical platyrrhines. Moreover, paleobiogeographic and phylogenetic inferences presented in the chapter support the "long lineage hypothesis" that proposes that some living lineages had representatives in Patagonia, with a dispersal route for a south-north evolutionary continuity during the Miocene. Finally the authors proposed a revised classification of the platyrrhines including living and extinct genera.

In **Chapter 4**, Aristide and Perez investigated statistically the phylogenetic history and the phenotypic diversification of New World monkeys, using molecular phylogenetic (species tree), geometric morphometric and comparative methods. They present results that suggest that the phenotypic differences among platyrrhines could be mainly the product of deterministic factors that generate stasis in size variation related to ecological diversity and random factors that generate shape differences that increase proportionally with phylogenetic distance. The two last chapters of this section include a genetic approach with authors that belong to the main two research lines dealing with the application of these techniques in primatology.

Chapter 5 shows an integration of cytogenetic methodologies in the post genomic era suggesting ways to be used in order to elucidate evolutionary relationships among primate species. In this regard, Nieves *et al.* develop the application of qualitative analysis such as Fluorescence *in situ* Hybridization (FISH), and studies of variability in mitochondrial and nuclear DNA polymorphisms to characterize intra and inter specific difference in non-human primates. They also review the Comparative Genomic Hybridization (CGH) method, which allows analysis of how much of the genome is shared among congeneric species and on what chromosomes differences are located.

Oklander *et al.* in **Chapter 6** investigated the geographical structure and evolutionary history of the black howler monkeys along population distributions in Argentina, analyzing nuclear microsatellite (STRs) genetic diversity from fecal samples. They found significant differences between populations. However their analysis shows that some populations share genetic characteristics and they could be pooled as clusters. Those populations are connected by rivers or riparian forests suggesting that black howler dispersion depends on their presence. This study contributes to the biological knowledge of the genus *Alouatta*, and could have direct and important applications in the field of conservation allowing researchers to define priority areas for establishing conservation units applicable to the species.

The third section of the book deals with the ecological challenges primates face and the ways in which they have adapted to these various pressures, including comparative studies in the same species, communication studies, comparison between captive and wild sets, and communication systems in howlers and capuchins.

Zunino and collaborators evaluate the effect of seasonality on the activity budget, food availability, and diet of black and gold howler monkeys in northern Argentina taking advantage of the relatively strong seasonality of the forest where they inhabit. The authors have found that seasonality seems to influence the activity budget and diet of *Alouatta caraya*, and they suggest that periods of time with low availability or absence of seasonal foods may be the key variable to determining the limits for primates in the geographic distribution toward southern latitudes. Furthermore, the study was conducted in a subtropical region, where physical and ecological constraints should differ from those faced by howler monkeys that live in tropical areas. These results contribute with data indicating that these animals can be quite plastic in their use of varying habitat types.

In **Chapter 8**, Fernández and colleagues specifically examine possibly one of the most well studied aspects in neotropical primates, their diet. However, they approach this topic using the nutritional ecology approach, which focuses on the importance of quantifying both the intake and the nutritional composition of food items. These authors suggest the implementation of new frameworks like the geometric framework to examine the patterns of nutrient prioritization among primates. The authors encourage researchers to employ adequate methodological approaches and analytical frameworks, such as the Geometric Framework, for exploring questions about the nutritional ecology of non-human primates.

In **Chapter 9**, Tujague and Janson review experimental and observational studies in Neotropical primates from the wild and captivity about the ability of primates to remember the locations of resources. They also present original results on experiments carried out with capuchins at the PN Iguazú that suggest that these primates are able to partially recall the spatial and temporal location and type of food items. The last two chapters of this section focused on the communication ecology of non-human primates.

Di Bitetti and Wheeler describe the acoustic structure and context of usage of the vocal repertoire of the black-horned capuchin monkey at the Iguazú NP in **Chapter 10**. They described over 30 distinguishable call types, and argued that the most common notes serve to regulate within-group spacing. The authors provide a picture of the range of acoustic variation and the range of conditions under which different vocalizations are used. The large vocal repertoire of capuchin monkeys is consistent with the hypothesis that the diversity of primate calls is positively correlated with group size and the complexity of social interactions.

In **Chapter 11**, Holzmann and collaborators described short-distance vocalizations in *Alouatta caraya,* which are naturally emitted short distance vocalizations used particularly in intra-group communication. They describe eight different types of short-distance calls and suggest that some of these vocalizations may function to reestablish contact between lost individuals. Being the first study of this kind, these results introduce a field that needs to be expanded and studied.

The last section of the book consists of four chapters that focus on diseases, conservation status of non-human primates and finally a proposal on what is needed to strengthen primatological studies in Argentina.

In **Chapter 12**, Kowalewski and collaborators review the presence of a cestode parasite in black and gold howler monkeys across their distribution (and brown howlers), analyzing *Bertiella* spp. infection patterns and provide new data onthese parasites in howler monkeys. The authors indicated the presence of this parasite at all studied sites, and suggested the use of *Bertiella* spp. prevalence as a rapid health index status for *Alouatta caraya*.

Agostini and colleagues focused **Chapter 13** on a review of the current status of conservation and threats of *A. guariba clamitans* in an endemic species of the Atlantic Forest of Brazil and Argentina. This species is categorized as vulnerable by the IUCN and critically endangered in Argentina by the SAREM (2012). Main threats for the species include the high susceptibility of howlers to recurrent yellow fever outbreaks and habitat loss. The authors described the results obtained in the brown howler workshop facilitated by the IUCN/SSC's Conservation Breeding Specialist Group (CBSG), and the application of Population Viability Analysis using *Vortex* and *Outbreak* software packages that confirm that the main threat was the yellow fever outbreak acting on such small populations of non-human primates.

In **Chapter 14**, Juárez and collaborators provide information on long-term studies of a cathemeral subspecies of owl monkey and these studies provide valuable behavioral, ecological, genetic and demographic data in the Formosa Province in northern Argentina. The Owl Monkey Project started in 1996 as a multi-disciplinary program on the Azara's owl monkey of the Argentinian Chaco. In this chapter the authors use long term data to elucidate factors underlying the demographic structure of different owl monkey groups inhabiting different types of habitats. They suggest that for example estimated densities for non-protected areas are higher than in protected forests, and size and range of groups, birth rates and age were similar between sites. The long term data provided by this research group contribute to the understanding of the behavior and the ecology of this species, as well as the evolution of social monogamy and male care.

Finally, Oklander *et al.*, in **Chapter 15**, review the perspectives on studies and the perspectives on conservation of primates in Argentina. This last review is highly important because all five primate species inhabiting Argentina have protected populations within national or provincial reserves, but the area covered by these reserves represents only 1% of the total estimated area occupied by these species. They also present tentative research lines needed in Argentina indicating which are the priorities needed to conserve non-human primates in our country.

Final remarks

Our idea with this volume was to compile a series of chapters that partially describes the situation of primatology in Argentina. It is clear that although Argentinian researchers are producing good information we still lack the intensification of studies in non-protected areas, most of our National Parks and with some species such as *Cebus paraguayanus* or *Sapajus cay*. Habitat degradation is a reality in northern Argentina. In this regard we need, as a group (possibly through the Asociación de Primatología Argentina (APrimA), to evaluate these threats that may impede future primatological research and to advice on how scientific research can contribute to conservation, especially with regard to determining specific habitat requirements. We hope that the increase in research being carried out on wild populations of non-human primates in Argentina will continue in future years.

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Part II

HISTORY, PHYLOGENY AND EVOLUTION OF PRIMATES

2 BRIEF HISTORY OF PRIMATOLOGY IN ARGENTINA

BREVE HISTORIA DE LA PRIMATOLOGÍA EN ARGENTINA

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Abstract. This chapter comes as a contribution for primatologists in training. The knowledge on the history, stages and milestones that characterize the history of primatology in the country will provide them with a stronger background when proposing new projects. At the same time it will allow them to understand more clearly the need to work with multiple sources of knowledge in order to strengthen the multi-disciplinary nature of primatological studies. In 1989, a group of young research scholars of primates of Argentina published the first brief review of the state of the art of primatological studies in our country. Those studies focus on monkeys as an experimental biological model in biomedical studies, for an early solution to human problems mostly. Primatological studies proper began to develop intensively as a way to know the species as biological entities in their natural areas with their species-specific characteristics. It is in the final years of the last century where primatology aimed at a better understanding of primate species both in the wild and in captivity. The taxonomy, ecology and ethology studies allowed them to corroborate, by census and survey works of troops in the natural geographic distribution, that there are five species in Argentina: Atelidae: brown howler (*Alouatta guariba clamitans*) and black and gold howler (*A. caraya*), Cebidae: *Aotus azarae* and two species of *Cebus* (*C. nigritus* = *Sapajus nigritus* and *C. paraguayanus* = *Sapajus cay*). Several research centers created and developed in the country over the years are: Centro Argentino de Primates (CAPrim); Programa Argentino para el Estudio de Primates (PARPRI) and Estación Biológica Corrientes (EBCo), among others. In the last decade, different lines of work constituted a guide for research topics in fragmented environments, as well as evolutionary cytogenetic analysis, comparative genetics, paleontology, behavior, diet, social structure, among others, highlighting the importance of primatology studies in Argentina.

Resumen. Este capítulo surge como contribución para los primatólogos en formación. Pensamos que conocer los antecedentes, las etapas y los hitos que caracterizan la historia de la primatología en el país les permitirá disponer de argumentos al momento de proponer nuevos proyectos a la vez que comprender con mayor claridad la necesidad de trabajar con diversas fuentes de conocimientos, a fin de afianzar el carácter multi y transdisciplinar de los estudios primatológicos. En 1989, un grupo de investigadores jóvenes estudiosos de los primates de Argentina, publican la primera breve revisión del estado alcanzado en los estudios primatológicos en nuestro país. En su gran mayoría se trataba

de trabajos que utilizaban monos como modelo biológico experimental en estudios biomédicos, para una pronta solución a problemáticas humanas. No eran áreas de la primatología propiamente dicha, la que con los años se comenzó a desarrollar en forma intensa dedicada a conocer las especies como entidades biológicas en sus ámbitos naturales con sus características especie-específicas. Es en los años finales del siglo pasado donde la primatología va tomando marco con estudios destinados a un mejor conocimiento de las especies de primates tanto en estado libre como en las especies mantenidas en cautiverio de exhibición o a nivel de colecciones de museos o estaciones de cría. A pesar de las décadas transcurridas no hay en la actualidad un *studbook* activo de especies de primates neotropicales o un registro oficial en el orden nacional o provincial para estas especies, asignatura pendiente importante que requiere un tratamiento integral dado los avances que en caracterizaciones de fauna se han logrado en todo estos años. El trabajo en museos brinda información más acotada: Museo de La Plata (MLP), colecciones de 41 ejemplares de especies de monos no humanos, mayormente representado *Alouatta caraya*; Museo Argentino de Ciencias Naturales «Bernardino Rivadavia» (MACN), refiere 191 ejemplares con un predominio de *Cebus* (46%), seguido por *Alouatta caraya* con tan solo un 18% y *Aotus azarae* con un 16% aproximadamente, mientras que tan solo uno o dos ejemplares representaron otras especies, a excepción de seis *Saimiri* sp. que corresponderían a un 6% del total de ejemplares allí reunidos e identificados. Otros, como el Museo Provincial «Florentino Ameghino» (MFA, Santa Fe), contaba con ejemplares de colecciones, que superaban levemente los 25 ejemplares y en el caso del Instituto Miguel Lillo (IML, San Miguel de Tucumán) el registro del año 1989 refería tan solo 17 individuos. Un trabajo posterior muestra en estos mismos museos nacionales una recuperación parcial de las colecciones remarcando la necesidad de una evaluación continua y actualización de las colecciones de primates del Nuevo Mundo en museos nacionales, centrando la atención en categorizar pieles y cráneos y comparando la distribución de los especímenes de acuerdo al género que pertenecen y al origen geográfico de procedencia, cuando lo hubiere. Por su continuidad en el tiempo e importante desarrollo en el ámbito nacional, los más destacados estudios fundacionales de la primatología en la Argentina fueron los aportes al conocimiento que brindó la escuela de paleontología. En la década de 1980 se publican distintas revisiones y actualizaciones bibliográficas sobre la cariología con valor diagnóstico en especie de los Monos del Nuevo Mundo, en particular de las especies autóctonas de la fauna de Argentina. Para ese entonces también la taxonomía, la ecología y la etología de las especies endémicas argentinas permitieron corroborar, por trabajos de censos y relevamiento de tropas en la distribución geográfica natural, que en su distribución más extrema austral los primates neotropicales llegan a Argentina con cinco especies: *Alouatta guariba* y *Alouatta caraya* como atélidos propios y endémicos de nuestra mastofauna, junto a *Aotus azarae* y dos especies de *Cebus* (*C. nigritus* = *Sapajus nigritus* y *C. paraguayanus* = *Sapajus cay*). Dos de estos primates (*Alouatta guariba* y *Aotus azarae*) se encuentran en el *Libro Rojo* en la categoría de «en peligro de extinción». Se hace referencia a los distintos centros de estudios que con el paso de los años se crearon y desarrollaron en el país: Centro Argentino de Primates (CAPrim); Programa Argentino para el Estudio de Primates (PARPRI); Estación Biológica Corrientes (EBCo); así como los trabajos que se fueron ampliando hasta llegar al plantel actual de primatólogos incorporados en distintos equipos de investigación y docencia en diferentes universidades nacionales y extranjeras que llevan adelante proyectos destinados a profundizar el conocimiento de las especies nativas y su relación con los recursos disponibles y la dinámica poblacional accediendo a una formación de grado y postgrado con reconocimiento internacional. En la última década distintas líneas de trabajo se constituyeron en temas guía para la investigación en ambientes fragmentados, particulares de los límites sur de la distribución que llega a Argentina tanto en análisis de citogenética evolutiva, genética comparada, comportamiento, dieta, estructura social; aspectos todos ellos abordados en las formas actuales, destacando los notables avances en el campo del estudio de las formas fósiles propias de la distribución más austral de los primates neotropicales.

Introduction

Most of the earlier work on Primatology in Argentina (*e.g.*, research projects, research lines, manuscripts) is still unpublished, restricted to specialists of a particular discipline or mentioned only in scattered articles. In an attempt to fill the information gap, here we provide an overview of the history of this field. We believe that this review is particularly useful for new primatologists, as the experience gathered points strongly to the importance of inter- and multidisciplinary approaches as source of novel ideas and analytical insights.

Some primate species of biomedical value

Attempts have been made to compile previous studies of primatology in Argentina and document the early efforts of pioneers in this field. In 1989, a team of young researchers, who were taking their first steps in the study of primates of Argentina, published a brief state-of-the-art literature review. Their objective, cited at the beginning of the article, reads: "After compiling the available information on primate studies conducted in our country, we have interviewed different research groups with background in Primatology" (Arditi *et al.*, 1989). Their work could be accomplished because at that time there were very few biologists working with primates and almost none of them were devoted to primatology. Indeed, monkeys were viewed as experimental models within a biomedical frame and results were extrapolated to humans (Mudry, 1980; Colillas and Ruiz, 1986). Such activities were not aimed at studying the primate species as biological entities with species-specific features in their natural environments; results were not analyzed within a theoretical bio-ecological framework or from a biogeographical approach. An example of this is the Centro de Investigaciones en Reproducción Humana y Experimental (CIRHE, Research Center for Experimental and Human Reproduction), which was established in the 1980s. It was (and still is) the only university center in Argentina and one of the few in the world equipped with the facilities required for rearing and holding non-human primates. The CIRHE, founded by Professor Dr. M.A. Mendizábal in March 1983, is among the 180 centers all over the world collaborating with the World Health Organization in reproductive health research; moreover, it is part of the academic unit of the Centro de Educación Médica e Investigaciones Clínicas (CEMIC). At that time, the monkeys kept in the CIRHE were considered to be *Cebus* spp., commonly known as *capuchino* (capuchin) by medical and pharmacological researchers and *caí* by local people. Now, as then, the specimens are born and raised under climate-controlled conditions; they are housed separately in the nursery, in common rooms for young monkeys and individual rooms for adults. Monkeys at birth are treated like human neonates; as they grow, they interact with each other to undergo socialization, and can successfully reproduce in captivity. Their life expectancy at the Center may exceed 30 years. The CIRHE provided valuable information on the reproduction of the genus *Cebus*, through an uninterrupted flow of articles published in specialist journals (Nagle, 1979; 1980; 1986; Nagle and Rosner, 1980; Nagle and Denari, 1982; 1983; Nagle *et al.*, 1989; Carballal *et al.,* 1987). In 1988, CEMIC inaugurated the Unidad de Neurobiología Aplicada (UNA, Applied Neurobiology Unit), for neurobiology and reproduction research, headed by Dr. J. Colombo. Arditi *et al.* (1989) reported that non-human primate species such as *Cebus*, *Saimiri*, *Callithrix*, and *Alouatta* were used as biomedical models to characterize the effect of viral load. Some of these species proved to be suitable for studying the Argentine hemorrhagic fever (Weissembacher *et al.*, 1978), showing pathologic features similar to those observed in humans. Likewise, capuchin monkeys were used during the 1980s to study their susceptibility to different strains of *Trypanosoma cruzi*, the etiological agent of Chagas' disease of major importance in the Americas (Falasca *et al.*, 1987, 1988). In addition, the latter species and males and females of *Saimiri* sp. from Bolivia were used to explore alterations in cranial morphology and dimensions, in the context of a nutritional study (Pucciarelli *et al.*, 1990).

Scientific knowledge and conservation of Neotropical primates gained increasing attention during the 1990s, with the focus being placed on management procedures for improving animal welfare in captivity and semi-captivity, including animal houses. However, it was not until the end of the last century that studies of non-human primate species from wild populations, zoos, breeding stations and museum collections gave rise to the field of primatology.

Primates on exhibit

At that time, little was known about primates in zoos or museums. Bianchini reported that the zoo of La Plata City harbored 26 monkeys, 65% of which were males. In the zoo of Buenos Aires City, Jacome recorded 60 individuals, of which 42% were males, 50% females and about 8% of unknown sex. The zoos of Córdoba and Mendoza provinces exhibited an undetermined number of Platyrrhini and Catarrhini species. Despite the missing information, it is possible that more individuals were housed at CEMIC, the animal house at the Facultad de Medicina, Universidad de Buenos Aires (Faculty of Medicine of the University of Buenos Aires) and the Centro para la Reproducción de Monos Pequeños (CRIMOP, Center for the Breeding of Small Monkeys) in Escobar—where Dr. Tramezzani coordinated experimental neurobiology.

It is worth mentioning that, even today, no studbook—genealogical record—of Neotropical primates is held by the Comité de Manejo Cooperativo de Especies de la Asociación Latinoamericana de Parques Zoológicos y Acuarios (ALPZA, Committee on Cooperative Species Management of the Latin American Association of Zoos and Aquariums), or by the Asociación de Zoológicos y Acuarios de la República Argentina (AZARA, Argentinian Association of Zoos and Aquariums) (ALPZA's coordinator Dr. A. Sestelo, pers. comm., 2014). Taking into account the large amount of information on non-human primate species in Argentina, a comprehensive approach combining and integrating the available data for each taxon is still a pending issue.

In regard to museum collections, the following information is available for the 1990s: 41 specimens of non-human primates at Museo de La Plata (MLP), of which 50% were *Alouatta caraya* (pers. comm., Lic. Bianchini); 191 specimens at Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN), of which 46% were *Cebus* sp., 18% *Alouatta caraya*, 16% *Aotus azarae*, 6% *Saimiri* sp. and a few other taxa (Dr. Brown); 25 specimens at Museo provincial “Florentino Ameghino” (MFA) in Santa Fe Province, and 17 specimens in the Instituto Miguel Lillo (IML) in San Miguel de Tucumán. Some years later, Martinez *et al.* (2005) reported that these museums expanded their collections of non-human primates, despite the fact that data availability depended on the particular interests of the different researchers. These authors stressed the importance of keeping updated catalogues of the New World genera, as well as integrating the taxonomic identification of all skulls and furs with their geographic distribution. Among the material surveyed in 1989, 35 specimens in the MFA and 32 specimens in the IML were identified at the genus level. In the MACN, 384 specimens from six countries that were assigned to 12 genera may actually belong to 15 genera based on the Groves' classification (2001), while the remaining three museums (MLP, MFA and IML) may have 123 specimens of 15 genera.

At present, phenotypic diversification related to environmental factors provides new insight into the evolution of extant non-human primates, particularly at the limits of the species' distribution (Perez *et al.*, 2011). In this framework, furs and bone pieces existing in the museums emerge as important sources of data. Pérez and Arístide, who are researchers in the Division of Anthropology of the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (Faculty of Natural Sciences and Museum, La Plata National University), have conducted the most recent studies based on skull morphometry (pers. comm., 2014). For each specimen they recorded species identification, sex, age, provenance, and, if feasible, collector's name. So far, they have examined the skulls and a few jaws of 220 and 30 well-preserved Neotropical primates from the collections of the MACN and MLP, respectively. It is a burgeoning area of research, which will contribute to a better understanding of the relationships between present-day taxa from different areas of our country, in order to reconstruct temporal changes in their geographic distributions.

Some members of the teaching staff at the Department of Genetics of the Facultad de Ciencias Veterinarias y Ambientales, Universidad J. Agustín Maza (Faculty of Veterinary and Environmental Sciences, J. Agustín Maza University), have also made interesting contributions. The veterinarian D. Ferré and the biologist N. Gorla (CONICET) are dedicated to Genetic Toxicology and Cytogenetics for animal welfare and human well-being. They and other researchers have formed an interdisciplinary working team as an initiative to improve management practices for non-human primates living either under natural conditions, in semi-captivity in breeding centers and rescue shelters, or in captivity for exhibition (Gorla and Ferré, 2012).

During the last 30 years, evolutionary researchers have dealt with the speciation process of present-day non-human primate species at their southern distribution limit. Although the results are presented in detail in other chapters of this volume, it is worthwhile to mention an early doctoral thesis involving the karyology of many endemic species such as *Alouatta caraya*, *Aotus azarae* and *Cebus* spp. (Mudry, 1983). It represented a cornerstone for later studies, which analyzed the genetic variability under different hypotheses using morphological, molecular, and cytomolecular tools (Delprat, 1992; Szapkievich, 2001; Ascunce, 2002; Martinez, 2003; Nieves, 2002; 2007; Steinberg, 2005; 2011; Fantini, 2010; Aristide, 2012; Alvarez-Gonçalvez, 2012). These studies were conducted in captive and semi-captive colonies or free-ranging populations of non-human primate species at their southernmost distribution, and aimed at relating behavior (*e.g.*, activity patterns), diet and social structure (*e.g.*, group dynamics) to morphological and genetic data (Giudice, 2000; Bruno, 2011).

Fossil record as a primary data source for primatology in Argentina

A major contribution to the knowledge of non-human primates of Argentina came from the field of paleontology. Since the beginning of the nineteenth century, Argentinian paleontologists have been aware of the existence of many fossil primate species. All the fossil Neotropical primates or Platyrrhini that have been so far identified—more than one dozen species—were found in Patagonia. About 20 million years ago, they lived in vast forests under climatic conditions similar to those preferred by the extant marmoset and spider monkeys (Fleagle and Tejedor, 2002). Marsupials and primates occurred in forests supporting high biodiversity in the area between Plaza Huincul and Zapala, in the province of Neuquén. At that time, the landscape was characterized by wide valleys with rivers flowing down to small lakes. The subsequent uplift of the Andes caused the desertification of the region, leading to the almost complete extinction of taxa.

At the end of the nineteenth century, Ameghino (1891) described a skull "resembling that of a human being". He postulated that it may have belonged to an ancestor of anthropomorphs and humans (Ameghino, 1891), but this was not supported by further evidence. Many pioneer expeditions to Argentinian Patagonia were carried out during the twentieth century by prominent Argentinian and foreign paleontologists such as Bordas (1942), Kragelievich (1951), Hoffstetter (1969), Hershkovitz (1977), Kay *et al.* (1998; 2001; 2004) and Feagle (1984); Feagle and Kay (1989) and Feagle and Tejedor (2002). They found and described important fossils of non-primate humans (for more information see *http://www.primate-sg.org/diversity.htm*).

Recently, Tejedor and his team from the Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco recovered teeth and mandible fragments from different platyrrhine species (Tejedor 2000; 2005). In 2006, they unearthed remains of the forehead, face, palate and teeth—slightly worn—of *Killikaike blakei* (Tejedor *et al.*, 2006), later synonymized with *Homunculus patagonicus* (Perry *et al.*, 2014). It belongs to a monotypical genus, represents an indicator of lineage and is the most ancient record known in South America. In this way, paleontology has added—and still does—(Tejedor 1996; 2000; 2013) valuable data to the information on the genetics, physiology and anatomy of present-day native species (Mudry, 1983; Zunino, 1987). Tejedor and Novo, this volume, provides a more detailed and comprehensive review of non-human primate paleontology in Argentina.

A better characterization of non-human primate species in Argentina

Since the 1980s, much work has been done on a wide variety of topics concerning present-day species. Studies have dealt not only with their ecology and behavior, but also with reproductive, digestive, and circulatory aspects, nervous system responses, and parasitological surveys. Reviews published between 1981 and 1984 deepened our knowledge of the karyotype of New World monkey species, in particular from Argentina. For the latter species, no previous genetic description was available from specimens captured alive and of known provenance or from monkey-derived cell lines obtained in the country (Mudry and Salum, 1981; Mudry *et al.*, 1984; Matayoshi *et al.*, 1986; Mudry and Nieves, 2010). Before the 1980s, Argentinian scientists in different national research centers developed projects for the characterization of primates *in vivo* and *in vitro*, from tissue cultures. Early in the 1980s there was evidence that a low number of Vero cell culture passages resulted in karyological uniformity. Marker chromosomes, which appeared from passage 12 to passage 15, showed different structural rearrangements that were analyzed using the G-banding staining method; the analysis indicated that these marker chromosomes detected with different G-bands rearrangements were the consequence of cells division in cultured conditions, them they were considered as originated *de novo* due to growth *in vitro* (Gorostiaga, 1999; OPS, 2002). The importance of this investigation lies in the fact that both the karyotypic and immunological characterizations—carried out by the Panamerican Zoonosis Institute (IMPAZ) in Buenos Aires—of the Vero line resulted in a local typification for virus vaccine production in Argentina.

In addition, many new research areas involving wild populations of species from Argentina, border countries and areas in Mexico (northernmost distribution limit of Neotropical non-human primates) were developed. These covered applied research (*e.g.*, conservation and management) and also basic science (genomic structure and its variation among species).

Taxonomic, ecological and behavioral studies using census-derived data obtained from surveys of troops revealed that neotropical primates at their southernmost distribution in Argentina are represented by the following species: *Alouatta guariba* and *Alouatta caraya*, which are endemic to our country, *Aotus azarae* and two *Cebus* species, *C. nigritus* (= *Sapajus nigritus*) and *C. paraguayanus* (= *Sapajus cay*) (Brown and Zunino, 1994). Of these, *A. guariba* and *A. azarae* are listed on the IUCN Red List as critically endangered, with their habitats being found in non-protected areas under the impact of anthropogenic activities (Ojeda *et al.*, 2012). Since 2012 the genus *Cebus* was divided in two genera: *Cebus* and *Sapajus* (Boubli *et al.*, 2012; Lynch Alfaro *et al.*, 2012a, 2012b.). While the authors support their taxonomic decision on the existence of multiple data sources such as variability in mitochondrial DNA markers, the biogeographical distribution and ecological character, the evidence of other research groups do not hold this subdivision of the genus or reallocations to species level. We believe that these decisions require greater consensus of researchers, whose agreement is sustained by a considerable amount of consistent evidence each other, thereby preventing changes in the taxonomy to overestimate the number of species (Jameson Kiesling *et al.*, 2015; Nascimento *et al.*, 2015).

National institutions for the development of new primatologists

Originally, the lack of an academic background in primatology at national universities made it difficult for students to pursue both undergraduate and postgraduate education in the discipline. At that time, the Argentinian universities provided a solid foundation in mammalogy, botany and/or renewable resources, but specific knowledge had to be completed abroad after obtaining the Ph.D.

In regard to the study of primates endemic to Argentina, teams composed of three to five young scientists dealt with research and development projects. Some years after we published our earlier work, different primatologists explored other research lines under diverse approaches, thus extending the spectrum of topics. The Centro Argentino de Primates (CAPrim, Argentinian Primate Center), which operated between 1980 and 2001, was first headed by Dr. O. Colillas, and then by other Argentinian primatologists such as the veterinarian Dr. J. Ruiz and Dr. G. Zunino, with the active collaboration of the ecologists Dr. A. Brown and Dr. D. Rumiz. CAPrim provided professionals not only from Argentina but also from other Latin American countries with a learning environment while they pursued their master's degree or doctoral degree. The center ran training courses in experimental field and laboratory (*e.g.*, genetics) settings, aiming at the characterization of specimens on exhibition, in semi-captivity or captivity, as well as of natural populations. Studies included either native species belonging to the genera *Alouatta*, *Cebus* and *Aotus* or species from neighboring countries such as Bolivia (*Saimiri* sp.), Paraguay (*Cebus* sp.) and Brazil (*Callitrhix* sp.), among others (Mudry, 1983; Brown, 1986; Zunino, 1987; Rumiz, 1987; Brown and Zunino, 1994; Fernández-Duque, 1996). Most of the studies carried out by L. Oklander before and after she obtained her Ph.D. at the University of Buenos Aires are detailed in another chapter of this book (see Oklander, 2007).

In 1996, the Mirikiná Project was launched in the province of Formosa to initiate studies on the ecology and behavior of *Aotus azarae*, headed by Dr. E. Fernández-Duque. This species attracted the interest of specialists working in the field because of its monogamous mating system and cathemeral activity pattern, particularly in Argentinian populations. Dr. E. Fernández-Duque, together with Dr. C. Juárez, among other national and foreign collaborators, has addressed a variety of research fields including genetics, behavior, demography and conservation. Moreover, they engaged in mentoring graduate and postgraduate students and in teaching postgraduate courses. At present, they keep on working in long-term projects. This scientific team is highly productive, with many articles being published (Fernández-Duque *et al.*, 2009; Juárez *et al.*, 2011; Fernández-Duque and Huck, 2013; Fernández-Duque, 2014; among others).

Dr. M. Di Bitetti completed his undergraduate degree in Biology at Universidad Nacional de La Plata and earned a Ph.D. from the Department of Ecology and Evolution at the State University of New York at Stony Brook in 2001. Currently, Dr. Di Bitetti is Director of the Instituto de Biología Subtropical (IBS), Facultad de Ciencias Forestales, Universidad Nacional de Misiones – UNaM (Subtropical Biology Institute, UNaM). Here, he has supervised many junior ecologists and developed numerous research projects on primates from northeastern Argentina such as *Cebus* and *Alouatta guariba*, dealing particularly with ecological, behavioral and socio-economic aspects (Di Bitetti, 1997; Agostini *et al.*, 2008; 2014). The primatologists Ingrid Holzmann and Celia Baldovino, who completed their postgraduate degrees in the early 2000s, are among the scientists supervised and/or trained in the Instituto de Biología Subtropical (IBS). Galliari, another young researcher, described in 2005 the appendicular skeletal maturation in captive-born squirrel monkeys (*Saimiri boliviensis*).

The Estación Biológica de Usos Múltiples (EBCo–MACN, Field Station for Multiple-use Purposes), was an academic and research institution created in 2001, which covered wider objectives than primatology *per se.* Indeed, its main goal was the protection of animals and plants of the province of Corrientes (northern Argentina) and the development of an environmental education plan, with activities being undertaken in the area surrounding the station. Here, studies of monkeys were directed by Zunino until 2011 and by Kowalewski thereafter (Kowalewski and Zunino, 2004; Zunino *et al.*, 2007; 2008). Many researchers completed their fellowship at the field station, such as Bravo, Kowalewski and Oklander, followed by Pavé, Perez Rueda, Fernández, Peker, and Raño. On the other hand, more than 20 national and foreign students accomplished their undergraduate and doctoral theses at the EBCo. To further illustrate the improved academic performance of students undergoing training at the station and the wide range of topics they investigated, it is worth mentioning a recent article in which various co-authors propose different conservation strategies for a primate species that is sparsely distributed in Argentina and has been devastated by the yellow fever (Agostini *et al.,* 2014). In the last decade, other teams composed of Argentinian and foreign researchers also contributed to the field of primatology (Nieves and Mudry, 2011; Kowalewski *et al.*, 2011; Pavé *et al.*, 2012; Fernández *et al.*, 2013). They taught postgraduate courses for national and international students, covering topics different from those included in the biology undergraduate education programs at universities of Argentina and other Latin American countries; in such context, this literature adds to the publications of Cabrera and Yepes (1940); Cabrera (1957; 1961), Schneider and Rosemberger (1996), Rylands *et al.* (1997), Groves (2001), Rylands and Mittermeier (2009), among others. Courses addressed a variety of updated issues involving taxonomic classification, diet, ecology (*e.g.*, social structure, population dynamics), behavior, genetics and evolution of primates. In this regard, results revealing the negative effect of habitat loss—due to increased anthropogenic pressure in the region—on changes in population size, led to the design of research projects that could be developed in natural field laboratories within the distribution range of neotropical primates (Zunino *et al.*, 2007; Oklander *et al.*, 2010; 2014; Pavé *et al.*, 2010; Bruno *et al.*, 2012; Milozzi *et al.*, 2012; 2013).

Currently, all the scientific teams mentioned above keep on contributing to the expansion of our understanding of Neotropical primates. The information gathered during recent years provides a more complete picture of their demography, behavior and factors related to disease transmission. At the same time, previous knowledge of primate species in Argentina serves as a basis for the development of new research lines dealing with more specific aspects.

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3

PLATYRRHINE EVOLUTION AND PALEOBIOGEOGRAPHY REVISITED

EVOLUCIÓN Y PALEOBIOGEOGRAFÍA DE LOS PLATIRRINOS: NUEVOS APORTES

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Abstract. The evolutionary history of platyrrhines, today widely distributed in the Neotropical Region, presents various controversial issues that have been debated and discussed during the last three decades. The notable increase in the fossil record has reoriented our previous hypotheses in benefitting from a better knowledge of the diversity, distribution and phylogeny of the platyrrhines in the past, as well as their relationships with the living forms. These advances led to alternative interpretation of the platyrrhine fossil record and evolutionary history, particularly regarding the early to middle Miocene Patagonian forms that were often discussed as a distinctive stock with primitive appearance, difficult to integrate with the living clades. Another more advanced group of primates appears in the middle Miocene La Venta region, Colombia. Their affinities are in several ways comparable to the Patagonian forms. In light of the new oldest record of South American primates, from the late Eocene of Santa Rosa, Peru, the scenario changes notably and new evidence strongly supports the inclusion of all Patagonian forms into the crown Platyrrhini, as they exhibit even more advanced morphologies than previously thought. The proposal presented here suggests the possible existence of a western paleobiogeographic corridor that may have connected the areas where platyrrhines inhabited, from the austral regions to the northernmost Neotropics. Fossiliferous sites in western Argentina, Chile, and Peru, ranging from the early to late Miocene, reinforce the idea of this paleobiogeographic region facilitating the dispersion of the Patagonian primates, especially when considering that Santa Rosa is also located to the west and its primates are possibly part of an extensive radiation that certainly occurred during pre-Miocene times.

Resumen. La interpretación de la historia evolutiva de los primates platirrinos, hoy distribuidos ampliamente en la Región Neotropical, ha originado controversias especialmente durante las últimas tres décadas. Las principales discusiones se centraron en la filogenia, donde las evidencias moleculares coincidieron finalmente en un alto porcentaje con las clasificaciones basadas en la morfología. Así, actualmente hay consenso en la existencia de tres familias: Atelidae (Atelinae: *Ateles*, *Lagothrix*, *Alouatta*, *Brachyteles*), Cebidae (Cebinae: *Cebus*, *Saimiri*; Callitrichinae: *Callimico*, *Saguinus*, *Leontopithecus*, *Callithrix*, *Cebuella*, *Mico*, *Callibella*) y Pitheciidae (Homunculinae: *Callicebus*; Aotinae:

Aotus; Pitheciinae: *Pithecia*, *Chiropotes*, *Cacajao*), y todos los representantes extintos. En este esquema, solo la posición de *Aotus* permanece controvertida, ya que las filogenias moleculares lo incluyen entre los cébidos, en tanto la morfología y características ecológicas y comportamentales concuerdan en que mantiene grandes afinidades con los pitécidos. El considerable incremento del registro fósil da cuenta hoy de 33 géneros extintos, más dos especies extintas de los actuales géneros *Aotus* y *Alouatta*. Esta diversidad no se condice con los 18 géneros vivientes reconocidos, aunque la distribución de los extintos tiene un rango latitudinal y temporal que excede la distribución de los vivientes. Pero más allá de las diferencias en el número de géneros, el registro fósil ha orientado nuestras previas hipótesis hacia un conocimiento más profundo de la diversidad, distribución y relaciones filogenéticas de las especies extintas, así como sus probables vínculos con las formas actuales. De este modo, surgieron alternativas opuestas en la interpretación del rol desempeñado por los primates extintos del Mioceno inferior a medio de la Patagonia [datados entre 20 y 15,7 millones de años (Ma)] en la evolución de los Platyrrhini, que se fundamentan en la diversidad y relativa modernidad anatómica de los fósiles del Mioceno medio de La Venta, Colombia (aproximadamente entre 11–13 Ma). En la Patagonia se registraron, hasta el momento, 8 géneros y 10 especies que representan a 3 subfamilias de platirrinos. En La Venta se hallaron 11 géneros, de los cuales la mayoría tiene similitudes morfológicas con las formas modernas, y serían los clados mayormente amazónicos. Siendo Patagonia y La Venta dos enclaves que preservan aproximadamente dos tercios de los géneros extintos, hallar una posible continuidad entre ambos enclaves permitiría comprender gran parte de la historia evolutiva de los platirrinos. La propuesta aquí presentada promueve la existencia de un corredor paleobiogeográfico a través del oeste sudamericano (CPOS, Corredor Paleobiogeográfico Occidental Sudamericano), extendido desde la Patagonia hasta alcanzar la cuenca amazónica, donde se diversificaron posteriormente gran parte de los clados actuales con sus variadas adaptaciones; la existencia de ambientes poco propicios para la dispersión de los primates a través del centro y este de Argentina en tiempos del Mioceno respaldaría esta hipótesis del CPOS, sumado al registro de numerosos yacimientos de mamíferos en ese sector occidental. La idea de una continuidad evolutiva y paleobiogeográfica entre los primates patagónicos, los de Amazonia y los del yacimiento colombiano de La Venta está respaldada también por los hallazgos aislados de primates fósiles que se produjeron en Chile, Bolivia y Perú, incluyendo en este último país a los más antiguos registrados recientemente, los cuales datan del Eoceno tardío, sumados a otros registros del Oligoceno inferior también recientemente reportados. Los novedosos hallazgos de Perú extienden el biocrón de los Platyrrhini unos 10 millones de años y denotan un origen africano de los ancestros platirrinos, hipótesis que por primera vez tiene evidencias sólidas en las similitudes morfológicas comparando el nuevo género descripto para Santa Rosa, *Perupithecus*, y un género del norte africano, *Talahpithecus*. De este modo, el conocimiento acerca de la relación entre los primates del Nuevo Mundo y los antropoideos del Paleógeno de África entra en una etapa de renovados estudios para comprender adecuadamente sus relaciones filogenéticas en la medida que los registros se incrementen, y nos abre un panorama más complejo sobre la radiación basal de los primates del Nuevo Mundo. Así quedan por responder numerosos interrogantes en torno a las formas más antiguas, y se desprenden hipótesis alternativas: los primates del yacimiento peruano del Eoceno de Santa Rosa podrían representar un *stock* antropoideo pre-platirrino que se diferenció en África a partir de ancestros antropoideos, o bien se considerarían platirrinos ya diferenciados a partir de los mismos ancestros africanos. Finalmente, también cabe la hipótesis de la posible diferenciación de los platirrinos en África, donde posteriormente se extinguieron, siendo *Talahpithecus* un representante de esa antigua radiación.

Introduction

The platyrrhine primates, or New World Monkeys (NWM) are a diverse group currently distributed in the Neotropical Region, but whose past populations expanded also into the Caribbean Greater Antilles and as far south as Patagonia. It is noted that in recent reviews of the biogeographic areas and transition zones in South and Central America (see Morrone, 2006), the Neotropical Region is recognized as part of the Holotropical Kingdom, which extends from southern Mexico to northeastern Argentina. Thus, the extinct Patagonian primates, well to the south, are part of a different biogeographic zone, the Andean Region that is part of the Austral Kingdom. Amidst the vast Neotropics, primates have diversified, occupying several adaptive ecosystems, exploiting a variety of diets and locomotor postures, as well as body sizes; however, all are basically arboreal forms.

In recent years, morphological and molecular studies have converged on a systematic arrangement of platyrrhines into 3 families, and the living representatives are as follows: Atelidae (Atelinae: *Ateles*, *Lagothrix*, *Alouatta*, *Brachyteles*), Cebidae (Cebinae: *Cebus*, *Saimiri*; Callitrichinae: *Callimico*, *Saguinus*, *Leontopithecus*, *Callithrix*, *Cebuella*, *Mico*, *Callibella*), and Pitheciidae (Homunculinae: *Callicebus*; Aotinae: *Aotus*; Pitheciinae: *Pithecia*, *Chiropotes*, *Cacajao*). Only the position of *Aotus* remains controversial, because the molecular studies suggest it is related to the Cebidae (Schneider, 2000), while the morphological evidence supports a pitheciid affinity (Rosenberger and Tejedor, 2013).

The platyrrhine fossil record is relatively diverse, but very scarce in comparison with other mammals occurring at the same paleontological sites. The oldest records are from Santa Rosa, Peru, of ?late Eocene age, followed by Salla, Bolivia, at 26 Ma, and the Patagonian and Chilean forms, 20–15.8 Ma. The southern platyrrhines from Chile and Argentinian Patagonia are recorded in what was considered the Austral Kingdom, out of the Neotropical Region, thus the informal name “Neotropical primates” attributed to modern platyrrhines would not be appropriate, for their evolutionary history includes an important record of 8 genera outside the Neotropics. Additionally, the study of Cenozoic evolution of Neotropical and Patagonian mammals does not provide information regarding their adaptive responses as members of separate kingdoms. Platyrrhine primates are African immigrants that arrived certainly during the Eocene, and they may have had a different response compared to native mammals such us metatherians and native ungulates. This is a field to explore that, until now, could only be discussed speculatively. However, a phylogenetic debate arose regarding the relationships between the Patagonian forms and Neotropical extinct and living platyrrhines, resulting in two alternative hypotheses that will be described below.

A total of 33 extinct platyrrhine genera have been described thus far from South America and the Caribbean Greater Antilles (Fig. 1), as well as three extinct species of two living genera. The famous locality of La Venta, Colombia, has 11 genera, while Patagonia is second in diversity, with eight genera. One genus is from Chile, four genera are from the Caribbean, and four from Brazil. The forms from La Venta are among the most modern anatomically, and they contrast with the primitive aspect of several taxa from Patagonia, and they also differ from the peculiar Caribbean forms, probably because the latter are island endemics. In addition, the oldest records from Bolivia are odd specimens with particular adaptations not easily compared with other extinct platyrrhines.

In the present work, an update of the platyrrhine evolutionary history and paleobiogeographic scenarios will be provided. In this context, the evolutionary continuity between Patagonian and Neotropical forms will be emphasized.

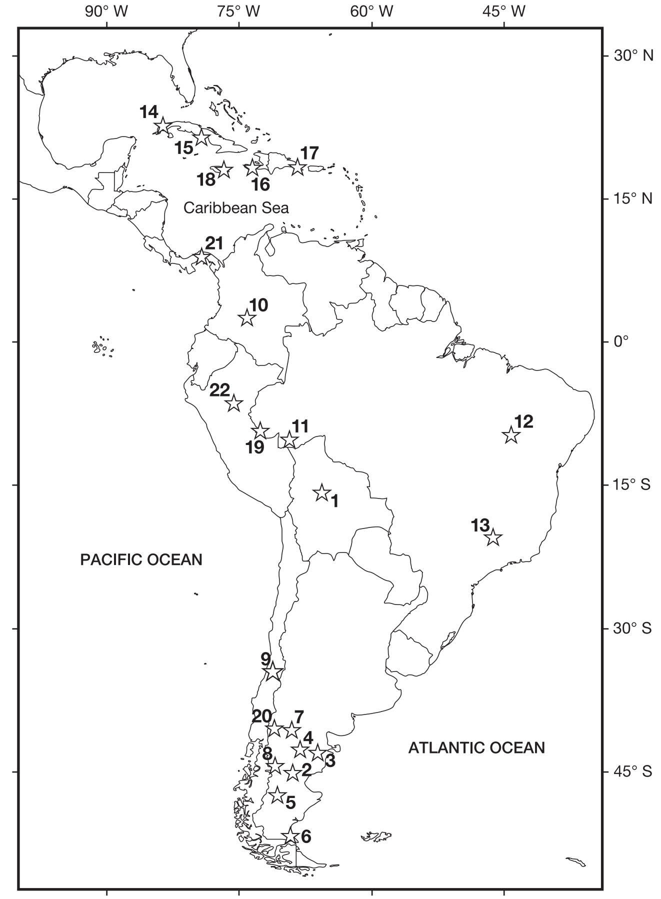

Figure 1. Map of South America and the Caribbean showing the primate sites. **1:** Salla, Bolivia (*Branisella*, *Szalatavus*); **2:** Gran Barranca, Chubut, Argentina (*Mazzonicebus*); **3:** Gaiman, Chubut, Argentina (*Dolichocebus*); **4:** Pampa de Sacanana, Chubut, Argentina (*Tremacebus*); **5:** Pinturas Formation, Santa Cruz, Argentina (*Soriacebus*, *Carlocebus*, gen. et sp. nov.); **6:** Coastal sites of Santa Cruz Formation (Killik Aike Norte, Cerro Observatorio, Estancia La Costa), Santa Cruz, Argentina (*Killikaike*, *Homunculus*); **7:** Cañadón del Tordillo, Neuquén, Argentina (*Proteropithecia*); **8:** Alto Río Cisnes, Chile; **9:** Abanico Formation, Chile (*Chilecebus*); **10:** La Venta, Colombia (*Cebupithecia*, *Stirtonia*, *Neosaimiri*, *Laventiana*, *Nuciruptor*, *Miocallicebus*, *Micodon*, *Lagonimico*, *Mohanamico*, *Patasola*, *Aotus dindensis*); **11:** Río Acre, Brasil (*Acrecebus*, *Solimoea*); **12:** Toca de Boa Vista, Bahía, Brasil (*Protopithecus*, *Caipora*, *Cartelles*, *Alouatta mauroi*); **13:** Lagoa Santa, Minas Gerais, Brasil; **14:** Pinar del Río, Cuba; **15:** Domo de Zaza, Cuba; **16:** Trouing Jérémie, Haiti (*Insulacebus*); **17:** Cueva de Berna and Padre Nuestro State Park, Dominican Republic (*Antillothrix*); **18:** Long Mile Cave and Jackson's Bay Cave, Jamaica (*Xenothrix*); **19:** Santa Rosa locality, Peru (*Perupithecus*); **20:** Cerro Bandera, Neuquén, Argentina (Platyrrhini indet.); **21:** *Panamacebus*, Las Cascadas Formation, Panama. **22:** *Canaanimico*, Contamana area, Peru. (Modified from Tejedor, 2013).

The platyrrhine fossil record

The oldest records

Until recently, the oldest record of platyrrhines were from Salla, Bolivia, at 26 Ma (see below). However, new and outstanding discoveries in the Santa Rosa local fauna, eastern Peru, are suggested to be ?late Eocene (Bond *et al.*, 2015), about 10 million years older. The fact that caviomorph rodents have also been found in Peru, in the Contamana area, which is dated at about 41 Ma, or late-middle Eocene (Antoine *et al.*, 2011), hinted that platyrrhines may have arrived much earlier, together with rodents.

The material from Santa Rosa comprises one complete upper molar, which is the holotype of the new genus and species *Perupithecus ucayaliensis* (Fig. 2), as well as two incomplete upper molars and one additional lower molar, unassigned. Surprisingly, the upper molars exhibit a primitive morphology, with some characters reminiscent of the living callitrichines (although callitrichine status as modern forms contradicts this resemblances), as well as the small size comparable approximately to the living *Callimico*.

But the most remarkable information that these upper teeth provide is the possible phylogenetic link with the late Eocene African anthropoid *Talahpithecus parvus*, from Libya (Jaeger *et al.*, 2010). So, this find not only contributes to extending the biochron of platyrrhines back at least 10 million years, but it also reinforces the hypothesized African link —largely supported by many scholars even in the absence of old platyrrhine fossils—and the concept is now improved. The phylogenetic analysis of Bond *et al.* (2015) placed *Perupithecus* and *Talahpithecus* as sister taxa, the stem of all other known platyrrhines.

The Santa Rosa lower molar is intriguing for its unusually primitive morphology, having a general primate appearance but with distinct characteristics that led us to suspect it does not match with any of the three isolated uppers. It has a low paraconid in the trigonid, imparting a triangular occlusal shape, and a well developed talonid with twinned entonconid-hypoconulid. Similarities shared with African forms are vague, and in the phylogenetic

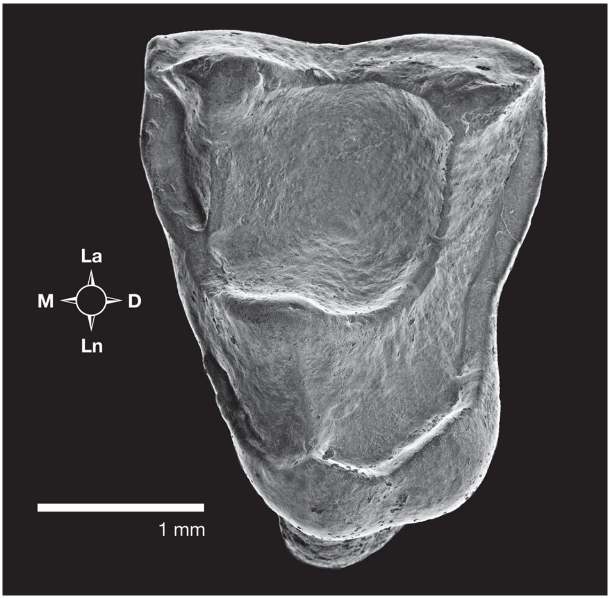

Figure 2. Occlusal view of CPI–6486 (LACM 6289/155085), M?1, holotype of *Perupithecus ucayaliensis*, from Santa Rosa, Peru (courtesy of Kenneth Campbell, Jr.). Letters indicate the position of the molar: mesial (M), distal (D), labial (La) and lingual (Ln).

analysis this tooth appears as a more basal branch relative to *Proteopithecus* and *Catopithecus*, fossils from the African latest Eocene.

The second oldest platyrrhines recorded come from the locality of Salla, Bolivia, with an age of 26 Ma (Kay *et al.*, 1998). The two genera described are *Branisella* and *Szalatavus* (Hoffstetter, 1969; Rosenberger *et al.*, 1991a; Takai *et al.*, 2000; Kay *et al.*, 2002), whose phylogenetic relationships have been largely debated, although in a recent work *Branisella* falls as stem to the remaining platyrrhines, living and extinct (see Bond *et al.*, 2015). The morphological patterns of both genera show a closer resemblance with callitrichines, or cebids *sensu lato*, such as subtriangular upper premolars and molars, extremely reduced hypocone, and conical p2, similar to *Callimico*. The geographic and temporal isolation of Salla, far from the richer primate sites of Patagonia and Colombia, impede any firm conclusions regarding the position of *Branisella* and *Szalatavus*. The associated mammalian fauna indicates open paleoenvironments with grasslands, including high-crowned mammals (MacFadden, 1990), a factor in the inference of semiterrestrial habits for *Branisella*, as suggested by Kay *et al.* (2002).

Patagonia

The southern forms from Patagonia have been a matter of discussion since their early discovery. Ameghino (1891) described the second fossil primate ever found in South America, *Homunculus patagonicus*, which he originally implicated in human evolutionary history. Currently, we know that *Homunculus* is one of the better represented fossil platyrrhines, including several remains of skulls, postcranium, and dentition (Fig. 3) (Tauber, 1991; Tejedor, 2000; Tejedor and Rosenberger, 2008; Kay *et al.*, 2012). Also, several other partial skulls have been recently reported as pertaining to *Homunculus*, but a deep taxonomic treatment was still not provided (Perry *et al.*, 2010; Kay *et al.*, 2012). All this material was collected in southeastern Santa Cruz Province, in the homonymous Santa Cruz Formation, dated at 16.5 Ma (late-early Miocene, Fleagle *et al.*, 1995; Tejedor *et al.*, 2006). This taxon shares similarities with the living *Callicebus* and the extinct *Carlocebus*, from the Pinturas Formation (see below), especially in the bunoid aspect of the molars, with prominent and broad cusps and developed hypocone. However, *Homunculus* exhibits a mandible acutely similar in shape to that of the living *Aotus* (Tejedor, 2000; Tejedor and Rosenberger, 2008); also, a more recently described mandible confirms the *Aotus*-like shape (Perry *et al.*, 2010). As suggested previously (Rosenberger, 1979, *et seq.*), this shared morphology reinforces by extension the possibility of phylogenetic relationships between *Aotus* and *Callicebus*, thus justifying their inclusion into the Pitheciidae.

The oldest records from Patagonia come from Chubut Province, with 3 genera dated to approximately 20 Ma (early Miocene). *Tremacebus harringtoni* was recovered in northern Chubut, at the locality of Sacanana, close to the town of Gan Gan (Rusconi, 1935), and has been described as related to *Aotus* especially because of the large orbit size and other traits of the skull, but the damaged teeth preserved in the holotype are broken, thus preventing further detailed comparisons (see Hershkovitz, 1974).

Dolichocebus gaimanensis was also recorded in Chubut, near Gaiman, in the eastern part of the province (Bordas, 1942; Kraglievich, 1951). The taxon includes an edentulous

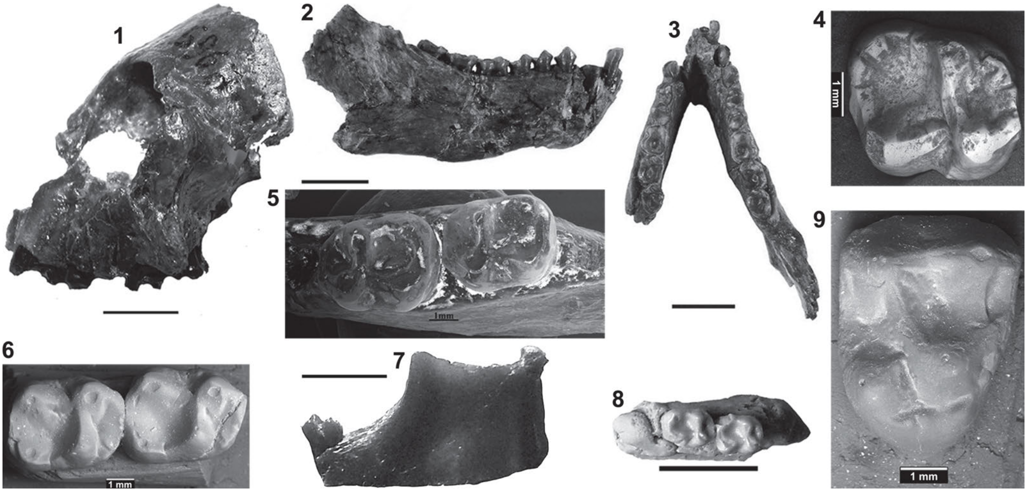

Figure 3. Specimens attributed to *Homunculus patagonicus*. **1:** MACN–A 5968, left partial skull; **2:** MACN–A SC5757 (neotype), right mandible in buccal view; **3:** Occlusal view of the neotype; **4:** MLP–55–XII–13–156, right m1; **5:** MACN–A 10403, left mandibular fragment with m1–2; **6:** MACN Pv SC336, mandibular fragment with m1–2 in occlusal view; **7:** MACN–A 5969^{a}, fragment of an ascending ramus with m2 and alveolous of m3 in lateral view; **8:** MACN Pv SC339, mandibular fragment with m1–2 in occlusal view; **9:** MACN Pv SC334, right M1 in occlusal view (modified from Novo, 2015).

cranium without mandible, and several isolated teeth found later and attributed to the genus (Kay *et al.*, 2008). *Dolichocebus* was described as related to the lineage of *Saimiri* (Rosenberger, 1979; Rosenberger and Fleagle, 1981; Tejedor, 2000) as a cebine, with shared traits such as the elongate skull, the shape of the orbits, and the narrow interorbital septum (but see Kay *et al.*, 2008). Also, the presence of an interorbital fenestra, as in *Saimiri* among the living cebines, has been a matter of discussion regarding its possible natural or artificial origin (see discussions in Rosenberger, 1979; Kay *et al.*, 2008), since it could have been a product of breakage during preparation. Based on the characteristics of the isolated teeth originally reported by Fleagle and Kay (1989), *Dolichocebus* was compared to *Saimiri* or callitrichines, but subsequent new findings (Kay *et al.*, 2008) led the authors change their view and suggested that *Dolichocebus* is a stem platyrrhine. There are relevant similarities between the dental characteristics of lower teeth of *Dolichocebus* and some specimens of *Neosaimiri*, from La Venta (see below).

Two isolated teeth were recovered in levels assigned to the Deseadan or Colhuehuapian sediments of Cerro Bandera, Neuquén Province (Kramarz *et al.*, 2012), a canine and a p4, both comparable with generalized primates from the Pinturas Formation, such as *Carlocebus*. The associated fauna indicate some elements from Deseadan deposits, while others are typically Colhuehuapian, thus obscuring the exact age of Cerro Bandera. It may be the oldest primate site in Argentina.

Among the Colhuehuapian primate remains from Patagonia, from levels at Gran Barranca, south-central Chubut Province, is a remarkable report involving the first description of *Mazzonicebus almendrae* by Kay (2010) and the recent finding of another specimen in a new locality for the genus (Novo *et al.*, 2017), who compared it to the slightly younger

Soriacebus ameghinorum (and the smaller species, *S. adrianae*) (Fig. 4) from the Pinturas Formation (Fleagle *et al.*, 1987; Fleagle, 1990; Tejedor, 2005a, b; Novo and Fleagle, 2015). The author named a new subfamily, Soriacebinae, to include both genera. Based on this comparison, Kay (2010) supported his view of the Patagonian platyrrhines of a stem radiation, that includes all the named genera except *Proteropithecia neuquenensis*, from the Collón Curá Formation in Neuquén Province (Kay *et al.*, 1998).

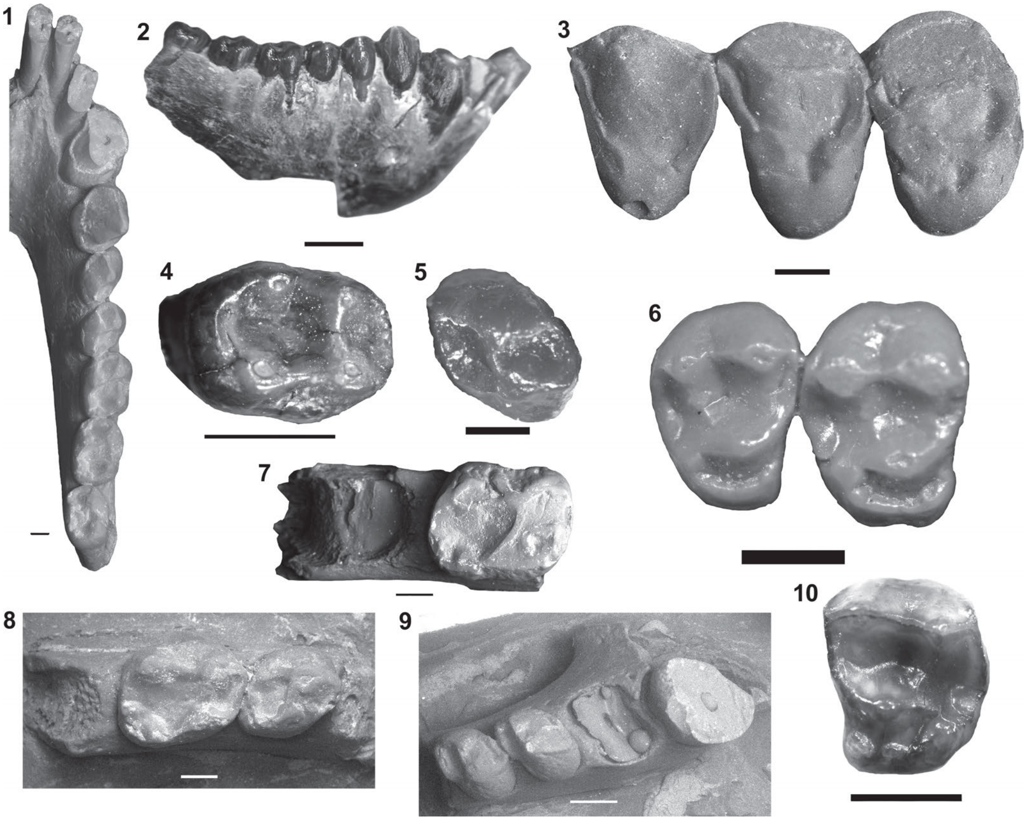

Figure 4. The genus *Soriacebus*. MACN Pv SC2, holotype of *S. ameghinorum*; right mandible in occlusal (**1**) and buccal (**2**) views. **3:** MACN Pv SC4, left maxillary fragment with P2–4 in occlusal view. m1 of the type of *S. ameghinorum* (4) and right p4 (**5**) in occlusal. **6:** Occlusal view of MACN Pv SC67, right M1–2 of *S. adrianae*. **7:** Occlusal view of MACN Pv SC5, an m1 of *S. adrianae*. **8:** MACN Pv SC344, left mandibular fragment with p4–m1 of *S. adrianae*. **9:** MACN Pv SC59, holotype of *S. adrianae* in occlusal view. **10:** MACN Pv SC269, left M1 of *S. adrianae* in occlusal view (modified from Novo, 2015).

Mazzonicebus is characterized by a pitheciine-like dentition, especially with its anterior teeth exhibiting procumbent incisors, large p2, and lower molars with a relatively quadrangular crown. This morphology closely resembles *Soriacebus*, with the exception of the latter's lower molars that are more elongate, uppers with the hypocone reduced in upper molars, and more robust and projecting canines. All these characters were discussed by Rosenberger *et al.* (1990) and Tejedor (2005a, b) to support the view of the pitheciine status of *Soriacebus*, especially based on the procumbent, compressed lower incisors and robust canines, as well as the posteriorly deep mandible. *Proteropithecia* is represented by rare isolated teeth, but it exhibits more derived pitheciine characters that led to Kay *et al.* (1998; 2008, *et seq.*) to hold the view that *Proteropithecia* is the only Patagonian taxon pertaining to the crown Platyrrhini, as part of the subfamily Pitheciinae.

Also from the Pinturas Formation come two species of the genus *Carlocebus*, *C. carmenensis* and *C. intermedius* (Fleagle *et al.*, 1987; Fleagle, 1990) (see Fig. 5). *Carlocebus* resembles *Homunculus* in general dental morphology, although *C. carmenensis* is larger and more bunodont. Along with the living *Callicebus*, *Carlocebus* and *Homunculus* may be parts of a single monophyletic clade.

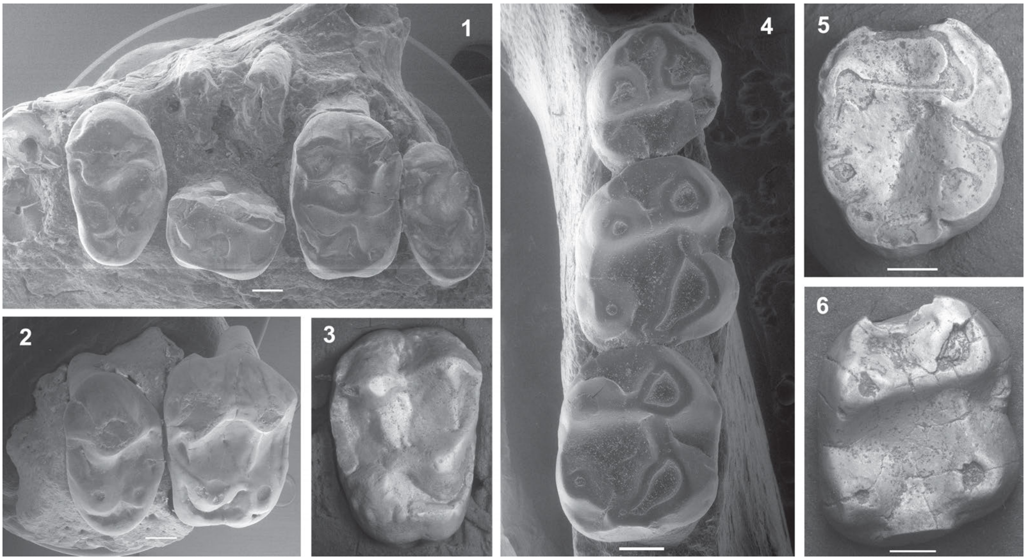

Figure 5. The genus *Carlocebus*. **1:** Occlusal view of MACN Pv SC400, maxilla with left P4–M3. **2:** MACN Pv SC230, maxillary fragment with P4–M1. **3:** MACN Pv SC254, right M2 in occlusal view. **4:** MACN Pv SC266, holotype of *C. carmenensis*, mandibular fragment with p4–m2 in occlusal view. **5:** MACN Pv SC248, right m3. **6:** MACN Pv SC370, M1 or M2 in occlusal view (modified from Novo, 2015).

The southernmost fossil primate ever discovered comes from the southeast of Santa Cruz Province, from the locality Killik Aike Norte (Santa Cruz Formation, late-early Miocene), on the northern bank of the estuary of the Gallegos River. It was named *Killikaike blakei* (Tejedor *et al.*, 2006) and includes a partial skull preserving an almost intact face with part of the dentition well preserved, and a second specimen, a piece of maxilla with M1–3 (Fig. 6). The face of *Killikaike* has notable similarities with the extant *Saimiri* in having an elevated frontal with large anterior brain volume (the frontal bone is complete and the brain mold is perfectly observable and measurable), as well as oval-shaped orbits with vertical expansion as seen in *Dolichocebus*, and with a relatively narrow interorbital septum. The anterior brain volume estimated for *Killikaike* is 1.6 ml, closer to the average in *Saimiri* (1.8 ml), in contrast to the lower average in *Callicebus* (1.0 ml), both of similar body size. These traits are remarkable because *Killikaike*, at 16.5 Ma (see Tejedor *et al.*, 2006), represents the oldest primate recorded with dramatic encephalization, several millions of years before our oldest known hominin ancestors and relatives. In contrast to these advanced traits, the face of *Killikaike* is still somewhat prognathous, indicating a primitive retention from anthropoid ancestors.

Kay and co-workers (Kay *et al*., 2008; Kay 2010; Kay *et al*., 2012; Perry *et al*., 2016) have questioned the validity of *Killikaike*, considering it as synonymous with *Homunculus* based on studies of new cranial material, but lacking detailed comparisons with a bona fide partial skull of *Homunculus*, MACN–A 5968, from the original Ameghino collection (see Tejedor and Rosenberger, 2008); this is the only skull that should be taken into account to assignate any other cranial specimen to *Homunculus*. In a recent publication, Perry *et al*. (2016) mentioned MACN–A 5968 without in-depth comparisons, especially regarding the orbits (not listed in their Table 2 of cranial measurements, crucial to estimate the relevant orbital size and shape, which are major characters in which *Homunculus* differs from *Killikaike*), or new illustrations (an old low-quality drawing was figured from the 1931 publication by Bluntschli). Therefore, we strongly support the validity of *Killikaike* as a different genus.

Finally, the youngest record of a platyrrhine in Patagonia is *Proteropithecia neuquenensis*, from deposits of Colloncuran age (15.7 Ma) in Cañadón del Tordillo, Neuquén Province (Pardiñas, 1991; Kay *et al*., 1998). Since the initial description, the isolated and rare teeth

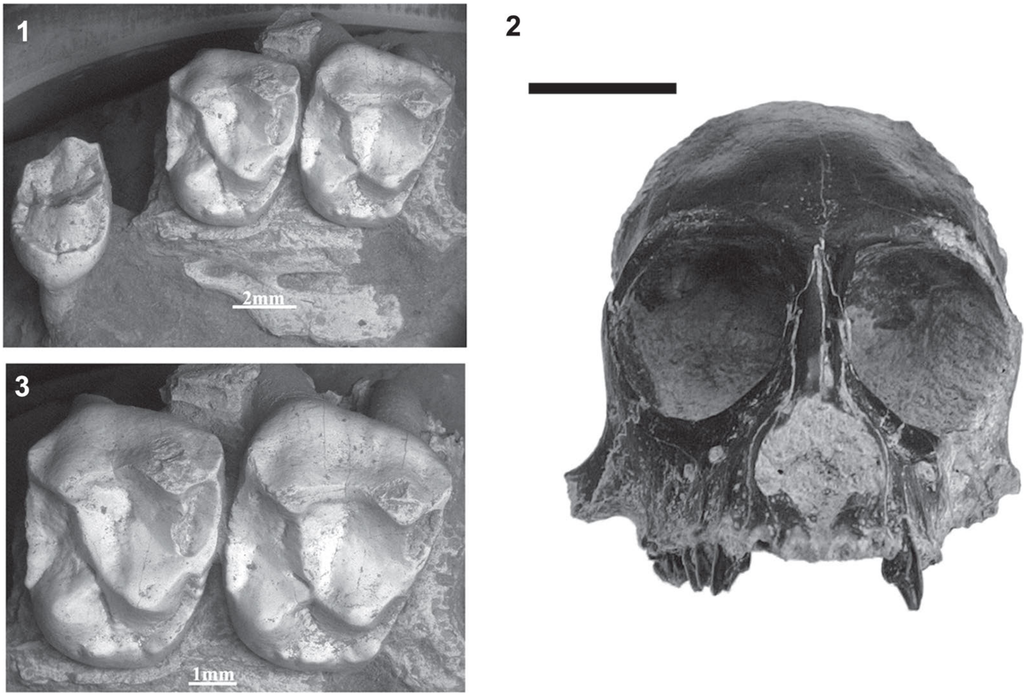

Figure 6. The genus *Killikaike*. **1:** MPM-PV 5000, maxillary fragment with M1–3. **2:** Holotype of *Killikaike blakei*, partial skull in frontal view.

of *Proteropithecia* have been undoubtedly assigned to the crown Pitheciinae, a view that contrasts with the alleged stem hypothesis for the remaining Patagonian platyrrhines (see Kay *et al.*, 2008, *et seq.*), because *Proteropithecia* shares several dental characters similar to or intermediate between *Soriacebus* and *Cebupithecia*, the latter two having been considered as pitheciines (see Tejedor, 2005). The incisors exhibit the characteristic compression and procumbence as in *Soriacebus* and *Mazzonicebus*, but the lower molars are more advanced toward the pitheciine pattern. Therefore, being from the same region and with an age difference of about 700,000 years, the probability of *Proteropithecia* (as the only crown platyrrhine from Patagonia) and *Soriacebus*/*Mazzonicebus* being convergent in morphology, as suggested by Kay (2010), is far from being supported.

Fossil platyrrhines from Chile

As part of this austral radiation, *Chilecebus carrascoensis* has been recorded in central Chile, south of Santiago, in sediments of the Abanico Formation, of Colhuehuapian age (20 Ma.; Flynn *et al.*, 1995; Sears *et al.*, 2008), thus correlated with the oldest Argentinian Patagonian records. *Chilecebus* is known from a skull preserving the upper dentition, which has unusual tooth proportions, given that the molars are large compared to the size of the palate, whereas the premolars show the typical cebine morphology, buccolingually elongated.

The upper molars have an unusual morphology, with large hypocones that are lingually displaced on a strong lingual cingulum, in combination with the presence of a buccal cingulum, a primitive trait. *Chilecebus* has a small P2, as seen in *Branisella*.

The southern regions of Chile, near the boundary of the Argentinian province of Chubut, have provided a single primate specimen dated to the middle Miocene. It is a primate talus coming from the Alto Río Cisnes region, well known since the report of Kraglievich (1930), and its affinities are with the Pinturas specimens of *Carlocebus* (Tejedor, 2003). Another primate findings from Alto Río Cisnes, corresponding to a mandibular fragment and an isolated premolar, were reported by Bobe *et al.* (2015).

The more modern radiation in the northern Neotropics

The fauna from La Venta, Colombia, and its primates have been known since the initial expeditions by Robert Stirton, from the University of California in Berkeley in the mid-20th century (Stirton, 1951). The age of these sediments ranges between 11.6 and 13.5 Ma (Flynn *et al.*, 1997), and the primates are anatomically more advanced than their Patagonian counterparts, resembling the adaptive pattern of several living clades; they include *Neosaimiri fieldsi* and *Laventiana annectens*, the genus *Stirtonia* with two species, as well as *Cebupithecia sarmientoi*, related to *Saimiri*, *Alouatta*, and pitheciines, respectively (Stirton, 1951; Hershkovitz, 1970; Kay *et al.*, 1987; Rosenberger *et al.*, 1991b; Takai, 1994). Several isolated teeth and mandibular remains have been recovered for *Neosaimiri fieldsi*, and they are morphologically very close to the living *Saimiri*. *Laventiana annectens* was described on the basis of a mandible with most of its dentition, and considered closely related to *Saimiri* (Rosenberger *et al.*, 1991b). Some authors, however, considered *Laventiana* as a junior synoym of *Neosaimiri* (Takai, 1994, Meldrum and Kay, 1997) due to the variability found in the distal sulcus of the lower molars, characteristic of the type of *Laventiana* but present with variable development in isolated specimens attributed to *Neosaimiri* by Takai (1994). Beyond these taxonomic controversies, it is clear that advanced cebines were present in La Venta by the middle Miocene with a morphology almost indistinguishable from that of *Saimiri*.

Cebupithecia is clearly related to living pitheciines, and exhibits most of the dental traits characterizing the living *Pithecia*, approaching the typical quadrangular pattern of the molars, with low occlusal relief but lacking crenulation, and the procumbent incisors and robust canines. Compared to the Patagonian pitheciines such as *Soriacebus* and *Mazzonicebus*, *Cebupithecia* has a "U-shaped" symphysis instead of a "V-shaped" arrangement seen in Patagonian pitheciines. Compared to living pitheciines, *Cebupithecia* still lacks the diastema between lower canines and lateral incisors, which should facilitate the processing of food during the powerful bite, as a space to lodge and split hard items (Rosenberger, 1992). Another pitheciine from La Venta is *Nuciruptor rubricae*, whose traits resemble *Cebupithecia* but with some distinctions such as the smaller canine; it also shares with *Cebupithecia* the unusually short talonid in the lower p3–4, the opposite adaptation of the living pitheciines that have more molarized p3–4 with long talonids. Notwithstanding, their attribution to the pitheciines has not been questioned (Meldrum and Kay, 1997), demonstrating that some unusual characters may appear even in taxa with clear affinities within living clades.

Stirtonia is an excellent example of modern morphologies among the Laventan primates. This genus has two species, *S. tatacoensis* (Stirton, 1951; Hershkovitz, 1970), the smaller, and *S. victoriae* (Kay *et al.*, 1987) whose resemblances with the living alouattines are remarkable. They are represented by mandibular, maxillary and dental remains showing the same adaptive pattern as *Alouatta*, such as the sharp crests on premolars and molars, developed buccal cingulum on upper molars, and the small trigonid and broad talonids on lower molars. This is the best evidence of a fully developed alouattine pattern in the middle Miocene.

Another relative of modern genera is *Miocallicebus villaviejai* (Takai *et al.*, 2001), although it is poorly represented by only a maxillary fragment with broken M2–3 and the root of M1. In general aspect, this specimen resembles the living *Callicebus*, only larger in size.

Mohanamico hershkovitzi (Luchterhand *et al.*, 1986) has been a matter of debate since the description of *Aotus dindensis* (Setoguchi and Rosenberger, 1987). Meldrum and Kay (1997) suggested that *Mohanamico* and *A. dindensis* are synonymous due to their similar morphology, size, and stratigraphic provenance, but new specimens described by Takai *et al.* (2009) reinforced the view that *A. dindensis* is a different taxon and certainly close to the living *Aotus*. The premolars and molars of *A. dindensis* differ from *Mohanamico* in the presence of a proportionally longer talonid with respect to the trigonid, where *Mohanamico* presents a pattern resembling callitrichines for the better developed trigonid and more elongated molars (Rosenberger *et al.*, 1990).

A taxon that deserved much attention is *Lagonimico conclucatus*, a crushed skull preserving the mandible and dentition, originally reported as a giant tamarin (Kay, 1994), but later compared to pitheciines mainly based on its mandibular morphology (Rosenberger, 2002). The teeth indeed have some callitrichine-like features, especially in the upper molars that have a triangular outline and lack a hypocone, and the premolar are slightly “waisted”. But the mandible lacks any callitrichine aspects; to the contrary, it is posteriorly deep as seen in *Callicebus*, instead of shallow as in cebids, and the ascending ramus is high and antero-posteriorly narrow, all characters that Rosenberger (2002) interpreted as pitheciine-like. *Lagonimico* represents a challenge in interpreting phylogeny because its mosaic of characters and large size warrant more in-depth studies.

Among possible callitrichines from La Venta, *Patasola magdalenae* was described by Kay and Meldrum (1997) as an intermediate between *Saimiri* and callitrichines, and it is certainly a cebid because of mixed characters of both groups. Also the poorly known *Micodon kyotensis* has been considered a callitrichine (Setoguchi and Rosenberger, 1985) of a small size, but it is represented by only an upper molar, an incisor and a lower premolar.

As mentioned by Tejedor (2013, p. 29): *“Es posible que esta frecuencia de caracteres compatibles con un patrón calitriquino se deba a una amplia radiación previa del grupo, que hasta hoy es prácticamente desconocido para toda América del Sur”.*

These callitrichine-like traits may have been a product of a previous radiation, which up to then lacked empirical evidence in South America, although their presence is now confirmed with the record of the Eocene Santa Rosa primates (Bond *et al.*, 2015). The cebid characters present in *Lagonimico*, as well as *Patasola*, *Mohanamico* and *Micodon*, may be resemblances shared with an older radiation in the northern Neotropics derived from a possible callitrichine-like ancestral pattern.

Additional discoveries in Peru and Panama

Recent expeditions to the Atalaya region, Cusco, Peru, have produced a platyrrhine talus that was originally reported as a cebine (Marivaux *et al.*, 2012), and dated to 18.65–16.5 Ma, thus “Pinturan” in age, or early Santacrucian (late-early Miocene). Also, recent reports of primate findings come from western Peru, in two new localities of early Miocene and middle-late Miocene age document the presence of fossil primates (Antoine *et al.*, 2014). The new genus *Canaanimico* was described for the Contamana area, Chambira Formation, with an age of 26.5 Ma (Marivaux *et al.*, 2016). *Canaanimico* is based on two upper molars and, following the authors, they may be related to *Soriacebus* and *Mazzonicebus*, from the early Miocene of Patagonia. Along with the Santa Rosa primates, these findings increase the interest in western Amazonia as a source of primitive platyrrhines.

The first fossil platyrrhines reported from the North American land mass come from Panama. With an age of 21 Ma, the new genus *Panamacebus* was recovered at Las Cascadas Formation in the Panama Canal Basin, and is based on seven isolated teeth described as cebines closer to *Saimiri* and *Cebus*, thus being part of crown Platyrrhini, even with their old age (Bloch *et al.*, 2016).

Caribbean and late Cenozoic of South America

Even though there are no native primates living today in the Caribbean islands, there is an outstanding record of unusual monkeys that lived at least since the early Miocene. For example, there is a single record from the early Miocene of Cuba, a talus named *Paralouatta* *marianae*, morphologically more primitive than the living *Alouatta,* and in some ways related to primitive traits seen in the talus of the living *Saimiri* (MacPhee *et al.*, 2003). However, the record mostly comes from the Pleistocene to Recent in Cuba, Haiti, the Dominican Republic, and Jamaica. These primates exhibit an unusual morphology, which is possibly due to their evolution in isolation during a long period of time after the land mass connection of GAARlandia (Greater Antilles and Aves Ridge; see Iturralde-Vinent and MacPhee, 1999) was interrupted during the early Oligocene, thus ending the contact between the Greater Antilles and northern South America.

Xenothrix mcgregory was discovered in Pleistocene levels from Jamaica (Williams and Koopman, 1952; Rosenberger, 1977; MacPhee and Horovitz, 2004), and is represented by several dental, mandibular and maxillary specimens with a distinctive morphology, including, among other features, low-crowned teeth with bulbous cusps, and the absence of third molars. It has been compared to pitheciids (Rosenberger, 2002) and more specifically with *Callicebus* (MacPhee and Horovitz, 2004), although the discussions are still open.

Another Antillean primate is *Antillothrix bernensis*, from the Dominican Republic, originally reported as *"Saimiri" bernensis* by Rímoli (1977), due to its similarities with the living squirrel monkey, then reallocated to the new genus (MacPhee *et al.*, 1995). There are specimens of *Antillothrix* recently described from Holocene caves of Dominican Republic, including three skulls and postcranial material (Kay *et al.*, 2011; Rosenberger *et al.*, 2011; Halenar *et al.*, 2017) with some phenetic similarities with cebines and pitheciids, although other traits are still debatable.

A new genus has been reported for the late Quaternary of Haiti, *Insulacebus toussaintiana* (Cooke *et al.*, 2011), that shows some dental traits comparable to some extinct Patagonian platyrrhines, as discussed by the authors. Cooke *et al.* (2011) suggest a relationship between *Insulacebus* and *Xenothrix*, as well as possible phylogenetic links with the living *Callicebus*, as proposed by MacPhee and Horovitz (2004), as well as *Aotus*.

Paralouatta varonai (Rivero and Arredondo, 1991) is a skull from a Pleistocene cave of Pinar del Río, Cuba, originally believed to be close to *Alouatta*. More remains were found later, including isolated teeth and a mandible (Horovitz and MacPhee, 1999), that were included in a monophyletic clade with *Callicebus* and *Xenothrix*. In a recent report, Rosenberger *et al.* (2015) have included *Paralouatta* in the same clade as *Protopithecus*, *Cartelles*, *Alouatta*, *Stirtonia*, and possibly *Solimoea*, thus reinforcing the alouattine status of *Paralouatta*.

Among the other primates from important biogeographic regions, there are scarce but informative remains from the Acre region, in western Amazonia. The Solimoes Formation is a late Miocene (Huayquerian SALMA) sedimentary basin containing at least three platyrrhine species only represented by isolated teeth. A lower molar is tentatively assigned to *Stirtonia*, an alouattine genus present in earlier levels of La Venta, Colombia. There is also a lower molar and a P3–4 that are representatives of the new species *Solimoea acrensis* (Kay and Cozzuol, 2006) that the authors suggested are related to the living atelines. The third species is a large cebine named *Acrecebus freileyi* (Kay and Cozzuol, 2006), with a morphological pattern seen in the upper molars of extant *Cebus*, although *Acrecebus* is considerably larger. At the moment, it is not prudent to suggest how these remains were related to other extinct or living platyrrhines with respect to their adaptive radiation, but they provide a strong indication of the presence of alouattines and cebines, the first since the middle Miocene of Colombia. *Solimoea*, however, is a matter requiring further discussion regarding the ateline status that the original authors claimed. There is clear evidence of a cebine of the *Cebus* lineage represented by *Acrecebus*, which is a remarkable record when seen in the context of the odd adaptations of *Cebus*. The bunodont aspect of the crown of the upper molar of *Acrecebus* is evidently an adaptation that took place well before the late Miocene because the thick enamel covering the occlusal surface is a novelty in platyrrhines, only present in the living *Cebus* and *Acrecebus*.

Extinct Pleistocene species have been described from younger sites in Brazil, in the eastern regions of the states of Bahia and Minas Gerais. *Protopithecus brasiliensis* (Lund, 1840; Hartwig and Cartelle, 1996; Rosenberger *et al.*, 2015) and *Caipora bambuiorum* (Cartelle and Hartwig, 1996) were described as giant atelids, and are represented by partial skeletons undoubtedly attributable to that clade. The specimen assigned to *Protopithecus* coming from a cave of Bahia is now being recognized as the new genus *Cartelles* (Halenar and Rosenberger, 2013), differing from the original postcranial remains of *Protopithecus* from Lagoa Santa, Minas Gerais, collected by Lund (1840). It is of historical interest that the specimens collected by Lund were the first platyrrhine fossils ever discovered, followed by *Homunculus* during the 1880s with the fieldwork of Carlos Ameghino. *Caipora* is an ateline closer to *Ateles*, whereas *Cartelles* shows particular adaptations resembling *Alouatta*, but also with traits exhibited by the living *Lagothrix*; however, the postcranium of *Cartelles* demonstrates clear adaptations to suspensory locomotion.

An extinct species of the living howler monkey, *Alouatta*, has been described by Tejedor *et al.* (2008) from a late Pleistocene cave in Bahia, Brazil. *Alouatta mauroi* is characterized by unusual dental proportions compared to living *Alouatta* species, including a huge P2 and upper canine not seen in living species.

Evolutionary history of platyrrhines reconsidered

The fossil record of platyrrhines is diverse and widely distributed across South America and the Caribbean. Thus, considering the morphological variations characterizing different clades, their phylogenetic relationships are still not fully resolved, as some authors claim. Major problems arise when we try to integrate geographically distant groups that we expect are part of a single adaptive radiation. Platyrrhines, in contrast to many South American mammal groups, lack an intracontinental connection through common morphology and adaptations, and all hypotheses regarding the origin of different clades and subsequent evolution are thus hotly debated subjects. The new reports from the late Eocene of Santa Rosa, Peru, more than 15 million years older than the oldest Patagonian fossils, are signs of the very early stages of platyrrhine evolution. They effectively refute the hypothesis that the Patagonian forms represent primitive morphologies as stem platyrrhines; instead, the new Peruvian findings reinforce the hypothesis that the Patagonian forms are crown platyrrhines. *Perupithecus* is morphologically similar to *Talahpithecus* from Libya, but the other two upper molars from Santa Rosa show a definitive callitrichine-like aspect, and the lower

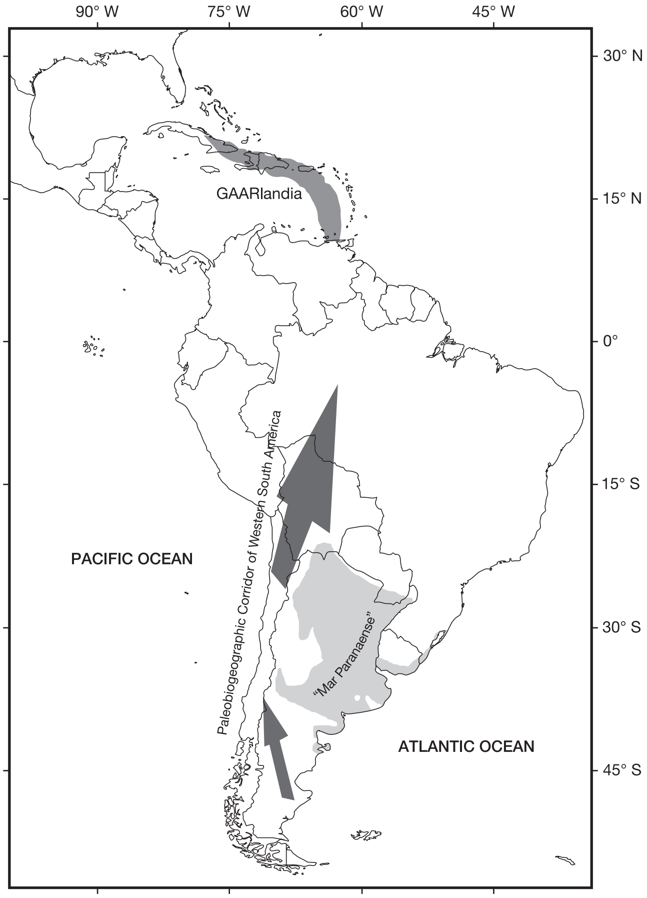

Figure 7. Map showing the early Oligocene land mass connection between the Greater Antilles and South America (GAARlandia), and the inland sea "Mar Paranaense" with the western paleobiogeographic corridor indicated by the arrows (modified from Tejedor, 2013).

molar is an odd specimen that still needs further analysis. This means that a very early stage of platyrrhine evolution probably involved a trend toward small body size and generalized morphology, a pattern seen in platyrrhines as a whole, but not in the more derived catarrhines of the Old World.

The discovery of the Santa Rosa primates has implications for the two current hypotheses of stem or crown status for the Patagonian platyrrhines (see Kay *et al.*, 2008; Rosenberger, 2010; Kay and Fleagle, 2010). These hypotheses need to be reconsidered in the light of the evidence of much older morphologies, thus reinforcing the advanced—not primitive—status of the Patagonian platyrrhines, some more easily related to the *Callicebus* lineage, or to pitheciines or primitive cebines (see Rosenberger *et al.*, 2009; Tejedor, 2013). Our new concept of platyrrhine origins supported by the oldest records from Peru has renovated the thinking on the evolutionary history of platyrrhines: they existed for at least 10 million years earlier than previously thought as a basal evolutionary radiation before evolving into the well adapted groups that gave rise to the living clades. The recently reported findings in eastern Peru add new western primate localities of the mid-Tertiary (Marivaux *et al.*, 2012; Antoine *et al.*, 2014). Given that Santa Rosa, in western Amazonia, is now the oldest region where platyrrhines appeared in the fossil record, we can support only the view of a western corridor (Paleobiogeographic Corridor of Western South America; *Corredor Paleobiogeográfico Occidental Sudamericano* in Spanish; see Tejedor, 2013; Tejedor and Muñoz-Saba, 2013; Novo *et al.*, 2017) that served as a route for dispersal of early platyrrhines. This is because the eastern and central parts of what is now Argentina was covered by an inland sea, the "Mar Paranaense" (Fig. 7; see also Tejedor, 2013). These primitive forms colonized Patagonia and, while the western corridor remained under favorable climatic, environmental and tectonic conditions during most of the middle Cenozoic, the southern Patagonian forms may have evolved into some living lineages that dispersed through that western corridor.

It is accepted that the Caribbean radiation of platyrrhines is another major event to explain, and they developed an unusual radiation of complex interpretation; this is because the possible land mass connections with northern South America (GAARlandia, see Iturralde-Vinent and MacPhee, 1999) have been interrupted since at least the early Oligocene (around 32 Ma). The Caribbean platyrrhines either dispersed from the south or from the north, this is, from South American ancestors that migrated through GAARlandia to the Caribbean, or vice versa. The remaining South American forms are not a significant factor because they come from central or northern South America, from areas where that crown platyrrhines inhabit today.

The aforementioned paleobiogeographic and phylogenetic inferences create a more promising scenario, making the "Long Lineage Hypothesis" (see Rosenberger, 2010; Tejedor, 2013) a more intuitive concept on platyrrhine evolution. In this sense, the Patagonian platyrrhines could have had a western dispersal route for a south-north evolutionary continuity. The main questions are now focused on platyrrhine origins as well as the initial radiation to understand adaptive trends in their early evolution, hopefully accompanied by new advances in the knowledge of paleoenvironmental conditions where this radiation took place. In this sense, tropical South America emerges as the place where fossils suddenly appeared after decades without a hope of finding them.

Following the above discussion of platyrrhine relationships, a revised classification of the platyrrhines—including living and extinct forms—is presented here (see **Appendix**).

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Appendix. Classification of the platyrrhine primates including living and fossil genera.

- Parvorder Platyrrhini E. Geoffroy, 1812
  - Superfamily Ateloidea Gray, 1825 (Rosenberger, Setoguchi and Shigehara, 1990)
    - Family Atelidae Gray, 1825
      - Subfamily Atelinae Gray, 1825 (Pocock, 1925)
        - *Ateles* E. Geoffroy, 1806
        - *Lagothrix* E. Geoffroy, 1812
        - *Brachyteles* Spix, 1823
        - †*Caipora* Cartelle and Hartwig, 1996
        - †*Protopithecus* Lund, 1838
      - Subfamily Alouattinae Trouessart, 1897 (Szalay and Delson, 1979)
        - *Alouatta* Lacépède, 1799
        - †*Stirtonia* Hershkovitz, 1970
        - †*Alouatta mauroi* Tejedor, Rosenberger and Cartelle, 2008
        - †*Cartelles* Halenar and Rosenberger, 2013
        - †*Paralouatta* Rivero and Arredondo, 1991
      - Subfamily *incertae sedis*
        - †*Solimoea* Kay and Cozzuol, 2006
    - Family Pitheciidae Gray, 1849 (Mivart, 1865)
      - Subfamily Pitheciinae Gray, 1849
        - *Pithecia* Desmarest, 1820
        - *Chiropotes* Lesson, 1840
        - *Cacajao* Lesson, 1840
        - †*Cebupithecia* Stirton and Savage, 1951
        - †*Soriacebus* Fleagle, Powers, Conroy and Watters, 1987
        - †*Nuciruptor* Meldrum and Kay, 1997
        - †*Proteropithecia* Kay, Johnson and Meldrum, 1998
        - †*Mazzonicebus* Kay, 2010
      - Subfamily Homunculinae Ameghino, 1894 (Rosenberger, Setoguchi and Shigehara, 1990)
        - *Callicebus* Thomas, 1903
        - †*Homunculus* Ameghino, 1891
        - †*Carlocebus* Fleagle, 1990
        - †*Miocallicebus* Takai, Anaya, Suzuki, Shigehara and Setoguchi, 2001

Appendix. (Continued).

- Subfamily Aotinae Poche, 1908
    - *Aotus* Illiger, 1811
    - †*Tremacebus* Hershkovitz, 1974
    - †*Aotus dindensis* Setoguchi and Rosenberger, 1987
- Subfamily *incertae sedis*
    - †*Xenothrix* Williams and Koopman, 1952
- Subfamily *incertae sedis*
    - †*Lagonimico* Kay, 1994

Family Cebidae Bonaparte, 1831

- Subfamily Callitrichinae Thomas, 1903 (Cabrera, 1958)
    - *Callithrix* Erxleben, 1777
    - *Cebuella* Gray, 1886
    - *Saguinus* Hoffmannsegg, 1807
    - *Leontopithecus* Lesson, 1840
    - *Callimico* Thomas, 1913
    - *Mico* Lacépède, 1799
    - *Callibella* van Roosmalen and van Roosmalen, 2003
    - †*Micodon* Setoguchi and Rosenberger, 1985
    - †*Patasola* Kay and Meldrum, 1997
    - †*Mohanamico* Luchterhand, Kay and Madden, 1986
- Subfamily Cebinae Bonaparte, 1831 (Mivart, 1865)
    - *Cebus* Erxleben, 1777
    - *Saimiri* Voigt, 1831
    - †*Dolichocebus* Kraglievich, 1951
    - †*Laventiana* Rosenberger, Setoguchi and Hartwig, 1991
    - †*Neosaimiri* Stirton, 1951
    - †*Killikaike* Tejedor, Tauber, Rosenberger, Swisher and Palacios, 2006
    - †*Acrecebus* Kay and Cozzuol, 2006
    - †*Panamacebus* Bloch *et al*., 2016
- Subfamily *incertae sedis*
    - †*Branisella* Hoffstetter, 1969
    - †*Szalatavus* Rosenberger, Hartwig and Wolff, 1991
- Subfamily *incertae sedis*
    - †*Chilecebus* Flynn, Wyss, Charrier and Swisher III, 1995

Family *incertae sedis*

- †*Antillothrix* Rímoli, 1977 (MacPhee, Horovitz, Arredondo and Jiménez-Vásquez, 1995)
- †*Insulacebus* Cooke, Rosenberger and Turvey, 2010

Family *incertae sedis*

- †*Canaanimico* Marivaux *et al*., 2016

?Parvorder Platyrrhini E. Geoffroy, 1812

- †*Perupithecus* Bond, Tejedor, Campbell, Chornogubsky, Novo and Goin, 2015

4 | PATTERNS OF PHYLOGENETIC DIVERGENCE AND PHENOTYPIC VARIATION AMONG GENERA OF PLATYRRHINI MONKEYS

PATRONES DE DIVERGENCIA FILOGENÉTICA Y VARIACIÓN FENOTÍPICA ENTRE GÉNEROS DE MONOS PLATIRRINOS

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Abstract. A great phenotypic diversification has occurred in some phylogenetic clades through a variety of biological processes during their evolutionary radiation. New World monkeys, the parvorder Platyrrhini of the order Primates, are an example of a major mammalian evolutionary radiation. Here, we study statistically the phylogenetic history and the phenotypic diversification of New World monkeys. To explore these subjects, we used molecular phylogeny (species tree), geometric morphometric and comparative methods. These approaches are very useful for studying phenotypic diversification in clades with a long phylogenetic history, such as platyrrhines. The comparative results allow us to depict a more complex picture of the phenotypic diversification among platyrrhines than has been achieved by previous studies. Specifically, we suggest that the phenotypic differences among platyrrhines could primarily be the product of both deterministic factors that generate stasis in size variation, and random factors that generate shape differences that increase proportionally with the phylogenetic distance.

Resumen. Algunos clados filogenéticos han sufrido una gran diversificación fenotípica a través de diversos procesos biológicos durante su radiación evolutiva. Los monos del Nuevo Mundo, parvorden Platyrrhini, son miembros del Orden Primates que constituyen un ejemplo notable de radiación evolutiva en mamíferos. Constituyen un grupo monofilético con una historia evolutiva relativamente larga de entre 20 y 30 millones de años en Sudamérica y el Caribe y que, evolucionando aisladamente de otros clados de primates, se diversificaron en unas 125 especies actuales agrupadas en quince géneros, existiendo además numerosos linajes extintos. Además, los platirrinos se diversificaron en una amplia gama de nichos ecológicos y en una gran variación en la forma craneana y el tamaño corporal. Trabajos previos sugirieron que la diversificación fenotípica en los platirrinos está determinada por la acción de factores selectivos-deterministas asociados a la ocupación de nichos ecológicos. Contrariamente, análisis recientes sugirieron que en algunos rasgos fenotípicos (p.ej. la forma craneana) la variación se acumuló proporcionalmente a la distancia filogenética debido a procesos aleatorios. El objetivo de este trabajo es, en primer lugar, inferir la historia de divergencia filogenética entre los géneros de platirrinos. Para esto se analizaron datos moleculares previamente publicados (5.329 pares de bases de ADN pertenecientes a 4 genes nucleares), obteniendo un árbol para cada gen mediante un método de Inferencia Bayesiana, y un árbol de especies utilizando el método de estimación bayesiana de árbol de especies (BEST). La estimación de una filogenia de las especies es un primer paso

fundamental para entender la diversificación fenotípica de un clado. En segundo lugar, exploramos la disparidad en la forma y el tamaño craneano, y el tamaño corporal. Para esto se utilizaron técnicas de la morfometría geométrica, midiendo 38 coordenadas 3D de *landmarks* y 64 de *semilandmarks* en 130 especímenes pertenecientes a una especie por género, y datos publicados de masa corporal. Finalmente, estudiamos el ajuste de la disparidad en tamaño y forma, y el patrón de divergencia filogenética a diferentes modelos evolutivos, particularmente a un modelo neutro de evolución estocástica (movimiento browniano) utilizando métodos comparativos filogenéticos (gráficos de disparidad a través del tiempo [DTT] y análisis de señal filogenética [estadístico *K*]). Los árboles estimados para cada gen individualmente resultaron concordantes con resultados previos que reconocen la división de los platirrinos en cuatro grandes clados: Atelidae, Pitheciidae, Callitrichinae y Cebinae + Aotinae. Dentro de cada clado, los árboles mostraron topologías similares para los géneros. Sin embargo, se observaron diferentes relaciones entre los cuatro clados principales según el gen analizado. Por otro lado, la estimación de un árbol de especies recuperó una topología reafirmando el agrupamiento en cuatro grandes clados, con alto soporte para todos los nodos (probabilidad posterior > 0,99) con la excepción del clado Cebinae + Aotinae que muestra una probabilidad posterior inferior (0,87). El análisis de la variación fenotípica indicó, por un lado, que la variación en el tamaño corporal muestra poca correspondencia con los cuatro principales clados. Contrariamente, la forma craneana muestra un patrón en donde la variación se agrupa de acuerdo a estos cuatro clados principales. Particularmente, los géneros de Callitrichinae muestran una forma craneana muy similar, mientras que los géneros de Atelidae y Cebinae muestran una diferenciación marcadamente mayor. El principal eje de variación en la forma craneana está determinado por un aumento en el tamaño relativo de la región facial asociado a una disminución en el tamaño de la bóveda craneana, siendo Atelidae y Cebinae + Callitrichinae extremos y opuestos en esta tendencia. El segundo eje de variación se asoció principalmente a un incremento en el tamaño relativo de las órbitas, siendo *Aotus* extremo en esta variación. Finalmente, el cálculo del estadístico *K* mostró la existencia de una fuerte señal filogenética en la masa corporal y en el tamaño y la forma craneanos, corroborando la importancia de la filogenia en la comprensión del patrón de variación fenotípica en los platirrinos. Sin embargo, se encontraron diferencias entre estos rasgos, ya que mientras la masa corporal y el tamaño craneano muestran valores más altos que lo esperado bajo un modelo de evolución Browniana, la forma del cráneo mostró valores que se ajustan a esta expectativa. Similarmente, el análisis de la disparidad a través del tiempo mostró que tanto la masa corporal como el tamaño craneano presentan valores de disparidad intraclado cercanos a cero, mientras que la disparidad en la forma craneana sigue la expectativa browniana. Estos resultados indican que la principal variación en tamaño se da entre clados, los cuales ocupan regiones del morfoespacio relativamente aisladas. En contraste, la variación en forma cambia proporcionalmente con la divergencia entre los géneros de acuerdo a un modelo representando procesos aleatorios. Nuestros resultados nos permiten describir una imagen de la diversificación fenotípica de los platirrinos más compleja que la propuesta por estudios previos. Específicamente, proponemos que las diferencias fenotípicas entre los platirrinos podrían ser producto principalmente de factores determinísticos que generan estasis en la variación del tamaño, y de factores aleatorios que generan diferencias en forma que se incrementan proporcionalmente con la distancia filogenética entre especies. Esta propuesta deberá ser explorada más profundamente en futuros estudios incorporando una mayor cantidad de datos morfométricos y ecológicos, junto con la utilización de modelos evolutivos que reflejen la aparente complejidad de la diversificación en el clado.

Introduction

New World monkeys, members of the Order Primates, parvorder Platyrrhini, are an example of a major mammalian evolutionary radiation. Platyrrhines are a monophyletic group with a relatively long evolutionary history of nearly 20–30 million years in South America and the Caribbean (Fleagle, 1999; Tejedor, 2008). This group invaded the continent at the Eocene–Oligocene transition and evolved in isolation from the Old World primates (Rosenberger, 2002; Tejedor, 2008; Wildman *et al.*, 2009). Within the Americas, they diversified into several lineages, comprising fifteen genera with approximately 125 extant species, and several extinct groups (Wilson and Reeder, 2005; Wildman *et al.*, 2009). Species diversified into a wide range of ecological niches (Fleagle, 1999; Norconk *et al.*, 2009) and great variations in cranial shape (Perez *et al.*, 2011) and body size, which range from small-size (approximately 100 g) to medium-size (approximately 10 kg; Fleagle, 1999; Norconk *et al.*, 2009).

Previous studies have suggested that ecological niche (*i.e.*, diet and life history) is the principal axis shaping the phenotypic diversification of platyrrhines, which is generally related to deterministic-selective factors (Fleagle, 1999; Marroig and Cheverud, 2001; Wildman *et al.*, 2009; Rosenberger, 2010). Conversely, recent analyses have suggested that variation among extant platyrrhines in some phenotypic traits—such as cranial shape—increases proportionally with the phylogenetic distance due to random factors (Perez *et al.*, 2011). Here, we study the phylogenetic history—(*i.e.*, the pattern and timing of divergence—of New World monkeys and its associated phenotypic diversification. Specifically, we statistically explore previous hypotheses on the evolutionary changes in size and shape among platyrrhine genera.

The aim of this work is threefold. First, we infer the history of phylogenetic divergence of the platyrrhine genera using molecular data and the Bayesian Estimation of Species Tree (BEST; Liu, 2008; Liu *et al.*, 2008; Knowles and Kubatko, 2010). Given the long evolutionary history of platyrrhine radiation, the estimation of a robust phylogeny that is independent of the phenotypic data is a necessary first step in understanding the factors responsible for its phenotypic diversification (Wiens, 2009; Freckleton *et al.*, 2011; Losos, 2011). Second, we explore the disparity in cranial size and shape, as well as in body size, among the platyrrhine genera using three-dimensional (3D) geometric morphometric techniques (Zelditch *et al.*, 2004; Mitteroecker and Gunz, 2009) and published data (Smith and Jungers, 1997), respectively. Geometric morphometrics allow us to study shape variation —(*i.e.,* changes in the relative spatial positions of reference points describing the anatomical landmarks, curves and surfaces of the skull—separately from size variation (Bookstein, 1997; Zelditch *et al.*, 2004; Gunz *et al.*, 2005; Mitteroecker and Gunz, 2009). Finally, we examine the fit of size and shape disparity and the pattern and timing of phylogenetic divergence to different evolutionary models using comparative methods (Blomberg *et al.*, 2003; Harmon *et al.*, 2003).

Material and methods

Samples

We studied one species within each of the fifteen extant genera of platyrrhines (Tab. 1; Wildman *et al.*, 2009). Species were chosen based on the availability of cranial samples for phenotypic analyses and the DNA sequences needed to infer the phylogenetic history independently of phenotypic data (Schneider *et al.*, 2001; Opazo *et al.*, 2006; Wildman *et al.*, 2009). Only adult specimens were included in our morphometric analyses, which were defined by the presence of an obliterated basioccipital synchondrosis and a completely erupted and functional dentition. Approximately equal numbers of male and female specimens were selected for each species and both sexes were pooled in the analyses. The mean shape for each platyrrhine species was calculated in order to obtain an adequate sample size for further statistical analyses. As this procedure could be problematic, we also employed alternative approaches, which yielded similar results (see Perez *et al.*, 2011).

Table 1. List of the 15 species used in the study, together with sample sizes (***n***) and phylogenetic clades in the Figure 2.

Species	**Clades**	***n***
Alouatta belzebul	Atelidae	5
Ateles belzebuth	Atelidae	5
Brachyteles arachnoides	Atelidae	7
Lagothrix lagotricha	Atelidae	9
Callicebus donacophilus	Pitheciidae	15
Pithecia irrorata	Pitheciidae	6
Cacajao calvus	Pitheciidae	4
Chiropotes satanas	Pitheciidae	5
Aotus azarae	Cebinae plus Aotinae	19
Cebus apella	Cebinae plus Aotinae	18
Saimiri sciureus	Cebinae plus Aotinae	5
Saguinus midas	Callitrichinae	5
Leontopithecus chrysomelas	Callitrichinae	6
Callithrix jacchus	Callitrichinae	18
Callimico goeldii	Callitrichinae	3

Molecular datasets and phylogenetic analyses

To study the phylogenetic relationships among the fifteen New World monkey genera, we analyzed molecular sequences obtained from GenBank for the platyrrhine species and one outgroup (*Homo sapiens*). The analyzed dataset comprised a DNA matrix with a total of 5.392 bp (Tab. 2), including four nuclear gene sequences: β2-microglobulin (*B2M*; 1.438 bp), β-globin (*HBB*; 1.224 bp), interphotoreceptor retinol binding protein (*IRBP*; 1.839 bp) and von Willenbrand factor (*vWF*; 891 bp). The sequences for each gene were aligned using ClustalW and were manually corrected with *BioEdit* 7.0.0 software (Hall, 2004) when necessary.

Table 2. Accession numbers of all sequences used in the phylogenetic analyses. β2-microglobulin (*B2M*; 1.438 bp), β-globin (*HBB*; 1.224 bp), interphotoreceptor retinol binding protein (*IRBP*; 1.839 bp) and von Willenbrand factor (*vWF*; 891 bp).

Species	***B2M***	***HBB***	***IRBP***	***vWF***
Alouatta	AF032048.1	AY279110.1	U18602.1	AF092837.1
Ateles	AF032087.1	AY279117.1	U18603.1	AF092813.1
Brachyteles	AF032051.1	DQ145531.1	U18605.1	DQ129680.1
Lagothrix	AF032054.1	AY279114.1	U18614.1	AF092830.1
Callicebus	AF069326.1	AY279119.1	U18613.1	AF092815.1
Pithecia	AF032072.1	AY279112.1	U18615.1	DQ129682.1
Cacajao	AF032078.1	DQ145529.1	U19748.1	AF092814.1
Chiropotes	AF032075.1	DQ145530.1	U18612.1	DQ129681.1
Aotus	AF032093.1	AY279113.1	U18601.1	AF092812.1
Cebus	AF032018.1	AY279115.1	U18611.1	AF092822.1
Saimiri	AF068765.1	AY279116.1	U18619.1	DQ129683.1
Saguinus	AF032024.1	DQ145533.1	U19752.1	AF092836.1
Leontopithecus	AF032036.1	DQ145532.1	AF362379.1	AF092832.1
Callithrix	AF068767.1	AY279111.1	U18606.1	AF092828.1
Callimico	AF032039.1	AY279118.1	U19749.1	AF092825.1

A gene tree was estimated for each gene using a Bayesian Inference (BI) method, as implemented in *MrBayes* version 3.1.2 (Ronquist and Huelsenbeck, 2003). *jModelTest* 0.1 (Posada, 2008) was employed to determine the most appropriate model of sequence evolution for each analyzed gene, following the Bayesian Information Criterion (BIC). The best fit model for *B2M, HBB,* and *vWF* genes was TPM3uf + G; and K80 + G for the *IRBP* gene. These models of sequence evolution were then implemented in the analyses. Two simultaneous runs were performed for each gene using Markov Chain Monte Carlo (MCMC) simulations for 2,000,000 generations. A sample frequency of 1,000 and a burn-in of 500 were used; convergence was determined using the program *Tracer* version 1.5 (Rambaut and Drummond, 2007).

A species tree for the complete molecular dataset was estimated using the Bayesian Estimation of Species Tree method implemented in the *BEST* software (Liu, 2008). *BEST* utilizes an MCMC algorithm to estimate the joint posterior distribution of gene trees and the underlying species tree under a hierarchical Bayesian model (Liu *et al.*, 2008). Two simultaneous analyses were performed using Markov Chain Monte Carlo (MCMC) simulations for 20,000,000 generations, with a sample frequency of 1,000 and a burn-in of 10,000. The convergence of the analyses was determined using *Tracer* version 1.5 (Rambaut and Drummond, 2007). The BEST approach has several advantages over other available approaches, including its ability to account for uncertainty in tree estimation and to calculate branch lengths or divergence times. Moreover, previous multi-locus simulated and real data analyses suggested that the *BEST* program estimates species trees more accurately than the Bayesian analysis of concatenated data (Leaché and Rannala, 2011).

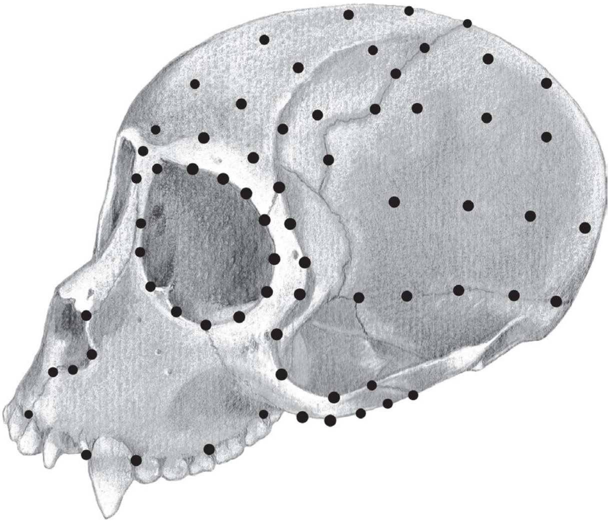

Figure 1. Cranial landmarks and semilandmarks recorded from New World monkeys using a 3D *MicroScribe* G2X digitizer. Drawing by Marina Perez.

Phenotypic variation analyses

Phenotypic disparity was measured as variation in cranial size and shape, as well as in body mass. Body mass (kg; BMass) data for each of the fifteen species were obtained from Smith and Jungers (1997). Cranial size (CS) and shape were measured from thirty-eight 3D coordinates of landmarks, and sixty-four 3D coordinates of semilandmarks obtained with a Microscribe G2X (Fig. 1; App. 1). Semilandmarks were defined between neighboring landmarks in several cranial bones and sutures. Special care was taken to ensure that these semilandmarks were restricted between the locations of fixed landmarks on different specimens to avoid the potential problem of assigning homology to semilandmarks (Wiley *et al.*, 2005; Polly, 2008). The centroid size (the square root of the summed squared distances from all landmarks and semilandmarks to the configuration centroid; Bookstein, 1991) was calculated for each specimen and then each species mean was obtained.

To analyze the cranial shape variation, coordinates of the obtained landmarks and semilandmarks were aligned by Generalized Procrustes Analysis (GPA; Rohlf and Slice, 1990; Bookstein, 1991). This procedure optimally translates, scales and rotates the superimposed configurations of landmarks and semilandmarks in order to eliminate non-shape variation (Rohlf and Slice, 1990). The semilandmarks were analyzed as equidistant points along ten curves and three surfaces (Fig. 1; App. 1; Williams and Slice, 2010). The use of equidistant points avoids some problems associated with the alternative sliding semilandmark method (*e.g.*, masking the shape of a curve). A Principal Component Analysis (Bookstein, 1991; Rohlf, 1993) was performed on the mean species shape variables (Procrustes shape coordinates) to reduce the dimensionality of the shape space. The principal component scores of the Procrustes shape coordinates are called Relative Warps (RW; Bookstein, 1991). RWs are calculated using a rigid orthogonal rotation that ensures that the Procrustes shape space is not deformed and the interobject distances are maintained.

Morphometric analyses were performed using *MorphoJ* 1.02j (Klingenberg, 2011) and *R* 2.13.0 (R-Development Core Team, 2011) software.

Comparative methods

We investigated the relationship between cranial size and shape, body size, disparity among platyrrhine species, and the pattern and timing of phylogenetic divergence using several comparative approaches. Particularly, we estimated whether the size and shape variables meet a Brownian motion model of evolution across the estimated phylogenetic tree (where phenotypic divergence is expected to increment proportionally with time) and explored the time pattern of morphometric variation.

First, we calculated the *K* statistic, proposed by Blomberg *et al.* (2003), for the size (LogCS and LogBMass) and shape (RWs) variation among the platyrrhine genera. The *K* statistic provides a univariate measure of the strength of the phylogenetic signal in the data and is computed as K = observed (MSE_0 / MSE) / expected (MSE_0 / MSE). The numerator MSE_0 is calculated as the mean squared error measured from the phylogenetic mean, and MSE is the mean squared error measured from the phylogenetic mean after first correcting for phylogenetic non-independence, assuming Brownian motion. The denominator is the expected MSE ratio under Brownian motion (Blomberg *et al.*, 2003). Values near 0 indicate a lack of signal, while values near 1 are expected if the trait evolved under a Brownian motion model (Blomberg *et al.*, 2003), thus resulting in a high phylogenetic signal. Losos (2008) pointed out that a value of *K* greater than 1 is evidence of evolutionary stasis. The null hypothesis of the absence of a phylogenetic signal is tested by calculating whether the observed *K* value is not greater than expected if the values for platyrrhine species were randomized among tips (Blomberg *et al.*,2003). The significance of the *K* statistic was assessed via permutation tests with 10,000 replications.

Finally, to explore the time pattern of size and shape variation, we calculated a disparity-through-time plot (DTT; Harmon *et al.*, 2003). This plot shows the changes in relative morphometric disparity along a phylogenetic tree. We calculated the morphometric disparity as the average squared Euclidean distance among all pairs of genera. The relative disparities for each subclade were calculated by dividing a subclade's disparity by the disparity of the entire tree (Harmon *et al.*, 2003). To describe the pattern of disparity through the phylogeny, we calculated at each node in our BEST tree the mean relative disparity of all subclades whose ancestral lineages were present at that time. Disparity values near zero imply that most of the phenotypic variation is partitioned among subclades rather than within each subclade (*e.g.*, among families, subfamilies, etc. rather than within each family, subfamily, etc.), whereas values near one imply the opposite, indicating that subclades have independently evolved to occupy similar places of morphological space (Harmon *et al.*, 2003). We compared the observed disparity through time to that expected if character

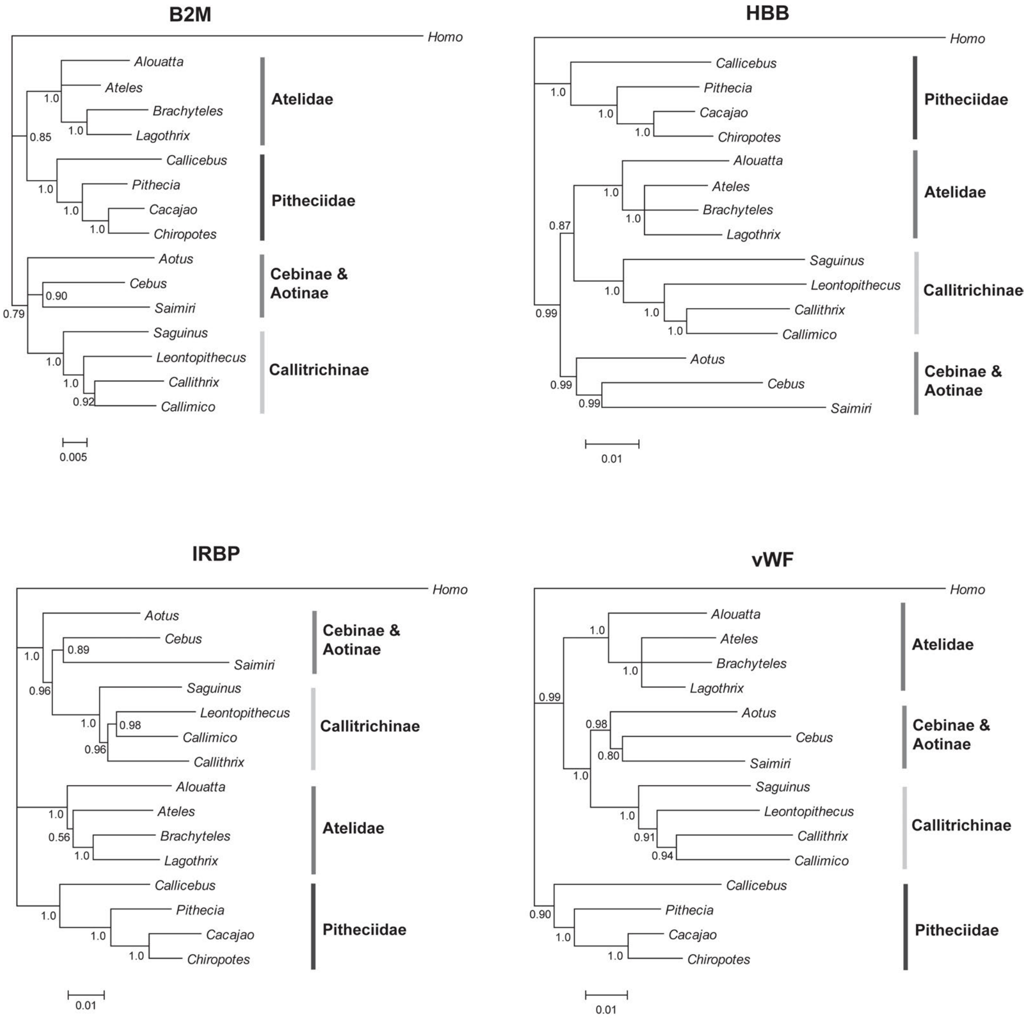

Figure 2. Gene tree phylograms obtained with *MrBayes* for the New World monkey genera for the nuclear gene sequences β2-microglobulin (*B2M*; 1.438 bp), β-globin (*HBB*; 1.224 bp), interphotoreceptor retinol binding protein (*IRBP*; 1.839 bp) and von Willenbrand factor (*vWF*; 891 bp). Numbers on branches represent posterior probability support.

evolution had followed a Brownian motion model of diversification by simulating character evolution 1,000 times across our tree (Harmon *et al.*, 2003).

The comparative statistical analyses were performed using the packages *picante*, *geiger*, *vegan* and *ape* for *R* 2.13.0 (R-Development Core Team, 2011).

Results

Phylogenetic history

The trees estimated individually for each of the four nuclear genes support (with high posterior probabilities) the previously recognized division of the platyrrhines into four clades: Atelidae, Pitheciidae, Callitrichinae and Cebinae plus Aotinae. These trees also display similar topologies for the genera within each major clade, compared to previous trees (Fig. 2; Opazo *et al.*, 2006; Wildman *et al.*, 2009). However, they are characterized by differences in the topological relationships among the four major clades. Whereas the *B2M* Bayesian tree shows a closer phylogenetic relationship between Atelidae and Pitheciidae with Cebidae being more distant, the *vWF* tree displays a closer relationship between Atelidae and Cebidae. The *HBB* tree supports a clade formed by Atelidae and Callitrichinae, while the *IRBP* tree fails to resolve any relationships among the four clades (Fig. 2). These single gene trees also differ in terms of branch lengths for each clade.

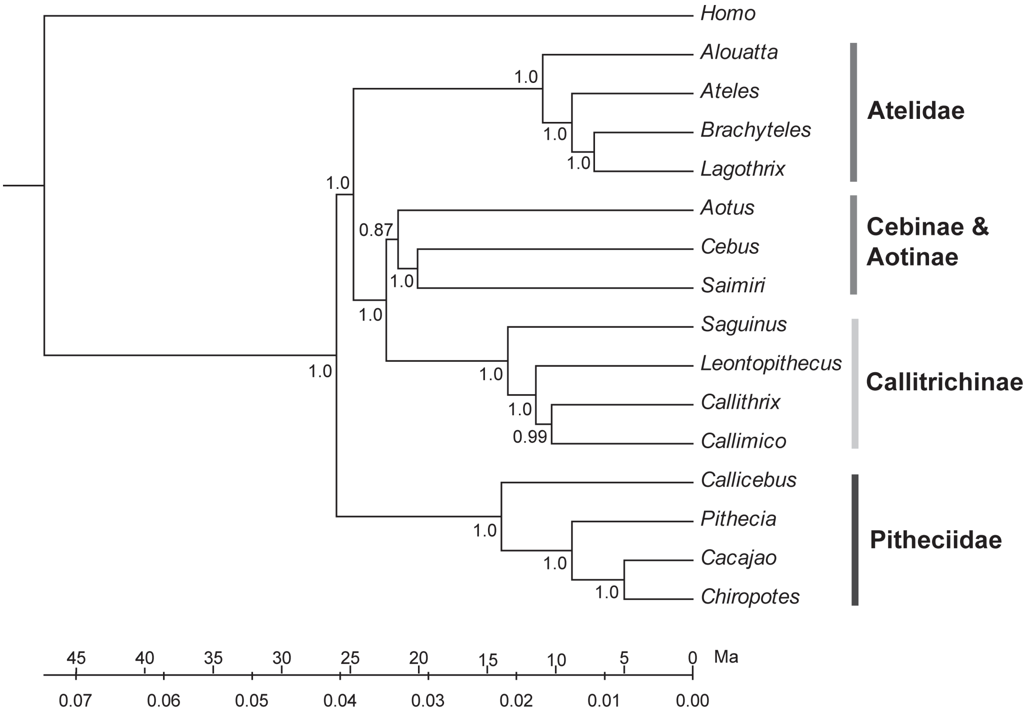

Figure 3. Phylogenetic species tree obtained with *BEST* for the New World monkey genera, combining B2M, HBB, IRBP and vWF gene sequences. Numbers on branches represent posterior probability support.

The *BEST* analysis of the combined four genes dataset yielded a tree in which most of the clades are strongly supported with high posterior probabilities (> 0.99), and only one terminal clade (*i.e.*, Cebinae and Aotinae) is characterized by a lower posterior probability (0.87; Fig. 3). This analysis supports the previous division of the platyrrhines into four clades and corroborates a closer phylogenetic relationship between Atelidae and Cebidae (Wildman *et al.*, 2009; Opazo *et al.*, 2006). Within the Atelidae, *Alouatta* is a sister group of a clade that includes *Ateles*, *Brachyteles* and *Lagothrix*, where *Brachyteles* and *Lagothrix* are clustered together. Within the Cebidae family, we find the Cebinae branch, which includes the *Cebus* and *Saimiri* genera as well as Aotinae, and the Callitrichinae branch, which is formed by the *Saguinus*, *Leontopithecus*, *Callithrix* and *Callimico* genera (Fig. 3).

Phenotypic variation

The ordination of the fifteen platyrrhine genera based on size variation (body mass and cranial centroid size) is shown in Figure 4.1. Although both variables are correlated, their relationship is not lineal. Moreover, the size ordination exhibits little correspondence with the four major phylogenetic clades (Fig. 3). Instead, this ordination shows a first group of platyrrhines smaller than approximately 1 kg in size comprising *Callicebus*, *Saimiri*, *Aotus* and the Callitrichinae genera. The second group contains four genera with sizes between approximately 2–3 kg including *Cebus* and the rest of the Pitheciidae genera. The third group includes the larger platyrrhines: the four Atelidae genera (approximately 6–9 kg). Conversely, the shape ordination based on the mean values of the first two RWs (approximately 64% of the total cranial shape variation among platyrrhine genera) shows a major correspondence with the four major phylogenetic clades (Figs. 3, 4.2). In addition, all Callitrichinae genera are very similar in shape, whereas the Atelidae and Cebinae genera show a major differentiation and the Pitheciidae genera display an intermediate shape differentiation (Fig. 4.2).

Shape variation along the first principal component or Relative Warp (RW) is demonstrated in Figure 4.3 as drawings of wireframe changes. This wireframe is composed of lines connecting landmark and semilandmark coordinates, which are transformed on the basis of RW loadings. The wireframe to RW1 shows changes that consist of a forward and downward growth in the facial region, a decrease in vault size, and a reorientation in the flexion of the cranial base. The Atelidae and Cebinae plus Callitrichinae clades are extreme in this variation (Fig. 4.2). The facial skeleton is larger in the Atelidae clade, whereas Cebinae and Callitrichinae show a more rounded cranial vault. The Atelidae clade also shows a foramen magnum that is backwards in orientation, whereas the Cebidae clade has a reorientation of the foramen magnum which is placed in a similar position to *Homo sapiens*, and is associated with the flexion of the cranial base. The RW2 primarily shows an increase in the relative orbital size, where *Aotus* is extreme in this variation (results not shown).

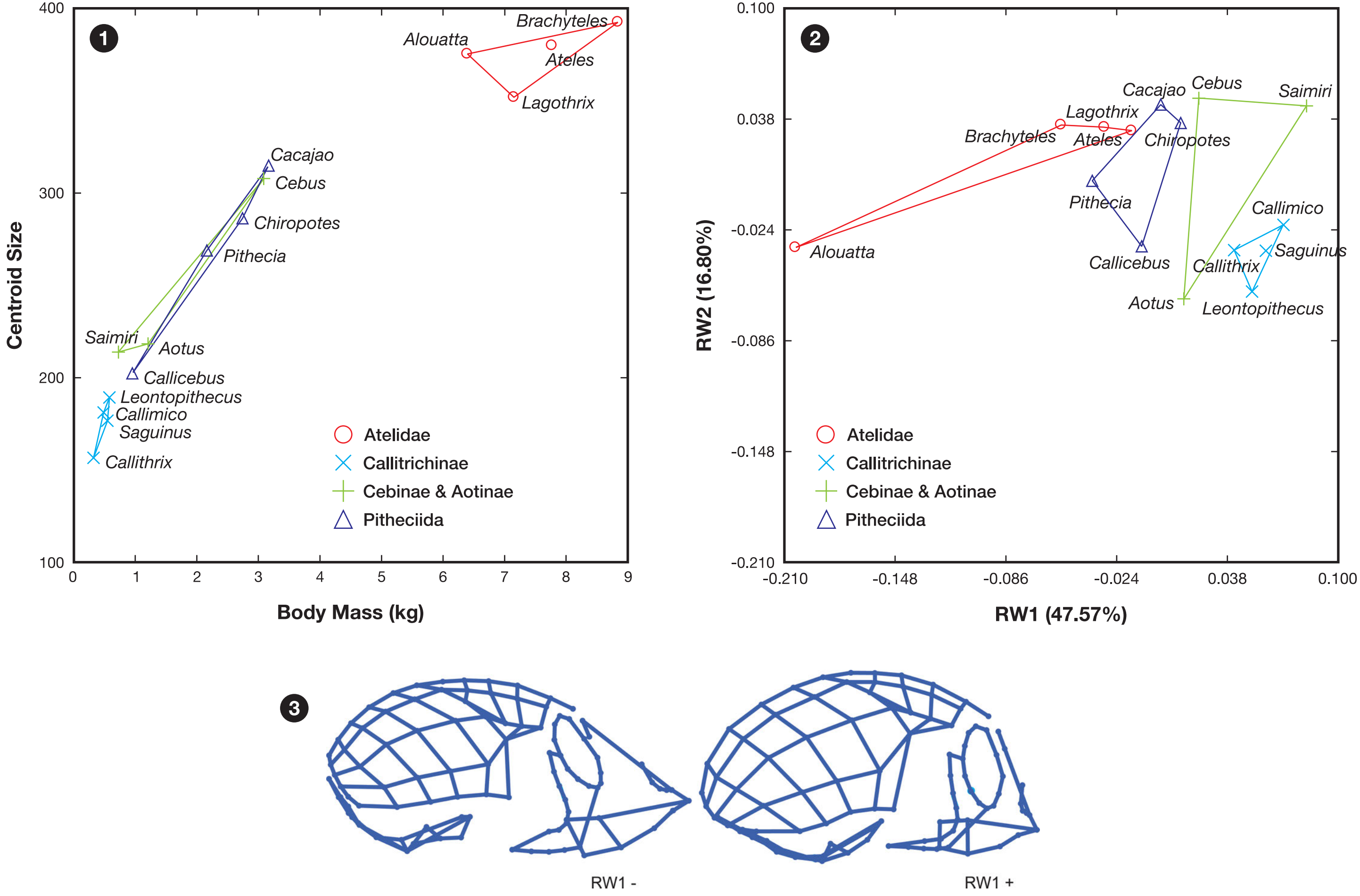

Figure 4. Ordination of the 15 platyrrhine genera in the space defined by the Body Mass and Cranial Centroid Size (**1**) and the first two RWs (**2**), as well as cranial shape changes along the first RW shown by wireframe changes (**3**). The dots represent the consensus individual for each genus.

Comparative statistical results

The *K* statistics revealed the existence of phylogenetic signal in body mass, cranial size and cranial shape variation (Tab. 3). Particularly, the LogBMass, the LogCS and RWs 1–3 show a significant phylogenetic signal. The use of the *K* statistic on the RWs is justified because they are linear combinations of the Procrustes shape coordinates aligned to the main direction of variation, and thus are expected to be proxies to phenotypically-relevant patterns. These results corroborate the importance of phylogeny in understanding the pattern of phenotypic variation observed among the platyrrhine genera. Moreover, the size and shape variables behave differently with respect to the *K* statistic (Tab. 3). For the LogBMass and LogCS variables, the *K* statistic is higher than what would be expected under a Brownian motion process, whereas for the RWs, it is nearly equal to the expectation (Tab. 3).

Table 3. Blomberg's *K* statistic and *P* values for Body Mass (LogBMass), Cranial Size (LogCS) and the first four relative warps (RW) for platyrrhines.

	K	***P***
LogBMass	2.1925	0.0001
LogCS	1.9403	0.0001
RW1	1.0053	0.0082
RW2	0.9689	0.0267
RW3	0.8426	0.0363
RW4	0.7505	0.1489

The disparity-through-time plots for the LogBMass, LogCS and RWs are shown in Figure 5. The DTT shows that both LogBMass (Fig. 5.1) and LogCS (Fig. 5.2) have values of average clade disparity near zero, whereas the shape variation has disparity values near the Brownian motion expectation (Fig. 5.3). These results indicate that most size variation occurs among the main platyrrhine clades, which tend to occupy more isolated regions of size space. In contrast, shape variation, both within and among the platyrrhine clades, changes proportionally with divergence among genera, according to the expectation under a hypothesis of shape evolution by unconstrained Brownian motion (*i.e.*, a random process). These results are concordant with the results in terms of the *K* statistic (Tab. 3) and with the visual size and shape ordination (Fig. 4).

Discussion

In this work, we studied the phenotypic diversification of the parvorder Platyrrhini, which began in the distant past (ca. 20 or 30 Ma; Opazo *et al.*, 2006; Rosenberger, 2010, Perez *et al.*, 2013). Given the antiquity of this diversification, it is very important to first obtain an independent estimation of the pattern and timing of the phylogenetic divergence

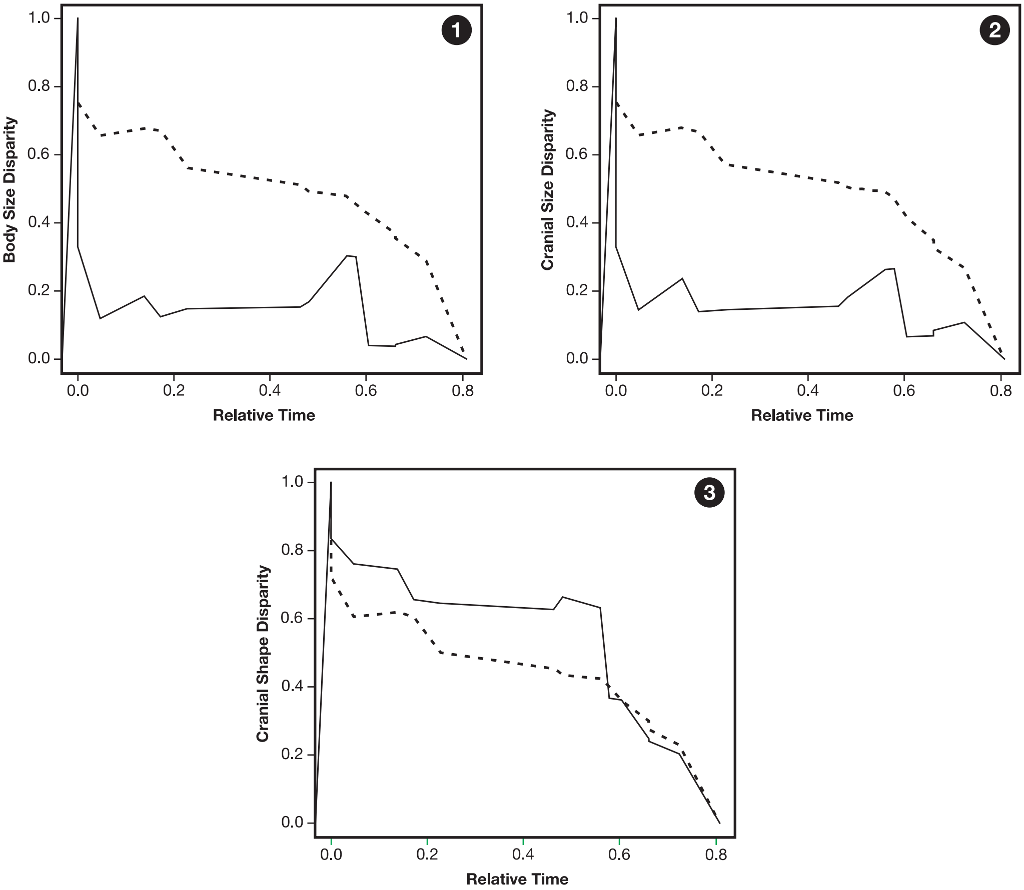

Figure 5. Relative disparity plots for the 15 platyrrhine genera for Log Body Mass (**1**), Log Cranial Size (**2**) and Cranial Shape (**3**). Time is expressed as a proportion of the total time after the first cladogenetic event inferred for the platyrrhines. Solid lines show actual size and shape disparity calculated for the clade; dashed lines show median expected disparity under the null hypothesis of Brownian motion evolution derived from 1,000 simulations.

among platyrrhines using molecular data before conducting any phenotypic diversification analyses. Several researchers have studied the phylogenetic relationships among platyrrhines using molecular data and gene tree estimation approaches, but have provided different views on the pattern and timing of platyrrhine divergence (see Schneider *et al.*, 2001; Horovitz *et al.*, 1998), a result also observed in our study (Fig. 2). These differences among single gene tree estimations can be explained by the fact that each gene may have an evolutionary history that differs from the underlying species tree, due to processes such as incomplete lineage sorting (Liu *et al.*, 2008; Knowles and Kubatko, 2010). More recently, techniques that employ information from multiple coding and non-coding DNA sequences, such as the Bayesian concatenation and Parsimony methods (Lemey *et al.*, 2009), have been used to estimate the platyrrhine phylogeny (Opazo *et al.*, 2006; Wildman *et al.*, 2009). However, these methods may not be useful for the estimation of a species tree if a species individual genes are polyphyletic (Liu *et al.*, 2008), as is true for the platyrrhine clade (Fig. 2). The Bayesian hierarchical model used in this study, which is based on the coalescent process, has been recently proposed for the estimation of robust species trees from multiple gene data (Liu, 2008; Liu *et al.*, 2008). Given the importance of estimating robust species trees in comparative studies of species with a long divergence history (Felsenstein, 1985; Wiens, 2009; Freckleton *et al.*, 2011; Losos, 2011), a species tree estimation based on the coalescent theory is a necessary first step for studies of phenotypic diversification on a macroevolutionary scale. A recent work using a similar approach arrived at similar conclusions (Perez *et al.*, 2012).

The pattern of phenotypic diversification during the branching process of the platyrrhines observed in our study is very interesting. Where previous studies have primarily focused on traditional morphometric methods for measuring cranial morphology variation—which are considered ineffective in describing separately the size and shape variation (Adams *et al.*, 2004; Zelditch *et al.*, 2004)—, our approach, which was based on geometric morphometric techniques, allows a more effective description of size and shape variation. Both size and shape disparity are extensive among platyrrhine genera. Particularly, size variation shows two extreme clades: Callitrichinae (approximately 200 – 900 g) and Atelidae, with genera that are ten orders of magnitude larger (approximately 6 – 9 kg) than the Callitrichinae genera. The pattern of cranial shape variation in platyrrhines corresponds to large differences in the relative position of the face, base, and cranial vault (Fig. 4). The Atelidae and Cebinae plus Callitrichinae clades are extreme in this shape variation (Fig. 4). The overall trends in shape variation among these clades can be summarized as follows: a) changes in the facial skeleton ranging from: a larger and more forward orientation in the maxillary region in the Atelidae clade, to a smaller and further downward orientation in the maxillary region in the Cebinae plus Callitrichinae clade, and b) changes in the neurocranial region ranging from: a smaller cranial vault and backward orientation of the foramen magnum in the Atelidae clade, to a more rounded cranial vault and downward orientation of the foramen magnum in the Cebinae clade.

Our analysis of size and shape variation using comparative methods suggests a more complex picture than previously suggested for the phenotypic diversification of platyrrhines during their phylogenetic history. Particularly, size and shape variation behave differently in our analyses. First, the body mass and cranial size variables depart from the Brownian motion expectation (Tab. 3). This suggests that the patterns of variation in these traits among platyrrhine genera may be shaped by an evolutionary stasis associated with deterministic factors. Therefore, phylogenetic conservatism —the retention of ancestral traits over time (Losos, 2008)—within the four main platyrrhine clades could be the most important characteristic of platyrrhine size diversification. Previous studies have suggested an evolutionary deterministic explanation for size variation (see Fleagle, 1999). Fleagle (1999) remarked on the association of body size with nutrient requirement, behavioral, and physiological variation. Primates in particular need a balanced diet of calories (energy), proteins, trace elements and vitamins for growth and reproduction. Fruits are high in calories, but, for small platyrrhines, proteins and vitamins are obtained primarily from insects, while for intermediate-large platyrrhines, these are obtained from leaves or seeds. In order to consume these different items, some platyrrhines have developed specific behavioral and physiological characteristics (*e.g.*, microorganisms in the digestive tract to break down cellulose in leaves), including a long digestive tract (Fleagle, 1999). In addition, diet plays a fundamental role in life history, as it provides the energy necessary for reproduction and growth.

Because cranial shape is related to numerous functions, including the acquisition and initial preparation of food—which has a direct association with ecological axes (Fleagle, 1999)—, it would be expected that shape variation in platyrrhines behaves similarly to size variation. Moreover, because different shape configurations may enhance the fitness of an individual, cranial shape may be subjected to selective pressures and therefore a pattern of evolutionary stasis similar to that seen for size variation would be expected. However, our analyses show that the cranial shape variation nearly fits the Brownian motion expectation. These results suggest that cranial shape differentiation among platyrrhines probably occurred via random processes during the branching process. Possibly only relatively small cranial shape changes associated with random processes characterized the platyrrhine diversification, causing a strong phylogenetic structure in the cranial shape disparity. Our shape variation results contrast both with our size variation results and with previous studies using traditional morphometric techniques (*e.g.*, Marroig and Cheverud, 2001). Cranial shape variation, which has been less explored on the macroevolutionary scale using model based approaches and geometric morphometric techniques, followed a clearly different pattern from size evolution. Additional studies are necessary to understand shape evolution on the macroevolutionary scale.

In summary, our approach allowed the study of the axes that are relevant for understanding the phenotypic diversification of platyrrhines. First, the BEST tree allowed an independent and robust estimation of the pattern and timing of phylogenetic divergence, which is a necessary first step for studies of phenotypic diversification at the macroevolutionary scale (Wiens, 1999; Losos, 2011). Second, methods in geometric morphometrics allowed shape and size, two important dimensions of phenotypic variation, to be studied separately (Bookstein, 1997; Zelditch *et al.*, 2004; Mitteroecker and Gunz, 2009). Finally, the comparative methods employed here allowed a detailed exploration of the pattern of phenotypic evolution (Blomberg *et al.*, 2003; Harmon *et al.*, 2003). This approach can be very useful for studying phenotypic diversification in clades with long phylogenetic histories. In the case of platyrrhine diversification, this approach allowed us to depict a more complex picture than has been suggested by previous studies. Specifically, we suggest that the phenotypic differences among platyrrhines could primarily be the product of deterministic factors, that generate stasis in size variation related to ecological diversity, and random factors, that generate shape differences that increase proportionally with phylogenetic distance. These proposals must be explored in the future by incorporating more comprehensive morphometric data and ecological variables.

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Appendix 1. Three-dimensional landmarks and semilandmarks (SL) for each cranial region and bone (Perez *et al.,* 2011).

Cranial region	Curve or surface	Landmark or semilandmark
Face	frontal curve	*Nasion (n)*
		Nasofrontale (nf)
	nasal curve	*Nasospinale (ns)*
	nasal curve	*SL nasal 1–2 (sl n1–2)*
	nasal curve	*Alare (al)*
	nasal curve	*Nasol (nl)*
	orbital curve	*Frontomalare anterior (fma)*
	orbital curve	*Sl orbitale 1 (sl o1)*
	orbital curve	*(SL) ectoconchion (ek)*
	orbital curve	*SL orbitale 2 (sl o2)*
	orbital curve	*Zygoorbitale (zo)*
	orbital curve	*SL orbitale 3 (sl o3)*
	orbital curve	*(SL) orbitale inferior (oi)*
	orbital curve	*SL orbitale 4 (sl o4)*
	orbital curve	*Dacryon (d)*
	orbital curve	*SL orbitale 5–6 (sl o5–o6)*
	orbital curve	*(SL) orbitale superior (os)*
	orbital curve	*SL orbitale 7 (sl o7)*
		Prosthion (pr)
		Premaxillare (pm)
		Canine (can)
		Premolare (prm)
		Ectomolare (em)
		Alveolon (alv)
		Palatine (p)
	malar curve 1	*Zygomaxillare anterior (zma)*
	malar curve 1	*SL malar inferior 1–3 (sl mi1–3)*
	malar curve 1	*Temporomalare inferior (tmi)*
	malar curve 2	*Frontomalare temporale (fmt)*
	malar curve 2	*SL malar superior 1–6 (sl ms1–6)*
	malar curve 2	*Temporomalare superior (tms)*

Appendix 1. (Continued).

Cranial region	Curve or surface	Landmark or semilandmark
Vault	frontal curve	*Glabella (g)*
	frontal curve	*Frontex (f)*
	frontal curve	*SL frontal medio 1–4 (sl fm1–4)*
	frontal curve/coronal curve/parietal curve	*Bregma (b)*
	coronal curve	*SL coronal 1–4 (sl c1–4)*
	frontal surface	*SL frontal 1–7 (sl f1–7)*
	parietal curve	*SL parietal medio 1–4 (sl pm1–4)*
	parietal curve/lambda curve/occipital curve	*Lambda (l)*
	lambda curve	*SL lambdica 1–4 (sl l1–4)*
	lambda curve/temporo-parietal curve	*Asterion (as)*
	temporo-parietal curve	*SL temporo-parietal 1–4 (sl tp1–4)*
	temporo-parietal curve	*Temperospheno (ts)*
	parietal surface	*SL parietal 1–12 (sl p1–12)*
Base	occipital curve	*SL occipital medio 1–3 (sl om1–3)*
	occipital surface	*SL occipital 1–4 (sl o1–4)*
	occipital curve	*Opisthion (o)*
		Basion (ba)
		Lateral foramen (lf)
		Spheno-occipital (so)
		Anterior ext audit meatus (amt)
		Posterior ext audit meatus (pmt)
		Porion (por)
		Anterior petrous (ap)
		Temporo-sphenoid-petrous (tsp)
		Temporo-occipito-petrous (top)

5 | PRIMATES FROM THE INSIDE: GENOMES AND CHROMOSOMES

LOS PRIMATES DESDE ADENTRO: GENOMAS Y CROMOSOMAS

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Abstract. Earlier studies on primates, carried out in Argentina in the 1980s, dealt with species level descriptions of zoo and animal house specimens. Some of these species were used as models in biomedical research because of their close relationship with humans, giving rise to basic and applied research such as species diagnosis, rabies and poliomyelitis vaccine studies and cell line characterization. New advances were made both methodologically and theoretically once the concept of population level was introduced in the discussion about what constitutes a species and their evolutionary relationships. By describing the heterochromatin polymorphisms present in some of the Argentinian species, a distinctive kariological characteristic for some Platyrrhini genera, this greatly expanded the knowledge of chromosomal rearrangements valuable from an evolutionary standpoint. At the same time, particular structural rearrangements were described, such as multiple sex chromosome systems. These were characterized by meiotic studies and genomic conservation analysis using Fluorescence *In Situ* Hybridization (FISH). At the molecular level, genetic studies of variability in mitochondrial and nuclear DNA polymorphisms contributed to our understanding of intra- and interspecific variability in primate species from Argentina. All these qualitative studies provided the basic knowledge to reach an interpretation of evolutionary processes at the family level and to propose a chromosome-based phylogeny incorporating other variables (genetic, ecological and ethological, among others). A second methodological leap was achieved by introducing Comparative Genomic Hybridization (CGH). By analyzing the genome of congeneric species, CGH determines what percentage of their genome is shared, and on which chromosomes differences are located. Currently, we approach studying primates by analyzing the structure and organization of a cell nucleus within the context of genome dynamics. Here, we discuss how the evolutionary process might occur and how primate genomes might be modulated.

Resumen. Los primeros estudios cromosómicos en primates en la Argentina se desarrollaron en la década de 1980, tratándose de descripciones a nivel de especie con ejemplares mantenidos en

zoológicos y bioterios en aquel momento. Algunas de estas especies se utilizaban como modelos de estudio biomédico por su cercanía filogenética con el hombre, dando lugar a investigaciones básicas y aplicadas; tal el caso del mono tití *Callitrhix jacchus*, que había demostrado ser sensible a la infección experimental con virus Junín. A partir del cultivo de linfocitos de sangre periférica de este mono neotropical se estableció en nuestro país la primera línea celular *in vitro* derivada de un primate sudamericano, identificada como HVB 4156. En los siguientes 20 años se produjo un salto notable, tanto metodológico como de marco teórico, al considerar el nivel poblacional en la discusión acerca de qué es una especie y cuáles son las relaciones evolutivas entre ellas. Se amplió el número de especies, géneros y familias en estudio, considerando las relaciones evolutivas ya no solo entre las especies con distribución geográfica en la Argentina, sino también las especies que habitan países limítrofes como Brasil, Paraguay y Bolivia. Se comenzaron a realizar estudios comparativos entre distintas poblaciones y colonias de cautiverio de las mismas especies a fin de identificar posibles polimorfismos regionales, locales e incluso interindividuales. Como todos sabemos, el establecimiento de la técnica de reacción en cadena de la polimerasa revolucionó el campo de la biología molecular, permitiendo analizar regiones acotadas y concretas del genoma. Se ampliaron así los estudios a nivel genético-molecular orientados a la resolución de las relaciones filogenéticas, en este caso particular, en Platyrrhini. Se publicaron las primeras filogenias moleculares de Primates del Nuevo Mundo empleando secuencias de distintas regiones nucleares y mitocondriales. La incorporación del análisis de distintas regiones de ADN mitocondrial (COII, Cytb y RC) mostró la presencia de dos clados mitocondriales de *Alouatta caraya* en simpatría en el extremo sur de su distribución, en Argentina. Estos nuevos hallazgos permitieron proponer a esta región, particularmente a la altura de Isla Brasilera, en Corrientes, Argentina, como un área de contacto secundario entre dos poblaciones de aulladores negros y dorados, previamente alopátricas, que se explicaría como producto de una expansión demográfica al principio del Holoceno. Se profundizó en el conocimiento de los reordenamientos de valor evolutivo en las especies de Argentina a partir de la descripción de polimorfismos de heterocromatina, característica distintiva en algunos géneros —como por ejemplo *Cebus*— y escasa en otros, como es el caso de *Alouatta*. Al mismo tiempo se describieron reordenamientos estructurales particulares, tales como los sistemas cromosómicos sexuales múltiples, caracterizados mediante estudios meióticos. Como característica distintiva en distintas especies de aulladores se demostró la presencia de distintos sistemas múltiples, como por ejemplo $X_1X_1X_2X_2/X_1X_2Y$ en *A. belzebul* y $X_1X_1X_2X_2/X_1X_2Y_1Y_2$ en *A. seniculus* y *A. caraya*. Al mismo tiempo, la Hibridación *In Situ* Fluorescente (FISH) permitió revelar reordenamientos intercromosómicos y continúa siendo el método primordial para revelar la organización del genoma. Desde hace unos años, los estudios de evolución cromosómica en mamíferos se han basado en la hibridación cruzada entre especies, también conocida como Zoo-FISH, y su uso se ha extendido aplicándose a múltiples especies. Los resultados de pintado cromosómico han posibilitado proponer cariotipos ancestrales de diferentes grupos, así como también los modelos a partir de los cuales estos cariotipos se habrían originado. Así, no solo han sido útiles para la caracterización de cada especie, sino que además la información proporcionada por la citogenética molecular se ha incluido como un carácter adicional en las reconstrucciones filogenéticas. Este abordaje citogenético moderno, dado por la combinación de técnicas citogenéticas clásicas y moleculares, permitió asimismo brindar un aporte fundamental en lo que respecta al manejo de especies en cautiverio. La caracterización citogenética a nivel de especie de ejemplares de cautiverio sin procedencia cierta se ha constituido en una herramienta importantísima a la hora de su reasignación y reubicación en distintos planteles, o incluso de tomar la decisión de quitarlos de los proyectos de reproducción en cautiverio por tratarse de animales híbridos. A nivel genético-molecular, los estudios de polimorfismos de variabilidad en ADN mitocondrial y nuclear aportaron a la caracterización de la variabilidad intra e interespecífica de las especies de primates de Argentina. Estos estudios cualitativos permitieron abordar la interpretación del proceso evolutivo a nivel de familias, donde entre otras cosas se llegó a proponer una filogenia cromosómica relacionada con otras variables taxonómicas y genéticas. Un nuevo salto metodológico y argumentativo lo brindó la Hibridación Genómica Comparativa (CGH). Surgida como una derivación de FISH, CGH es la única metodología de análisis que enfatiza y resalta las diferencias genómicas entre las muestras estudiadas, a la vez que permite obtener información cuantitativa sobre esas diferencias. De esta manera, ya no solo se estudiaban los primates a nivel evolutivo sino también a nivel de estructura y dinámica genómica. Como consecuencia de la necesidad propia de la disciplina de avanzar más allá de los límites establecidos, recientemente consideramos la incorporación de una nueva dimensión espacial de análisis a través de FISH–3D. Esta moderna aplicación de FISH complementa el estudio a nivel de dinámica del genoma, ya que permite el análisis de la estructura y organización del núcleo celular, considerado desde los modelos actuales de arquitectura nuclear una organela altamente compartimentalizada. Hoy se estudia a los primates desde un abordaje que permite el análisis de la estructura y organización del núcleo celular en un contexto de dinámica del genoma. En este marco, se discute la forma en que el proceso evolutivo ocurriría, así como la modulación a la cual está sometido el genoma de estos primates.

Why and what for?

When we were invited to participate with our investigation on evolutionary cytogenetics in the context of advances in primatology in Argentina, it took some time to decide how best to address this issue. We all agreed that the best approach was, first, to introduce the questions driving our research and the prevailing paradigms underlying each study; and second, to describe the questions or points of view raised by our results and to discuss shifts in some of the paradigms considered.

Our earlier, karyotypic analyses of primate species in Argentina were based on a single analysis framework: "one karyotype-one species-one geographic distribution". However, the results of cytogenetic characterization obtained for species from Argentina as well as bordering countries indicated a high degree of chromosome variability suggesting this analysis framework would not apply to all primates. Therefore, we extended the framework to include neotropical primates evolution and made comparative studies using both cytogenetic and molecular data from nuclear and mitochondrial DNA. At this point, we considered chromosome evolution as the most likely mechanism for generating diversity and speciation in primates. This paradigm was subsequently used as the basis for research on the evolution of neotropical primates in the following decades.

It soon became evident that the diversity of divergence patterns could not be explained by a single mechanism of chromosome speciation. The wealth of data gathered over the past 30 years allows us to propose new hypotheses about the processes and/or mechanisms that could have led to the present-day diversity of primates, thus contributing to a more integrated understanding. Currently, we focus on combining structural and functional genomic characteristics of neotropical primates.

Background

In the earliest 1960s, the diversity of higher organisms was frequently characterized based on morphological criteria, which, in general, were associated with different phases of the life-cycle in a given environment. In turn, genetic cell heritage and chromosome information contained in the nucleus have played—and still do—a key role in the characterization of individuals. The earliest studies of karyotypic characterization of non-human primates began in the 1960s. At that time, cytological characters concerning the structure of the eukaryotic chromosome, including shape, size and number, were used as a tool to solve taxonomic problems. Some taxonomists (Hershkovitz, 1984) began to consider intra- and inter-chromosomal differences as useful for analysis and subsequent classification. Studies of population and evolutionary genetics, taken together with viewpoints on the concept of species, introduced the karyotype as a new variable for species characterization. The routine karyological analyses included somatic chromosomal number, absolute and relative chromosome sizes, centromere position, satellite position, and marker chromosomes. Shortly afterwards, new characteristics were added, *i.e.*, differential staining patterns of chromosome bands (G-, Q-, NOR-, R-, T-, FPG and DAPI- banding, among others) with structural and functional implications, along with distribution and proportion of heterochromatin (C-banding, restriction enzymes) (Caspersson *et al.*, 1970; Dutrillaux and Lejeune, 1971; Seabright, 1971; Sumner, 1971; Schweizer, 1976).

In this context, pioneer work on cytogenetic characterization of monkey-derived material were also performed in Argentina. One of the most important advances was the characterization of cell lines, such as the Vero line (*in vitro* culture of kidney cells from *Chlorocebus aethiops*, an Old World primate, used for rabies and poliomyelitis vaccine studies). Bianchi and Ayres (1971) used C- and G-banding to reveal the presence of centromeric and extra-centromeric heterochromatin and to describe marker chromosomes at different cell passages. At that time, the variability in chromosome number with increasing cell line passages was considered to be infinite, as reported in international journals (Rodova *et al.*, 1979; Savelyeva and Mamaeva, 1987; Tsareva *et al.*, 1989). In the 1980s, it was acknowledged that a low number of passages ensured karyological uniformity, and that marker chromosomes showing different types of structural rearrangements (detectable by C-banding) appeared between passages 12 and 15.

In those years, various studies of karyological characterization focused on primate models used in biomedical research, such as *Callithrix jacchus*, a species that was found to be susceptible to experimental infection by Junín virus (Weissenbacher *et al.*, 1979). Developed in Argentina, lymphocyte cultures from *C. jacchus* represented the first *in vitro* cell line derived from a South American primate, identified as HVB 4156 (Mudry *et al.*, 1981). Until then, karyotypic studies of other primates were usually made for diagnosis at the species level. These specimens were held in captivity and used as model organisms for basic and applied biomedical research due to their close phylogenetic relationship with humans (De Boer, 1972; Chiarelli *et al.*, 1979; Dutrillaux, 1979).

By the next decade, research interests expanded to incorporate an evolutionary approach. Data included morphological, karyological and environmental variables, resource availability, diet, behavior, social structure and geographic distribution. Indeed, many studies were developed within different theoretical frameworks concerning, for example, phylogenetic relationships. Traditional cytogenetic techniques, used to answer specific questions, began to be used in combination and thus provide new information for species diagnosis. This led to a more comprehensive picture of organismal evolutionary history.

Genetic variability and cytogenetic diagnosis as cornerstones

With the addition of population level analyses in 1980, debates concerning the species concept and possible evolutionary relationships began to clarify. This heralded major improvements in methodological and theoretical investigation. Such a change in approach was facilitated by a collaborative agreement with zoos. We now had the opportunity to taxonomically characterize primates in captivity by providing zoos with a cytogenetic characterization of their specimens, a useful tool for colony management. The karyological analyses resulted in reassigning some individuals that had been classified as a different species based on traditional phenotypic criteria (Nieves *et al.*, 2003; Steinberg *et al.*, 2014a). This characterization also facilitated access to material for genetic studies on speciogenic processes in neotropical primates, where new objectives and novel methodologies could be tested.

Under the original framework of "one karyotype-one species-one geographic distribution", we explored similarities and differences in the chromosomes of platyrrhine families, genera and species to understand the underlying evolutionary relationships among them. In Argentinian species, describing heterochromatin polymorphisms, a distinctive feature for some genera, contributed to our understanding of evolutionarily significant chromosomal rearrangements. Particular structural rearrangements were identified, such as those leading to multiple sex chromosome systems. The Argentinian species *Alouatta caraya*, *Alouatta guariba*, *Aotus azarae*, *Cebus libidinosus* and *Cebus nigritus* from the Platyrrhini families Cebidae and Atelidae were thoroughly investigated. Chromosomal rearrangements and polymorphisms were analyzed by karyotyping and occurrence frequency to find out how many of them are shared. This assesses the amount of divergence between species and serves as markers for each genus (Mudry, 1982; Mudry *et al.*, 1984; Matayoshi *et al.*, 1986, 1987; Mudry, 1990; Mudry *et al.*, 1994; Ponsà *et al.*, 1995; Rahn *et al.*, 1996; among others). Moreover, other South American species and genera distributed outside Argentina (*Alouatta* sp., *Aotus* sp., *Ateles* sp., *Cebus* sp., *Callithrix* sp., and *Saimiri* sp.) were also characterized cytogenetically. The analyses revealed great diversity and prompted new questions on the evolutionary history of Platyrrhini, supporting the need to revise testing hypotheses. For the genus *Cebus*, which was intensively studied by us, we applied different methods that provided complementary information on its genome. Results were analyzed using an array of approaches and provided insights into understanding present-day patterns of diversity within the genus (Mantecón *et al.*, 1984; Mudry de Pargament *et al.*, 1985; Mudry *et al.*, 1987, 1991; Ferrucci *et al.*, 1995; Martinez *et al.*, 2002, 2004).

In addition, the electrophoretic isoenzyme analysis and the characterization of polymorphisms in nuclear and mitochondrial DNA also provided information that shed light into the intra- and interspecific genetic variability of some primate species from Argentina (Szapkievich *et al.*, 1998, 2002; Ascunce *et al.*, 2003; Szapkievich and Mudry, 2003;

Ascunce *et al.*, 2007). We found a low number of chromosome polymorphisms in the black and gold howler monkey (*Alouatta caraya*) both from the wild (southern distribution limit) and captivity, but the molecular genetic studies highlighted information that could be useful for adequately managing howler monkey groups. For example, we conducted karyological and kinship studies on specimens confiscated from the illegal pet trade, which were kept in semi-captivity at the Centro de Reeducación del Mono Aullador Negro (CRMAN), La Cumbre, Córdoba. In this center howler monkeys were rescued, rehabilitated and released under controlled conditions in a landscape composed of patchwork exotic forest. Paternity and kinship data accounted for behavioral dynamics (*e.g.*, hierarchy of dominance or expulsion), which seemed contradictory to observations from the wild. Consequently, we demonstrated kinship between two females of the same troop and confirmed its absence in another female who had been expelled from the group despite being thought to be the offspring of a dominant female. This scenario is consistent with a linear hierarchy among dominant females, as would be expected in the wild (Bruno, 2011 and references in it). Our studies have also contributed to the knowledge of the species' behavioral ecology, indicating a high plasticity to withstand environmental conditions different from those in its native range.

Paradigm shifts

At the beginning of the 1990s, molecular genetics became increasingly relevant in many scientific disciplines, contributing to a conceptual shift reflected in our studies. Our goal was to answer questions such as: how much genetic variability is in natural populations? Which demographic, historical and ecological events might have affected genetic variability? In which species or populations might they have occurred? These questions were addressed in a broader evolutionary context, rather than aiming to purely characterize genetic variability *per se*. Moreover, we assumed that the most probable mechanism giving rise to the diversity and speciation in primates would be chromosome evolution.

Molecular studies in neotropical primates began with the characterization of protein variants at both the generic and specific levels in different species, mostly belonging to the *Cebus*, *Saimiri*, *Aotus* and *Alouatta* genera. Blood phenotypes frequency was analyzed using agglutination tests, and the allelic variants at different enzyme loci (Carbonic anhydrase, Lactate dehydrogenase, Glyoxalase, Esterase D) were characterized using horizontal electrophoresis (Schneider *et al.*, 1991; Sampaio *et al.*, 1996). The earlier morphological studies divided Platyrrhini into 16 taxa (Ford, 1986; Rosenberger and Strier, 1989; Kay, 1990). This classification prevailed until the advent of molecular genetic analysis techniques. The PCR technique (Polymerase Chain Reaction) revolutionized the field of molecular biology, allowing the analysis of well-defined, delimited genomic regions. In the mid 1990s, this technique was applied to molecular genetic studies of Platyrrhini phylogeny (Schneider and Rosenberger, 1996; Schneider, 2000; among others). Although some taxonomic problems could be resolved, it also opened up new controversies. For example, some authors have recently proposed to separate the number of species and subspecies in the genus *Cebus* into two different genera: *Cebus* and *Sapajus* (gracile and robust capuchins, respectively) (Silva Jr., 2001; Lynch-Alfaro *et al.*, 2012; Ruiz-García *et al.*, 2012). In the *Saimiri* and

Ateles genera, molecular data coupled with structural rearrangements such as inversions and translocations provided strong support for phylogenetic inferences and traditional taxonomic classification (Nieves *et al.*, 2005; Steinberg *et al.*, 2009).

Likewise, combined data sources were gradually applied in the field of Cytogenetics to obtain more reliable results. In particular, molecular-based Fluorescence *In Situ* Hybridization (FISH) is a technique commonly used to detect and localize the presence of specific DNA sequences. FISH is based on hybridizing a labeled chromosomal fragment (probe) with a DNA target and is performed on interphase nuclei or metaphase chromosomes. Among other applications, it is valuable for distinguishing qualitatively between DNA samples of different origin.

The cross-species chromosome painting (Zoo-FISH) technique has been increasingly used for about 15 years in studying chromosome evolution in mammals (Consigliere *et al.*, 1996; Richard *et al.*, 1996; Bigoni *et al.*, 1997; Müller *et al.*, 1999; Neusser *et al.*, 2001; among others). Chromosome painting has proven to be efficient for visualizing interchromosomal rearrangements (translocations) and remains the method of choice for revealing global genome organization. Moreover, chromosome painting data have led a number of researchers to propose ancestral karyotypes for different groups, and have been used to generate models accounting for the origin of their karyotypes. Thus, this technique is useful for species characterization and provides molecular cytogenetic data used as additional characters for phylogenetic reconstructions (Medeiros *et al.*, 1997; de Oliveira *et al.*, 2002, 2005, 2012; Nieves *et al.*, 2005; Stanyon *et al.*, 2008; Steinberg *et al.*, 2014b; among others).

Although FISH is useful for answering several cytological questions, multiple experiments are needed to characterize a full karyotype when comparing different species. Indeed, specific probes for a particular chromosome or chromosomal region are rather difficult to obtain. In the case of neotropical primates, hybridization has most often been conducted with human-derived probes, with results indicating partial and particular differences. In other words, most of the available information on the genome of Platyrrhini is based on our current knowledge of the human genome. In addition, FISH analysis of chromosomal homeologies in closely related species may mask the complexity of important genomic events. It is uninformative regarding the interpretation of rearrangements that are either complex or involve small translocated fragments, failing to detect what makes a species karyotype and genome unique (Stanyon *et al.*, 2008). Therefore, a slightly different approach should be adopted to investigate and highlight the peculiarities of each species with respect to its closely related relatives.

Besides assessing chromosomal composition and the degree of homeology between related species, chromosome painting has been applied to whole genomes using Genomic *In Situ* Hybridization (GISH), *e.g.*, when comparing hybrid species to their putative parental species. Comparative Genomic Hybridization (CGH), which is a FISH-based technique, has emerged as the only analysis tool highlighting genomic differences between the studied samples that is able to provide quantitative information on these differences. It was initially developed for molecular cytogenetic analysis of human solid tumors in relation to healthy cells (Kallioniemi *et al.*, 1992), but its use has expanded beyond human medical clinics to be applied to evolutionary and descriptive studies of different organisms (Toder *et al.*, 1998;

Traut *et al.*, 1999; Dunham *et al.*, 2002; Sahara *et al.*, 2003; Neusser *et al.*, 2005; Nieves *et al.*, 2010; Fantini *et al.*, 2011). At that time, variants of fluorescence *in situ* hybridization (GISH, CGH) started to be used to study the degree of molecular differentiation between sex chromosomes in neotropical primates. CGH is useful because it reveals differences between genomes that cannot be detected by other molecular cytogenetic techniques. Moreover, it allows a quantitative approach for an evolutionary analysis of the genome. For these reasons, CGH is chosen for the analysis of hybrids and closely related species showing differences in genome size.

It took decades to build our knowledge of chromosome structure in different primate species, shifting from characterizing the shape, number and distribution of chromosomes in the karyotype to characterizing their full genome. Here, as in many other areas of science, the road to progress was long and winding rather than smooth and straight, and strongly influenced by context and circumstances. This journey, understood as a collective and historical process, is relevant because it accounts for both changes in perspectives and expansion of interpretations, leading to the questions we ask today.

In keeping pace with the aforementioned technological advancements, as well as the need to overcome cytogenetic boundaries, we are working on incorporating a new spatial analysis tool, the tentatively named 3D–FISH. This modern application of FISH complements genome level study, since it allows analysis of the structure and organization of the highly compartmentalized cell nucleus (Foster and Bridger, 2005; Bártová and Kozubek, 2006). Thus, the comparison between chromatin arrangements in the nucleus of different species provides valuable information on evolutionarily conserved, and functionally relevant, structures of the nuclear architecture (Neusser *et al.*, 2007).

This combination of methodological improvements led to conceptual shifts and new approaches and interpretations, bringing together different disciplines to contribute to the growth of primate cytogenetics.

How we got to know *Alouatta caraya*

Earlier cytogenetic studies on *Alouatta caraya* (ACA) were conducted in the mid 1960s, using standard staining techniques (Giemsa conventional staining) (Egozcue and Egozcue, 1965; 1966). These studies reported a diploid number of 2N = 52 and XX/XY sex chromosomes. In the 1970s, the development of chromosome banding techniques such as G-, C-, NOR- and restriction enzymes led to characteristic banding patterns for ACA. These studies revealed structural features of the chromosomes for the first time, from both wild specimens of the Corrientes Province (within the species' natural distribution range) and in captive specimens (Mudry, 1982; Mudry *et al.*, 1984). In addition to contributing to the *A. caraya* taxonomic diagnosis, these methods were used to make the first interspecific cytogenetic comparisons. The results indicated that ACA has a higher degree of chromosome homeology with *A. seniculus* than with *A. palliata* or *A. belzebul* (Mudry *et al.*, 1994).

Towards the end of the 1990s, interdisciplinary approaches to taxonomic diagnosis were novel, and we were the first to use biochemical markers (analysis of blood protein electrophoretic patterns) in ACA. These markers enabled assessment of inter- and intraspecific variability within the genus, phylogenetic relationships, and possible divergence times for the different groups. Analyzing the patterns revealed genetic differences between ACA and congeneric species but not between the ACA populations on each side of the Paraná River (Szapkievich *et al.*, 1998; Szapkievich, 2001). The latter result indicates that the Paraná River does not act as a barrier to gene flow for these ACA populations, in contrast to that observed for other *Alouatta* species distributed along the Amazon and Orinoco rivers, such as *A. seniculus* and *A. belzebul* (Ayres and Clutton-Brock, 1992).

The inclusion of mitochondrial DNA markers (COII, Cytb and control region) in the analysis supported the presence of two mitochondrial clades occurring in sympatry at the southern extreme of the species' Argentinian distribution (Ascunce *et al.*, 2007). These new findings suggested that its southern distribution limit, particularly at the latitude of Isla Brasilera in Corrientes Province, is an area of secondary contact between two previously allopatric populations. This would be the result of a demographic expansion that took place at the onset of the Holocene (15,500–7,000 years ago). Subsequently, we applied nuclear markers (microsatellites) to populations located at this southern limit, and found evidence for a bottleneck event prior to the demographic expansion (Ascunce *et al.*, 2007; Ruiz-Garcia *et al.*, 2007). We also found low genetic diversity, which is of critical importance when designing conservation and management strategies.

All the data from cytogenetic and population genetic studies were obtained from somatic cell tissues (blood, fibroblast, cells found in feces, etc.). When analyzing a species, the studies in somatic cells provide only a portion of the information that can be obtained. For example, the characterization of a species' karyotype is not complete until the corresponding germ cell studies are performed, thus confirming or refuting the information obtained from the somatic tissue. The first studies of germ cells in genus *Alouatta* were performed in the late 1980s, revealing the presence of multiple chromosomal sex-determining systems, including $X_1X_1X_2X_2/X_1X_2Y$ in *A. belzebul* (Armada *et al.*, 1987) and $X_1X_1X_2X_2/X_1X_2Y_1Y_2$ in *A. seniculus* (Lima and Seuánez, 1991), both species previously thought to have XX/XY systems. Subsequent meiotic studies performed in ACA males showed a $X_1X_1X_2X_2/X_1X_2Y_1Y_2$ multiple sex chromosome system which is homeologous to that of *A. seniculus*, but had previously been undetected by mitotic analysis (Rahn *et al.*, 1996; Mudry *et al.*, 1998; 2001). This sex chromosome system was characterized as a translocation between chromosomes ACA7 and Y.

The development of FISH has made it possible to refine karyotypic comparisons between congeneric species, as they exhibit extremely rearranged karyotypes (Schneider *et al.*, 2001; Dobigny *et al.*, 2004). It has also contributed to a better understanding of the origin of these sexual systems. Thus, in the South American howler species, the autosomal pair involved in the Y-autosome translocation shares homeology with portions of human chromosomes 3 and 15 (Stanyon *et al.*, 1995; Consigliere *et al.*, 1996; Mudry *et al.*, 2001; de Oliveira *et al.*, 2002). This Y-autosome translocation would have occurred early, and only once, in the evolutionary history of the genus, with some authors suggesting $X_1X_1X_2X_2/X_1X_2Y_1Y_2$ as the most ancestral sex chromosome system (de Oliveira *et al.*, 2002). However, further studies in Mesoamerican species using these techniques indicated that the multiple sex chromosome systems found in *A. pigra* and *A. palliata* arose from a different autosomal pair (Steinberg *et al.*, 2008), suggesting an independent origin for the sex chromosome system in those groups.

Recently, molecular markers (Cytb) and chromosome markers (FISH-based homeologies and G-bands) were combined in the same analysis to create a phylogeny (Steinberg, 2011; Steinberg *et al.*, 2014b). The Mesoamerican species *A. pigra* and *A. palliata* were included in this phylogenetic analysis for the first time. In contrast to previous studies, ACA clustered together with species of the *seniculus* group (*A. sara*, *A. macconelli* and *A. seniculus arctoidea*) instead of being grouped with *A. belzebul*, thus supporting the results obtained from the FISH analysis (Stanyon *et al.*, 2011). *A. belzebul* was recovered as the most basal species within the clade of the Mesoamerican species, suggesting that the ancestral sex system in this group would be $X_1X_1X_2X_2/X_1X_2Y$ rather than $X_1X_1X_2X_2/X_1X_2Y_1Y_2$, as previously postulated.

Where are we going?

Like us, many researchers have "dissected" the genome of Platyrrhini using different approaches, at different but complementary levels of analysis. Cytogenetic studies in Platyrrhini have shown that it is a heterogeneous group in which most chromosomal divergence occurred following what was an early, rapid divergence between the presently recognized major taxonomic divisions (between 20.8 and 35 Mya) (Stanyon *et al.*, 2004; 2008). The speciation process might have been accompanied by chromosomal rearrangements, mainly fusions, fissions, inversions and translocations and major variations in heterochromatin regions (Seuánez *et al.*, 1986; Matayoshi *et al.*, 1987; Ponsà *et al.*, 1995; Mudry *et al.*, 2001; Seuánez *et al.*, 2005; Nieves *et al.*, 2011; de Oliveira *et al.*, 2012). Among the karyotypic peculiarities of the Platyrrhini, the presence of multiple sex chromosome systems in males of the genera *Alouatta*, *Aotus*, *Callimico* and *Cacajao* (Seuánez *et al.*, 1989; Moura-Pensin *et al.*, 2001; Solari and Rahn, 2005; Steinberg *et al.*, 2008, 2014b), constitutes an outstanding characteristic of the genome structural organization. The great diversity in karyotypes does suggest the participation of chromosomal rearrangements in the evolutionary changes of primates, giving rise to the present forms.

In the last 50 years, the speed of scientific progress, the blurring of the boundaries between different disciplines and the ongoing need for developing highly specialized and sophisticated knowledge and skills, have led to new theoretical frameworks, paradigm shifts and proposals for using transdisciplinary approaches to solve problems that have previously been discussed based only on a few data sources. Our working hypotheses for studying primate cytogenetics have also changed over time. While our research has historically focused on the evolutionary divergence process in Platyrrhini, we now look to continue this work using modern methods and in an interdisciplinary fashion, studying genome organization.

We study the genomic structure, composition and interaction between species and genera native to Argentina and other South and Central American countries. For example, Figure 1 illustrates how we have used classical cytogenetics to analyze karyotypes and marker chromosomes of different species, as well as how we have described different rearrangements involved in the evolutionary process of many genera. Then, we improved our knowledge of evolutionarily significant chromosomal rearrangements adding the Fluorescence *In Situ* Hybridization (FISH) technique, thus creating more precise identification of interspecific homeologies. However, these studies were qualitative only and somewhat limited, highlighting the need for quantitative analysis as well. This became possible by incorporating the Comparative Genomic Hybridization (CGH) technique, which allowed us to investigate the relationship between euchromatin and heterochromatin and to analyze karyotypic differences in other variables between the species of interest.

Our original framework of "one karyotype-one species-one geographic distribution" proved invalid due to the high variability resulting from different methodologies; likewise, "chromosomal speciation as the mechanism for explaining the diversity of primates" became doubtful due to the high diversity and variability at different levels of structural complexity. In light of results obtained by us over 25 years, we no longer consider chromosomal speciation as the only mechanism for the present-day pattern of diversity in neotropical primates; most likely, a specific set of events would have led to the complex current pattern of Platyrrhini diversification.

We must also consider how our results contribute to the definition and concept of species. The concept of a species is an integral part of the evolutionary theory and requires an understanding of taxonomy (Eldredge, 1993). 20 definitions compete for the meaning of

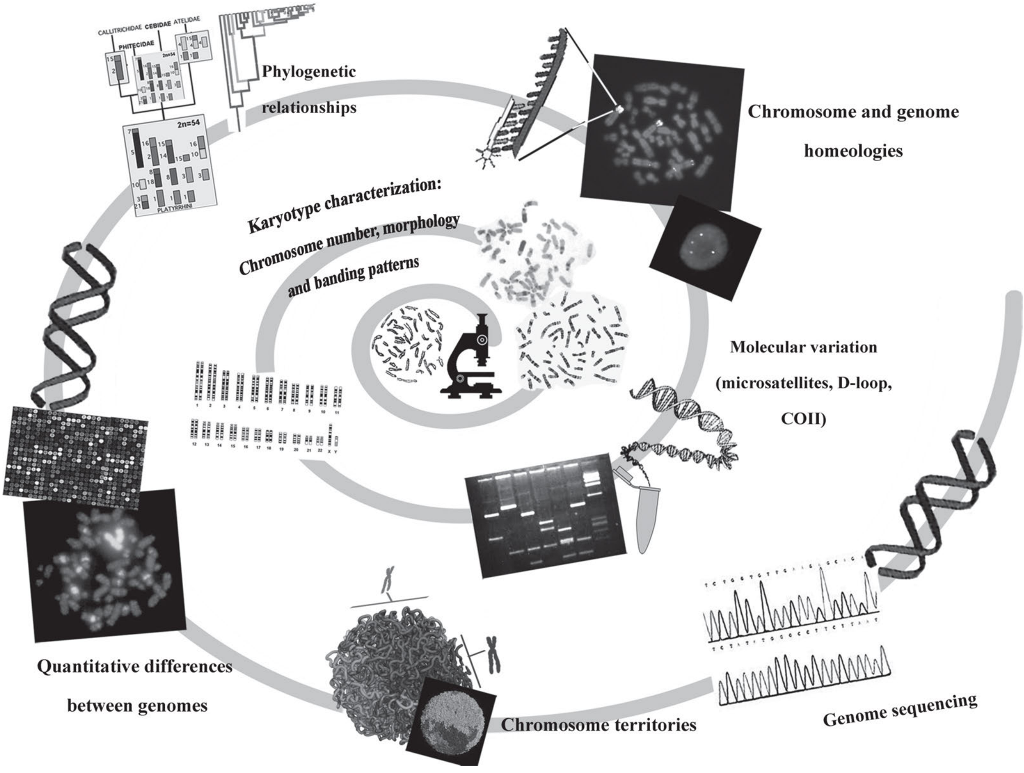

Figure 1. Illustration of the methodological progression developed through the years that accompanied the changes in the theoretical framework. Reading from the center of the spiral to the periphery.

species (Hey, 2001) but a comprehensive understanding of the species concept must go beyond its meaning, given the gravitas of its use. Rather, the way we think about a set of individuals determines how we conceive and define a species, as well as the questions being asked. This is particularly true for our research, where we make an account of the reciprocal effect of such interaction on our work over time. Our investigations, like those conducted by others, were initially based on the biological concept of species, defined as a reproductive community, a gene pool and a genetic system (Mayr, 1963). At that time, for example, we focused on the characterization of the karyotype and on genetic features determining reproductive discontinuity between sets of individuals (*i.e.*, species).

However, we have extended this characterization by incorporating new data and retaining the "classical" species concept to a greater or lesser extent. The new results and novel technologies led to changes in the paradigm, which, in turn, modified the concept of species. The phylogenetic assumptions of the past used different data while the current ones are usually based on a few nuclear and/or mitochondrial genes. As a result, the dominant positions rely on historical, rather than reproductive, relationships. Subsequently, species are now regarded as evolutionary entities whose cohesion is defined by their similarities, in genetic and, to a lesser extent, morphological traits.

In many cases, changes in the questions asked, and approaches taken led to purely reductionist interpretations of the results, since over-specialization may result in a single type of evidence. A current example is seen in *Cebus*: after 50 years of being considered a single genus, it has been proposed we divide it into two, *Cebus* and *Sapajus*, as inferred from the analysis of just one data source—the mitochondrial Cytb gene (Lynch-Alfaro *et al.*, 2011, 2012). According to the authors, this division is also strongly supported by further evidence including morphological, ecological and physiological data. However, we believe that the importance of the Cytb gene marker has been overestimated. Nonetheless, a debate about this taxonomic issue and its implications for the evolutionary study of *Cebus* is beyond the scope of the present work. Our position is that, although some reductionism is inevitable in science, the pursuit of knowledge requires a holistic approach encompassing different disciplines.

Clearly, such a controversial matter will be difficult to solve and we should look to our ultimate goal—to diligently study each level of complexity, identifying the properties and characteristics that emerge from; striving to interpret the evolutionary process.

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6

GENETIC VARIABILITY IN ARGENTINIAN HOWLER MONKEYS (*ALOUATTA CARAYA*): A NATURAL PROCESS OR AN ANTHROPIC EFFECT?

VARIABILIDAD GENÉTICA EN MONOS AULLADORES EN ARGENTINA (*ALOUATTA CARAYA*): ¿UN PROCESO NATURAL O UN EFECTO ANTRÓPICO?

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Abstract. Black and gold howler monkeys (*Alouatta caraya*) are the most southerly primates in the Neotropical region. Currently, they are threatened by the process of forest loss and fragmentation induced mainly by the expansion of the agricultural frontier, notably for cattle ranching and soy production. Previous studies suggest that habitat fragmentation modifies the dispersal patterns of black and gold howler monkeys, and might reflect a trend towards a reduction in their ability to disperse. Howler monkeys are very important from an epidemiological point of view because they are considered sentinels of yellow fever virus. Populations of this species have been seriously threatened by the yellow fever virus, with reported deaths in Argentina and Brazil. In the current study, we present preliminary results on the genetic variation in several black and gold howler populations in Argentina. We studied nine *A. caraya* populations from various provinces of northern Argentina, and one population from Paraguay. With the aim of characterizing the level of genetic variability, and the processes that determined the actual genetic structure, we analysed eight already-described microsatellites or STRs (short tandem repeats). We found significant differences between populations (FST = 0.105, $p < 0.001$). However, our analysis showed that there are some populations that share genetic characteristics and that these could be pooled as clusters. These populations are connected by rivers and riparian forests suggesting that dispersion may have had taken place through them. Our results not only contribute to the biological knowledge of the genus *Alouatta*, but also have direct and important applications in the field of conservation by identifying priority areas for establishing conservation units for *A. caraya*.

Resumen. Los monos aulladores negros y dorados (*Alouatta caraya*) son los primates neotropicales con distribución más austral. Actualmente están amenazados por el proceso de pérdida y fragmentación de los bosques, provocado principalmente por la expansión de la frontera agrícola, en particular para la cría de ganado y la producción de soja. Estudios previos sugieren que la fragmentación del hábitat modifica los patrones de dispersión de estos primates y podría reflejar una tendencia hacia una

reducción de su capacidad para dispersarse. Todas las especies de mono aullador son muy sensibles al virus de la fiebre amarilla y sufren un alto grado de mortalidad cuando están infectadas. Por lo tanto, se consideran especies centinelas para la detección temprana del virus. Las poblaciones de esta especie han sido seriamente atacadas por el virus de la fiebre amarilla, con muertes registradas en Argentina y Brasil. En consecuencia, un estudio del estado actual de la variabilidad genética y la conservación de estas poblaciones silvestres es de gran importancia desde el punto de vista epidemiológico. En el presente trabajo se presentan los resultados preliminares del estudio de la variabilidad genética en varias poblaciones de aulladores negros y dorados de Argentina. Se estudiaron nueve poblaciones de *A. caraya* de las provincias de Chaco, Corrientes, Formosa y Misiones del norte argentino y una población de Paraguay que habitan áreas con diferentes grados de aislamiento y modificación ambiental. Con el objetivo de caracterizar el nivel de variabilidad genética y los procesos que determinan la estructura genética actual, analizamos ocho microsatélites o STRs (*short tandem repeats*) previamente descriptos para la especie. Encontramos diferencias significativas entre las poblaciones (FST = 0.105, $p < 0.001$). Sin embargo, nuestro análisis muestra que hay algunas poblaciones que comparten características genéticas y que se podrían agrupar en tres grupos o *clusters*. Uno de estos *clusters* está representado mayoritariamente en las poblaciones Yacutinga, Piñalito, Yapeyú y Garupá, todas ubicadas en el río Paraná río arriba de la represa de Yacyretá o en la proximidad del río Uruguay. Las poblaciones más representativas del segundo *cluster* son la población de Paraguay y de Isla Brasilera (Argentina), que también están ubicadas en el río Paraná, pero aguas abajo de la represa de Yacyretá. El último *cluster* está muy representado por la población de San Alonso que se encuentra en los esteros del Iberá y está muy lejos de cualquier río importante (alrededor de 100 km al norte hasta el río Paraná y 120 km al este del río Uruguay). Las poblaciones de Guaycolec y PN Chaco mostraron un componente intermedio y alto de este tercer grupo. Además, ambas poblaciones están localizadas al norte del río Paraná y más cerca del río Paraguay que del río Paraná. Vale la pena mencionar que PN Chaco es la única población que contiene componentes considerables de los tres *clusters* y no mostró ninguna diferenciación en los análisis estadísticos de la población de Garupá. Esto no sigue los patrones que explican la estructura genética para todas las demás poblaciones analizadas. Bajo tales circunstancias, la explicación más parsimoniosa es que individuos de *A. caraya* hayan migrado desde Garupá hacia Parque Nacional Chaco. Esto solo podría ser factible mediante alguna forma de intervención humana, como la liberación de monos capturados. La represa de Yacyretá está ubicada muy cerca de la población de Garupá, y durante su construcción se capturaron y rescataron a muchos monos de áreas que iban a ser inundadas, y luego fueron transportados a otros sitios y liberados. Si los monos fueron reubicados en áreas protegidas como PN Chaco, esto podría explicar la variación genética observada para esta población. Las diferencias genéticas encontradas entre los tres *clusters* no se explican por aislamiento por distancia. En cambio, nuestros resultados sugieren la existencia de mayores tasas de dispersión entre las poblaciones conectadas por bosques continuos y una diferenciación o reducción del flujo genético entre poblaciones separadas por paisajes antrópicos modificados. Además, las características genéticas de una población sugieren que probablemente en el pasado hubo individuos de otra población genéticamente distinta. Por lo tanto, la estructura genética de las poblaciones de *A. caraya* se ve afectada por efectos antrópicos. Es probable que otros primates y no primates arbóreos que dependen de la continuidad de los bosques para dispersarse tengan efectos antrópicos inducidos similares sobre la diversidad genética, lo que podría resultar en una incapacidad para responder a los desafíos de las nuevas presiones selectivas.

Este estudio destaca la importancia de los ríos y bosques para la dispersión de algunos mamíferos, ya que las poblaciones que conforman los *clusters* están conectadas a través de los ríos o bosques ribereños, sugiriendo que la dispersión de *Alouatta caraya* se produce a través de los mismos. Nuestros hallazgos contribuyen a la comprensión de las características genéticas de muchas poblaciones de monos aulladores negros y dorados. Sin embargo, es necesario realizar más investigaciones sobre la estructura genética de estas poblaciones mediante muestreo y análisis adicionales de las secuencias mitocondriales para proporcionar información adicional que ayude a orientar futuras decisiones de ordenación de la conservación dentro del área geográfica de esta especie. Además de contribuir con el conocimiento biológico del género *Alouatta*, los resultados de este estudio tienen aplicaciones directas y relevantes en el campo de la conservación, permitiendo definir zonas prioritarias para establecer unidades de conservación para la especie.

Introduction

The black and gold howler monkey (*Alouatta caraya*) lives in the northeast of Argentina, southern Brazil, and adjacent parts of Bolivia and Paraguay (Brown and Zunino, 1994). This species has the southernmost distribution of all living Neotropical primates, and constitutes an important educational and conservation icon for the inhabitants of northeastern Argentina (Zunino and Kowalewski, 2008). Black and gold howlers, despite being described as a species at risk of extinction (CITES Appendix II, IUCN 2015), are considered threatened by the indiscriminate habitat clearance currently occurring on the southern edge of its geographical range (Brown and Zunino, 1994; Peres, 1997; Zunino and Kowalewski, 2008). Nowadays, in the Argentinian provinces of Formosa, Misiones, Salta, Corrientes, and Chaco, entire forest fragments of are being clear-cut for agriculture and cattle ranching (UMSEF, 2014). Several studies mention the capacity of this species to survive in fragmented and impoverished habitats, including forests that have suffered selective logging (Bicca-Marques, 2003; Zunino *et al.*,2007; Bicca-Marques *et al.*,2009). Although *A. caraya* groups still inhabit degraded and fragmented forests, the real capacity of howlers to endure under these levels of habitat degradation in the long-term is still unknown.

Habitat reduction and fragmentation trigger secondary processes in populations, including restrictions on dispersion, resource depletion and exposure to pathogens. All of these can generate a decrease in the effective population size and a reduction in the genetic variability that may lead to local extinction. The disappearance of local populations can quickly lead to wider-spread regional extinctions if populations are separated by distances large enough to prevent re-colonization of empty habitats or which present physical or anthropogenic barriers that reduce both movement and survivorship of dispersing individuals (Lacy, 1992).

Genetic variability of a species is essential for its long-term survival. When the genetic diversity of a population is reduced it decreases its evolutionary potential, reducing the ability to respond to future environmental challenges. It is therefore important to study levels of genetic variability in endangered species, especially when, like the black and gold howler, these species must confront rapid and extensive habitat fragmentation.

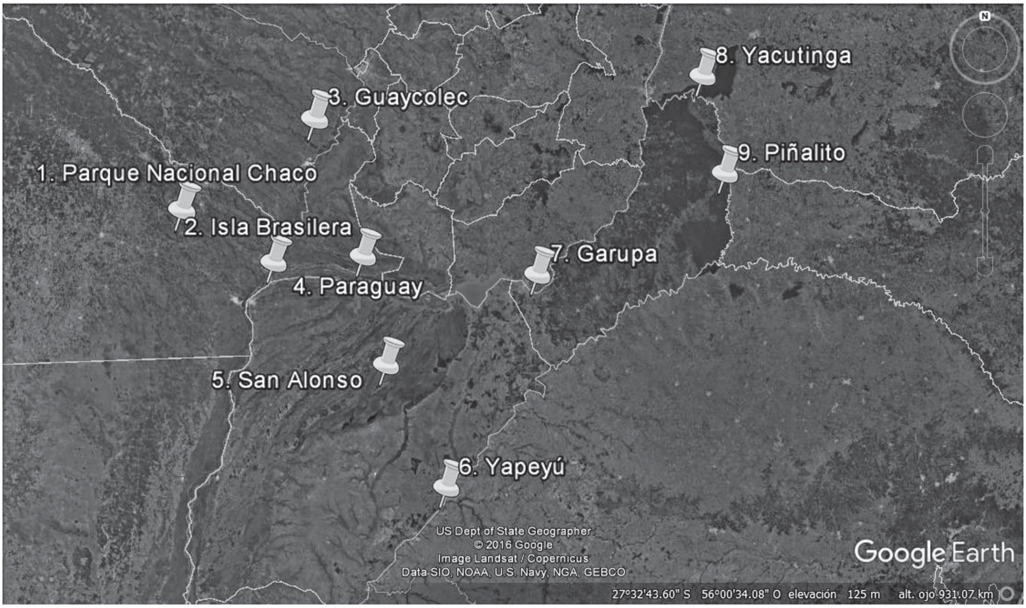

Figure 1. Geographic location of black and gold howler monkey (*Alouatta caraya*) populations sampled for this study.

All species of howler monkey are very sensitive to the yellow fever virus, and suffer a high degree of mortality when infected. They are therefore considered sentinel species for early detection of the virus (Rifakis *et al*., 2006). Consequently, a study of the current state of genetic variability and conservation of these wild populations is of great importance from the epidemiological point of view.

In summary, genetic data is relevant because determining genetic variability and population dynamics provides important insights concerning dispersal patterns at varying geographic scales, and this enables an evaluation of both, how dispersal might have affected historical gene flow (Prugnolle and de Meeûs, 2002), and which sub-specific population units are of greatest importance for the effective management and conservation of the species concerned (O'Brien *et al*.,1985).

Today, it is considered that molecular genetic methods are an essential tool for management and decision-making in the conservation of endangered species (Avise and Hamrick, 1996; Smith and Wayne, 1996; Avise, 2000). Phylogeography offers a mean to reconstruct the historical processes that triggered the current geographic distribution of biodiversity (Avise *et al*., 1987). For *A. caraya* previous phylogenetic analyses using mitochondrial DNA (mtDNA) revealed the coexistence of two divergent haplogroups in sympatry or in close proximity at the confluence of the Paraná and Paraguay rivers. This suggested that this would be an area of secondary contact between formerly divergent allopatric populations (Ascunce *et al*., 2007). This observation also suggested that, since the early Holocene, migration routes of the species have gone through unflooded forests and seasonally-flooded forests on the banks of the Paraná and Paraguay rivers (Szapkievich, 2001).

Microsatellites have proven to be an extremely valuable tool for the study of population genetics and mating systems, among others, in a wide range of organisms (Zane *et al.*, 2002). With these markers it is also possible to detect geographical genetic structure using the individual as the unit of analysis (Pritchard *et al.*, 2000). Such structure is not necessarily the signature of vicariance or long-term isolation of populations. In fact, any reduction in gene flow that allows for the divergence of allelic or haplotype frequencies will be found with this methodology (Zink and Barrowclough, 2008).

In the current study, we used eight microsatellite markers to interpret contemporary patterns of genetic variability and genetic divergences or similarities between *A. caraya* populations.

Specifically, we were interested in whether populations of *A. caraya* are structured according to rivers and their associated forests to test the hypothesis that rivers and riverside forests act as biological corridors for dispersal of *A. caraya* populations (Brown and Zunino, 1994).

If the dispersion medium used are those forests bordering rivers, the gallery forest (Brown and Zunino, 1994), and the matrix of remnant arboreal vegetation in areas now containing plantations or cattle (Marsh, 2003), it is expected that the ongoing processes of fragmentation and reduction of habitat within the geographic range of Argentinian *A. caraya* populations will increase their dispersal costs.

In this study, we sampled eight populations covering almost the entire geographical range of *A. caraya* in Argentina and one from Paraguay (N = 111, Tab. 1, Fig. 1). We analysed populations from the provinces of Chaco, Corrientes, Formosa and Misiones inhabiting areas with different degrees of isolation and environmental modification. Our results are relevant in identifying priority areas in which to establish conservation units for the species.

Table 1. Black and gold howler monkey populations studied, and *Structure* clustering.

Population number	Country	Province	Locality/Name	Latitude	Longitude	Clusters 1	Clusters 2	Clusters 3	No. of individuals
1	Argentina	Chaco	Parque Nacional Chaco	26° 47' 29.38" S	59° 37' 51.75" W	0.305	0.210	0.485	9
2	Argentina	Chaco	Isla Brasilera	27° 18' 48.82" S	58° 38' 47.33" W	0.959	0.018	0.023	47
3	Argentina	Formosa	Guaycolec	25° 58' 12.26" S	58° 10' 37.86" W	0.238	0.036	0.725	12
4	Paraguay		Paraguay	27° 16' 30.56" S	57° 41' 1.63" W	0.964	0.017	0.019	5
5	Argentina	Corrientes	San Alonso	28° 18' 26.48" S	57° 27' 26.99" W	0.008	0.010	0.982	10
6	Argentina	Corrientes	Yapeyú	29° 26' 42.72" S	56° 47' 59.28" W	0.010	0.859	0.131	9
7	Argentina	Misiones	Garupá	27° 28' 0.74" S	55° 49' 35.65" W	0.169	0.809	0.022	6
8	Argentina	Misiones	Yacutinga	25° 34' 28.01" S	54° 4' 31.36" W	0.023	0.887	0.090	4
9	Argentina	Misiones	Piñalito	26° 30' 0.00" S	53° 50' 0.00" W	0.011	0.935	0.054	9

Populations sampled and methods

Sampling

Faecal samples were collected non-invasively from each population in Argentina with exception of Isla Brasilera, from which DNA samples had already been obtained during previous studies (Oklander *et al.*, 2014). Two different faecal samples were collected from each individual immediately following their defecation, and preserved at room temperature in solid NaCl (Oklander *et al.*, 2004) until DNA extraction. DNA from five individuals from Paraguay was also available (Oklander *et al.*, 2009). Using faecal samples avoids invasive methodology, and so prevents any undesirable impact on study animal behaviour. In addition, it nullifies the possibility of injuries or infectious diseases transmission between the animal and the sample collector.

This study complied with current Argentinian laws and was conducted with permission from the National Resources Board, Fauna and Flora Department, Corrientes Province, Argentina. We adhered to the American Society of Primatologists' (ASP) Principles for the Ethical Treatment of Non-Human Primates (*https://www.asp.org/society/resolutions/EthicalTreatmentOfNonHumanPrimates.cfm*).

Laboratory procedures

A total of 111 individuals were genotyped in this study. DNA was extracted from faecal samples from 3 months to 1 year after collection, using the *QIAmp Stool* kit (*QIAGEN*). We used a multiple tubes procedure: five DNA extractions were carried out at the same time for every faecal sample, including negative controls. These five extractions were then pooled to provide a template for PCR (Taberlet *et al.*, 1996). To prevent possible contamination, the extraction procedure was performed in a sealed room dedicated to DNA extraction from forensic samples, hair, and faeces. Extractions were performed in a laminar flow hood with negative pressure. We used aerosol resistant tips fitted into automatic pipettes for all DNA extractions and PCR preparations. All plastic ware was disposable.

Species-specific (STRs) markers for *A. caraya* (Oklander *et al.*, 2007) were used. Three independent amplifications from each pooled extract were performed for eight STRs: AC14, AC17, AC45, AB7, D8S165, D17S804, LL1118, LL157 (Gonçalves *et al.*, 2004; Oklander *et al.*, 2007). Genotyping polymerase chain reaction (PCR) reactions were performed using the two-step multiplex PCR method described in Arandjelovic *et al.* (2009), with slight modifications.

In the initial multiplexing step, all microsatellite loci were amplified in a single reaction in a final volume of 25 µl using 5 µl of the extraction pool in faecal samples, including *Go Taq* buffer (*Promega*) 1.75 mm $MgCl_2$, 0.2 mm each dNTP, 1U *GoTaq* DNA polymerase (*Promega*), 4 pmol of each forward primer bearing an M13 tail, 4 pmol of each reverse primer and 0.4 mg/ml bovine serum albumin (BSA).

PCR thermocycling was performed in a *Verity* (*Applied Biosystems*) thermocycler with the following parameters as initial denaturation for 4 min at 94° C, 30 cycles of 45 s at 94 °C, 90 s at 58–60 °C, and 60 s at 72 °C, and a final extension of 5 min at 72 °C.

The second PCR consisted of single-plex PCRs carried out as above, but with a final

Table 2. Genetic diversity indices for each population.

Population/Stats	1	2	3	4	5	6	7	8	9	Mean	s.d.	Total
Mean He	PNCh	Isla	Guay	Paraguay	SAlon	Yape	Garu	Yacu	Piña	0.425	0.069	0.475
s.d.	0.380	0.454	0.472	0.447	0.280	0.457	0.367	0.468	0.503	0.164	0.044	0.223
Mean number of alelles	0.181	0.276	0.216	0.209	0.281	0.280	0.298	0.253	0.191	3.028	0.792	7.500
s.d.	2.750	4.750	3.625	2.750	2.250	3.250	2.500	2.250	3.125	1.069	0.632	4.567
Mean Theta (Θ)	0.886	3.059	1.598	1.165	1.389	1.165	1.309	1.165	1.553	0.761	0.194	9

Mean He: Mean expected heterocigocity; **s.d.:** standard deviation; **1. PNCh:** Parque Nacional Chaco (Chaco, Argentina); **2. Isla:** Isla Brasilera (Chaco, Argentina); **3. Guay:** Guaycolec (Formosa, Argentina); **4. Para:** Paraguay; **5. SAlon:** San Alonso (Corrientes, Argentina); **6. Yape:** Yapeyú (Corrientes, Argentina); **7. Garu:** Garupá (Misiones, Argentina); **8. Yacu:** Yacutinga (Misiones, Argentina); **9. Piña:** Piñalito (Misiones, Argentina).

volume of 12.5 μl and the following modifications: 5 of 5:100 diluted first multiplex PCR product was used as template, half the amount of $MgCl_2$. Additionally, each single-plex PCR contained a single primer pair: 4 pmol of each reverse primer and a 4 pmol of M13 labelled with a fluorescent dye (6–FAM) on its 5' end (Schuelke, 2000; Oklander *et al.*, 2007).

The cycling conditions were as above. The PCR products were combined and electrophoresed on an *ABI PRISM 310 Genetic Analyser*. Alleles were sized relative to an internal size standard (ROX-labelled HD400) using *ID–X Gene Mapper* Software version 1.2 (*Applied Biosystems*). Homozygous genotypes were replicated four additional times each. All amplification assays included negative controls.

Population genetics analyses

Intra-population analysis. The program *Arlequin* version 3.5.1.3 (Excoffier and Lischer, 2010) was used to assess genetic diversity indices as expected heterozygosity (He), number of alleles, mean Theta (Θ), of each population. Levene (1949) tests were used to evaluate departure from Hardy–Weinberg equilibrium, with *P* values estimated with a Markov chain method for all loci (Forecasted chain length: 10^6, Dememorization steps: 10^5). The presence of null alleles was tested with the program *Cervus* 3.0.3 (Kalinowski *et al.*, 2007).

Inter-population analysis. To investigate the genetic relationship among populations, two types of analyses were performed. First, an analysis of molecular variance (AMOVA), based on allele frequency information and computation of pair-wise multi-locus Fixation indexes (FST and RST) values among populations, as well as Inbreeding coefficient (FIS) values were performed using the software *Arlequin* version 3.5.1.3 with 1,000 permutations, and allowing a missing data level of 0.08.

Finally, the genotypic population structure was investigated using the Bayesian approach implemented in *Structure* version 2.3.4 (Pritchard *et al.*, 2000). This software calculates the proportion of membership of each individual to the inferred K clusters (K = number of potential parental populations). The most likely value of K is the smallest value of K that

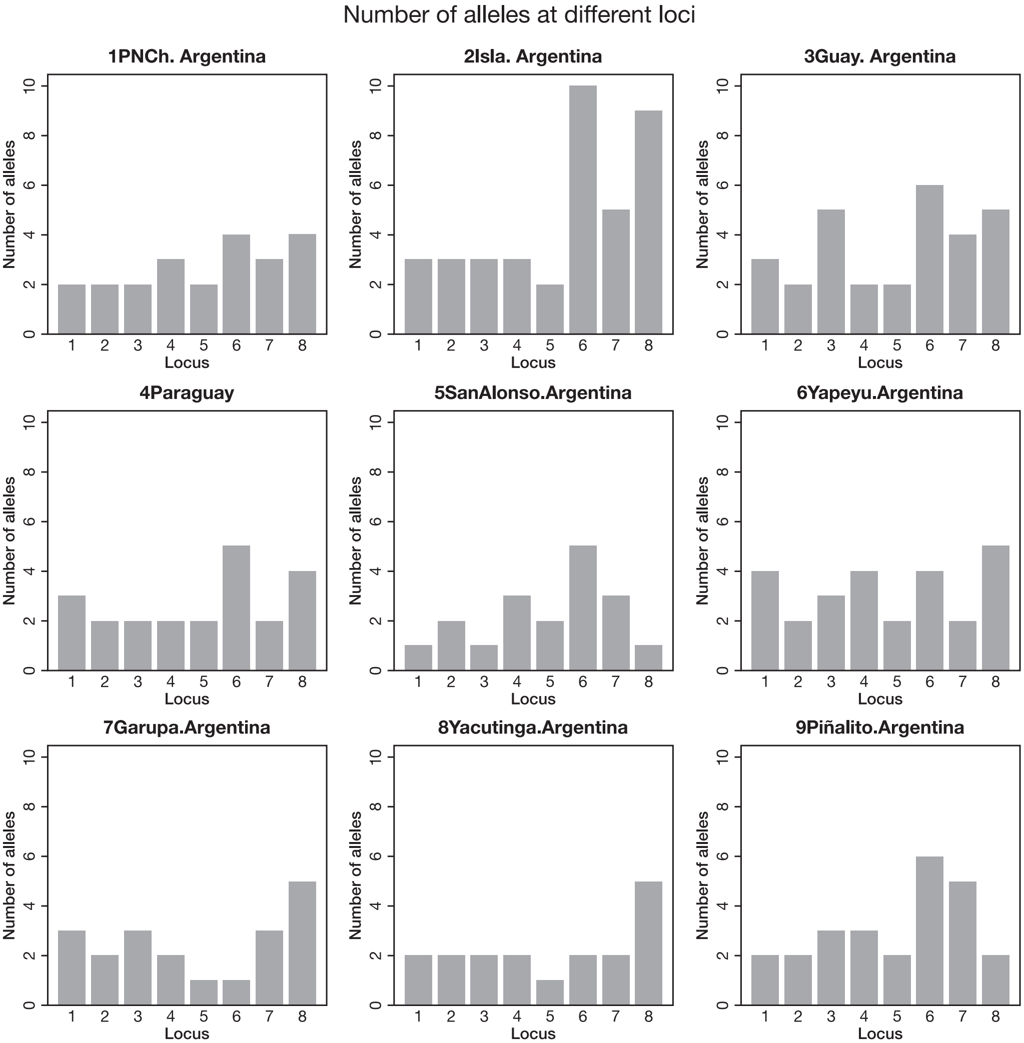

Figure 2. Histogram showing number of alleles for each marker in each population of *Alouatta caraya*.

captured the most structure [maximum value of L(K)] in the data. However, it had been shown that in many cases L(K) may still increase slightly, even after the most likely K is reached (Evanno *et al.*, 2005), making it difficult to infer K solely based on the highest values of L(K). The most likely value for the number of populations (K) was determined using the program *Structure Harvester*, which enables the Evanno method to be implemented (Earl, 2012). The Evanno method has been applied in many studies, and can successfully detect the most likely number of clusters (Neophytou *et al.*, 2010; Gu *et al.*, 2013). Ten iterations for parental populations (K) from two to nine were set and 10,000 burning followed by 20,000 Markov Chain Monte Carlo simulations were performed for each round. The Admixture model, correlated allelic frequencies and *locprior* model were chosen. Data analysis was refined using *Clumpp* software (Jakobsson and Rosenberg, 2007), and bar plotting was performed with the help of *Distruct* software (Rosenberg, 2004). We analyzed possible correlations between geographic and genetic distances via a Mantel test with the help of the software *Arlequin* 3.5.1.3 (Excoffier and Lischer, 2010).

Results

From the 111 individuals assayed, a total of 60 alleles for 8 loci were identified. The number of alleles per locus ranged from 3 to 16, with an average of 7.5. No genotypic linkage disequilibrium was detected among the locus-pairs across all populations. Figure 1 shows the distribution of study populations, numbered sequentially from west to east.

Intra-population analysis

For the 9 populations, all loci were in Hardy-Weinberg equilibrium. The genetic diversity indices for each population are summarised in Tab. 2.

We analysed the allele distribution per locus (Fig. 2), the mean number of alleles per locus, the mean He and the mean Theta (ΘH) between populations (Tab. 2). STR LL 1118 (Locus 6, Fig. 2) showed the most variability, varying between 1 (Garupá) and 10 (Yacutinga). In contrast, STR AB7 (Locus 5, Fig. 2) only showed one or two alleles in all studied populations. San Alonso and Yacutinga were the populations with lowest mean number of alleles, while Isla Brasilera population had the highest. The average He was 0.425, with the lowest found in the San Alonso population (0.280), and the highest in Piñalito (0.503). Some of these results could be explained by the differences in sample size, however the population of Yacutinga, with the smallest sample size, exhibited the second larger Mean Theta (a value which quantifies genetic diversity) for these howler populations.

Table 3. Pairwise FST/p value.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	*								
2	**0.112+-0.00**	*							
3	**0.062+-0.00**	0.000+-0.00	*						
4	**0.160+-0.00**	**0.052+-0.00**	0.010+-0.00	*					
5	0.000+-0.00	0.000+-0.00	0.000+-0.00	0.001+-0.00	*				
6	0.034+-0.00	0.000+-0.00	0.001+-0.00	0.001+-0.00	0.000+-0.00	*			
7	**0.409+-0.00**	0.001+-0.00	0.003+-0.00	***0.044+-0.00***	0.000+-0.00	**0.752+-0.00**	*		
8	0.018+-0.00	0.003+-0.00	0.015+-0.00	0.008+-0.00	0.003+-0.00	**0.050+-0.00**	**0.050+-0.00**	*	
9	0.012+-0.00	0.019+-0.00	0.039+-0.00	0.008+-0.01	0.000+-0.00	**0.078+-0.00**	**0.415+-0.00**	**0.364+-0.00**	*

Bold: Non-significant diferences; **bold italic:** Marginal differences. **1.** Parque Nacional Chaco (Chaco, Argentina); **2.** Isla Brasilera (Chaco, Argentina); **3.** Guaycolec (Formosa, Argentina); **4.** Paraguay; **5.** San Alonso (Corrientes, Argentina); **6.** Yapeyú (Corrientes, Argentina); **7.** Garupá (Misiones, Argentina); **8.** Yacutinga (Misiones, Argentina); **9.** Piñalito (Misiones, Argentina).

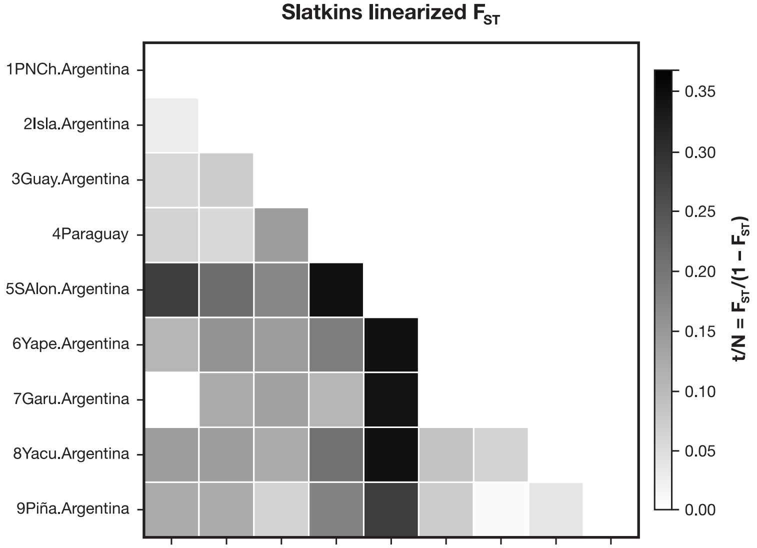

Figure 3. Slatkin linearized F_{ST}s between *Alouatta caraya* populations. The darker the greater value of F_{ST}s. **1. PNCh**, Parque Nacional Chaco (Chaco, Argentina); **2. Isla**, Isla Brasilera (Chaco, Argentina); **3. Guay**, Guaycolec (Formosa, Argentina); **4. Para**, Paraguay; **5. SAlon**, San Alonso (Corrientes, Argentina); **6. Yape**, Yapeyú (Corrientes, Argentina); **7. Garu**, Garupá (Misiones, Argentina); **8. Yacu**, Yacutinga (Misiones, Argentina); **9. Piña**, Piñalito (Misiones, Argentina).

Inter-population analysis

The results of AMOVA showed genetic differentiation among the sampled populations (FST = 0.105, $p < 0.000$). No statistically significant differences were observed for FIS = 0.041 ($p = 0.072$), indicating no genetic inbreeding within populations.

Pair-wise multi-locus comparisons showed interesting results that could help elucidate dispersal patterns between the studied populations (Tab. 3).

Genetic differences between populations did not correlate with geographical distances between fragments (Fig. 1; Mantel test, regression coefficient = –0.003, $p = 0.788$).

Group pairwise FSTs showed only one population that differed significantly from all others (San Alonso), being the most genetically differentiated (Tab. 3). All other populations had significant differences with one or more populations (Tab. 3, Fig. 3). The differences observed allow the arrangement in two groups of populations: those in the upper left extreme and those in the lower right extreme of the table/figure (Tab. 3, Fig. 3). These two groups of populations occur in forests near the confluence of the Paraná and Paraguay rivers (PN Chaco, Isla Brasilera, Guaycolec and Paraguay), and upstream of the Yacyretá dam on the Paraná River, and in the Uruguay River basin (Garupá, Yacutinga, Piñalito and Yapeyú). However, the Garupá population showed no significant differences from populations in either groups and, surprisingly, was not different from the PN Chaco population despite its distance (over 380 km in a direct line).

Analysis of genetic substructure using *Structure* showed that the nine populations were best represented by three clusters. A value of K = 3 best fit our dataset, and was selected for

Table 4. Evanno table. Best K by Harvester for *Structure*.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
2	10	-2547.52	1.015	—	—	—
3	**10**	**-2489.84**	**8.492**	**57.68**	**74.85**	**8.814**
4	10	-2507.01	26.321	-17.17	40.97	1.556
5	10	-2483.21	26.716	23.8	27.31	1.0222
6	10	-2486.72	33.688	-3.51	62.34	1.8505
7	10	-2552.57	49.786	-65.85	3.97	0.0797
8	10	-2622.39	106.097	-69.82	48.46	0.4567
9	10	-2643.75	191.546	-21.36	—	—

Reps: Repetitions; **Bold:** Best K values.

all further analysis (Tab. 4, Fig. 4). One of these clusters (cluster 2, Tab. 1; white, Fig. 5) is mostly represented in the Yacutinga, Piñalito, Yapeyú and Garupá populations, which are all located on the Paraná River upstream the Yacyretá dam, or in proximity to the Uruguay River. Secondly, Paraguayan and Isla Brasilera (Argentina) populations, which are also located on the Paraná River, but downstream the Yacyretá dam, showed a high component of cluster 1 (Tab. 1; black, Fig. 5).

The last cluster (cluster 3, Tab. 1; grey, Fig. 5), is highly represented by the San Alonso population, which is located in a wetland and is a long way from any major river (around 100 km north to the Paraná River and 120 km east to the Uruguay River). Guaycolec and PN Chaco populations showed a medium to high component of this cluster; and both

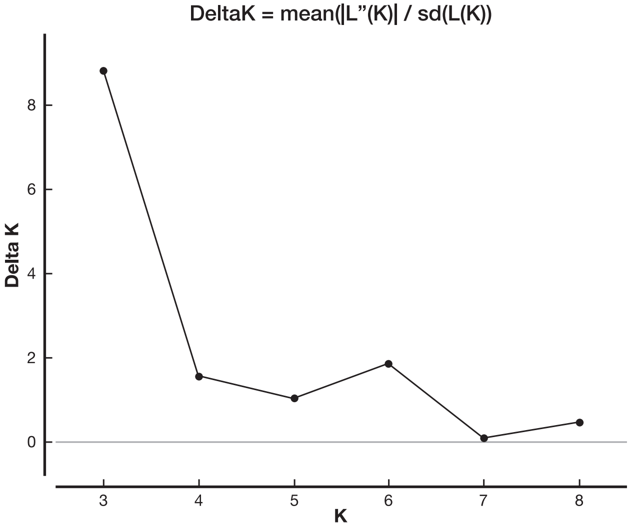

Figure 4. Graph of **Delta K** for **K** value (Evanno *et al.*, 2005) by Harvester for *Structure*. L: Likelihood; sd: standard deviation.

populations are located north of Paraná River and closer to the Paraguay than to the Paraná River (Fig. 1). It is worth mentioning that PN Chaco is the only group that contains considerable components of all three clusters.

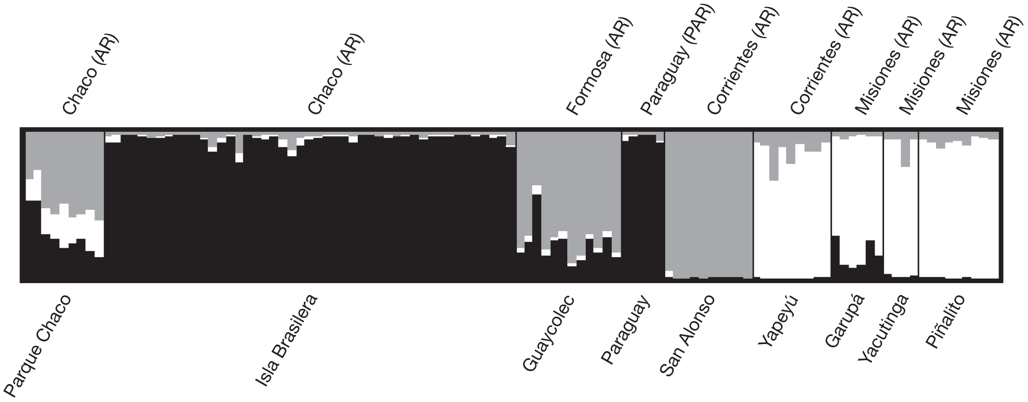

Figure 5. *Structure* clustering of *Alouatta caraya* populations. **Cluster 1:** black; **cluster 2:** white; **cluster 3:** grey.

Discussion

The differences in allele frequencies between populations may be due to the action of evolutionary forces over time, and therefore can often function as an archive of the evolutionary history of populations (Hedrick, 2005). Differences in allele frequency between populations constitute the genetic structure. Therefore, in species with high genetic structure, there are pronounced differences in allele frequencies between populations. In contrast, species with low genetic structure show no or very few differences in allele frequencies between populations.

If populations are genetically differentiated it may indicate that their sizes are small (indicating intense genetic drift) and/or that there has been little gene flow (dispersion) between these populations for a long period of time. Natural selection can also cause differences, but it only generates differences in related gene adaptations. Degradation and fragmentation of natural habitats as a result of increased anthropic impacts, such as large-scale clearing for agricultural purposes, urbanization and industrialization has placed at risk both biodiversity and the balance of ecosystems worldwide (Primack and Rodrigues, 2001). Loss of genetic variability and inbreeding depression typically characterize populations living in areas post-forest fragmentation, and are therefore likely to have highly deleterious implications for the long-term viability of animal populations. Few data are available on the influence of habitat fragmentation on genetic variation in free-ranging Neotropical primates (Grativol *et al.*, 2001; Bastos *et al.*, 2010; Oklander *et al.*, 2010).

Our results showed that *Alouatta caraya* populations are genetically differentiated FST = 0.105 $p < 0.001$. In other words, from the total genetic variation found in the species, approximately 10.5% is due to differences between populations (while 89.5% of the variation is found within populations). The population that exhibited the lowest genetic diversity was San Alonso's. This population was also significantly different from all other analysed populations, suggesting the existence of higher dispersal costs to/from the wetland were this population inhabits due to strong environmental restrictions. Clustering analysis linked San Alonso to PN Chaco and Guaycolec, indicating some role of the *A. caraya* populations near the Paraguay River in the historical origin of the current wetlands population. However, phylogeographic analyses are needed to confirm this possibility.

Two populations on the Paraná River, Isla Brasilera and Paraguay, are grouped in all our analyses. They are both downstream from the Yacyretá dam and are connected to each other via the river and its riparian forests. Both also share variability with the two populations upstream on the Paraguay River (PN Chaco and Guaycolec).

Yacutinga and Piñalito are the populations surrounded by largest areas of continuous forest in the current study; there are neither significant differences between them, nor between them and the Yapeyú and Garupá populations. They had the highest levels genetic diversity in the study, suggesting a widespread gene flow occurs through them. Both results of FST and genetic clustering indicate that the four populations upstream of the Yacyretá dam on the Paraná River and on the Uruguay River share genetic similarities. Overall, the Province of Misiones has relatively high forest coverage when compared to other northern Argentinian provinces (UMSEF, 2014). As a result, the populations located in this province (Garupá, Yacutinga and Piñalito) could be directly connected by forests and riparian forest (Fig. 1).

Downstream of the Yacyretá dam, the landscape is strongly modified by clearing and urbanization, and this may contribute to genetic differentiation between Garupá and the populations near the confluence of the Paraná and Paraguay rivers. In the area of the Yacyretá dam, the Paraná River is only some 70 km distant from the Uruguay River, and most of the intervening land is still covered by forest. This could also explain the greater similarities between the Garupá and Yapeyú (downstream on the Uruguay River) populations, than between the Garupá and Paraguayan or Isla Brasilera populations of *A. caraya*.

Finally, the population at PN Chaco possessed components from all three clusters, and showed no differentiation from the populations downstream from the Yacyretá dam (Isla Brasilera, Paraguay) and Guaycolec, near Paraguay River, but also showed no differentiation from Garupá in the FST analyses.

This does not follow the patterns that explain the genetic structure for all the other analysed populations. Under such circumstances, the most parsimonious explanation is that individual(s) from the Garupá *A. caraya* groups migrated into this population. This could only happen feasibly via some form of human intervention, such as a release of captured monkeys. The Yacyretá dam is located very close to the Garupá population, and during its construction many monkeys were captured and rescued from areas that were going to be flooded, and were then transported to other sites and released. If monkeys were relocated to protected areas such as PN Chaco this could explain the genetic variation observed for this population. As is common with such construction-related releases (Harrison-Levine *et al.*, in press), there seems to have been no follow-up to see if the translocated primates survived. But, based on genetic evidence, it seems likely that some of the dam rescue survivors had descendants at the new site. However, this is just a hypothesis and further investigations have to be made in order to test it.

Overall, our results using multi-locus genotype data supports the hypothesis that rivers and riverside associated forests act as biological corridors for movement of individuals between populations of *A. caraya* (Brown and Zunino, 1994). Moreover, those populations connected by continuous forest share more similarities with each other than do populations connected by water courses that have had their riverside vegetation anthropogenically modified. This illustrates the great importance of the forest, including riverside forest, for the dispersion of this species.

We observed a differentiation of the populations upstream and downstream of the Yacyretá dam on the Paraná River, possibly related to the anthropogenic modifications of the habitat within the riparian forest that could cause isolation by interrupting natural fauna dispersal corridors.

Ascunce *et al.* (2007) suggest a demographic expansion of *A. caraya* at the beginning of the Holocene. These authors analyzed populations located on or near the Paraguay and Paraná rivers and proposed that the southern portion of the distribution of the species, the area comprising northeastern Argentina and southern Paraguay, is characterized by the presence of two differentiated mitochondrial clades in sympatry or close proximity probably. They suggested this was caused by secondary contact between previously allopatric populations. Our STRs data also showed two differentiated clusters at the area around the confluence of the Paraguay and Paraná rivers. In addition, we found another cluster that links the populations upstream of the Yacyretá dam on the Paraná River and those on or near the Uruguay River. This could simply be due to the fact that we sampled populations on this area and the authors of the other study did not have samples from these locations.

The genetic differences found between the three clusters are not explained through isolation by distance. Instead our results suggest the existence of higher dispersal rates between populations connected by continuous forest, and differentiation or reduced gene flow among populations separated by anthropic modified landscapes. In addition, the genetic characteristics of one population suggest that probably individuals of another, a genetically distinct, population were released there in the past. Therefore, the genetic structure of *A. caraya* populations is affected by anthropic effects. Other primates and arboreal non-primates that depend on forest continuity to disperse are likely to show similar anthropic-induced effects on genetic diversity, possibly resulting in an inability to respond to the challenges of new selective pressures.

In this study we highlight the importance of rivers and forests for the dispersion of *A. caraya*. Our findings contribute to the understanding of the genetic characteristics of many black and gold howler populations and the results of this study could have direct and important applications in the field of conservation. However, further investigations on population genetic structure through additional sampling and analysis of mitochondrial sequences is necessary to provide the additional information that will help guide future conservation management decisions within the geographical range of this species.

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Part III

BEHAVIOR, ECOLOGY AND COMMUNICATION IN PRIMATES

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7 | EFFECT OF CLIMATIC SEASONALITY AND FOOD AVAILABILITY ON THE ACTIVITY BUDGET AND THE DIET OF BLACK AND GOLD HOWLER MONKEYS (*ALOUATTA CARAYA*)

EFECTOS DE LA ESTACIONALIDAD CLIMÁTICA Y DE LA DISPONIBILIDAD DE ALIMENTO EN EL PATRÓN DE ACTIVIDAD Y LA DIETA DE MONOS AULLADORES NEGROS Y DORADOS (*ALOUATTA CARAYA*)

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Abstract. The activity budget and the diet of primates are influenced by climatic seasonality and day length. Here, we examined the effect of seasonality and food availability on the activity budget and diet of black and gold howler monkeys (*Alouatta caraya*). These monkeys are living near the southern limit of their geographic distribution in a subtropical environment, near a transitional limit to being a warm and temperate climate. Their habitat is characterized by seasonal fluctuations in temperature and day length. In the winter months, days are colder and about three hours shorter than in the summer. Fall and spring are intermediate in terms of temperatures and day length. There was a marked seasonality in resource availability, mainly in seasonal foods: new leaves, flowers, and fruits. Non-seasonal foods, mostly mature leaves, were less variable and more abundant throughout the year. The monkeys' activity budget and diet composition differed between seasons. Their time spent resting was highest in the summer and lowest in the spring. Their time spent moving and engaging in social activities was highest in the spring, the season with the greatest availability of seasonal foods. Temperature had no influence on their activity budget. There was a negative relationship between the abundance of non-seasonal foods and time spent feeding, and also between the abundance of seasonal foods and time spent resting. The abundance of seasonal foods positively correlated with time spent moving and engaging in social activities. During the spring, their diet was dominated by new leaves. In the summer and early fall, their diet consisted mainly of mature leaves and fruits. Our results indicate that seasonality influences both the activity budget and diet of *Alouatta caraya* living near the southern limit of their distribution. The periods of time with low availability or absence of seasonal foods may be the key variable in determining the limits for primate geographic distribution towards the southern latitudes.

Resumen. El patrón de actividad y la dieta de los primates están influenciados por la estacionalidad climática y la longitud del día. En este estudio examinamos el efecto de la estacionalidad climática y la disponibilidad de alimento en el patrón de actividad y la dieta del mono aullador negro y dorado (*Alouatta caraya*) viviendo en cercanía al límite sur de su distribución geográfica, en un ambiente subtropical cerca de la transición a un clima templado cálido. El sitio de estudio se localiza en la provincia de Corrientes, Argentina (27° 30' S, 58° 41' W) y se caracteriza por bosques altos, semideciduos y fragmentados, bosque ribereño en galería, bosque abierto con palmeras y pastizales. Durante 2004 se estudiaron 2 grupos de monos aulladores (N = 13 individuos) habitando, cada uno, un fragmento de bosque alto. El hábitat de los monos presenta fluctuaciones estacionales en temperatura y longitud del día. En invierno, los días son más fríos y alrededor de 3 horas más cortos que en verano. Otoño y primavera son estaciones intermedias en términos de temperatura y longitud del día. Para estimar la disponibilidad de recursos alimenticios en el área de estudio se analizó la composición florística y la estructura de la vegetación mediante parcelas (de 20 × 20 m; N = 45) en los fragmentos utilizados por los grupos de estudio, y además se realizaron estudios fenológicos mensuales en 14 especies vegetales que forman parte de la dieta de los monos aulladores. Para registrar los datos comportamentales se utilizó el muestreo focal continuo en individuos adultos y juveniles durante 4 días al mes por grupo de estudio. Se obtuvieron 631,3 horas de observación. Los datos fueron analizados utilizando modelos lineales mixtos. En el sitio de estudio se registraron 46 especies vegetales (42 árboles y 4 lianas). Hubo una marcada estacionalidad en la disponibilidad de recursos, principalmente en alimentos estacionales: hojas nuevas, flores y frutos. Los alimentos no estacionales, principalmente hojas maduras, fueron menos variables y más abundantes a lo largo del año. Hubo baja disponibilidad general de recursos entre mayo y julio (fines de otoño – mediados de invierno). Los monos consumieron recursos alimenticios de 36 especies vegetales (32 árboles y 4 lianas). El patrón de actividad y la composición de la dieta difirieron entre las estaciones. El tiempo invertido en descanso fue máximo en verano (8,7 ± 1,1 hs) y mínimo en primavera (6,5 ± 1,1 hs). El tiempo invertido en movimiento y actividades sociales fue máximo en primavera (movimiento = 2,7 ± 0,6 hs; social = 1,5 ± 0,5 hs), la estación con mayor disponibilidad de alimentos estacionales. La temperatura no afectó el patrón de actividad. Se obtuvo una relación negativa entre la abundancia de alimentos no estacionales y el tiempo invertido en alimentación, así como entre la abundancia de alimentos estacionales y el tiempo invertido en descanso. La abundancia de alimentos estacionales se correlacionó positivamente con el tiempo invertido en movimiento y en actividades sociales. Durante la primavera, la dieta estuvo dominada por hojas nuevas. En verano y otoño temprano, la dieta consistió principalmente en hojas maduras y frutos. Nuestros resultados indican que la estacionalidad climática afecta el patrón de actividad y la dieta de *Alouatta caraya* viviendo en cercanía al límite sur de su distribución. Los períodos de tiempo con baja disponibilidad o ausencia de alimentos estacionales pueden ser la variable clave para determinar los límites en la distribución geográfica hacia latitudes del sur de los aulladores.

Introduction

In climatic terms, seasonality corresponds with annual oscillations of variables such as temperature and precipitation, which have repercussions on resource abundance (food and water availability), costs of thermoregulation, and intra-annual variation in predation risk (Brockman and van Schaik, 2005). Habitats with seasonal fluctuations pose diverse and conflicting ecological challenges to animals (Kappeler, 2002). However, animals exhibit a wide range of behavioral, physiological, and life history responses to cope with these ecological challenges (Brockman and van Schaik, 2005). Several studies have indicated that the activity budget (*i.e.*, the proportion of the activity time invested to different behavioral categories) and diet of primate species, like other mammals living in tropical, subtropical or temperate regions, are influenced by climatic seasonality and day length (rodents: Stokes *et al.*, 2001; Larrucea and Brussard, 2009; ungulates: Roberts and Dunbar, 1991; primates: Clutton-Brock, 1977; Hill *et al.*, 2003; Alberts *et al.*, 2005; Grueter *et al.*, 2009; Strier and Mendes, 2009; Van Doorn *et al.*, 2010; Agostini *et al.*, 2012; Ferrari and Hilário, 2014). For example, studies of the population of *Papio cynocephalus ursinus* at the Hoop Nature Reserve in South Africa focused on the considerable variations in day length (between 9.8 and 14 h), and found that the time baboons spent feeding, moving, grooming, and resting were significantly positive functions of day length (Hill *et al.*, 2003).

Food quality, composition, and abundance are considered among the most important factors regulating the activity budget in primates, though seasonality in day length, temperature, and precipitation may interact with food availability and thermoregulatory processes. The majority of studies related to the effects of seasonality on howler monkeys (Di Fiore and Campbell, 2007) are concentrated in tropical regions where seasons depend on the amount of precipitation (van Schaik and Pfannes, 2005). Towards temperate regions (*i.e.*, zones in which temperature varies seasonally), other climatic variables, such as day length and temperature, become more important than the annual cycle of precipitation. Howler monkeys (*Alouatta* spp.) are one of the most widespread neotropical primate genera, inhabiting a diverse range of habitats and climates (Crockett and Eisenberg, 1987; Di Fiore and Campbell, 2007). Northern Argentina and southern Brazil are the southernmost geographic limits of the black and gold howler monkeys (*A. caraya*). Coincidentally, this limit includes the transition between subtropical and warm temperate zones (Bicca-Marques and Calegaro-Marques, 1994; Brown and Zunino, 1994). In northern Argentina, the summer is characterized by having longer days (14 h *vs.* 11 h) and higher temperatures than the winter (34 °C *vs.* 11 °C). Therefore, winter is a critical season in terms of food availability and thermoregulatory costs for howler monkeys (Rumiz *et al.*, 1986; Zunino, 1986; Kowalewski and Zunino, 2004).

Adaptations contributing to the success of howler monkeys include flexibility in diet, behavior, and social organizations (Eisenberg, 1980; Chapman and Balcomb, 1998; Di Fiore and Campbell, 2007; Garber *et al.*, 2009). Howler monkeys present a wide inter- and intraspecific variation in their dietary composition (Di Fiore and Campbell, 2007; Norconk *et al.*, 2009). For example, the contribution of fruits to their diet ranges from 5% in *A. belzebul* to 59% in *A. fusca*, and 66% in *A. pigra* (Bonvicino, 1989; Chiarello, 1994; Pavelka and Knopff, 2004; Koch and Bicca-Marques, 2007).Within species, this ranges from 12–49% in *A. palliata* (Glander, 1978; Estrada, 1984). Beyond these differences in diet composition, field studies have revealed that howler monkeys prefer foods with higher rewards in terms of energy, protein, and digestibility. Even when these foods are less abundant and more ephemeral, howler monkeys still prefer new leaves, flowers, and fruits (Milton, 1979, 1980, 1981; Silver *et al.*, 1998; Di Fiore and Campbell, 2007). However, in some populations of howler monkeys, like *A. caraya* in northern Argentina, individuals are constrained by the relatively little energy provided by their diet, which is largely composed of leaves (between 46–70%), requiring longer times for digestion, and hence are defined as energy-minimizers (Nagy and Milton, 1979; Milton, 1980; Zunino, 1986; Bravo and Sallenave, 2003; Kowalewski, 2007; Pavé, 2013). The theory of energy maximization (Schoener, 1971) predicts that energy-minimizers should spend as little time foraging as possible to meet their energetic needs.

In studying the diet of howler monkeys, foods are usually categorized as being seasonal (new leaves, flowers, and fruits) or non-seasonal (mature leaves; Milton, 1979, 1980). Seasonal foods are characterized by having a high protein/fiber ratio (new leaves), a high content of readily assimilable energy under the form of non-structural carbohydrates (flowers and fruits), and as being ephemeral, patchily-distributed, and scarce. On the other hand, most non-seasonal foods, primarily the mature leaves, are considered difficult to digest. Although they offer lower levels of readily available energy, they are evenly distributed and abundant (Milton, 1979, 1980). Despite this increased time needed for digestion, mature leaves contribute to the diet, both with protein and simple sugars, and in this way could help to balance energy and nutrient intake (Behie and Pavelka, 2012).

While seasonality is an important determinant of activity levels, previous studies on *Alouatta caraya* from northern Argentina have almost exclusively focused on seasonal variation in food availability and life history parameters (Rumiz *et al.*, 1986; Kowalewski and Zunino, 2004; Pavé *et al.*, 2012; Pavé, 2013). In contrast, little attention has been paid to how the seasonal variation in day length might limit the active period of howler monkeys at temperate regions (see Bravo and Sallenave, 2003, and Kowalewski, 2007, for studies at flooded forests in northern Argentina). Such information is particularly scarce for the population living on the mainland, where annual values of temperature are more extreme at the same latitude and altitude (Rumiz *et al.*, 1986).

In this study, we present data on the floristic composition and vegetation structure of the forest, estimations of monthly food availability, and the activity budget and dietary composition of wild black and gold howler monkeys (*Alouatta caraya*) living in mainland forest fragments at a temperate region during an 11-month period. We evaluate the effects of seasonal temperatures and food resource availability on the activity budget and diet of black and gold howler monkeys. In this regard, we predict that: 1) when temperature is low, howler monkeys will conserve energy by increasing the time resting; 2) when the availability of high-quality food increases, howler monkeys will increase the time invested in localizing them; 3) the time invested consuming high-quality foods will be positively associated with their availability.

Material and methods

Study site

Field work was carried out in 2004 between January–May and July–December in two forest fragments of the Riachuelo river basin (27° 30' S, 58° 41' W), located 25 km southeast of the city of Corrientes, Argentina (Fig. 1). The landscape is flat, the elevation

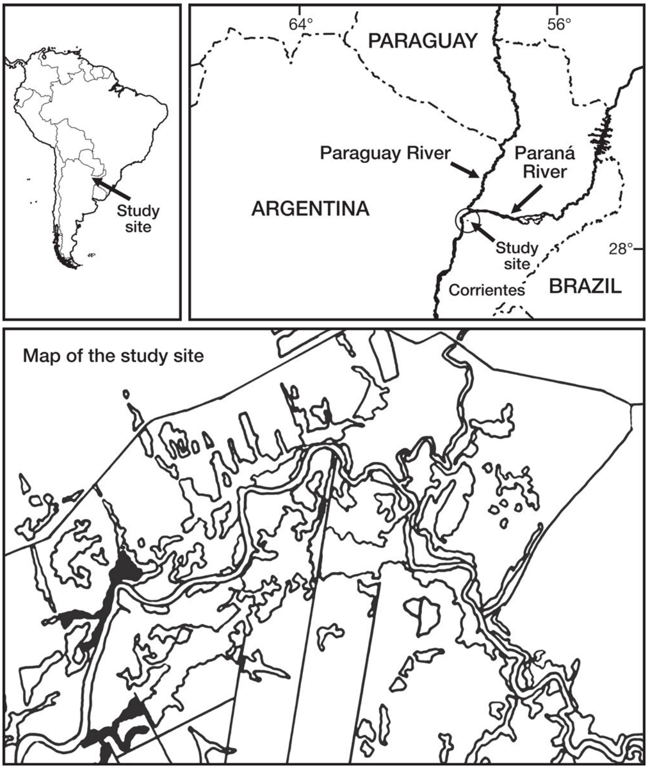

Figure 1. Map indicating the area of the Riachuelo river in Corrientes Province, Argentina, and the forest fragments, habitat of the howler monkeys (in black).

oscillates between 70–80 m above the sea level, and the terrain is a wide sedimentation basin along the Paraná River and its tributaries.

Information about temperature and precipitation were provided by the National Weather Service (Servicio Meteorológico Nacional; *http://www.smn.gov.ar*), corresponding to the Corrientes Aero meteorological station, located 15 km north from the study site and at the same altitude. Long-term (1990–99) mean (± SD) annual temperature is 21.3 °C ± 4.03 °C, and mean annual rainfall is 1440 ± 99.37 mm. During 2004, the mean annual temperature was 21.2 °C ± 4.04 °C (range = 14.9 °C – 27.5 °C) and the total annual rainfall was 1115 mm with a mean monthly rainfall of 122.2 ± 86.20 mm (Fig. 2). Rain occurred throughout the year, but decreased during the winter (June–August). For example, the monthly rainfall was between 80–195 mm in the summer, spring, and fall, and between 35–50 mm in the winter, the dry season at the study site. As opposed to the tropics, the dry season at our study site is the coldest period, where the mean minimum temperature in July was 11.0 °C. The summer months (December–February) are wet and warm, with the mean maximum temperature of 34.4 °C occurring in January. Fall (March–May) and spring (September–November) are transitional seasons with intermediate values in recorded of temperature. According to the life zone ecology classification (Holdridge, 1967), the study site

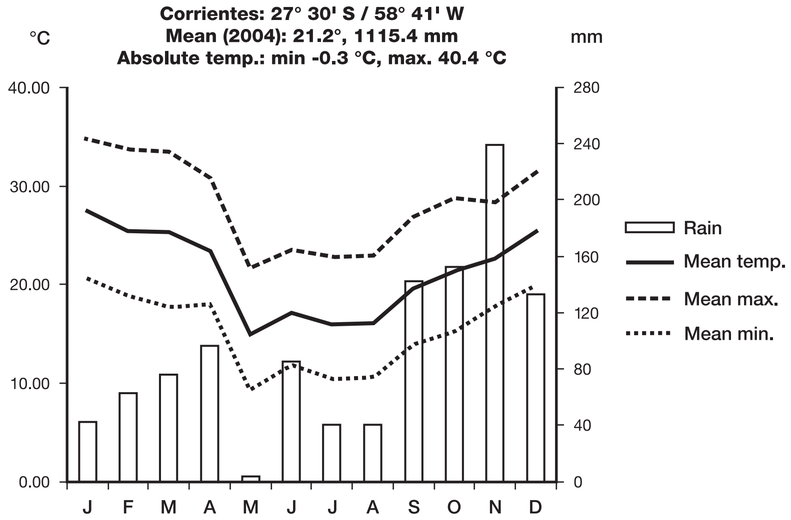

Figure 2. Monthly variation of temperature (mean, maximum, and minimum) and precipitation of the area of the Riachuelo river during the year of study, 2004. Source: Servicio Meteorológico Nacional, Estación Corrientes Aero, Argentina.

is located close to the limit between the subtropical and warm temperate zone, and between the ranges of sub-humid and moist forest. The site has a savanna landscape with a mosaic of palms, low forests, tall forests in fragments, and clear-cut areas that are dominated by grazing fields that developed after the tall forests were cut. Howler monkeys permanently inhabit only the tall forest, with such forest accounting for only five percent of the study area (Zunino *et al.*, 2007). Fragments of tall forest, ranging from 1–10 ha in size, are located on hillocks along the creeks and banks of the Riachuelo river.

Forest characteristics and food availability

We recorded data from all trees, including shrubs, with a diameter at breast height (dbh) ≥ 10 cm in 45 random plots of 20 m × 20 m (20 plots in fragment 1, and 25 in fragment 2), including: species names, height, type of fruit (fleshy or dry), and dbh of all the trees within the home range of the howler monkey groups under study. Additionally, lianas with a dbh > 5 cm were counted in 15 random plots of 10 m × 10 m (seven in fragment 1, and eight in fragment 2). Plants were identified to species level whenever possible. For identification of species, we used a reference herbarium and a field guide (López *et al.*, 1987). Several species were identified with the help of the Instituto de Botánica del Nordeste (IBONE, Corrientes, Argentina).

To estimate food availability, we selected 14 species known to be food sources for howler monkeys at the study site (Rumiz *et al.*, 1986). Each month,we randomly selected 10 individual plants of each species to estimate the abundance of new leaves, mature leaves, flowers, and fruits. Buds were categorized as new leaves, while the syconia of *Ficus* trees were considered flowers when light green and bright, and fruits when dark green-brownish and opaque. We used a subjective scale of abundance from 0–4, where 0 indicates absence and

4 that the plant part is full in the entire crown. The value of 4 was the highest possible combined score for vegetative structures (new and mature leaves) and for reproductive structures (flowers and fruits). For each species, we estimated the specific availability (availability index) by multiplying the mean value of abundance by the mean basal area. To calculate the availability of each potential food, we averaged the specific availabilities. To analyze howler preferences, foods were grouped as high quality or seasonal (new leaves, flowers, fruits), and low quality or non-seasonal (mature leaves, barks). Although some mature leaves may contain relatively high protein/fiber ratios and should be classified as high-quality foods (Milton, 1981; Behie and Pavelka, 2012), all mature leaves were categorized as low-quality food because there is no information available about their chemical composition from our study site with which to discriminate the existence of specific differences.

Activity budget and diet

We recorded the activities and diets of individuals from two groups of howler monkeys inhabiting two forest fragments that are separated by 800 m. Group 1 occupied a fragment of 6.8 ha and group 2 a fragment of 11.7 ha. Both groups were previously under observation for several months and were habituated to the presence of observers. We identified individuals by age, sex, and natural and/or artificial markings (color anklets and ear tags). We assigned age categories following Rumiz (1990). At the beginning of the study, group 1 contained one adult male, two adult females, and two juvenile males. Group 2 contained two adult males, two adult females, two juvenile males, and two juvenile females. Both groups used all of the area of their fragments and do not have permanent neighboring groups in the same fragment.

Data were collected by applying the focal-animal sampling technique (Altmann, 1974) for eight days each month (four days in each group) for 11 months, between January–December, 2004. Data from June were excluded from the analysis due to heavy storms during the sampling period that hampered most of the observations. Each sample consisted of one session lasting between 40–50 minutes on different individuals which were randomly selected (up to 20 minutes were used to locate the next individual). Samples with less than 40 minutes of data were not included in the analyses, and the rest of samples with less than 60 minutes were converted proportionally to one hour. Behavioral data were recorded from dawn until dusk. The proportion of time invested in each behavioral category was controlled by the duration of the daily activity period of howler monkeys, which varied across the seasons. This type of control was made in previous studies (Hanya, 2004a; Ferrari and Hilário, 2014). The mean duration of the daylight period in each season was 14 h in the summer (range = 13.5–14 h; from 06:00–20:00), 11 h in winter (range = 10.5–11 h; from 07:00–18:00), 13 h in the spring (range = 12.5–13.5 h; from 06:00–19:00), and 12 h in the fall (range = 11–12.5 h; from 07:00–19:00). Information regarding the hours of sunrise and sunset were obtained from the Servicio Nacional de Hidrografía Naval (National Hydrographic Service; *http://www.hidro.gov.ar*).

Behaviors were grouped into four mutually exclusive categories: feeding, moving, resting, and social activities. Feeding was defined as the amount of time it took for an individual to manipulate the food, move it into its mouth, chew, and swallow. Feeding also included

Table 1. Plant species surveyed, and percentage of time feeding by howler monkeys. Species marked (*) were included in the phenological study. References: **L**, leaves; **NL**, new leaves; **ML**, mature leaves; **Fl**, flowers; **Fr**, fruits.

Species	Fruit type	Mean Height (m)	Density (ind./ha)	Parts eaten	% of diet
Trees and shrubs					
Celtis spp. (two species) (*)	Fleshy	6.31	4.44	NL, L, Fr	19.19
Gleditsia amorphoides (*)	Dry	9.82	86.67	NL, ML, Fl	11.92
Eugenia uniflora (*)	Fleshy	5.17	68.33	NL, ML, Fr	9.59
Maclura tinctorea (*)	Fleshy	9.92	14.44	NL, ML, Fl, Fr	6.16
Ficus luschnathiana (*)	Fleshy	13.83	10.00	NL, ML, Fl, Fr	5.46
Erythrina crista-galli (*)	Dry	8.75	17.78	NL, ML, Fl, Fr	3.87
Sapium haematospermum (*)	Dry	5.89	39.44	NL, ML, Fr	3.11
Sorocea saxicola	Fleshy			NL, ML, Fl, Fr	2.18
Acrocomia aculeata	Fleshy	9.00	0.56	Fr	2.08
Mircyanthes pungens (*)	Fleshy	12.47	25.00	NL, ML, Fr	1.99
Phytolacca dioica (*)	Fleshy	16.00	5.00	NL, ML, Fl	1.95
Nectandra angustifolia (*)	Fleshy	10.29	9.44	NL, ML, Fr	1.63
Tabernaemontana catharinensis	Fleshy	7.88	8.89	NL, ML	1.48
Geoffroea decorticans	Dry			NL, ML, Fl	1.23
Enterolobium contortisiliquum (*)	Dry	13.11	10.56	NL, ML, Fl	1.07
Citrus aurantum	Fleshy			NL, ML	0.91
Hovenia dulcis	Fleshy			Fr	0.71
Unknown Mirtaceae	Fleshy	4.00	1.11	ML, Fr	0.69
Chrysophyllum marginatum	Fleshy	7.07	22.78	ML	0.61
Rapanea laetevirens	Fleshy	9.71	3.89	NL, ML	0.43
Handroanthus heptaphyllus (*)	Dry	15.20	2.78	NL, Fl	0.35
Hexachlamis edulis	Fleshy	8.88	4.44	ML, Fl, Fr	0.33
Astronium balansae	Dry			HM	0.27
Copernicia alba	Fleshy	9.80	24.44	Fr	0.25
Chloroleucon tenuiflorum	Dry	10.50	1.11	ML	0.13
Achatocarpus praecox	Dry	8.02	17.78	ML	0.12
Allophylus edulis	Fleshy	5.44	8.89	ML	0.05
Ruprechtia laxiflora (*)	Dry	7.14	3.89	NL	0.05
Melia azedarach	Fleshy	6.50	1.11	NL, ML	0.04
Tessaria integrifolia	Dry	8.07	8.33	ML, Fl	0.04
Zanthoxylum rhoifolium	Dry	8.00	3.33	Fr	0.02
Sideroxylon obtusifolium	Fleshy	14.00	0.56		
Albizia inundata	Dry	11.67	3.33		
Eugenia burkartiana	Fleshy	6.50	3.33		
Guarea macrophylla	Fleshy	7.00	0.56		
Luehea divaricata	Dry	12.00	0.56		
Patagonula americana	Dry	10.78	5.00		
Peltophorum dubium	Dry	10.00	0.56		
Schinopsis balansae	Dry	17.00	0.56		
Scutia buxifolia	Dry	6.63	6.67		
Sebastiania brasiliensis	Dry	6.76	21.11		
Lianas					
Forsteronia glabrescens (*)	Fleshy		12.31	NL, ML, Fl, Fr	21.8
Other lianas (3 species)	Fleshy			NL, ML, Fl, Fr	0.71

the time involved in short movements between bites (less than 5 seconds). A feeding bout finished when the animal had spent at least 10 seconds without manipulating another food item, it began to eat another food type, or had changed its activity. During feeding, we record the plant species and the part consumed (new leaves, mature leaves, flowers, fruits). Movement included all horizontal and vertical displacement lasting at least 5 seconds. Resting included the time in which the animals remained inactive. Social activities included the time engaged in grooming, play, copulation, vocalizations, and agonistic behaviors.

A sampling period usually consisted of two or three days of phenological records and four days of observation of each group. A total of 631.3 h of observation were used in this study. For analyses, individual data were pooled regardless of age or sex, as these differences were outside of the scope of this study. We used linear mixed models that controlled for repeated measures on the same two groups in different months, as a way to evaluate potential changes in activity budget associated to the annual cycle of temperature and day length. In these analyses we used the ID of each individual as a random (intercept) factor and the seasons as fixed factors. We grouped the monthly records into four seasonal periods: summer (December–February), fall (March–May), winter (July–August), and spring (September–November). We performed stepwise multiple regression analyses to show the relationships among monthly time invested on each activity (dependent variable), temperature, and the availability of seasonal and non-seasonal foods (independent variables). No data transformations were necessary because all residuals where within two standard deviations of the mean value. To detect whether preferences existed for different types of food, we used Spearman rank correlations between times spent feeding on each food category (seasonal, non-seasonal) with their abundance. Additionally, we tested the existence of an association between day length and the time devoted to each activity through correlation analyses. We used mean monthly values of all of the individuals in the correlation and regression analyses

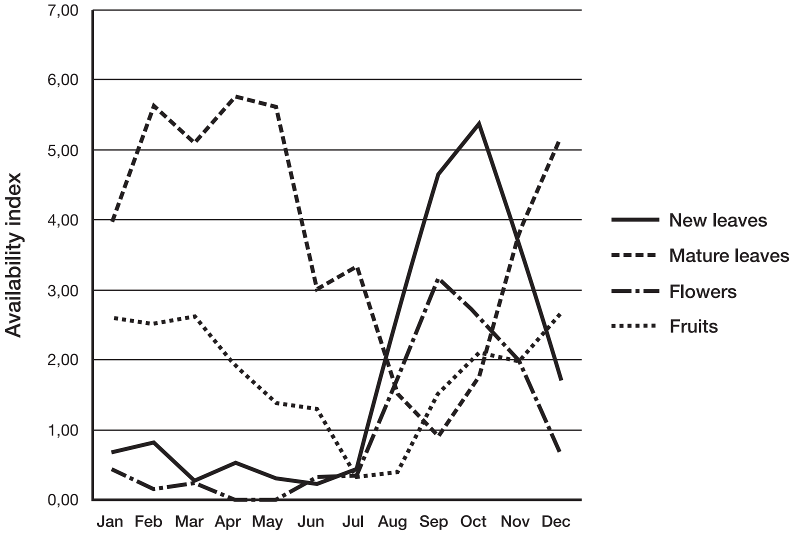

Figure 3. Availability index of plant parts throughout the year at the area of the Riachuelo river.

because we did not detect significant differences between groups in activity budget. In all analyses, the upper level of significance was 0.05. Statistical analyses were performed in *IBM SPSS Statistics* 19.

Results

Forest characteristics and food availability

The study site has the physiognomy of a semideciduous forest consisting of several strata that are difficult to identify. The canopy reached a maximum height of ~17 m (8.49 ± 3.70 m), with 67% of the trees being shorter than 10 m. We identified 42 species of trees and four species of lianas, 35 of which accounted for 94.4% of the howler diet (Tab. 1). The mean density of trees was 447.8 ± 240.5 individuals/ha. The fragments showed evidence of selective cutting, where open areas with both old and recent tree stumps were common. The presence of exotic plants, cattle, trails, and the absence of trees of economic importance indicated a considerable amount of human activity.

The 14 plant species included in the phenological study accounted for 78.2% of the howler monkeys' diet (Tab. 1). All of the plant parts they consumed showed variation in their abundance throughout the year (Fig. 3). The availability of new leaves and flowers peaked between September and October (spring), whereas fruits peaked between October and April (mid-spring to mid-fall). As expected, the availability of mature leaves was less fluctuating than that of seasonal foods, as the leaves were present throughout the year, with their lowest availability being at the end of winter and early spring. Seasonal foods showed lower annual availability than non-seasonal ones, and were scarce or absent during the first

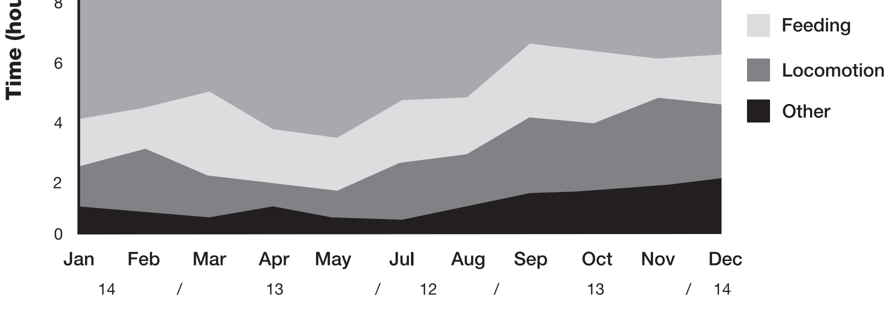

Figure 4. Monthly diurnal activity budget expressed in hours by day to *Alouatta caraya* in the area of the Riachuelo river. The activity "other" refers to social activities. The x-axis shows the months of the year and the mean daylight period by month and season.

Table 2. Linear mixed model for mean hours (± standard deviation) engaged in different activities during each season for *Alouatta caraya* in the area of the Riachuelo river. F-value (n = 22; df = 18.3; * = $p < 0.05$; ** = $p < 0.01$; ns = non-significant). Summer (December to February), fall (March to May), winter (July to August), and spring (September to November).

Activity	Summer	Fall	Winter	Spring	F-value
Resting	8.7 (1.1)	8.9 (0.6)	7.4 (0.3)	6.5 (1.1)	**
Feeding	1.5 (0.4)	1.9 (0.5)	2.0 (0.2)	2.1 (0.6)	ns
Locomotion	2.0 (0.8)	1.1 (0.3)	1.9 (0.3)	2.7 (0.6)	**
Social	1.2 (0.7)	0.8 (0.2)	0.7 (0.3)	1.5 (0.5)	*

seven months of the year (Fig. 3). Phenology also revealed a pattern of reduction in overall food availability between May and July (late fall to mid-winter). New leaves, flowers, and fruits reached minimum values in the first half of winter (June–July).

Activity budget and diet

On an annual basis, howler monkeys spent 14.9% of their daily time feeding, 15.4% moving, 60.3% resting, and 8% engaged in social activities. On a monthly basis, the daily hours that howler monkeys spent on each activity varied. Feeding activities varied from 1.3 h in November to 2.9 in March (1.9 ± 0.44 h), time spent moving varied from 0.8 h in April to 3.0 in November (1.9 ± 0.7 h), resting times varied from 5.4 h in September to 9.9 in January (7.8 ± 1.2 h), and time engaging in social activities varied from 0.4 h in July to 1.9 in December (1.0 ± 0.5 h; Fig. 4). When activities were grouped by season (Tab. 2), we found that the length of the activity period (time spent engaging in feeding, moving, social activity, and resting) was consistent with the daylight period. For example, the shortest mean activity period occurred in the winter (July–August) and the highest mean activity period occurred in the summer (December–February). However, the time spent engaging in each activity was not significantly correlated with the hours of daylight ($p > 0.05$; Fig. 4). The time spent feeding was shortest in the summer (December–February), the season with more hours of daylight. Additionally, the hours of daylight were similar in the spring and fall. Nevertheless, the time spent resting was lowest in the spring and highest in the fall, and the hours spent moving were highest in the spring and lowest in the fall.

The stepwise multiple regression analyses showed that temperature did not influence the time spent feeding, moving, resting, or engaging in social activities (Tab. 3). The time spent feeding decreased when the availability of non-seasonal foods was high. On the other hand, when the availability of seasonal foods was high, the time invested in resting decreased, while the time spent moving and engaging in social activities increased.

The howler monkeys consumed plant parts from 32 species of trees and shrubs and 4 species of lianas. The top ten plant species (Tab. 1) accounted for 85% of the annual diet. Annually, howler monkeys distributed their time feeding as follows: 16.6% on new leaves, 49.9% on mature leaves, 2.1% on flowers, and 31.2% on fruits. The hours spent each day

Table 3. Stepwise multiple regression analysis of influence of temperature and food availability (independent variables) on the activity budget (dependent variable) to *Alouatta caraya* in the area of the Riachuelo river. Standardized regression coefficients are shown. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, ns = non-significant.

Time	Temp. (°C)	Food availability	
		Seasonal	Non-seasonal
Feeding	ns	ns	-0.69*
Moving	ns	0.69*	ns
Resting	ns	-1.56*	ns
Social	ns	1.03**	ns

eating different plant parts varied across the year from 0–1.8 (0.38 ± 0.5 h) for new leaves, from 0.2–1.8 (0.91 ± 0.6 h) for mature leaves, from 0–0.1 (0.03 ± 0.05 h) for flowers, and from 0–1.8 (0.61 ± 0.6 h) for fruits (Fig. 5). During the early part of spring (September), the time spent eating new leaves increased, which coincided with the shortest recorded time eating mature leaves (Tab. 1). Most fruits were eaten between the spring and late fall months. Flowers accounted for 5.0% of the feeding time in the spring and were occasionally consumed during the rest of the year.

We found significant positive correlations between time feeding and food availability for new leaves, flowers, and fruits. There was a non-significant correlation between abundance and consumption of mature leaves. Significant negative correlations were found between the consumption of matures leaves and the availability of new leaves and flowers (Tab. 4).

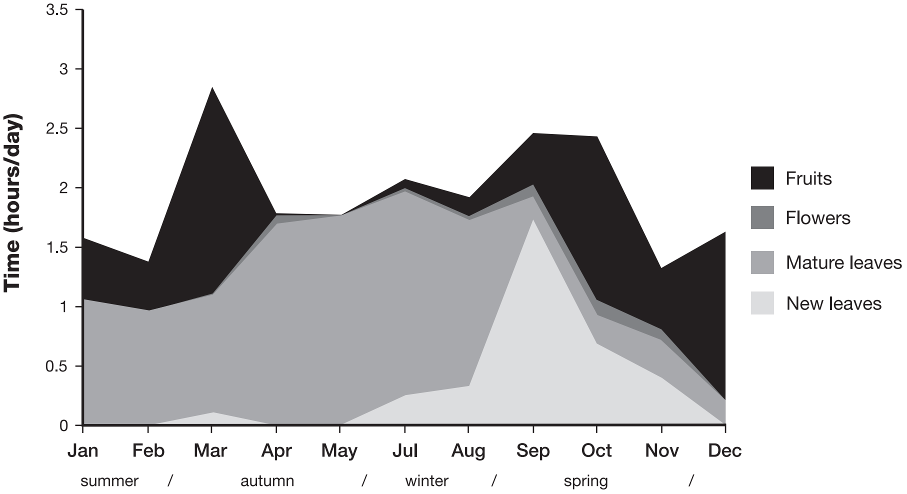

Figure 5. Average daylight hours consuming different plant parts for *Alouatta caraya* in the area of the Riachuelo river.

Table 4. Spearman rank correlation comparing time feeding and availability of plant part categories. * = $p < 0.05$; ** = $p < 0.01$.

Time feeding	**Plant parts availability**			
	New leaves	**Mature leaves**	**Flowers**	**Fruits**
New leaves	0.78 (**)	-0.87 (**)	0.90 (**)	-0.37
Mature leaves	-0.80 (**)	0.49	-0.76 (**)	-0.36
Flowers	0.94 (**)	-0.74 (**)	0.92 (**)	-0.13
Fruits	0.22	0.03	0.14	0.63 (*)

Discussion

The overall activity budget of the black and gold howler monkeys in the area of the Riachuelo river fell within the range reported for other populations within the southern latitudes (Bicca-Marques and Calegaro-Marques, 1994; Bravo and Sallenave, 2003; Kowalewski, 2007; Agostini *et al.*, 2012; Pavé, 2013). Several studies of other primate species, including *Theropithecus gelada* (Dunbar, 1992), *Macaca fuscata* (Hanya, 2004a, b), *Semnopithecus entellus* (Sayers and Norconk, 2008), *Papio ursinus* (Van Doorn *et al.*, 2010), and *Callithrix flaviceps* (Ferrari and Hilário, 2014) have suggested an association between variations in temperature and daylight period across seasons with the animal's activity budget and dietary composition. For example, variation in temperature has been shown to affect the activity budget of Japanese macaques (*Macaca fuscata*) in a coniferous forest at Yakushima, Japan (Hanya, 2004a). When the temperature is low, their time spent traveling and feeding decreases, however no changes are observed in their time spent engaging in social interactions and resting. Howler monkeys in the area of the Riachuelo river adjusted their total activity period to the daylight period that varied between the different seasons, with a maximum of 3 hours between summer and winter. Similar results were obtained with *Callithrix flaviceps* at two sites in southeastern Brazil (Ferrari and Hilário, 2014). These marmosets adjusted their activity budget to the daylight period by decreasing their activity time in the winter by 2.35 hours, when compared with the summer. Additionally, at our study site, we detected significant differences in the activity budget between seasons. Howler monkeys rested longer in the summer (65%) and less in spring (51%). They invested more hours moving during the spring months (21%) and fewer hours during the fall (8.7%). Also, the time engaged in social activities was at a maximum in the spring (11.7%) and at a minimum in the winter (5.8%).

Our prediction that the daily inactivity patterns of black and gold howler monkeys would increase with decreasing temperatures was not supported. This suggests that the low temperatures during the coldest season in the study were not low enough to significantly affect their energy and modify their activity patterns, as it occurs in populations of *Macaca fuscata* inhabiting some of the coldest environments of Japan (Hanya, 2004a, b). However, previous information, including hourly temperature at the study site has suggested that howler monkeys do modify their activity schedules in accordance with temperatures during the extreme seasons. In the summer, howler monkeys begin to feed and move at sunrise, but during the colder days of winter, they remain inactive for one or two hours after sunrise, exposed to the sun whenever possible (Zunino, 1986). This behavior reinforces the idea that black and gold howler monkeys perform activities, possibly of high thermoregulatory cost, during optimum ranges of temperature, as suggested by Bicca-Marques and Calegaro-Marques (1998).

Howler monkeys reduced their time spent resting and increased their time spent traveling when the abundance of seasonal food was high (spring season), suggesting an active search of this type of food. Contrary to our prediction however, the time invested in feeding was slightly higher in the spring compared with other seasons, though we did not find a relationship between the abundance of seasonal foods and the time spent feeding on them (Tab. 3). This result may indicate that howler monkeys consume different foods despite their availability, possibly balancing the energy income with the intake of different types of nutrients (Schoener, 1971; Milton, 1979, 1980; Felton *et al.*, 2009). The time devoted to social activities was higher when seasonal food availability increased, reinforcing the idea of a possible energetic and time restriction during periods of scarcity of seasonal food.

Milton (1979) pointed out that all howler monkey species occupy the same dietary niche. In this sense, the time spent feeding on leaves by *Alouatta caraya* at our study site (66.5%) was higher than the values reported for most of the studies in other species of *Alouatta* in the tropics, which range between 25–54% (Milton, 1980; Chapman, 1988; Estrada *et al.*, 1999; Palacios and Rodriguez, 2001; Pinto, 2002), and is more comparable with the time spent feeding on leaves for *A. caraya* at other study sites in the subtropics (60.9% [Bicca-Marques and Calegaro-Marques, 1994], 64.0% [Bravo and Sallenave, 2003] and 82.4% [Prates and Bicca-Marques, 2008]). The time spent feeding on new leaves (16.6%) was low compared with time reported for *A. palliata* (34–63.5%) (Glander, 1978; Milton, 1980; Estrada, 1984; Larose, 1996; Stoner, 1996) or *A. seniculus* (39.4–45%) in the tropics (Gaulin and Gaulin, 1982; Neves and Rylands, 1991). At our study site, howler monkeys preferred seasonal foods when available; however, their ability to subsist on non-preferred foods (mature leaves) may explain the capacity of howler monkeys to occupy seasonal subtropical or warm temperate habitats besides the tropics. Some studies have supported the preference of howler monkeys for new leaves and fruits based on their high content of energy and proteins (Milton, 1980), and a low amount of secondary compounds (Glander, 1978). Additionally, howler monkeys have the capacity to process large amounts of fiber-rich foods in their enlarged cecum (Nagy and Milton, 1979; Lambert, 1998), an ability that increases their survival in environments where mature leaves serve as the primary source of food for several months.

We detected some noticeable differences in the diet of black and gold howler monkeys compared with studies in other howler species. For example, at our study site, fig trees were common (10 ind./ha), but their contribution to their diet (5.46%) was low in comparison to the rates of consumption exceeding 20% of the time spent feeding in *A. palliatta* (Milton, 1980; Estrada *et al.*, 1999; Serio-Silva *et al.*, 2002), *A. seniculus* (Gaulin and Gaulin, 1982), and *A. pigra* (Silver *et al.*, 1998). Fig trees are categorized as asynchronous, but climatic conditions or day length may act as limiting factors for asynchrony (Shanahan *et al.*, 2001). Our phenological records indicated that the cycle of fig trees was not asynchronous and was similar to the other tree species surveyed. On the other hand, some authors have suggested that lianas might be an important source of food for primates in Africa and Asia (Leighton and Leighton, 1983; Preece, 2006; Moscovice *et al.*, 2007; Takenoshita *et al.*, 2008; Marshall *et al.*, 2009). Lianas seem to contribute little to the diet of howler monkeys in the tropics (Silver *et al.*, 1998; Estrada *et al.*, 1999; Asensio *et al.*, 2007). However, our results indicate a heavy use of lianas as a food source by black and gold howler monkeys, reaching peaks of 30% and 40% of the feeding time during the fall and winter months, respectively. It is likely that in some extreme habitats, such as our field site, lianas play an important role as a source of food during periods of shortage.

Howler monkeys spent less time resting and more time moving in the spring, when their time spent consuming new leaves and flowers was at its highest. Two (not mutually exclusive) explanations are possible: 1) new leaves are patchily distributed food resources (Chapman *et al.*, 2003; Milton, 1980), or 2) howler monkeys are selective when eating new leaves due to differences in specific quality (Milton, 1979, 1981). We lack necessary information regarding the distribution pattern of specific plant species, relative to the existence of a selective behavior. Some evidence supports these possibilities, as howler monkeys did not eat new leaves from certain species of plants during the peak in spring, but consumed mature leaves of these species in other seasons.

Pavelka and Knopff (2004) observed that *A. pigra* spent less time moving during the folivorous months. If we considered only mature leaves, the most folivorous season in our study site was fall, when the time spent moving was minimal, whereas if we considered only new leaves, the most folivorous season was spring, when the time spent moving was the highest. This behavior indicates a strong influence of food quality on the activity budget of *Alouatta caraya* at our study site. Most of the production of new leaves is concentrated in the spring, the season when howler monkeys invest most of the time feeding on new leaves despite the presence of mature leaves.

Conclusions

Black and gold howler monkeys in the area of the Riachuelo river seem to face a critical period of food scarcity, including the non-preferred foods like mature leaves during the three months corresponding to winter (June–August). Possibly, the length of the scarcity period is one of the most important factors defining the limits of the southern distribution of howler monkeys. During this period, however, howler monkeys adopt a behavioral strategy by adjusting their activity budget to the daylight period. Beyond the variability observed in the patterns of activity and changes in the diet, possible emerging evidence for the influence of seasonality on howler monkeys at the study site is the grouping of births observed in the fall and early winter (Kowalewski and Zunino, 2004; Pavé, 2013). To evaluate the influence of seasonality from another point of view, it would be important to examine whether infants born in seasons outside of certain periods are less likely to survive or if howler monkeys go through some nutritional stress during the winter.

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8 DIET AND NUTRITIONAL ECOLOGY OF PRIMATES IN ARGENTINA: CURRENT KNOWLEDGE AND NEW DIRECTIONS

DIETA Y ECOLOGÍA NUTRICIONAL DE LOS PRIMATES EN ARGENTINA: CONOCIMIENTO ACTUAL Y NUEVAS DIRECCIONES

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Abstract. Platyrrhines are characterized by a broad diversity of behavioral and morphological adaptations that facilitate the exploitation of diverse plant and animal resources. Whereas feeding ecology has long been a major topic of primate field studies and focused on time spent feeding to describe primate diets, the recent past has witnessed a growing interest in nutritional ecology, as researchers recognize the importance of quantifying both the intake and the nutritional composition of food items. Differences in biomass and handling time compromise the effectiveness of using time spent feeding as proxy of the contribution of each food item to the diet. Here, we review the literature on the diet of the three genera of Argentinian primates—*Alouatta*, *Cebus* and *Aotus*—and discuss their feeding strategies according to their digestive physiology, activity patterns, and diet breadth. We compare the nutrients in foods eaten by primates, and indices of diet composition, such as dietary quality. We use right angle mixture triangles to illustrate the variety of nutrients of the diets of these primates in a comparative context. Finally, we suggest new directions for investigating the nutritional ecology of primates in Argentina. For example, new frameworks like nutritional geometry have been successfully used in primatology to test hypotheses on nutrient regulation and food choice. These methods can be used to examine the patterns of nutrient prioritization among Argentinian primates.

Resumen. Los primates platirrinos presentan una amplia diversidad de adaptaciones morfológicas y comportamentales que les permiten especializarse en el consumo de diferentes recursos vegetales y animales. Este es uno de los tópicos más estudiados en Primatología, generalmente referido como «ecología alimentaria» y en donde se utiliza el tiempo que los individuos invierten en manipular e ingerir los diferentes alimentos para describir su alimentación e inferir estrategias (por ejemplo, folivoría y frugivoría). En los últimos años ha crecido el interés en la ecología nutricional a partir

del creciente reconocimiento de la necesidad de cuantificar tanto la ingesta como la composición nutricional de los alimentos. Las diferencias en la biomasa y los tiempos de manipulación de los diferentes alimentos comprometen la efectividad de utilizar al tiempo invertido en alimentación como un indicador de la contribución relativa de cada alimento a la dieta de los individuos. En este capítulo se realiza una revisión de la literatura publicada sobre dieta en los tres géneros de primates que habitan en la Argentina —*Alouatta*, *Cebus* y *Aotus*— y se discuten sus estrategias alimentarias en relación a la fisiología, patrón de actividades y amplitud de la dieta. Asimismo, se compara la composición nutricional de los alimentos ingeridos por estos primates, así como otros parámetros, como el índice de calidad de la dieta (*dietary quality index*). Por otro lado, se utilizan los gráficos de triángulos de mezcla de nutrientes para ejemplificar, de manera comparativa, la variedad de la composición nutricional de la dieta de estos primates. Finalmente, se sugiere la utilización de nuevos enfoques y marcos teóricos en los estudios de ecología nutricional en primates, como la utilización de la geometría nutricional, para poner a prueba hipótesis sobre la regulación individual de nutrientes y la elección de alimentos. Utilizando los enfoques tradicionales para analizar la ecología de la alimentación de los tres géneros de primates argentinos, es decir, utilizando datos sobre el tiempo dedicado a la alimentación en diferentes recursos, se observan patrones típicos género-específicos. Los monos mirikiná (*Aotus*) se caracterizan por una dieta basada principalmente en frutos y, en menor medida, en hojas, flores y probablemente insectos; los monos aulladores (*Alouatta*) tienen una dieta basada en frutos y hojas; y los monos capuchinos (*Cebus*) se alimentan principalmente de insectos y frutos, pero también de hojas. Sin embargo, cuando se analiza la variabilidad de la dieta en relación a la estación del año, emergen patrones diferentes. Debemos enfatizar que estos datos se calculan sobre la base de porcentajes de tiempo dedicados a la alimentación de diferentes recursos y no sobre las cantidades estimadas de alimentos ingeridos. Esto puede conducir a un error, ya que el tiempo dedicado a la alimentación de un determinado recurso no equivale a la biomasa ingerida, especialmente en recursos que requieren largos tiempos de manipulación. En particular, se ha demostrado que utilizando el tiempo de alimentación como variable para describir la dieta se tiende a subestimar el consumo de frutos y sobrestimar el consumo de hojas y flores. La diversidad en la composición de la dieta puede no estar necesariamente relacionada con diferencias en la adquisición de nutrientes, dado que diferentes individuos pueden satisfacer necesidades nutricionales similares utilizando distintos alimentos como recursos. Comprender la elección de alimentos desde una perspectiva nutricional requiere no solo un marco adecuado para analizar e interpretar datos complejos, sino también observaciones y análisis detallados. Para esto se necesita poder cuantificar precisamente el tiempo de alimentación, las tasas de ingesta de los alimentos (por ejemplo, bocados ingeridos por unidad de tiempo), el tamaño y el peso del bocado ingerido. La revisión de la literatura sobre ecología alimentaria de primates argentinos realizada en el presente capítulo sugiere que es necesario realizar estudios nutricionales en más localidades y en más especies de primates de Argentina. La mayoría de los datos de alimentación disponibles para las especies que habitan este país derivan de estudios que no fueron diseñados para analizar la ecología nutricional de las especies estudiadas y, por lo tanto, no están utilizando metodologías adecuadas para recolectar la información necesaria, como estimaciones de la cantidad diaria de alimento ingerido por los individuos y la ingesta diaria de nutrientes. Sin este tipo de información básica no es adecuado sacar conclusiones sobre las estrategias nutricionales de los primates silvestres. Con este capítulo se espera fomentar a los investigadores a emplear enfoques metodológicos y marcos analíticos adecuados, como la geometría nutricional, para explorar cuestiones sobre la ecología nutricional de los primates no humanos en la Argentina.

Introduction

Diet is tightly linked to all aspects of life history, including growth, reproduction, reproductive cycling, and survival. In fact, diet and nutrition have an important effect on ontogeny and life history traits in mammals, and in primates in particular (Leigh, 1994), with protein and energy intake significantly affecting female fitness (Lee, 1987; Altmann, 1991, 1998, 2006; Emery Thompson *et al.*, 2007; Terasawa *et al.*, 2012; Emery Thompson, 2013). Reproductive fitness has been directly linked to energy balance (using C-peptides of insulin as a measure of energetic condition) in mangabeys (McCabe and Emery Thompson, 2013) and chimpanzees (Emery Thompson *et al.*, 2012). Energy intake also has been related to the fecundity and reproductive success of wild female baboons (*Papio cynocephalus*), and energy shortfalls (as proportions of a calculated "optimum") were found to account for 81% of female reproductive success, measured as the number of surviving juveniles (Altmann, 2006). Likewise, seasonality in conceptions and conceptive success have been associated with fluctuations in food availability and female diet and nutritional state (great apes: Knott, 2005; *Semnopithecus entellus*: Koenig *et al.*, 1997).

Moreover, studies of primate nutrition are important because many of the key adaptations that define the evolutionary history of different primate groups are associated with critical changes in diet, foraging strategies, and digestive physiology (Leonard and Robertson, 1994; Leonard *et al.*, 2003). Due to the high diversity of diets, habitats, and ecological niches occupied by primates worldwide, feeding patterns have been the subject of primatological studies over several decades and have been related to physiology, morphology, ontogeny, ecology, behavior and sociality (Milton, 2006; Lambert, 2011; Chapman *et al.*, 2012; and references therein). Primate feeding ecology seeks to understand how individuals choose, acquire, and process their foods. Traditionally, dietary and feeding ecology have been approached from an ecological viewpoint, focusing on how foraging and feeding are influenced by the spatial and temporal distribution of resources (Clutton-Brock, 1977; Chivers and Herbert, 1978; Montgomery, 1978; Hladik *et al.*, 1980; Milton, 1980; Oates, 1987). However, more recently, new frameworks such as the geometric framework of nutrition have been developed to bridge the gap between the study of primate phenotypes (*e.g.*, behavior, morphology, physiology) and ecology (Raubenheimer *et al.*, 2009). In this sense, nutritional ecology studies the interactions of individuals with their heterogeneous environments, and analyzes individual-level mechanisms driving primate foraging strategies and nutritional goals within an ecological and evolutionary framework (Milton, 2006; Raubenheimer and Boggs, 2009; Raubenheimer *et al.*, 2012; Marshall *et al.*, 2013).

In this chapter, we review the literature in relation to the diet of the three genera of Argentinian primates—*Alouatta*, *Cebus* and *Aotus*—and discuss their feeding strategies according to their digestive physiology, activity patterns, diet breadth, and dietary quality. We use right angle mixture triangles (Raubenheimer, 2011) to illustrate the differences in the nutrients of the foods eaten by these primates in a comparative context. Finally, we suggest new directions for investigating the nutritional ecology of primates in Argentina.

Feeding ecology of Argentinian primates

The five primate species inhabiting Argentina are grouped in three genera belonging to the Cebidae family: *Aotus*, *Cebus* and *Alouatta*, which are characterized by marked ecological differences (Zunino and Kowalewski, 2008). They reside in a wide range of habitats from savanna to dense forest, and consume a variety of food items from several plant and animal species (Zunino and Kowalewski, 2008).

Traditionally, primates have been classified according to their feeding strategies, such as folivory, frugivory, or insectivory, which relate to specific patterns of food acquisition and digestive strategies. From an anatomical view, there are specialized structures involved in food acquisition, chewing, and digestion (Parra, 1978; Chivers, 1978; Chivers and Hladik, 1980). The digestive strategies depend on the anatomy of the digestive system that allows an individual to extract energy and nutrients from food, and that requires a series of physical and chemical processes. For example, it has been suggested that among New World primates such as callimicos, marmosets, and pitheciins, masticatory and digestive adaptations including an elongated hindgut, relatively long food transit time for their body mass, and specialized features of the anterior and posterior dentition have enabled these primates to exploit difficult to digest resources that are present year-round (*e.g.*, fungi for callimicos, exudates for marmosets, immature fruits with hard mesocarps for sakis) (Norconk *et al.*, 2009; Porter *et al.*, 2009; Power, 2010). In addition to anatomical specializations of their masticatory and digestive systems, many primate species have developed behavioral and cognitive solutions that serve to increase foraging efficiency or reduce energetic requirements (Milton, 1978, 1980, 1998; Strier, 1992; Garber and Brown, 2006; Di Fiore and Suarez, 2007; Janson, 2007; Ganas *et al.*, 2008; Garber and Dolins, 2010; Asensio *et al.*, 2011; Lambert, 2011). These include adopting a highly selective feeding pattern in which particular plant parts or species are consumed in order to balance nutrient intake and avoid possible toxic effects caused by ingesting secondary metabolites found in a single plant part; increasing the time devoted to behaviors that conserve energy such as resting; and using goal-directed travel between feeding sites to minimize locomotor costs (Milton, 1980, 1998; Janson and Boinski, 1992; Garber and Hannon, 1993; Lambert, 1998, 2011; Janson, 2014). In the following sections we describe the available information on feeding patterns, anatomical characteristics and foraging strategies of Argentinian primates.

Aotus azarae. Owl monkeys, also called *douroucoulis* or *mirikinás*, range from Panama to northern Argentina and from the Andes to the Atlantic Ocean. They are known for concentrating their activity during the night, with peaks at dawn and dusk (only one species shows cathemerality), for being one of the few socially monogamous primates species, and for showing biparental care (Wright, 1981; Kappeler and van Schaik, 2002; Fernández-Duque, 2006). They form small groups, with only one breeding pair, one infant, one or two juveniles and, occasionally, a subadult (Fernández-Duque, 2006). Owl monkeys in Argentina live in the gallery forests of the provinces of Formosa and Chaco (Brown and Zunino, 1994), in territorial groups ranging from two to six individuals (Fernández-Duque, 2011). While quantitative descriptions of diet composition and foraging strategies for the strictly nocturnal *Aotus* spp. ranging from Panama to Paraguay are scarce, studies of cathemeral owl monkeys (*A. a. azarae*) in Argentina have provided some information on their diurnal diet (Wright, 1985; Arditi, 1992; Giménez and Fernández-Duque, 2003; Giménez, 2004; Fernández-Duque, 2011). They are primarily frugivorous (45–66% of their time devoted to feeding on fruits), but leaves (15–41%), flowers (1–14%) and insects are also part of their diet (Arditi, 1992; Giménez and Fernández-Duque, 2003; Giménez, 2004) (Tab. 1, Fig. 1.1). In northeastern Argentina, the annual diet of one group of owl monkeys was described by Arditi (1992), who observed them feeding on leaves (41.3%), fruits (44.9%) and flowers (13.7%), but this pattern was very seasonal, possibly due to changes in food availability. In winter and fall, leaves were the principal item ingested, but in the winter monkeys only fed on mature leaves (51.4%), whereas in the fall they spent more time feeding on young leaves (27.4%) than mature leaves (15.3%) and also included items such as shoots (10.2%). In the summer, fruit consumption accounted for 65.3% of their feeding time, whereas mature leaves only accounted for 16.8%. During the three seasons covered by this study, owl monkeys also consumed flowers (fall: 6.8%, winter: 11.8%, and summer: 12.0%). In this same population, owl monkeys were observed eating insects and fungi (Giménez and Fernández-Duque, 2003), but that was not quantified in the earlier study.

According to Kay's body mass threshold model (Kay, 1984), owl monkeys would be expected to be mainly insectivorous, and include a higher percentage of insects than leaves in their diet (as a source of protein) due to their relatively small body mass (990–1,550 g) (Fernández-Duque, 2004, 2011). However, it is difficult to observe insect-eating in nocturnal and cathemeral primates, thus owl monkeys might be more insectivorous than reported (Norconk *et al.*, 2009). On the other hand, *Aotus* is characterized by a gastrointestinal tract comparable to that of other frugivores (*i.e.*, with little overall structural specialization) (Chivers and Hladik, 1980), but the proximal portion of the colon is expanded and there are sacculations along its entire length (Hill, 1960; Chivers and Hladik, 1980; Stevens and Hume, 1995), thus showing possible capacity for microbial fermentation of fiber from leafy material for use as an energy source. Finally, as reported in several Neotropical nutritional studies, flowers and inflorescences are a good source of protein (crude protein: 16.8% (% dry matter) [Norconk *et al.*, 2009]; available protein: 15.8 ± 5.5% [Righini, 2014], 17.5 ± 6.6% [Fernández, 2014]), and owl monkeys may ingest them to complement their nutrient requirements when other sources are not available.

In addition to dietary seasonal variation, owl monkey food selection is also affected by the temporal variation in plant nutritional characteristics. Owl monkeys (*A. azarae*) in Paraguay were found to consume leaves more frequently in the late afternoon (average leaf-eating time during three months: 45 minutes per day) than in the morning (14 minutes/day), early afternoon (2 minutes/day), or night (5 minutes/day) (Ganzhorn and Wright, 1994). This leaf-eating pattern was related to the finding that, on average, crude protein content in the leaves of ten dry forest tree species was 13% higher in the afternoon than in the early morning, and soluble carbohydrate concentration also was higher at 16:00 hrs (5.9 ± 1.5%) than at 06:00 hrs (4.9 ± 1.2%) (Ganzhorn and Wright, 1994). These diurnal feeding patterns have been noted in other primates such as spider monkeys (Chapman and Chapman, 1991) and chimpanzees (Carlson *et al.*, 2013).

***Alouatta* spp.** The genus *Alouatta* is distributed from 21° N to 30° S from Mexico to Argentina and thus has the largest geographical distribution among Neotropical primates. There are two species in Argentina: brown (*A. guariba*) and black and gold howler monkeys (*A. caraya*). Brown howler monkeys are endemic to the Atlantic Forest of South America, ranging from Bahia and Espírito Santo in the north, to Rio Grande do Sul, Brazil, and the south of Misiones province, in Argentina (Cordeiro da Silva, 1981; Kinzey, 1982). The black and gold howler monkey, *A. caraya*, is the southernmost species (Di Fiore *et al.*, 2011), and its geographic range includes Paraguay, south of Brazil, north and east of Bolivia, and northern Argentina (Brown and Zunino, 1994). Both species have similar body weights (black and gold howlers: average adult female = 4.33 kg, average adult male = 6.42 kg; brown howlers: average adult female = 4.55 kg, average adult male = 6.18 kg; reviewed in Di Fiore *et al.*, 2011). The two species live in sympatry in few areas along their distribution, one of them ("El Piñalito") is found in the Atlantic Forest of the Misiones province in northeastern Argentina (Agostini *et al.*, 2008). *A. caraya* live in cohesive social groups including 1–4 adult males, several adult females and juveniles, and groups can contain up to 21 individuals (Rumiz, 1990; Bravo and Sallenave, 2003; Kowalewski and Zunino, 2004; Di Fiore *et al.*, 2011) and present bisexual dispersal (Rumiz, 1990; Oklander *et al.*, 2010). Average group size of *A. guariba* is 4.9 individuals (Chiarello, 1993a, b), varying from 2 to 11 individuals (Di Fiore *et al.*, 2011).

Compared to many Old World primates, the diet of New World monkeys contains smaller amounts of mature and immature leaves (Norconk *et al.*, 2009; Campbell *et al.*, 2011) and the fruits have lower amounts of protein than in some parts of Africa such as Madagascar (Ganzhorn *et al.*, 2009). Howler monkeys represent a notable exception. Folivorous primates possess a variety of physical adaptations that promote, through symbiotic microbial fermentation and mechanical action, the degradation of the structural and chemical defenses of plants, as the enlargement of the hindgut to promote microbial fermentation (Parra, 1978; Chivers, 1978; Chivers and Hladik, 1980). Despite the fact that howler monkeys lack extreme modifications in gut morphology like colobines (Milton, 1998), they possess adaptations for hindgut fermentation, such as hindgut sacculations and longitudinal bands, which increase digesta retention in the gut, favoring microbial degradation (Clemens and Philips, 1980; Milton, 1984; Lambert, 1998). Moreover, howler possess molars with high shearing crests that contribute to the efficient processing of leafy material (Kay, 1975, 1990). Howler monkeys are also described as behavioral folivores, since it has been suggested that they follow a strategy of energy minimization, with short day ranges, long resting time every day and highly selective feeding patterns (Milton 1978, 1979; Strier, 1992). For example, mantled howlers (*A. palliata*) in Costa Rica were reported to avoid the leaves of many common tree species, while seeking out the leaves of specific rare tree species (Glander, 1981).

It is commonly reported that for howler monkeys living in highly seasonal environments, leaves may account for more than 80% of monthly feeding time (Milton, 1980; Estrada, 1984; Zunino, 1986; Galetti *et al.*, 1987; Chiarello, 1994; Pavelka and Knopff, 2004). For example, at Riachuelo (Corrientes, Argentina), black and gold howlers spent 91.3% of their time feeding on leaves during the winter (75.5% mature leaves, 15.8%

young leaves), whereas in the summer leaves only accounted for 42.4% of their feeding time (41.7% mature leaves and 0.6% young leaves) (Delgado, 2005; Tab. 1). There are few data on *A. guariba*'s diet in Argentina, however one study in southeastern Brazil reported that brown howlers spent 73% of their time feeding on leaves, 12% on flowers, and 5% on fruits (Chiarello, 1994). For howlers living in sympatry, at El Piñalito both *A. caraya* and *A. guariba* have similar dietary patterns, feeding mainly on leaves (> 60% of annual feeding time, divided almost equally between young and mature leaves), but including fruits (19–24%, mainly ripe), flowers (~6%) and other items such as pine cones (3–7%) (Agostini *et al.*, 2009) (Tab. 1, Fig. 1.2).

Figure 1.2 shows the diet characteristics of Argentinian howlers in terms of time spent feeding on structural plant parts (*e.g.* leaves, petioles), reproductive plant parts (*e.g.*, fruits, seeds, flowers), and animal matter. Overall, the pattern confirms the typical classification of howler monkeys as folivores-frugivores. However, separating the data by season highlights certain differences, such as a high reliance on leaf-eating for *A. caraya* during the fall and the winter, which are periods of low fruit and flower availability and high availability of mature leaves. Moreover, it can be noted that the dietary range is wider for *A. caraya* than for *A. guariba*, since it spans seasonally from totally leaf-based diets to high percentages of frugivory. This also agrees with the fact that black and gold howlers tend to have a more diverse diet than brown howlers, as observed at El Piñalito, where during 11 months, brown howlers consumed 97 different food items from 40 plant species, whereas *A. caraya* fed on 144 food items from 56 plant species (Agostini *et al.*, 2009). This difference in dietary diversity may or may not translate to nutritional differences in their diets as these howlers may be meeting similar nutritional needs by consuming different foods (Twinomugisha *et al.*, 2006; Rothman *et al.*, 2007).

***Cebus* spp.** Along with howler monkeys (genus *Alouatta*), capuchins (genus *Cebus*) are one of the genera with the widest distribution among Neotropical primates (Platyrrhini), comprising forest habitats from Honduras in Central America to northern Argentina in the south of the continent (Wilson and Reeder, 2005). Capuchins inhabit all types of Neotropical forests; principally they prefer the canopy, but they descend to the understory or to the ground to feed and travel. All *Cebus* species lives in social groups, ranging from 5 to 44 individuals, with several adult males and females, and their offspring (Di Bitetti, 2001a; McCabe, 2005; Janson *et al.*, 2012). They are characterized by strong hierarchical within-group dominance relationships (Janson, 1985; Di Bitetti, 1997).

Cebus is a genus with a high level of genetic, phenotypic, morphological, ecological, and behavioral variation, which has generated major controversy about the taxonomy of the genus (Lynch-Alfaro *et al.*, 2012; Arístide *et al.*, 2013; Wright *et al.* 2014, Nieves *et al.*, this volume). In this chapter we will refer to species inhabiting Argentina as *Cebus libidinosus* (in the cloud forests of the Andes, the Yungas, in northwestern Argentina—Salta and Jujuy provinces—and southeastern Bolivia) and *C. nigritus* (inhabiting the Atlantic forest of Brazil, from Rio Doce in Minas Gerais State, extending south along the coast to the northern part of Misiones Province in Argentina and part of Rio Grande do Sul State, Brazil).

Capuchin monkeys, together with squirrel monkeys (*Saimiri* spp.), are the two most "omnivorous" platyrrhines, including fruits, flowers, leaves and other plant parts, invertebrates and vertebrates (such as squirrels, other small mammals, frogs, lizards and birds) (Izawa, 1975; Terborgh, 1983; Galetti, 1990; Ferreira *et al.*, 2002; Resende *et al.*, 2003) in their diet (Tab. 1, Fig. 1.3). In general, capuchins are described as opportunistic foragers that use their "manipulative abilities and strength" to obtain foods that are unavailable to other species (Fleagle, 1999). At Iguazú National Park (Misiones, Argentina), where they have been studied continuously from 1991, capuchin monkeys occupy large home ranges of 70–250 ha, and it has been suggested that, in the lean season, when they face food scarcity, capuchins usually increase their range and explore areas not recently visited (Di Bitetti, 2001b). At this site, they are reported to spend 70–90% of their active time searching for food, and although they have a mainly fruit-based diet, they spend more time looking for arthropods than foraging and feeding on fruits (Di Bitetti, 2001a). Protein-rich arthropods appear to be a very important component of capuchins' diet, to the point that a population crash (> 70% of the individuals died) was recorded for *C. capucinus* on Barro Colorado Island, Panama, due to unusually heavy rainfall during December 2010, which eliminated the arthropod peak expected every year (Milton and Giacalone, 2014).

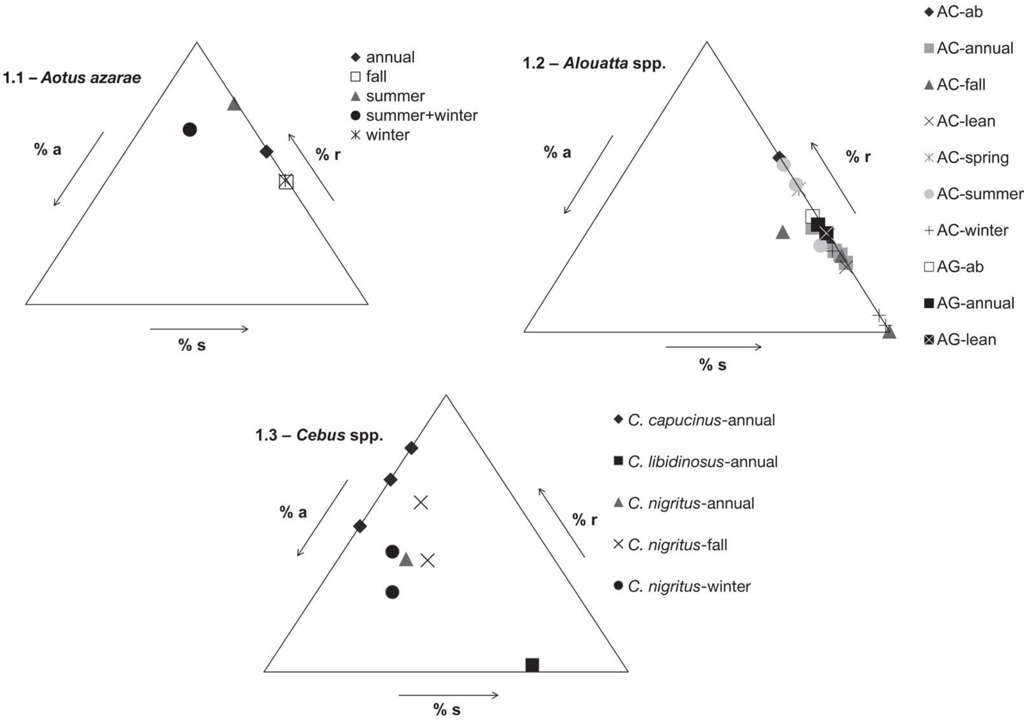

Figure 1. Diets of **1.** *Aotus azarae*, **2.** *Alouatta* spp., and **3.** *Cebus* spp., in terms of time spent feeding on different items. Axes represent the percentage of time devoted to feeding on **a)** animal matter, **r)** reproductive plant parts (fruits and flowers), and **s)** structural plant parts (leaves, shoots, and petioles). Percentages increase in the direction of the arrows. Abbreviations: **AC**, *Alouatta caraya*; **ab**, abundant season; **AG**, *Alouatta guariba*. In **Figure 1.3**, dietary data on *Cebus capucinus* in Costa Rica are reported for comparative purposes.

Nevertheless, due to the great variability in the diets of capuchin groups living in different habitats or even belonging to the same population (Di Bitetti, pers. comm.), categorizing capuchin feeding strategies is complex. Chapman and Fedigan (1990) reported great differences among neighboring groups at Santa Rosa National Park, Costa Rica, to the point that one of them could be classified as frugivorous (81.2% fruit, and 16.9% insect), whereas the neighbor group's diet was composed of high amounts of invertebrates (53% fruit and 44.3% insects), fitting an insectivorous pattern. These groups rarely ate flowers and leaves (less than 3%). Brown and Zunino (1990) followed one capuchin group for two consecutive years and found conspicuous differences in the time spent feeding on certain foods among years (Tab. 1). For example, in the fall of 1987, capuchins spent 61.6% of their time feeding on fruits, while the following fall only 39.7%. The same occurred during the winter: in 1987 they spent half of the time (20.2%) eating fruits than in 1988 (41.7%). These authors also found major differences between the two capuchin species inhabiting Argentina in the consumption of bromeliad leaves. These items accounted for 72.3% of the annual feeding time of *C. libidinosus* inhabiting the Yungas (Salta Province), but only 2.2% of the annual feeding time of *C. nigritus* in Misiones (Brown and Zunino, 1990). In part, these discrepancies might be due to the differences in habitat structure, plant composition, and food availability at different sites; however, since in most cases the abundance of certain plant species does not predict their consumption, it has been suggested that other factors, such as taste preference, or cultural predisposition and local traditions, play a role in capuchin food choice (Chapman and Fedigan, 1990; Miller, 1998). We argue that strategies of nutrient mixing and regulation also have an important influence on individual foraging and feeding habits and thus could possibly contribute to this between-group variation.

Dietary quality

The relationship between body mass, basal metabolism, and dietary quality is summarized by the Jarman/Bell principle (Bell, 1971; Jarman, 1974). Since small animals have low absolute energy requirements, but high requirements per unit weight, they must feed on “high quality” (*i.e.*, relatively low in fiber and plant secondary compounds, and high in easily digestible energy) foods, even if these are not as abundant in the environment as “lower quality” foods (Gaulin, 1979). Attempts to analyze the diet-body mass relationship in primates, however, have been problematic due to the variety of diets shown by different taxa, and to the lack of precision associated with the use of broad categories such as “frugivores”, “folivores”, and “insectivores”. Sailer *et al.* (1985) proposed an index of dietary quality (*dq*) which takes into account the relative energy and abundance of different food types in primate diets that may allow comparability. The index ($dq = 1s + 2r + 3.5a$) is based on the percentage of plant structural parts (s), reproductive parts (r), and animal prey (a) in the diet. The coefficients for s, r, and a were estimated by the authors using regression equations and scaled to body mass. *Dq* ranges from 100 to 350, with low values representing diets based on abundant nutrient-poor resources (*e.g.*, fibrous leaves), and high values corresponding to diets based on nutrient-rich foods (*e.g.*, animal material). Using data from 72 primate species, Sailer *et al.* (1985) found a significant negative correlation between dietary

Table 1. Diet composition (values represent percentage in the diet based on feeding times) and characteristics of feeding ecology studies of *Alouatta* spp., *Aotus azarae*, and *Cebus* spp.

Genus	Species	Season	Sex	BM(F)	BM(M)	L	ML	YL	F	RF	UF	FL	AM	Others	Behavioral data collection technique	N groups	Study length	References and location
Alouatta	*caraya*	Annual	both			**64**			**19**			**6**		3% pine cones / 7% bark	scan sampling	2	December 2006 – November 2007, 383 hrs (N = 35 days) and 351 hrs (N = 34 days)	1, P
		Abundant				**58.92**	17.84	30.45	**23.49**	19.22	3.67	**6.71**		9.65% bark	scan sampling	2		
		Lean				**73.86**	49.24	12.79	**11.38**	6.32	4.19	**3.56**		3.07% bark / 7.51% pine cones	scan sampling	2		
		Annual				**76.15**			**21.17**			**2.68**			scan sampling		267 hrs, 5–6 days per season during 1 year	2, F
		Summer				**34.55**	28.05	6.5	**50.82**			**0**		1.04% petioles / 13.51% shoots	scan sampling			
		Winter				**86.58**	77.1	9.48	**2.36**			**0**		11.11% petioles	scan sampling			
		Fall				**89.94**	63.22	26.72	**0.26**			**0**		9.79% petioles	scan sampling			
		Spring				**63.37**	41.72	21.65	**25.19**			**8.27**		3.15% petioles	scan sampling			
		Annual		4.4	6.7	**63**	51	12		18	1	**12**		4% shoots	scan sampling	2	8 months	3, IB
		Summer		4.4	6.7	**42.37**	41.71	0.66	**57.63**			**0**			scan sampling	2	January – December 2004, 1181 hrs, 10 days/months (5 each group)	6, COR
		Fall		4.4	6.7	**72.56**	72.56	0	**26.28**			**0**		1.145% petioles	scan sampling	2		
		Winter		4.4	6.7	**91.27**	75.51	15.76	**4.84**			**1.01**		2.88% petioles	scan sampling	2		
		Spring		4.4	6.7	**50.67**	26.03	24.64	**34.34**			**15**			scan sampling	2		
		Annual		4.4	6.7	**45**	4	41	**24**			**17**		10% shoots	scan sampling	2	December 2003 – November 2004, 12 months, full day follow	8, IB
		Summer	AM	4.4	6.7	**47.44**	4.35	43.09	**30.03**	9.04	6.75	**2.29**		9.9% shoots / 3.48% petioles / 0.11% bark	scan sampling	2		
		Summer	AF	4.4	6.7	**53.51**	10.83	42.68	**21.45**	6.92	1.16	**5.76**		17.49% shoots / 0.63% petioles	scan sampling	2		
		Fall	AM	4.4	6.7	**42.7**	7.12	35.58	**35.38**	12.6	9.81	**2.79**		6.63% shoots / 2.69% bark	scan sampling	2		
		Fall	AF	4.4	6.7	**46.1**	7.58	38.52	**26.97**	17.77	13.89	**3.88**		6.68% shoots / 2.48% petioles	scan sampling	2		
		Winter	AM	4.4	6.7	**49.21**	9.65	39.56	**23.07**	7.72	2.11	**5.61**		18.6% shoots / 1.14% petioles / 0.26% bark	scan sampling	2		
		Winter	AF	4.4	6.7	**53.51**	10.83	42.68	**21.45**	6.92	1.16	**5.76**		17.48% shoots / 0.63% petioles	scan sampling	2		
		Spring	AM	4.4	6.7	**43.4**	2.61	40.79	**2.09**	47.72	0.07	**47.65**		5.97% shoots / 0.67% petioles / 0.07% bark / 0.07% soil	scan sampling	2		
		Spring	AF	4.4	6.7	**45.7**	3.94	41.76	**1.73**	46.83	0.13	**46.7**		4.60% shoots / 0.4% petioles / 0.6% bark / 0.13% soil	scan sampling	2		
		Summer + spring	both	4.4	6.7	**33**	13	20	**56**	33	23	**4**		7% shoots / 1% others	scan sampling	2	September 2007 – February 2008, 6 months, 3 full day follows per month	11, COR
		Annual		4.33	6.7	**54.58**	39.17	15.41	**26.72**	0				15.88% petioles / 2.78% shoots				14, COR
	guariba	Annual		4.55	6.18	**62**			**24**	6		**6**		1% others / 7% pine cones	scan sampling	2	December 2006 – November 2007, 352 hrs (N = 43 days) and 308 hrs (N = 33 days)	1, EP
		Abundant				**59.09**	19.89	30.05	**31.13**	25.47	2.6	**8.68**			scan sampling	2		
		Lean				**65.74**	49.62	11.45	**10.92**	10.74	0.18	**0.9**		22.23% pine cones	scan sampling	2		

Table 1. (Continued).

Genus	Species	Season	Sex	BM(F)	BM(M)	L	ML	YL	F	RF	UF	FL	AM	Others	Behavioral data collection technique	N groups	Study length	References and location
Aotus	*azarae*	Annual				**41.34**			**44.91**			**13.75**			scan sampling		262 hrs, 5–6 days per season during 1 year	2, F
		Summer				**16.81**	16.81	0	**65.33**			**12.03**		6.3% shoots	scan sampling			
		Winter				**51.37**	51.37	0	**35.74**			**11.88**		0.46% shoots / 0.46% fiber	scan sampling			
		Fall				**42.75**	15.3	27.45	**40.11**			**6.86**		10.19% shoots	scan sampling	1		
		Summer + winter				**15**			**66**	1		**1**		observed eating insects and fungi			2 months	7, F
						40			**16**	33		**33**		observed eating insects			2 months	13, PH*
Cebus	*libidinosus*	Annual							**2.9**			**0**	24.9	72.3% bromeliad leaves	scan sampling	1	May–December; June–November	4, ER
	nigritus	Annual				**16.4**			**37.2**	3.9		**3.9**	40.3	2.2% bromeliad leaves	scan sampling	1	Fall and Winter 1987 & 1988	4, IG
		Fall				**11**			**61.6**				26	1.4% bromeliad leaves	scan sampling	1	Fall 1987	
		Fall				**23.7**			**39.7**			**1**	34.7	1% bromeliad leaves	scan sampling	1	Fall 1988	
		Winter				**16.6**			**20.2**			**9**	50	4.2% bromeliad leaves	scan sampling	1	Winter 1987	
		Winter				**12.5**			**41.7**			**2.1**	42.7	1% bromeliad leaves	scan sampling	1	Winter 1988	
	capucinus	Annual							**81.2**				16.9		10-min focal animal	1	20 months	5, SR*
		Annual							**53**				44.3		10-min focal animal	1		
		Annual							**69.8**				29		10-min focal animal	1		
		Annual		2.3	3.2													9, SR*
		Annual											42.9	57% plant parts (= fruit mesocarp / seeds)	2-min instantaneous	1	10 months	12, LS*
	olivaceus	Annual												51% plant parts	30 seconds each ½ hour		1 year	10, HP*
		Annual												62% plant parts	30 seconds each ½ hour		1 year	10, HM*

References: 1) Agostini 2009; **2)** Arditi 1992; **3)** Bravo and Sallenave 2003; **4)** Brown and Zunino 1990; **5)** Chapman and Fedigan 1990; **6)** Delgado 2005; **7)** Gimenez and Fernández-Duque 2003; **8)** Kowalewski 2007; **9)** McCabe 2005; **10)** Miller 1998; **11)** Raño 2010; **12)** Urbani 2009; **13)** Wright 1985; **14)** Zunino 1989. **Sites: SR:** Santa Rosa, Costa Rica; **LS:** La Suerte, Costa Rica; **HP:** Hato Piñero, Venezuela; **HM:** Hato Masaguaral, Venezuela; **IG:** Iguazú, Argentina; **ER:** El Rey, Argentina; **F:** Formosa, Argentina; **PH:** Presidente Hayes, Paraguay; **EP:** El Piñalito, Argentina; **COR:** Riachuelo, Corrientes, Argentina; **IB:** Isla Brasilera, Chaco, Argentina. **(*)** Studies outside of Argentina. **BM:** Body mass **(F)** females and **(M)** males. Percentages are based on time spent feeding on different items, **L:** Leaves; **ML:** mature leaves; **YL:** young leaves; **F:** fruit; **UF:** unripe fruit; **RF:** ripe fruit; **FL:** flower; **AM:** animal matter.

quality and body weight, yet only 43.3% of the variance in dietary quality was explained by body weight (Sailer *et al.*, 1985).

Although this index is considered very coarse because there is a lot of within and between species variation in nutritional composition, we used it to analyze the characteristics of the diet of Argentinian primates. We found that *C. nigritus* exhibited the highest value (*dq* = 242) (Tab. 2), indicating that this species' diet is heavily based on animal matter and fruits. The same species in Brazil, however, showed lower values (*dq* = 198), mainly because ingestion of vertebrates and invertebrates was reported to occur in lower amounts. The index for *C. libidinosus* was lower than that of *C. nigritus* since at this site in the Yungas province Brown and Zunino (1990) observed frequent ingestion of bromeliad leaves. Capuchins were followed by owl monkeys, with an index of 158.6, an intermediate value between *Cebus* and *Alouatta*; it is possible however that the true value is higher due to the ingestion of insects, which has been reported at some sites but not quantified (Wright, 1985; Gimenez and Fernández-Duque, 2003). Both species of howler monkeys in Argentina were characterized by almost identical low indices (~136), but *A. caraya* showed variability, ranging from 126.7 to 160, based on data reported in different studies. The diet of these two howler species in Brazil suggests high similarity with Argentinian howlers, since the average *dq* (~134) did not differ from the average value shown by *Alouatta* spp. in Argentina. Again, we should stress that these data are calculated based on percentages of time spent feeding on different resources, and not on the estimated amounts of foods ingested. The latter can potentially lead to an error, as time spent feeding on a certain resource does not equate to amount ingested, especially in the case of items requiring long handling times.

Table 2. Average (± SD) dietary quality indices (***dq***) calculated for the five Argentinian primate species and for the same species inhabiting different sites in Brazil. The index (*dq* = 1s + 2r + 3.5a) is based on the percentage of time spent feeding on plant structural parts (s), reproductive plant parts (r), and animal prey (a) (Sailer *et al.*, 1985).

	Argentina			**Brazil**		
	dq	**SD**	**n**	***dq***	**SD**	**n**
Alouatta caraya	135.94	14.09	5[a]	134.98	11.17	4[b]
Alouatta guariba	136.00		1[c]	133.57	10.70	9[d]
Aotus azarae	158.66		1[e]			
Cebus libidinosus	165.25		1[f]			
Cebus nigritus	241.85		1[f]	198.53	1.31	2[g]

[a]Zunino, 1989; Bravo and Sallenave, 2003; Kowalewski, 2007; Raño, 2010; Agostini, 2009; [b]Rimoli *et al.*, 2012; Ludwig *et al.*, 2008; Bicca-Marques and Calegaro-Marques, 1994; [c]Agostini, 2009; [d]Martins, 2008a, b; Pereira, 2008; Jardim and Oliveira, 2000; Cunha, 1994; Lunardelli, 2000; Galetti *et al.*, 1994; Santos, 2007; Miranda, 2004; [e]Arditi, 1992; [f]Brown and Zunino, 1990; [g]Galetti and Pedroni, 1994; Rimoli and Ferrari, 1997.

Nutritional ecology of Argentinian primates: a geometric approach

One important goal of nutritional ecology is to determine how food nutritional variation can influence the food choices and feeding patterns of free-living primates. In this sense, the Geometric Framework for nutrition (GF) relates nutrient composition of foods with animal responses (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993, 1997). The GF is a nutritionally explicit, multi-dimensional model in which variables such as different food components, amount of ingested nutrients, and nutritional characteristics of available foods are viewed in geometric space. Observed patterns of nutrient intake can be compared with patterns predicted by dietary models such as the protein leverage hypothesis, energy maximization, and nutrient mixing, which posits that primate foraging decisions represent an attempt to obtain balanced diets based on independent macro and micronutrient regulation (Raubenheimer *et al.*, 2009; Simpson and Raubenheimer, 2012).

This framework has been recently applied to studies of the feeding ecology of non-human primates in the wild (spider monkeys: Felton *et al.*, 2009a, b; gorillas: Rothman *et al.*, 2011; chacma baboons: Johnson *et al.*, 2013). This approach requires detailed data on the absolute amounts of nutrients eaten by the individuals, and it is difficult to find this information in the literature, due to the difficulty of collecting this type of data in the field. A useful alternative in these cases is represented by the right-angled mixture triangles (RMTs), whereby a graphical analysis can be performed when only data on the nutritional composition of the diets are available (Raubenheimer, 2011; Raubenheimer and Rothman, 2013). In these two-dimensional geometric plots, food composition can be compared according to the concentration and ratio of different nutrients.

Right-angle mixture triangles. We performed a meta-analysis, gathering data from multiple feeding ecology studies (published papers, theses, and dissertations) in Argentina. First we compiled a list of the plant parts and species consumed by each primate species, and then we searched for information on the corresponding nutritional chemistry. Few studies report the nutritional composition of Argentinian primate foods (Zunino, 1989; Brown and Zunino, 1990; Gonzales-Valentin, 2010; Fernández, 2014); thus, when needed, we used phytochemical data for the same plant genus or species reported in published Neotropical primate nutritional ecology studies outside of Argentina (*i.e.*, Silver, 1997; McCabe, 2005; Felton, 2008; Milton, 2008). This method can be problematic because food nutritional chemistry can dramatically vary over time and space (Chapman *et al.*, 2003; Rothman *et al.*, 2012; Carlson *et al.*, 2013), but we simply do this for illustrative purposes.

To illustrate the composition of the foods consumed by *A. caraya*, *A. azarae*, and *Cebus* spp., we plotted the data using a right-angled mixture triangle, with carbohydrates and lipids (% of dry weight) on the **x** and **y** axes, respectively, and crude protein on the third, implicit, axis (Fig. 2). Despite the different sample sizes, the most noticeable pattern is that several foods consumed by howler monkeys were characterized by a higher concentration of protein than foods consumed by the other primates, whereas foods consumed by capuchins were mostly characterized by a high concentration of carbohydrates and relatively low protein. In fact, the average food protein:carbohydrate ratio was 0.76:1 (range: 0.006–4.82)

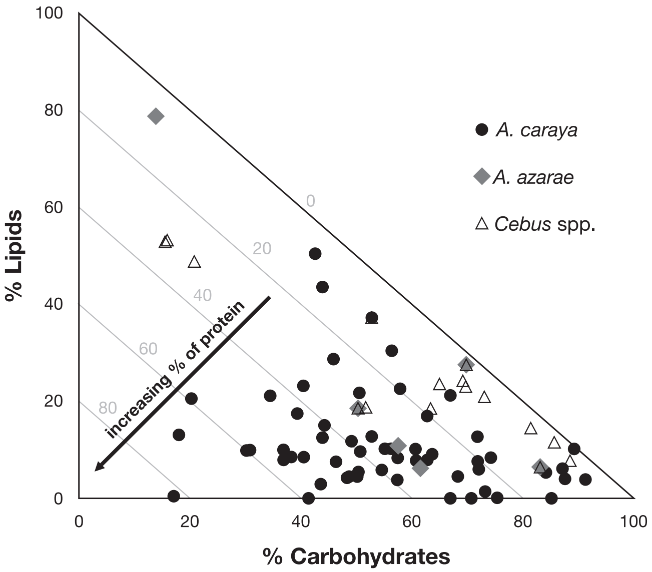

Figure 2. Right-angle mixture triangle representing the carbohydrate, lipid, and protein (implicit axis) content of the diet of capuchins (*Cebus* spp.), owl monkeys (*A. azarae*) and howler monkeys (*A. caraya*) in Argentina.

for *A. caraya*; 0.43:1 (range: 0.03–2.02) for *Cebus* spp.; and 0.39:1 (range: 0.03–0.61) for *A. azarae*. The majority of the plant parts consumed by all primate species had a high carbohydrate:lipid ratio (> 1), and only some items consumed by *A. azarae* (*i.e.*, *Trichilia catigua* fruits) and *Cebus* spp. (*e.g.*, *Luehea divaricata* seeds) were particularly rich in lipids.

Feeding ecology *vs.* nutritional ecology

A traditional approach to analyze the feeding ecology of the three genera of Argentinian primates, *i.e.*, using data on time spent feeding on different resources, revealed typical genus-specific dietary patterns. Owl monkeys are characterized by a diet mainly based on fruits, and to a lesser extent on leaves, flowers, and probably insects; howler monkeys have a leaf- and fruit-based diet; and capuchins feed largely on insects and fruits, but also on leaves and other plant parts. These differences are conspicuous in the dietary triangles (Fig. 1.1–1.3), where points representing the diets of these three genera clearly occupy separate areas of the triangle. However, different patterns start to emerge if we analyze separately the diets by season, and a high variability in feeding patterns is clearly recognized. For instance, a population of *A. caraya* in Riachuelo, Corrientes can switch from a leaf-based diet (> 80% feeding time) in winter and fall (Delgado, 2005), to a fruit-enriched diet (only 33% of feeding time on leaves) in the summer (Raño, 2010). The latter is just an example of how a change in the temporal scale of the study could reveal opposite feeding patterns.

To understand food choice from a nutritional perspective, an adequate framework to analyze and interpret complex data is necessary, and also detailed observations and analyses are required. Specifically, it is essential to be precise in assessing the actual amounts and nutrient composition of the food ingested and daily nutrient intake of individuals. First, it has been shown that traditional measures of time spent feeding are not adequate to describe actual feeding patterns in primates, since they do not reflect the amount of food consumed, tending to underestimate fruit consumption and overestimate leaf and flower intake (Gaulin and Gaulin, 1982; Oftedal, 1991; Chivers, 1998; Amato, 2013; Righini, 2014; Fernández, 2014; Garber *et al.*, 2015). Quantification of feeding time, ingestion rates, bite size, and weights of the items ingested per bite must therefore be implemented in all primate nutritional ecology studies (Schuelke *et al.*, 2006; Felton *et al.*, 2009c; Rothman *et al.*, 2012). Second, data on the nutritional composition of plant parts included in the diet are necessary to calculate daily nutrient intake of individuals. Common generalizations (*e.g.*, mature leaves contain more fiber and less protein than young leaves) need to be taken cautiously, since there is high variation in the phytochemical content of different items (depending on plant species, individual plant, location on the plant, time of day, season, site, habitat) (Oftedal, 1991; Chapman *et al.*, 2003; Worman and Chapman, 2005; Houle *et al.*, 2007; Carlson *et al.*, 2013).

Many studies of Neotropical primate feeding ecology focus on dietary and foraging strategies of howler monkeys throughout their geographical range, and a recent publication on the sampling effort in Neotropical primate diet studies revealed a significant taxonomic bias toward this genus, with 37.4% of the 290 studies across 17 countries having *Alouatta* spp. as subject of the research (Hawes *et al.*, 2013). Despite the high amounts of dietary information on this genus, howler monkeys (together with *Brachyteles* spp.) have been traditionally considered the “folivore model” for Neotropical primates (Milton, 1980; Di Fiore *et al.*, 2011), due to observations of increased time spent feeding on leaves at least during certain seasons of the year. However, it has been demonstrated (Garber *et al.*, 2015) that dietary patterns can change drastically when using data on amounts of food ingested. For example, based on time spent feeding, black howler monkeys (*A. pigra*) in Campeche, Mexico were found to consume annually 50.6% leaves, 40.2% fruits, and 7.6% flowers; however when using the estimated amount of foods ingested (based on feeding rates) the pattern was completely reversed, since fruits were the most consumed item (58.2%), followed by leaves (37%), and flowers (4.7%) (Righini *et al.*, 2017). Analogously, structural plant parts accounted for 56% (47% leaves and 9% shoots) of the annual feeding time (averages of seasonal patterns) of *A. caraya* inhabiting Riachuelo (Corrientes Province, Argentina), and fruits and flowers for 43% of their feeding time (36.4% and 6.4%, respectively); however, as reported for *A. pigra*, according to the biomass consumed (dry weight), fruits resulted to be the most consumed items in *A. caraya*'s diet (59.5%), followed by leaves (29.3%), shoots (9.5%) and flowers (1.1%) (Fernández, 2014) (Fig. 3).

Concluding remarks and future directions

The majority of the available feeding data for Argentinian primates (such as those reported in Tab. 1, with few exceptions) derive from studies that were not designed to analyze the nutritional ecology of the studied species, and thus have not used adequate methodologies to gather needed information such as estimations of daily amount of food ingested by focal animals and daily nutrient intakes (Felton *et al.*, 2009c). These are the basic data required to draw conclusions about the nutritional strategies of wild primates. Nonetheless, recent awareness on the importance of collecting more adequate feeding data (Felton *et al.*, 2009c; Rothman *et al.*, 2012) is currently producing new and important results for howler monkey nutritional ecology (Amato, 2013; Aristizábal, 2013, Fernández, 2014; Righini, 2014). For example, daily nutrient balancing and protein regulation appear to have an important influence on howler foraging strategies and food choice (Amato, 2013; Fernández, 2014; Righini, 2014).

Our review of the literature on feeding ecology of Argentinian primates demonstrates that there is a pressing need for nutritional studies in more locations in Argentina and on more species. While many feeding ecology studies have focused in Mesoamerica, there are strong differences between countries, and the majority of primate diet studies are being carried out in Brazil, Peru, and Costa Rica (Hawes *et al.*, 2013). In particular, for genera such as *Aotus* spp., much work still needs to be done, since the sampling effort devoted to its feeding ecology is very low, even at continental scale, including species found outside of Argentina (Hawes *et al.*, 2013). For *Cebus* spp. the situation looks more promising, since this genus is much more studied, and data are especially abundant for species such as *C. albifrons* and *C. apella* (Hawes *et al.*, 2013). Despite the difficulty of collecting precise data on feeding rates, bite size, and weights of the ingested foods, the fact that this type of data are beginning to increase and accumulate (see McCabe, 2005; Vogel, 2005; and McCabe and Fedigan, 2007 for *C. capucinus*) will allow, at least for some primate species or for specific plant parts, to extrapolate (*e.g.*, from feeding time to food mass ingested) and gather useful insights on the nutritional goals of different primate species. We encourage

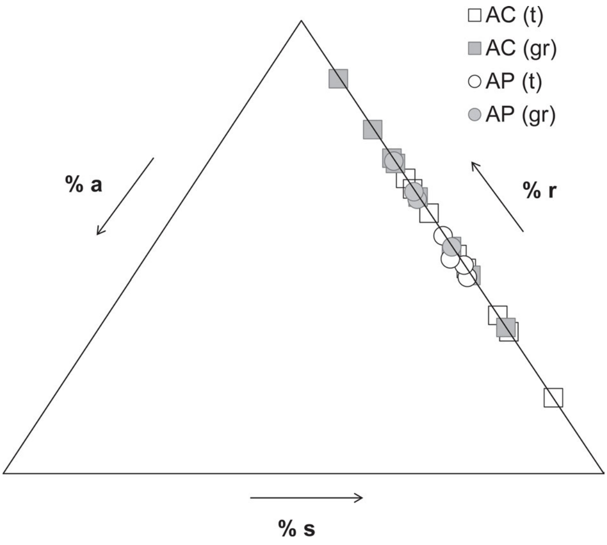

Figure 3. Diets of *Alouatta caraya* (**AC**) and *Alouatta pigra* (**AP**) in terms of time spent feeding on different plant parts (**t**) and in terms of plant biomass (dry weight) consumed (**gr**). Axes represent percentage of **a**) animal matter, **r**) reproductive plant parts (fruits and flowers), and **s**) structural plant parts (leaves, shoots, and petioles). Percentages increase in the direction of the arrows.

researchers to employ adequate methodological approaches and analytical frameworks, such as the Geometric Framework, for exploring questions about the nutritional ecology of non-human primates in Argentina.

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9 | FORAGING COGNITION IN NEOTROPICAL PRIMATES: INTEGRATING STUDIES FROM THE WILD AND FROM CAPTIVITY

COGNICIÓN DURANTE EL FORRAJEO EN PRIMATES NEOTROPICALES: ESTUDIOS EN VIDA SILVESTRE Y EN CAUTIVERIO

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Abstract. Individual foraging behavior includes exploration and use of information related to resources. While memory of food location has long been suspected to be a selective factor in the evolution of increased intelligence, experiments and observations in the wild and in captivity provide strong evidence that primates remember other critical "features" of resources that may influence the probability of finding and revisiting them. Several studies indicate that primates are able to remember the location of a resource, to anticipate the quantity of reward to be obtained and to plan routes that efficiently visit multiple resources. There is less evidence for primate cognition of temporal patterns of fruit ripening. A few studies suggest that some species know that fruits of the same type ripen relatively synchronously and also remember the quantity of ripe and unripe fruit, information that can be used to predict ripening intervals and adjust the timing of revisits to individual food resources. Because most of these studies came from Old World species, the aim of the present chapter is to review experimental and observational studies in Neotropical primates from the wild and captivity on these topics, giving special attention to studies carried out with capuchin monkeys in Argentina.

Resumen. El comportamiento individual de forrajeo incluye la exploración y el uso de información relacionada a los recursos alimenticios, incluyendo la búsqueda, la preparación y el consumo de los mismos. A su vez, la cognición implicada en el forrajeo comprende una serie de mecanismos de procesamiento de información que permite a los individuos tomar decisiones adecuadas acorde a la situación, para explotar de la mejor manera posible la heterogeneidad del ambiente. Clásicamente los investigadores han asumido que los animales forrajean al azar sin un conocimiento sobre dónde buscar el alimento, o al revés, que poseen un conocimiento perfecto sobre la localización y la disponibilidad de los sitios en donde alimentarse. Pero ninguno de estos dos extremos es lo que ocurre en realidad. Se han propuesto dos modelos para explicar las estrategias espaciales de los primates no-humanos durante el comportamiento de forrajeo: 1) los mapas topológicos o basados en rutas de forrajeo, donde el individuo utiliza segmentos de rutas conectados a través de marcas en el terreno que son usadas como puntos para cambiar la dirección de desplazamiento, y 2) los mapas basados en

coordenadas o mapas Euclidianos, donde se utiliza la ubicación de rasgos sobresalientes del ambiente como referencia para establecer relaciones geométricas entre ellos. En cualquiera de los dos casos, los individuos codifican información basándose en rasgos fijos o puntos de información del ambiente para orientarse en el espacio, y esta información debe ser recordada y recuperada para poder decidir los movimientos diarios. Mientras que la memoria para localizar el alimento ha sido considerada un factor selectivo en la evolución de una mayor inteligencia, experimentos y observaciones en cautiverio y en vida silvestre proveen evidencia contundente de que los primates recuerdan otros rasgos críticos de los recursos que pueden influenciar la probabilidad de encontrarlos y revisitarlos. Numerosos estudios indican que los primates son capaces de recordar la localización de un recurso, de anticipar la cantidad de recompensa que obtendrán y de planificar rutas para visitar múltiples sitios de manera eficiente. Sin embargo, existe escasa evidencia sobre el conocimiento que los primates poseen acerca de los patrones temporales de maduración de frutos. Pocos estudios sugieren que algunos primates conocen que los frutos de una misma especie fructifican de manera sincrónica, a la vez que recuerdan las cantidades de fruta madura e inmadura presentes, información que puede ser usada para predecir los intervalos de maduración y así ajustar los tiempos de revisita a los recursos individuales. Dado que la mayoría de los estudios sobre cognición durante el comportamiento de forrajeo provienen de especies del Viejo Mundo, el objetivo del presente capítulo es revisar los estudios observacionales y experimentales en cautiverio y vida silvestre publicados sobre estos temas para los primates neotropicales, con énfasis en los estudios llevados adelante en monos caí o capuchinos (*Cebus = Sapajus nigritus*) en Argentina. Ofrecemos una revisión de las cinco hipótesis más relevantes sobre los mecanismos involucrados en la cognición durante el forrajeo y los estudios que las apoyan. Dichas hipótesis están enmarcadas dentro de tres grandes temas de investigación: 1) el conocimiento espacial relacionado a la habilidad para localizar los recursos de alimento; 2) la habilidad para recordar las cantidades de recompensa a obtener en cada sitio de alimentación; y 3) el conocimiento temporal en relación a la variación de los recursos en los sitios de alimentación a través del tiempo. Las cinco hipótesis son las siguientes: 1) los primates recuerdan la relación espacial entre sitios relevantes del terreno (o recursos alimenticios); 2) los primates utilizan mapas mentales apropiados en relación a la tarea de forrajeo a la cual se enfrentan; 3) los primates utilizan información almacenada en la memoria sobre las recompensas que obtuvieron con anterioridad en determinados sitios, y organizan sus rutas de forrajeo en relación a esta información; 4) los primates son capaces de recordar los cambios en la disponibilidad de los recursos en el espacio y en el tiempo; y 5) los primates son capaces de demorar una respuesta ante determinado evento en función de obtener una mejor recompensa en el futuro. Todas las hipótesis enumeradas tienen un importante sustento en la literatura, con excepción de las hipótesis 2 en lo que respecta a la existencia de una representación espacial de la localización de los sitios con alimento basada en mapas de coordenadas, y la hipótesis 4, referida a la capacidad de los primates de recordar los cambios en la disponibilidad de los recursos en el espacio y en el tiempo. Existe evidencia de que algunas especies (en particular humanos, chimpancés y bonobos) son capaces de codificar información en mapas de coordenadas. Algunos autores sugieren que esta habilidad se debe a una diferente capacidad de memoria en relación a aquellas especies que codifican la información en la forma de mapas topológicos. Sin embargo, la capacidad de los primates para codificar la información en la forma de mapas no ha sido documentada aún de manera exhaustiva. Con respecto a la capacidad de recordar cambios espacio-temporales en la disponibilidad del alimento, los estudios que existen provienen de un mismo proyecto y sitio de estudio: el Parque Nacional Iguazú, Argentina.

Se ha sugerido que los sistemas especializados de memoria podrían haber evolucionado en conjunto con la capacidad de los primates de demorar una respuesta ante un determinado evento en función de obtener una mejor recompensa en el futuro. Si esto fuera así, algunos tipos de memoria que aún se consideran exclusivos de los humanos podrían estar presentes en otras especies, como por ejemplo la memoria episódica, que sería un factor clave para aquellas especies que necesitan recordar la variación espacial y temporal de los recursos, evitando así las revisitas a sitios que ya se han agotado o a sitios que han sido descubiertos recientemente. Esta memoria es un sistema de memoria de largo plazo que incluye la recuperación de un evento pasado que está integrado por componentes múltiples entre los que se encuentran, en el caso de un evento de alimentación, un componente de lugar o «dónde» ocurrió el evento, «qué» alimento fue consumido, «cuándo» fue encontrado y con «quién» estaba el individuo durante el mismo. Estudios realizados en el Parque Nacional Iguazú sugieren que los caí recuerdan los componentes *dónde*, *qué*, y posiblemente *cuándo*, de un evento pasado de visita a un recurso. Esperamos que futuras investigaciones en el sitio puedan brindar mayor información sobre la capacidad de los caí de utilizar este tipo de memoria.

Introduction

Every day, foraging animals need to find enough resources of sufficient quality (*e.g.*, adequate energy density, supplying micronutrient demands, yet not too difficult to digest). Foraging behavior can be understood as the search for, localization of, and consumption of food (Boyer *et al.*, 2003). Search may be viewed as solving the problem of what to do in a multiple exploration scenario in which resources are depleted in time and space. In this scenario, search becomes a serial problem that requires the ability to keep track of past movements to avoid losing time and energy in considering alternatives that have already been exploited (De Lillo *et al.*, 1997). In this case, an animal is expected to have some kind of "mental map": an internal representation of external information about spatial and temporal environmental structure (Godfrey-Smith, 2001). This information will be processed and used to solve multiple problems of daily life, including, for example, finding food and mates or avoiding predators (Riba I Campos, 1997; Rehbein and Moss, 2002; Lahitte *et al.*, 2003). Foraging cognition comprises the set of mechanisms of information processing that allow individuals to make decisions that "optimally" (understood as "best within constraints") exploit known environmental heterogeneity (Godfrey-Smith, 2001). Frequently researchers have either assumed that animals have no knowledge of where to look for food and forage randomly, or that animals have perfect knowledge of the location and availability of feeding sites. Neither of these extremes is probably common (Garber, 2000). Also, the social environment can influence the types of information available to animals that forage in dispersed or cohesive groups, including decisions concerning where to search, whom to follow, whom to avoid, an assessment of individual differences in competitive ability or dominance, and the costs and benefits of remaining in a food patch jointly occupied by others (Garber, 2000; Garber *et al.*, 2009).

Researchers of wild and captive primates generally agree that primates have some kind of mental representation of their environment and that they keep track of multiple kinds of information about their resources. Many studies show that primates move toward food patches in a manner that is consistent with goal-directed behavior that implies spatial knowledge about food location (Garber and Dolins, 1996; Platt *et al.*, 1996; Garber and Paciulli, 1997; Janson, 1998; Pochron, 2001; Cunningham and Janson, 2007), the quantity of reward to be obtained (Garber and Paciulli, 1997; Janson, 1998), and some kind of temporal knowledge about ripening states (Janmaat *et al.*, 2006a; Anzelc, 2009). Observations in the wild show that primates travel faster and more directly toward more productive resources, suggesting that they anticipate both where and how much resource may be obtained (*e.g.*, Janson, 1998; Pochron, 2001, 2005; Janmaat *et al.*, 2006a; Noser and Byrne, 2007a, b; Cunningham and Janson, 2007; Valero and Byrne, 2007). In some cases primates appear to anticipate food quantity even when this varies over time, as long as there are predictable cues about changes in its availability (Janmaat *et al.*, 2006b, 2013a, b).

Several studies indicate that wild primates are more efficient in finding food than predicted by random search models (*e.g.*, Garber and Hannon, 1993; Janson, 2000; Milton, 2000; Cunningham, 2003; Valero and Byrne, 2007, and also see Zuberbühler and Janmaat, 2010 for review). Although such non-random searches often coincide with taking the most direct route to the closest available (or most preferred) resource, such an intuitive solution is not always adopted. Deviations from straight line travel to the closest resource may happen for several reasons: 1) the shortest route is not always the most efficient one (Di Fiore and Suarez, 2007), 2) animals could combine different goals in one single route (Cunningham and Janson, 2007; Janson, 2007), and 3) they could be monitoring food without exploiting it (Sigg and Stolba, 1981; Janmaat *et al.*, 2006a; Garber and Porter, 2014).Whatever the short-term criteria that affect route selection between one goal and the next, there is little evidence that primates minimize distance or optimize foraging returns across sequences of multiple goals (Janson, 2014).

Spatial memory for locating food has long been suspected to be a selective factor in the evolution of primate increased intelligence (Milton, 1981). In the case of South American primates, the high diversity of plant foods in tropical forests, combined with the low density of any one species and their clumped distributions in space and time could have been a major selective force in advanced cerebral complexity in taxa such as Cebidae, Pithecidae and Atelidae (Milton, 1981, 1988). This extreme diversity offers a complex environment to frugivores (Suarez, 2003) because the number of fruiting trees will be high but individual trees are ephemeral, varying in maturation state and ripening rates across seasons that may vary from year to year (Janson and Emmons, 1990; Chapman *et al.*, 1999b). Non-fruit resources used by primates also show seasonal variation in availability and may be as patchy and important as fruits to particular primate species (*e.g.*, flowers: Janson *et al.*, 1981; Garber, 1988). The ability to remember these features and to travel efficiently among feeding patches would help to minimize energy expenditure by reducing the time and energy spent searching for food items in a random pattern (Janson, 1998; Milton, 2000). In her study, Milton (1980) estimates that green and ripe fruits of twelve random selected species were available only 3.7 months a year, while ripe fruits of particular species were available for only 1.1 months. Further, ripe fruits were available on individual trees for only 0.8 month a year, reinforcing the constant need to update information to remember locations of trees and their states of ripeness.

The present chapter reviews experimental and observational studies on foraging cognition from wild and captive Neotropical primates, giving special attention to studies carried out with capuchin monkeys in Argentina. In this review, we provide a broad perspective of the most relevant hypotheses tested in three major topics of research: spatial knowledge in relation to the ability to locate resources, the ability to remember quantities of reward in each location, and temporal knowledge related to variation of resources at a given site across time (*e.g.*, ripening states).

Cognitive processes underlying foraging behavior

Multiple cognitive processes are associated with memory (Fagan *et al.*, 2013). Successful foraging requires more than simply remembering the spatial location of a number of food trees. It depends on a set of integrated cognitive skills (Zuberbühler and Janmaat, 2010) including at least two types of memory: ***spatial memory***, which encodes spatial relationships or configurations, and ***attributes memory***, which encodes the attributes of local features.

Spatial information allows an animal to reduce uncertainty with regard to its position with respect to geographical objects or locations (*e.g.*, trees), while attribute information allows animals to reduce uncertainty with regard to location-dependent characteristics of the objects (*e.g.*, quantity or type of food of a tree: Fagan *et al.*, 2013). Another aspect of attribute memory is the time passed since a location was last visited, and this aspect is especially useful in avoiding recently explored areas (Van Moorter *et al.*, 2009). In addition, there are some attributes that are also time-dependent but not location-dependent (*e.g.*, changes in the rate of ripening of fruiting trees according to the weather: Janmaat *et al.*, 2006b).

Two general models have been proposed to explain non-human primate spatial strategies: route-based maps and coordinate-based maps. Foragers that encode information in either of these two models rely on fixed features or information points of the environment to orient in space (Urbani, 2009).

A coordinate-based mental map or Euclidian map (Tolman, 1948; O'Keefe and Nadel, 1978; Poucet, 1993; Normand and Boesch, 2009) is a spatial representation in which the geometric forager encodes locations of salient features of the environment in a way that preserves the relationships between them. Using such a mental map, a forager is expected to compute relatively accurate distances and directions from their current location to their goal, and travel using direct routes and novel shortcuts, that is, minimizing distances between consecutive goals.

In contrast, a topological or route-based mental map is associated with the ability of a forager to navigate to and relocate locations using known travel segments that connects into networks via landmarks used as switch points (Milton, 1980; Di Fiore and Suarez, 2007). Landmarks are visual images or olfactory cues that can be used as guides or beacons (Garber, 2000). Poucet (1993) suggested that, by using a route network, animals should be able to navigate effectively even with only a minimal understanding of the general spatial relationships among nodes in the network. Mental representation of the routes linking the nodes would require only storage of a series of linked views so that the animal would recognize the route during travel, and these would be reinforced by repeated use of the routes.

Table 1. Most important hypotheses and sub-hypotheses about mechanisms involved in foraging cognition in Neotropical primates tested in studies from the wild and from captivity.

Hypothesis H0 to H5

Null hypothesis

H0: Primates use a random or sensory search strategy without use of knowledge of location, productivity or temporal characteristics of previously-visited resources.

Spatial knowledge: location and maps

H1: Primates remember the spatial relation between landmarks (resources).
    H1a: Primates visit resources that are closer than predicted by random search.
    H1b: Primates reach a resource by goal-directed travel.
    H1c: Primates travel faster toward known resources.
    H1d: Primates revisit fruiting trees according to a preferential pattern.
    H1e: Primates plan their routes while moving between resources (multi-step movements).

H2: Primates adopt mental maps appropriate to the scale of foraging task encountered.
    H2a: Primates have a coordinate-based spatial representation of the location of feeding sites in their home range.
    H2b: Primates have a route-based spatial representation of the location of feeding sites in their home range.

Spatial Knowledge: quantity

H3:Primates use information about prior rewards obtained at a determined location and organize their foraging route according to this information.
    H3a: Primates preferentially visit resources with more (previous) reward.

Temporal knowledge

H4: Primates can trace changes in resource availability in time and space (attribute memory).
    H4a: Primates can anticipate seasonal availability of a given resource.
    H4b:Primates can anticipate short-term changes in availability due to weather.
    H4c: Primates can anticipate short-term changes in availability due to time elapsed since a prior visit to a resource.
H5: Primates discount future resource availability.
    H5a: Discounting applies equally to all resources, regardless of type or amount.
    H5b:Discounting is greater for resources that are less predictable.

Taking into account all these mechanisms we reviewed studies from the wild and from captivity that analyze these topics. We offer readers a summary of their most important findings about mechanisms involved in foraging cognition in Neotropical primates (Tab. 1).

Spatial knowledge: remembering location of resources and quantity of reward

Janson and Byrne (2007) reviewed observational support for resource cognition in wild animals. They pointed out that the detection ability of an animal for its resources is a vital parameter needed to model the foraging process and that it can be estimated experimentally (Janson and Di Bitetti, 1997). Detection distance will vary between resource types

(Noser and Byrne, 2007a) and between different sizes of the same resource type (Janson and Di Bitetti, 1997). Once we have knowledge about the detection ability of a primate species, there are several behavioral criteria that support the notion that one or more foragers anticipate the location and use of resources that are not yet within detectable range, thus implicating the use of spatial memory. First is a non-randomly straight-line trajectory toward apparent goals (Byrne *et al.*, 2009), richer goals (Pochron, 2001; Cunningham and Janson, 2007) or previously-visited resources (Janson, 1998) compared to "random" search or movement models. Second, higher travel speed is expected along trajectories toward known resources than toward unknown ones (Janson and Di Bitetti, 1997; Janmaat *et al.*, 2006a; Noser and Byrne, 2007a). In the case of group foraging, speed is expected to increase as the group approaches known resources because subordinates can avoid aggressive competition within the resource by arriving there before the dominants (*e.g.*, Di Bitetti and Janson, 2001).

The ability of individuals to remember the value of a resource from previous visits is assumed in most models of animal foraging and operant-conditioning, but anticipating the future values of a resource may require integrating several complex and dynamic variables. In the case of fruit ripening in a tree, the expected amount of ripe fruit will be a function of at least four variables: 1) the amounts of ripe and unripe fruit in the tree when the forager last left the resource and the ability of the forager to obtain access to the resource (*i.e.*, individual foraging success), 2) the time since the forager last visited the resource, 3) the rate and pattern of fruit ripening, and 4) the chance that another forager has depleted the resource between the focal forager's last visit and the current one (Janson, 2007).The first three of these are parameters that a forager could learn with high precision through past and current experience: the last may be indexed roughly by correlates of competitor presence (central versus peripheral location in a home range, *e.g.*, Peres, 1989, loud calls, *e.g.*, Whitehead, 1987; Teixeira da Cunha and Byrne, 2006). In terms of behavioral changes, researchers have used the increased directedness or speed of primate groups toward more rewarding food patches as evidence of anticipation of both the resource's location and of its expected value (Janson, 1998; Janmaat *et al.*, 2006a).

Apart from studying the ability of primates to remember the location of resources and quantity of reward, some researchers are beginning to ask about primates' ability to recall past events and plan for the future. The ability to plan a foraging route (Noser and Byrne, 2007a; Valero and Byrne, 2007; Noser and Byrne, 2010) or to delay the response to some event in order to get a better reward in the future (temporal discounting: Tobin and Logue, 1994; Stephens and Anderson, 2001; Stevens *et al.*, 2005a, b) are examples of this ability, and should also be linked with the ability to remember ripening states and organize their visits to trees as a result of these memories.

Temporal knowledge: remembering ripening states

An area of foraging cognition about which we have no more than small hints for any species is temporal knowledge (Janson and Byrne, 2007). As we can see in Tab. 2, the amount of information related to this in Neotropical primates is still rare.

There is little available evidence that New or Old World primates are good at discriminating the maturing state of fruiting trees, apart from quantity (Janmaat *et al.*, 2006a; Cunningham and Janson, 2007; Janmaat *et al.*, 2012), or to make decisions about fruit ripening according to climate conditions (Janmaat *et al.*, 2006b). Janson (1998) found that capuchins avoid revisiting fruit sources within about 24 hours of a previous visit, but did not test for their ability to discriminate among different replenishment rates. Temperature and solar radiation influence ripening rates of fruits as well as the maturation of insect larvae inside them (Houle, 2004), making the replenishment of edible fruits in a patch somewhat predictable. A unique study on free-ranging mangabeys in Africa (Janmaat *et al.*, 2006b) suggests that they respond to such differences by revisiting fruit-bearing trees more frequently when temperatures and solar radiation are higher; this behavioral adjustment was not a simple consequence of moving less in cold or rainy weather, because visitation rates to non-resource trees did not change.

Ripe fruits are ephemeral and frugivores must continuously update their knowledge of the locations (spatial memory) of edible fruit (attribute memory of trees). Ripe fruit in particular trees only appears at certain times in the year, and when it does, many animals compete over this sweet and energy-rich food (Houle *et al.*, 2006). Because not all rainforest trees carry fruit every year, and sometimes skip one or more years (Chapman *et al.*, 1999a), frugivores would benefit from relearning, every year, which individuals are producing fruit and which ones are not (Janmaat *et al.*, 2013b).

Despite such variation in fruit production between individuals and years, many rainforest tree species have a reproductive strategy that causes different trees of the same species to fruit simultaneously within a clustered time period (van Schaik *et al.*, 1993). Primates could use this synchrony to increase their fruit finding efficiency by learning the temporal patterns of fruiting (Janmaat *et al.*, 2013a, b). After discovering fruit in one tree, primates can switch to an “inspect all strategy” and start inspecting other trees of the same species. Indications for the use of such a “synchronicity-based inspection strategy” (Janmaat *et al.*, 2013a, b) were first found in Japanese macaques and later in grey-cheeked mangabeys (*Macaca fuscata*, Menzel, 1991; *Lophocebus albigena*, Janmaat *et al.*, 2012), but are currently lacking for New World primates.

Support for resource cognition in Neotropical primates

Several observational and experimental studies from the wild have compared observed patterns of animal movement with null models to test hypotheses 1 to 3 (Tabs. 1 and 2). Another group of studies carried out experiments in the wild and in captivity to test the ability of monkeys to use attribute knowledge while foraging for resources (hypothesis 4, Tabs. 1 and 2). In contrast, the use of temporal knowledge by Neotropical primates has rarely been tested.

There are studies for at least some aspects of each of the hypotheses (1 to 5) about foraging cognition. Of the 40 studies (35 scientific publications, four Doctoral theses, one Master's thesis) almost half are observational and the rest experimental. More studies are carried out in the wild rather than in captivity.

In the wild, observational studies use time-based location sequences to take account of monkey movements and visits to food resources throughout their home range (see Tab. 2) and then compare those movements with mechanistic or statistical null models. Experimental studies insert some kind of device in the monkey's environment (see Tab. 2) and by manipulating types of food, quantity and location, they test for specific cognitive mechanisms affecting a monkey's choice while foraging (hypotheses 1a to 1b, hypothesis 3). The ones that use speed to test for spatial memory (hypothesis 1c) compare the values between sites that have foods of differing preference (Anzelc, 2009) or different quantities of ripe and unripe fruit (Tujague, 2013).

Evidence from captivity comes from experiments only. In this case, there are more methodological paradigms. Researchers frequently use artificial feeders with food hidden inside installed with different arrangements, and with different purposes according to the hypotheses being tested (Menzel *et al.*, 1985; MacDonald *et al.*, 1994; De Lillo *et al.*, 1997; Potì, 2000; Beran *et al.*, 2008). Some studies use boards or chambers where the monkeys have to remove food placed at different locations (Ludvig *et al.*, 2003; Dolins, 2009). Finally, another methodological paradigm is the use of radial mazes (Andrews, 1988) or modified versions of radial mazes (Platt *et al.*, 1996; Tujague, 2013). In all these cases, the study subject interacts with three dimensional space and searches for hidden food.

Sample sizes in most studies are small. Only one study in the wild collected data on three groups of the same species (*Cebus nigritus = Sapajus nigritus*) (Tujague, 2013), and only three studies in captivity tested more than three or four individuals (Andrews, 1988: eight individuals of two different species, *Callicebus moloch* and *Saimiri sciureus*; Platt *et al.*, 1996: 16 individuals of two different species, *Leontopithecus rosalia* and *Callithrix kuhli*; Dolins, 2009: 16 individuals of *Saguinus oedipus oedipus*; Fichtner Gomes and Bicca-Marques, 2012: eight individuals of *Cebus nigritus*). The rest of the studies worked with few individuals or one group only.

Although some species are the focus of many studies, the total taxonomic diversity investigated is large. The number of species tested is 20 (discounting synonymies such as *Cebus nigritus*, *Cebus apella nigritus* and *Cebus apella* in Argentina and south-eastern Brazil). Most genera of Neotropical primates are represented, with the exception of *Brachyteles* (Atelidae), *Cacajao* (Pithecidae), and *Aotus*, *Callimico*, and *Cebuella* (Cebidae).

There is a lot of evidence for hypotheses 1 to 3 (although only a few studies give support to hypothesis 2a in Neotropical primates), but there is very little information about hypothesis 4 (all coming from the same study site: the Caí Project, Iguazú National Park, Argentina). The Caí Project (*caí* means monkey in Guarani, the language of local native populations) has studied capuchin behavior at Iguazú National Park since 1991 (Janson *et al.*, 2012) and is a collaboration between foreign and Argentinian researchers working on diverse topics from ecology to cognition. Studies at Iguazú have been carried out on the same population (and also on the same group, although Tujague (2013) included two more groups) but using two complementary designs: observations of monkey movements and visits to target fruiting trees according to qualitative observations of ripe and unripe fruit (Tujague, 2013) and experiments of capuchins' movements while visiting food platforms (Janson, 1996, 1998, 2007).

Table 2. Support studies to the most important hypotheses and sub hypotheses referred in Table 1. Authors, year of publishing, species involved, type of research (observational= **Obs** or experimental= **Exp** in wild or captivity= **Capt.**) is explained for each study. **BE** = Biological Station, **Dep.** = Department, **MC** = Medical Center, **ND** = No data available for Neotropical primates, **NP** = National Park, **RC** = Research Center, **RS** = Research Station.

Hi	Study	Species	Type of study	N groups	Site
H1	De Lillo *et al.*,1997	*Cebus apella*	Exp Capt.	4 indiv.	Consiglio Nazionale delle Richerche, Italy
	Dolins 2009	*Saguinus oedipus oedipus*	Exp Capt.	16 indiv.	University of Stirling Primate Unit, U.K.
	Garber and Dolins 1996	*Saguinus mystax*	Exp Wild	1	Padre Isla, Peru
	Garber and Paciulli 1997	*Cebus capucinus*	Exp Wild	1	La Suerte BE, Costa Rica
	Platt *et al.*, 1996	*Leontopithecus rosalia / Callithrix kuhli*	Exp Capt.	16/16 indiv.	University of Nebraska, Omaha Callitrichid RC, U.S.A.
	Ludvig *et al.*, 2003	*Saimiri sciureus*	Exp Capt.	3 indiv.	SUNY Downstate MC, Brooklyn, USA
	MacDonald *et al.*, 1994	*Callithrix jacchus jacchus*	Exp Capt.	4 indiv.	Metropolitan Toronto Zoo, Canada
	Menzel *et al.*, 1985	*Saguinus fuscicollis*	Exp Capt.	5 indiv.	Dep. of Psychology, Stony Brook, USA
	Presotto and Izar 2010	*Cebus nigritus*	Obs Wild	1	Carlos Botelho State Park (PECB), Brazil
	Tujague 2013	*Cebus libidinosus* (*Sapajus cay*)	Exp Capt.	2	La Plata Zoo, Argentina
H1a	Janson 1998	*Cebus apella*	Exp Wild	1	Iguazú NP, Argentina
	Valero and Byrne 2007	*Ateles geoffroyi yucatanensis*	Obs Wild	1	Otochma'ax Yetel Kooh reserve, Yucatan, Mexico
	Garber and Hannon 1993	*Saguinus mystax / S. fuscicollis*	Obs Wild	1 mixed	Río Blanco RS, Peru
H1b	Anzelc 2009	*Pithecia pithecia*	Obs Wild	1	Brownsberg Nature Park, Suriname
	Cunningham and Janson 2007	*Pithecia pithecia*	Obs Wild	1	Round Island, Guri Lake, Venezuela
	Garber 1989 / Garber and Hannon 1993	*Saguinus mystax / S. fuscicollis*	Obs Wild	1 mixed	Río Blanco RS, Peru
	Garber and Dolins 2010	*Saguinus mystax / S. fuscicollis*	Obs Wild	1 mixed	Ometepe BE, Nicaragua
	Janson 1998	*Cebus apella*	Exp Wild	1	Iguazú NP, Argentina
	Milton 1981	*Ateles geoffroyi / Alouatta palliata*	Obs Wild	2	Barro Colorado Island, Panama
	Suarez 2013	*Ateles belzebuth*	Obs Wild	1	Yasuní NP, Ecuador
H1c	Anzelc 2009	*Pithecia pithecia*	Obs Wild	1	Brownsberg Nature Park, Suriname
	Tujague 2013	*Cebus nigritus* (*Sapajus nigritus*)	Obs Wild	3	Iguazú NP, Argentina
H1d	Cunningham and Janson 2007	*Pithecia pithecia*	Obs Wild	1	Round Island, Guri Lake, Venezuela
	Tujague 2013	*Cebus nigritus* (*Sapajus nigritus*)	Obs Wild	3	Iguazú NP, Argentina
H1e	Janson 2007	*Cebus apella nigritus*	Exp Wild	1	Iguazú NP, Argentina
	Valero and Byrne 2007	*Ateles geoffroyi yucatanensis*	Obs Wild	1	Otochma'ax Yetel Kooh reserve, Yucatan, Mexico
H2	Garber 1989 / Garber and Hannon 1993	*Saguinus mystax / S. fuscicollis*	Obs Wild	1 mixed	Río Blanco RS, Peru
	Janson 2007	*Cebus apella nigritus*	Exp Wild	1	Iguazú NP, Argentina
	Milton 1981	*Ateles geoffroyi / Alouatta palliata*	Obs Wild	2	Barro Colorado Island, Panama
	Robinson 1986	*Cebus olivaceus*	Obs Wild	12	Fondo Pecuario, Venezuela
	Tujague 2013	*Cebus* (*Sapajus*) *nigritus*	Obs Wild	3	Iguazú NP, Argentina
	Valero and Byrne 2007	*Ateles geoffroyi yucatanensis*	Obs Wild	1	Otochma'ax Yetel Kooh reserve, Yucatan, Mexico
H2a	Potì 2000	*Cebus apella*	Exp Capt.	4 indiv.	Consiglio Nazionale delle Richerche, Italy
	Presotto and Izar 2010	*Cebus nigritus*	Obs Wild	1	Carlos Botelho State Park (PECB), Brazil
	Urbani 2009	*Cebus capucinus*	Exp Wild	1	La Suerte BE, Costa Rica

Table 2. (Continued).

Hi	Study	Species	Type of study	N groups	Site
H2b	Anzelc 2009	*Pithecia pithecia*	Obs Wild	1	Brownsberg Nature Park, Suriname
	Di Fiore and Suarez 2007	*Ateles belzebuth* / *Lagothrix poeppigii*	Obs Wild	2	Yasuní NP, Ecuador
	Garber 1989 / Garber and Hannon 1993 / Garber 2000	*Saguinus mystax* / *S. fuscicollis*	Obs Wild	1 mixed	Río Blanco RS, Peru
	Garber and Jelinek 2005	*Alouatta palliata*	Obs Wild	1	Ometepe BE, Nicaragua
	Garber and Dolins 2010	*Saguinus mystax* / *S. fuscicollis*	Obs Wild	1 mixed	Amazon Basin, Northeastern Peru
	Garber and Porter2014	*Saguinus fuscicollis weddelli*	Obs-Exp Wild	1	Callimico, Pando, Bolivia
	Janson 1998	*Cebus apella*	Exp Wild	1	Iguazú NP, Argentina
	Milton 2000	*Alouatta palliata*	Obs Wild	1	Barro Colorado Island, Panama
	Porter and Garber 2013	*Saguinus fuscicollis weddelli*	Obs Wild	1	Callimico, Pando, Bolivia
	Presotto and Izar 2010	*Cebus nigritus*	Obs Wild	1	Carlos Botelho State Park (PECB), Brazil
	Shaffer 2013	*Chiropotes sagulatus*	Obs Wild	1	Upper Essequibo Conservation Concession (UECC), Southern Guyana
	Suarez 2003	*Ateles belzebuth*	Obs Wild	1	Yasuní NP, Ecuador
	Suarez *et al.*, 2014	*Ateles belzebuth*	Obs Wild	3 indiv.	Yasuní NP, Ecuador
	Urbani 2009	*Cebus capucinus*	Exp Wild	1	La Suerte BE, Costa Rica
H3	Beran *et al.*, 2008	*Cebus apella*	Exp Capt.	7 indiv.	Language Research Center of Georgia State University, U.S.A.
	Fichtner Gomes and Bicca-Marques 2012	*Cebus nigritus*	Exp Wild	8 indiv.	Porto Alegre, Brazil
	Garber 1988	*Saguinus mystax* / *S. fuscicollis*	Obs Wild	2	Río Blanco RS, Peru
	Garber *et al.*, 2009	*Saguinus imperator* / *S. fuscicollis*	Exp Wild	2	Parque Zoobotânico, Rio Branco, Brazil
H3a	Andrews 1988	*Callicebus moloch* / *Saimiri sciureus*	Exp Capt.	8 / 8 indiv.	California Primate RC, U.S.A.
	Cunningham and Janson 2007	*Pithecia pithecia*	Obs Wild	1	Round Island, Guri Lake, Venezuela
	Garber and Paciulli 1997	*Cebus capucinus*	Exp Wild	1	La Suerte BE, Costa Rica
	Janson 1998	*Cebus apella*	Exp Wild	1	Iguazú NP, Argentina
	Janson 2007	*Cebus apella nigritus*	Exp Wild	1	Iguazú NP, Argentina
H4	Janson 1998	*Cebus apella*	Exp Wild	1	Iguazú NP, Argentina
H4a	ND				
H4b	ND				
H4c	Janson 1996	*Cebus apella nigritus*	Exp Wild	1	Iguazú NP, Argentina
	Tujague 2013	*Cebus* (*Sapajus*) *nigritus*	Obs Wild	3	Iguazú NP, Argentina
H5	Bourjade *et al.*, 2012	*Cebus apella*	Exp Capt.	2 indiv.	Primatology Center, University of Strasbourg, France
	Stevens *et al.*, 2005b	*Saguinus oedipus* / *Callithrix jacchus*	Exp Capt.	4/4 indiv.	Primate Cognitive Neuroscience Laboratory, Harvard University, U.S.A.
H5a	McKenzie *et al.*, 2004	*Saimiri sciureus*	Exp Capt.	2 indiv.	University of Western Ontario, Canada
	Ramseyer *et al.*, 2006	*Cebus apella*	Exp Capt.	6 indiv.	Primate Center of the Louis Pasteur University, Strasbourg, France
H5b	Stevens *et al.*, 2005a	*Saguinus oedipus* / *Callithrix jacchus*	Exp Capt.	6/5 indiv.	Primate Cognitive Neuroscience Laboratory, Harvard University, U.S.A.

Janson's (1998, 2007) experiments on capuchin monkeys at the study site show that their spatial decisions are sensitive to the amount of food at feeding platforms. In his early experiments, Janson (1998) installed 31 provisioning platforms arranged in 17 sites over an area of 1 km^2 in the home range of the Macuco group within the capuchin home range. Platforms were provisioned with 10 to 80 tangerines per platform site: quantities of fruit at platforms were changed during the study according to two different period designs. Observers followed the capuchin group from dawn to dusk and recorded at 15-minute intervals the spatial location of the geographical center of group, in addition to all visits and durations of fruit-feeding activities, both at provisioning platforms and natural fruit trees. These data were plotted to summarize the patterns of movement during each experimental period. These patterns were compared with two null models of group movement that assume no explicit spatial or temporal knowledge: the step model and the geometric model (see Janson, 1998 for model details). If patterns of movement generated by a null model with straight-line travel cannot be made consistent with observed data, then observations of straight-line travel alone could be used to validate the assumption of spatial memory, although not whether it is organized according to a Euclidean or landmark map (Janson, 1998). The study group did not move in a way consistent with any of the null models tested: the capuchin group moved significantly more often toward closer platforms and in straighter lines than expected by any random search model. Results of logistic regression showed that the amount of food reward was a significant predictor of site choice, but when the ratio of reward to cost (number of tangerines divided by distance to target site) was included along with the other variables, it was not a significant predictor of target choice. Apparently the study group did not simply compare the sites one by one and choose the most profitable one available, although both distance and reward amount were important factors in target site choice.

Janson (2007) explored the observed tradeoff between distance and reward (Garber, 1989; Janson, 1998) more directly. Three feeding sites were installed, each consisting of three platform sites (to reduce potentially high levels of within-group competition, *e.g.*, Di Bitetti and Janson, 2001). The three sites were arranged in an oblique triangle, with one corner distinctly closer to the neighboring one than to the opposite one. Rewards at the corners were arranged so that regardless of the starting corner, the group had a choice between a closer, less rewarding site versus a more distant, more rewarding site. Logistic regression was used to analyze the chance that one or the other site was visited first, as a function of the sites' absolute or relative distances and rewards. No explanatory model that treated the two sites as strict alternatives could explain the choices made by the group. Instead, the results suggested that members of this group integrate information about the sites' rewards, travel distances, relative spatial geometry and perhaps potential food competition in deciding which of two alternative foraging ***routes*** they would use. Based on estimates of energetic expenditure from captive studies, Janson (1988) calculated that an average adult capuchin would expend about 123.9 kJ per km in travel costs, or 0.124 kJ per meter. Using this information he concluded that capuchin monkeys in his experimental design did not use the longer “detour” route unless the energy gained from extra food outweighed the energy cost of extra travel (Janson, 2007).

Capuchins at Iguazú also appear to be somewhat sensitive to time since the last visit to a resource. The Macuco group studied by Janson (1996, 1998) avoided revisiting a feeding site at intervals shorter than the experimentally imposed reinforcement delay of 24 hours established in the experiment.

Evidence of more subtle adjustment of the timing of intervals between visits was obtained by Tujague (2013), using Janmaat's method (2006a) to record visits by known capuchin groups to pre-selected target trees, as a function of the tree's amount of ripe and unripe fruit. Tujague recorded capuchin visits to target trees without evident visual and olfactory fruit ripeness cues every day and analyzed their patterns of return. Ripe plus unripe fruit quantity was scored 0–4 for each tree according to the percentage of branches containing fruit: 0 (no fruit), 1 (1–25%), 2 (26–50%), 3 (51–75%), 4 (76–100%) (Janmaat *et al.*, 2006a). She divided the crown into four quadrants following the cardinal points and visually estimated the average percentage of branches containing fruit. A tree of category 4 had all the branches containing fruit. When a tree did not have branches in one of the quadrants, then that quadrant was assigned as category 0. For species in which it was possible to distinguish between the phenophases of ripe and unripe fruit, the number of branches containing each phase was counted separately and averaged to produce a final percentage for each phenophase for that tree crown. During periods of 2 months for each group in different years (2008/09 for Macuco group; 2009/10 for Gundolf group; 2010/11 for Rita group), revisit intervals for 406 target trees were analyzed according to ripe and unripe fruit quantity, using a Generalized Linear Mixed Model (R Development Core Team, 2010). Results suggest that Iguazú capuchins used information about quantity and also ripening state of fruit when deciding to revisit trees. The time until the next revisit for a given tree decreased independently with increasing amounts of both ripe and of unripe fruit in the crown at the end of the visit (GLMM; effect of ripe fruit on revisit interval, controlling for effect of unripe fruit and random effects of individual tree, sample year and study group: Chi-square = 8.845, df = 1, P = 0.003; effect of unripe fruit on revisit interval, controlling for effect of ripe fruit and random effects of individual tree, sample year and study group: Chi-square = 3.50, df = 1, P = 0.06). Although results for unripe fruit do not quite reach statistical significance, it seems that capuchins may anticipate the ripening of unripe fruit in a tree crown: they shortened their revisit intervals when a tree had more unripe fruit to mature prior to a future visit, even though the monkeys did not consume the unripe fruit. Future detailed analysis will explore these results.

Despite the likely confounding and unmeasured traits of each tree (fruit nutritional composition, ripening rates, relative risk of predation, etc.), this observational study gives evidence that capuchins may anticipate the amount of ripe (and probably unripe) fruit available in a tree based on past experience.

It is interesting to note that both studies (experimental and observational) at the site have arrived at similar conclusions in relation to the monkeys' memory capacities and their ability to remember the quantity of the food reward. The most important difference between Janson's experiments and observations carried out by Tujague is the suggestion that capuchins are sensitive to differences in the amount of ripe and unripe fruit in natural food resources and are able to adjust their revisits to target trees according to this information.

Revisit intervals were shorter when quantities of food were high but they were shortest in particular when quantities of ripe fruit were high. These findings and the results from Janson (2007) suggest that the capuchins have an ability to plan their foraging routes, and it raises the possibility that monkeys may have the capacity for strategic planning, defined as the behavioral process that deals with future cause and effect consequences of an actual decision that an individual is going to make (Steiner, 1979), and may even be able to remember specific unique past events and use these memories to cope with future foraging decisions.

Discussion

With the exception of hypothesis 2a (primates have a coordinate-based spatial representation of the location of feeding sites) and 4 (primates can trace changes in resource availability in time and space), the rest of the hypotheses tested have found strong support in the literature.

There is evidence that some primate species, in particular humans, chimpanzees, and bonobos, may encode spatial information in the form of a coordinate-based map (see Menzel *et al.*, 2002; Normand and Boesch, 2009; Normand *et al.*, 2009). Normand and Boesch (2009) suggested that the difference between these few species that use a coordinate-based map and other arboreal and non-arboreal primates which encode spatial information in the form of a topological map (see Tab. 2) is memory capacity. Chimpanzees and bonobos have very large home ranges filled with a high density of trees and often located in relatively flat terrain with few obvious long-range landmarks visible from the ground; with > 10,000 trees as possible landmarks, recalling and updating a network of travel routes might not be feasible for even complex primate brains. However, the map capacities of primates have not been well-documented, and it is possible that smaller monkeys may also possess coordinate maps of their home ranges, even if they also use landmarks as nodes between preferred travel routes when available.

In addition, there are several reasons why a forager might not take the direct route between resources, leading researchers to conclude they lack coordinate-based spatial representation. The forager could be monitoring information about other resources and monitoring their availability, checking routes seeking safer ones, avoiding areas with high predation risk, or just monitoring home-range edges for the presence of conspecific groups (Garber and Porter, 2014; Porter and Garber, 2013). The three studies that supported a landmark-based mental map in Neotropical primates are studies in small-space scale, suggesting that ecological factors play an important role in the development of this kind of map.

The use of locational memory may also be sensitive to environmental effects. It has been shown that primate groups can vary from strong reliance on spatial memory to weak or no demonstrable use of spatial memory: during the period of most pronounced scarcity of resources, white-faced sakis (*Pithecia pithecia*) shifted their diet to use small unproductive and dispersed food items. At this time, they showed little evidence of the use of spatial memory, which contributed little to determining the travel routes and patch choices of the study group (Cunningham and Janson, 2013). In contrast, during the period of resource abundance, they showed strong evidence of memory of resource location and value

(Cunningham and Janson, 2007). The critical factor is not scarcity or abundance of resources *per se*, but rather the uniformity of the resource distribution.

Specialized memory systems may have evolved in concert with the hypothesized reward discounting mechanisms to support foraging on foods with distinct spatial and temporal distributions (Long and Platt, 2005). If this is the case, then some types of memory that are still thought to be exclusively human could be present in other animals, for example episodic memory. We can define episodic memory as a system of long term memory that includes retrieval of a past event which integrates multiple components and that belongs to the personal past of the individual. This definition in terms of consciousness makes it impossible to demonstrate episodic memory in animals. That is the reason that authors refer to episodic-like memory or EM when studied in non-human animals (Clayton *et al.*, 2003). This EM is classically divided into four components: 1) the "what" component of the retrieved event (for example, a type of food), 2) the "where" component (for example, the location of the food), 3) the "when" component (the moment of the day when the past event took place), and in some cases an additional criterion, 4) the "who" component (for example, who supplied the food) (Schwartz and Evans, 2001; Clayton *et al.*, 2003). The EM is a key factor for species that need to remember spatial and temporal variation of resources, to avoid revisiting depleted sites or revisit resources recently discovered (Janmaat *et al.*, 2012). EM has been proved in birds (Clayton *et al.*, 2003; González-Gómez *et al.*, 2011; Flores-Abreu *et al.*, 2012). In non-human primates there is evidence for what-where memory (Hampton *et al.*, 2005, see also Tab. 2), suggestive evidence of memory of specific prior feeding experiences (Janmaat *et al.*, 2013a, b), and weak and variable evidence for the ability to plan future actions (Bourjade *et al.*, 2012). Studies about EM and ability to plan the future in capuchins are rare (Basile *et al.*, 2009, Bourjade *et al.*, 2012), although the separate components of EM have been analyzed. Capuchins avoid consuming a non-preferred but available food to obtain a preferred food in the immediate future (Bramlett *et al.*, 2012), they are sensitive to differences in food quantity (Beran *et al.*, 2008), and learn fast about reversion contingences (Anderson *et al.*, 2008). The results we have found in Iguazú National Park suggest that capuchins use at least the "where" and "what" components of EM during their visits to fruiting trees or experimental platforms, and probably the "when" component. We hope future research at the site will highlight more aspects about capuchins' ability to use this type of memory and also to plan for future actions.

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10 | THE VOCAL REPERTOIRE OF THE BLACK-HORNED CAPUCHIN (*CEBUS* [*SAPAJUS*] *NIGRITUS*): AN ACOUSTIC AND CONTEXTUAL ANALYSIS

REPERTORIO VOCAL DEL MONO CAÍ (*CEBUS* [*SAPAJUS*] *NIGRITUS*): UN ANÁLISIS ACÚSTICO Y CONTEXTUAL

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Abstract. We describe the vocal repertoire of black-horned capuchins (*Cebus* [*Sapajus*] *nigritus*) at Iguazú National Park, Argentina, in terms of the calls' acoustic structure and the contexts in which different calls are emitted. Audio recordings of vocalizations obtained in natural contexts were supplemented with calls recorded when subjects were presented with food or predator decoys. We conducted spectrographic analysis of digitized calls to describe their acoustic structure. Multivariate analyses revealed over 30 distinguishable call types. Some of these are discrete types but other calls grade with other forms. An examination of the contexts of production provided insights into the functions of the calls. The most commonly-produced call is a "contact note" that apparently serves to regulate within-group spacing. Two varieties of loud calls ("whistle series") are used by lost individuals, as well as for communication between groups. Food-associated vocalizations are produced by animals feeding on clumped resources. Playback experiments showed that these calls attract listeners towards the food source. Black-horned capuchins give distinct call types to aerial and terrestrial predators; listeners respond to these different call types with predator-specific behaviors. Calls associated with terrestrial predators are frequently used in the absence of predators in a functionally deceptive manner. Vocalizations given in social contexts include several calls given by aggressors and in response to aggression, estrous vocalizations and copulation calls, vocalizations directed to newborn infants, calls that are used to coordinate group movement, and vocalizations associated with a reunion display among males. Infants appear to go through a babbling period; their calls develop into adult-like calls as they mature. The black-horned capuchin vocal repertoire is consistent with suggestions that a large number of call types are favored in closed habitats and in complex social milieus, and provides insights into some of the under-studied aspects of the socioecology of this Neotropical primate.

Resumen. La comunicación entre individuos es un aspecto fundamental del comportamiento social. La comunicación vocal representa una parte importante, sino la mayor, del repertorio de señales que intervienen en la comunicación en la mayoría de los primates arborícolas. Sin embargo, descripciones completas del comportamiento vocal de los primates, basadas en análisis acústicos detallados y descripciones de los contextos ecológicos y sociales en que las vocalizaciones son producidas son aún escasas, incluso para muchos de los taxa más estudiados. En este capítulo describimos el repertorio vocal del mono caí (*Cebus* [*Sapajus*] *nigritus*) en el Parque Nacional Iguazú, Argentina, en función de la estructura acústica y del contexto de emisión de los llamados. El objetivo de este capítulo es describir el rango de variación acústica del repertorio vocal del mono caí, así como el rango de condiciones ecológicas y sociales bajo las cuales las diferentes vocalizaciones son usadas. En base al contexto de emisión de las vocalizaciones y de la respuesta de otros individuos, sugerimos algunas hipótesis acerca de las funciones de algunas de las vocalizaciones. Obtuvimos registros sonoros de animales focales (identificados individualmente por sus rasgos físicos) y, en forma oportunística, de las vocalizaciones emitidas en condiciones naturales. Estos registros fueron suplementados con vocalizaciones registradas bajo condiciones experimentales en las que los sujetos fueron expuestos a fuentes de comida o a maquetas de depredadores. Hicimos un análisis espectrográfico de archivos digitales de las vocalizaciones para describir su estructura acústica. Para ello, 411 vocalizaciones de 7 individuos fueron clasificados a priori en 20 categorías distintas en función de cómo sonaban a los autores o en base al contexto de emisión. Análisis multivariados (análisis de componentes principales y funciones discriminantes) permitieron discriminar acústicamente a la mayoría de estas vocalizaciones en base a 10 variables acústicas relacionadas a la duración y la frecuencia de los sonidos. Un análisis más detallado de un subconjunto de vocalizaciones, las llamadas de series de silbidos (*whistle series*), permitió encontrar diferencias acústicas entre ellas en función de cuatro contextos de emisión: 1) individuos alimentándose de fruta (1104 llamados de 24 individuos); 2) individuos forrajeando (135 llamados de 16 individuos); 3) individuos perdidos (64 voces de 8 individuos); y 4) infantes produciendo series de silbidos cuando se encontraban separados de sus madres (85 llamados de 3 infantes). En este análisis usamos 21 medidas distintas de duración, frecuencia y conteo (número de sílabas) de cada una de las 1388 series de silbos analizados. Análisis multivariados permitieron discriminar la mayor parte de las series de silbidos en función del contexto de emisión de las mismas. Estos análisis, en combinación con experimentos usando fuentes artificiales de comida y maquetas de depredadores, así como vocalizaciones registradas en forma ocasional en contextos particulares, revelaron la presencia de más de 30 tipos de vocalizaciones distintas en el repertorio del caí. Algunos de estos llamados constituyen tipos discretos, sin formas intermedias con otras voces del repertorio; otras vocalizaciones difieren en forma gradual. Examinar el contexto de emisión de las distintas vocalizaciones ayuda a entender la posible función de las mismas. El llamado más frecuentemente emitido es una «nota de contacto» breve y de baja amplitud que aparentemente sirve para regular las distancias entre los individuos. Dos variedades de llamados de gran amplitud que suenan como una áspera serie de silbos son usados cuando algunos animales pierden contacto con su grupo y para comunicarse con otro grupo, en cuyo caso el encuentro intergrupal termina en corridas y agresiones. Los llamados asociados a comida (tanto *grgrs* como series de silbos) son producidos por individuos que se alimentas en fuentes de alimento agregadas, especialmente frutos. Experimentos de *playback* de estas vocalizaciones atraen a los oyentes hacia el emisor. Grabaciones de sonidos en condiciones naturales, así como las obtenidas durante experimentos con maquetas de depredadores, permitieron determinar que los caí emiten distintos llamados de alarma para los depredadores aéreos y terrestres. Los oyentes responden a estos llamados con comportamientos específicos para cada tipo de depredador: mirar hacia abajo o correr hacia arriba cuando se emiten alarmas a depredadores terrestres (carnívoros), y mirar hacia arriba u otear el horizonte o buscar refugio en un lugar con cobertura vegetal cuando se emiten señales a depredadores aéreos (águilas). Sin embargo, para ambas vocalizaciones la respuesta más frecuente fue mirar al emisor de la misma. Además, los llamados asociados a depredadores terrestres fueron frecuentemente usados de una forma funcionalmente engañosa en ausencia de depredadores. Las vocalizaciones producidas en contextos sociales incluyen llamados emitidos por agresores, chillidos (*squeals*) y gritos (*screams*) producidos en respuesta a agresiones, vocalizaciones de celo y de cópula, voces dirigidas a infantes recién nacidos, llamados usados para coordinar los desplazamientos del grupo, y llamados asociados a *displays* de encuentro entre machos. Los infantes parecen pasar por un período de «balbuceo», y sus voces se asemejan paulatinamente a las voces adultas a medida que maduran. El repertorio vocal de los caí es más diverso que el de otras especies de primates neotropicales, aunque comparable al de otras especies del género *Cebus*. La gran diversidad de voces registradas en el caí y en otras especies de *Cebus* es consistente con la hipótesis de que los ambientes donde la visibilidad es limitada (bosques) favorecen un mayor número de vocalizaciones, aunque da mayor sustento aún a la hipótesis de que la diversidad de vocalizaciones de los primeras está correlacionada positivamente con el tamaño del grupo y la complejidad de las interacciones sociales. El análisis del repertorio vocal de una especie, tanto en función de la estructura acústica como del contexto de emisión de los sonidos, constituye el punto de partida para comprender varios aspectos de la comunicación vocal, incluyendo cómo los emisores usan estas vocalizaciones para alterar el comportamiento de los receptores; por qué los receptores responden a estas señales; los mecanismos próximos de emisión de estas voces; la ontogenia de la producción, el uso y la percepción de estas voces; y el posible uso de vocalizaciones en combinaciones novedosas. Futuros estudios del repertorio vocal del mono caí en contextos naturales y experimentales brindarán respuestas más detalladas a estos aspectos de la comunicación vocal de los primates.

Introduction

Communication is a crucial aspect of social behavior, with vocal communication accounting for much or most of the communicative repertoire for most arboreal, forest-living primates (Snowdon, 1997). However, complete descriptions of vocal behavior based on detailed acoustic analysis and the broader contexts in which the calls are produced are lacking for most primate species, including many of the best-studied taxa. Such descriptions of a given species' vocal repertoire are important because they serve as an ethogram of vocal behavior and provide a starting point for more detailed analysis of the species' communicative behavior, as well as understanding the evolution of communication in a comparative, cross-taxon perspective. For example, documentation of the repertoire is a necessary first step towards understanding the extent to which variation in acoustic characteristics across call types might be related to variation in underlying motivational states (*e.g.*, Hammerschmidt and Fischer, 1998), or whether call types given alone in some contexts might be combined in novel ways in other contexts (*e.g.*, Robinson, 1984; Cäsar *et al.*, 2013). Likewise, analysis of the form and contexts of vocalizations provides a starting point for the generation of hypotheses regarding their function (*e.g.*, Owren and Rendall, 2001). Across species, comparisons of vocal repertoires of closely related taxa can provide insight into the degree to which vocal production is phylogenetically conserved (*e.g.*, Cleveland and Snowdon, 1982; Fuller, 2014), whereas details regarding the size and nature (*e.g.*, graded *vs.* discrete) of the repertoire are important for understanding how these characteristics are evolutionary shaped by ecological and social factors across taxa (Marler, 1975; McComb and Semple, 2005; Krams *et al.*, 2012).

In this chapter, we describe the vocal repertoire of wild black-horned capuchins (*Sapajus nigritus*, taxonomically synonymous with *Cebus nigritus* or *Cebus apella nigritus*) in terms of the acoustic structure of the calls and the context of their occurrence. This work is not aimed at providing a definitive classification of the black-horned capuchin's vocal repertoire and should be considered a preliminary descriptive work. The objective of this chapter is to provide a picture of the range of acoustic variation and the range of conditions under which different vocalizations are used. By describing the acoustic structure of the vocalizations and their relationship to other calls in the repertoire and to other calls in related species of New World monkeys, we can obtain some insights on the evolution of certain call types. By describing the context of occurrence of the different vocalizations and the response of other animals to the calls, we will suggest some tentative hypotheses for the function of some of these calls.

Methods

Study site and study subjects

Data presented here were collected in natural and experimental contexts in Iguazú National Park, Argentina by MDB between August 1997 and December 1998, supplemented with further data on predator-associated calls in experimental contexts collected by BCW periodically from 2003 to 2012. The black-horned capuchin is one of the eight species currently recognized within the genus *Sapajus*, commonly referred to as tufted or horned capuchins (Rylands and Mittermeier, 2013). Tufted capuchins are medium-sized (2.5–5 kg) omnivorous primates that inhabit a variety of forest habitats in tropical and subtropical South America. The study subjects were one wild group (Macuco group) of 24–27 individuals during the first study period, which subsequently fissioned into multiple groups during the second study period (see Janson *et al.*, 2012). All subjects were easily identifiable. See Janson *et al.* (2012) for further details on the study site and subjects.

Recording of vocalizations and context of occurrence

During the first study period, MDB followed focal individuals and recorded their calls on CrO_2 tapes using a *Sennheiser* directional shotgun microphone (MKH815T) connected to a *Marantz* PMD–222 cassette recorder. Following a continuous focal animal sampling protocol (Altmann, 1974) we dictated on the microphone the gross and detailed activities of the focal animals (see descriptions below) and indicated each time a focal animal vocalized. Focal animals were chosen opportunistically but we tried as much as possible to get an even representation of all individuals in the group, in different behavioral contexts and spread among the different seasons. Focal animals were usually 2–12 m from the microphone. On a few occasions, recordings of some rare vocalizations (*e.g.*, calls given during intergroup encounters and mating, and by animals lost or trying to coordinate group movements) were obtained *ad libitum* from non-focal animals and/or from distances > 12 m. These non-systematic samples were not treated as focal animal samples and are thus not used to estimate calling rates.

Gross behavioral categories were used (following Janson, 1988) to describe the group and the focal animal's general activity, and fine behavioral categories to describe in more detail the activity of the focal animal. The gross behavioral categories were defined as: feeding (when the focal animal was eating fruit or other clumped resources like concentrated insect larvae), foraging (when it was looking for, pursuing or consuming dispersed food items like arthropods and bamboo shoots), resting, traveling, and other (*e.g.*, intergroup encounters, copulations). Finer activity categories were also indicated: manipulate substrate, grab insect, inspect substrate, ingest (substrate and prey type identified when possible), drink, scan, sit, lay, stand, move, play, groom, aggression, sex. Particular body postures or displays that accompanied vocalizations were also indicated. To describe the social context of the focal animal, we dictated onto the tape at the start of a recording session and at regular intervals (at least one minute apart): the identity and proximity of the nearest neighbor, the gross activity of nearby individuals, the number of individuals within 10 m of the focal animal, and the focal animal's spatial position within the group (following Janson, 1990; Di Bitetti and Janson, 2001).

In addition to observations of vocal behavior in natural contexts, we also performed experiments using predator decoys (snakes, felids, and raptors; see Wheeler, 2010b) and novel food sources placed on feeding platforms (see Di Bitetti, 2003, 2005; Wheeler, 2009), with predator decoys being used primarily in the second study period. Call exemplars obtained at feeding platforms by MDB were used in the acoustic analyses, whereas calls given to the different predator models were categorized by ear. MDB obtained 86.5 hours of tape recordings, of which 37% were focal animal samples of animals doing their normal activities, 29% were focal animal samples taken at the feeding platforms and the rest were divided between recordings of the discovery experiments and *ad libitum* samples. The sample was biased in terms of context of occurrence of the vocalizations due to the difficulty of obtaining vocalizations from focal animals under certain circumstances (*e.g.*, when feeding at fruit trees, see Di Bitetti, 2001a). For this reason we are not providing a full description of calling rates for each vocal type across all circumstances. However, we estimated calling rates based on focal animal samples for particular vocalizations under some circumstances.

To describe the vocalizations produced by the capuchins and to compare the different calls and determine if they differ in their acoustic parameters, we used different univariate and multivariate statistical techniques. Because some of the vocalizations produced by the capuchins are very simple (*e.g.*, contact notes) and because we wanted to measure a series of acoustic variables that allowed us to compare all the calls at once, we used a small number of acoustic variables (Tab. 1). We did not use any measure of amplitude nor any real measure of the frequency modulation of the calls except for very crude approximations (variables six and eight in Tab. 1). For this reason, this analysis is very conservative, as potential differences among different call types are underestimated. Future tests may further discriminate some call subtypes or find significant differences between calls that we were not able to discriminate in this study.

Table 1. Acoustic variables used in the multivariate analyses of tufted capuchin vocalizations.

Variable	Variable description	Units
1	Number of discontinuous elements in the call (interrupted by a silence)	None
2	Initial peak frequency of the call	Hertz
3	Peak frequency at end of the call	Hertz
4	Maximum peak frequency of the call	Hertz
5	Minimum peak frequency of the call	Hertz
6	Range of spectral energy of the call or band width (variable 4 – variable 5)	Hertz
7	Peak frequency on the mean spectrum of the call[1]	Hertz
8	Change in frequency from start to end of call (variable 2 – variable 3)	Hertz
9	Call duration	Seconds
10	Duration of the longest element of the call	Seconds

[1] The frequency spectrum depicting the distribution of energy from the start to the end of the call was produced with the program ***Avisoft*** and, from this spectrum, the frequency where the peak energy was located was automatically entered in an ***Excel*** file with the dynamic data exchange feature of ***Avisoft.***

Using *Avisoft-SASLab Pro*, we measured the 10 acoustic variables listed in Tab. 1 on 411 calls from 7 individuals, including three adult/subadult males, two adult females, a juvenile female, and two infants. Because this is a rather small number of acoustic variables, potential differences among different call types may be underestimated. The present study should thus not be considered a definitive description of the full repertoire, and we encourage more detailed analyses that may identify further call types or subtypes or, alternatively, demonstrate graded continuity between call types.

These 411 vocalizations were classified a priori into 20 different call types according to how they sounded to one of the authors (MDB) or according to their context of occurrence. Calls given in a similar context but that sounded different were considered different calls. Some calls sounded similar but were used in very different contexts; in this case, we classified them *a priori* as different call types to test if there could be subtle acoustic differences based on context (*e.g.*, Owren *et al.*, 1997). In addition to these 20 call types, we describe a few vocalizations that were not included in the analysis, either for lack of recordings from the individuals used in this analysis (aerial predator alarm calls, estrous vocalizations) or because the acoustic variables chosen could not be accurately measured on calls showing large variability and noisiness (screams and squeals). Fortunately, these vocalizations are extreme discrete types and are easily discernible by ear, although it is possible that unidentified subtypes exist.

Acoustic measurements were taken on spectrograms of calls digitized with sample frequencies of 11.025 kHz or 16 kHz. We made fast Fourier transforms of the digitized sounds using the following spectrogram parameters: FFT-length = 256, Frame (%) = 100, Hamming window, and 50% overlap. The frequency resolution for the calls digitized at 16.000 Hz was 81 Hz and for those digitized at 11.025 Hz was 55 Hz. The time resolution was 8.00 ms for the vocalizations sampled at 16.000 Hz and 11.61 ms for those sampled at 11.025 Hz. Although there was a difference in the frequency and time resolution of vocalizations digitized with a different sampling rate, there was no systematic bias on the measurements obtained on the calls obtained with these different sampling rates (M. Di Bitetti, unpublished results). Vocalizations were filtered before obtaining the acoustic measures if background noise, insects or bird sounds of high amplitude were present on band frequencies not comprised by the capuchin's call. We used the finite impulse response (FIR) filter as well as high-pass, low-pass or band-pass filters (depending on the location of the noise), after inspecting the frequency range of the call, to avoid filtering frequency bands that corresponded to the focal animal vocalization. Time and frequency measurements of the calls were automatically transferred to an *Excel* (version 7.0, *Microsoft*) spreadsheet (using the dynamic data exchange feature of *Avisoft*) by clicking on the spectrogram with a bounded reticulate cursor that follows the peak amplitude of the call.

To see if there were acoustic differences among the different calls, we used two complementary multivariate techniques. First, because the different acoustic variables may be correlated to some extent, we used a principal component analysis (PCA) to reduce the acoustic variation to a smaller number of variables (first three principal components). We used ANOVA and *a posteriori* Tukey HSD tests to compare all the calls and test whether or not they differed in their values on the first three principal components. Second, we used a discriminant function analysis (DFA) to see how well the different vocalizations were correctly assigned to their call type based on measurements of their acoustic variables. In this analysis we used the same acoustic variables indicated above. To do the DFA, we split the original sample evenly into two sub-samples of equal size. One sub-sample was used as a training sample to construct the discriminant functions. The other half (test sample) was used to see how well the calls were assigned to their original *a priori* type.

Whistle series are given in at least five different contexts, and we were particularly interested in knowing if the whistle series (WS) produced by capuchins while feeding belong to a different acoustic type (food-associated WS, see results). We thus made a similar multivariate analysis on all the WS of good recording quality (N = 1388 calls) obtained from all individuals in the group in four different contexts: 1) feeding on fruits (N = 1104 calls from 24 individuals), 2) foraging (N = 135 calls from 16 individuals), 3) when an animal was lost (N = 64 calls from 8 individuals), and 4) infants producing WS in an apparent attempt to be retrieved by their mother or allomother (N = 85 calls from 3 infants). The aim of this analysis was to determine if different WS variants were associated with the different contexts of call emission, something that was not clearly revealed by the analysis described above (see Results). Given that WS are highly tonal and structurally similar across the different contexts of production, we were able to use a larger number of acoustic variables in this analysis. For the acoustic analysis we used only those calls that had a good signal to noise ratio and had no overlap with calls from other individuals. For the acoustic analyses we digitized the vocalizations with a sample frequency of 11.025 kHz (17 individuals) or 16.000 kHz (8 individuals). To obtain spectrograms of the calls we made fast Fourier transforms of the digitized sounds using the following spectrogram parameters: FFT-length = 256, Frame (%) = 100, Hamming window, and 50% overlap. As described above, we digitized the spectrograms with different frequency and time resolutions because the sounds of different individuals were digitized using two different sampling rates. A PCA and a cluster analysis using several WS given by the same individual during one feeding bout showed that the different sampling rates did not introduce any systematic bias in the sample.

Twenty-one measurements of time, frequency, and counts were obtained from each of the 1388 whistle series used in this analysis (Tab. 2). All time and frequency measurements were obtained on the fundamental of the vocalization. The measurements were transferred to an *Excel* spreadsheet as described above. For data on the peak amplitude of the mean frequency of the call or a syllable, we used the standard marker cursor to select the portion of the spectrogram for which we wanted to estimate the mean spectrum. The free reticulate cursor was used to click on the frequency value with the peak amplitude. From the 21 measurements obtained with the procedures explained above, we obtained 16 variables that were used in the multivariate analyses (see details in Di Bitetti, 2001a).

To see if there are acoustic differences in the WS given in different contexts or by different individuals, we also used PCA and DFA as described above. To test if whistle series given in different contexts differ in their values on the principal component axes, we used the mean values per individual in each context to avoid the problem of having different sample sizes for different individuals, which may bias the results. These mean values per individual were used to test for differences among WS given in different contexts using ANOVA. We used a DFA to see how well WS given in different contexts or by different individuals were correctly assigned to their contexts of emission or to the individual that called, based on the measurements of their acoustic variables. In this analysis we used the same 16 variables indicated above (see Di Bitetti, 2001a for details). We also split the sample evenly into two subsamples, one of which was used to construct the discriminant functions, and the other was used as the test sample. A goodness-of-fit test was then used to test if the observed classification of calls according to context of occurrence or individuals agreed with that expected under the null hypothesis of random assignment of calls to the group centers.

Before conducting parametric tests (*e.g.*, ANOVA) we checked if assumptions were met and, when necessary, we transformed data to meet those assumptions. We conducted statistical tests with the program *JMP*® (version 3.2.2, SAS Institute, 1997).

Table 2. Acoustic variables measured on 1388 whistle series.

Variable	Variable taken on	Description of variable
1	Whole call	# syllables
2		# *grgrs*
3		Time & frequency at start of call
4		Time and frequency at end of call
5		Max freq = highest frequency of the call
6		Min freq 1 = lowest frequency on whistles (grgrs or initial contact note not included)
7		Min freq 2 = lowest frequency on call (grgrs or whistles but not initial contact note)
8		Dominant (peak) frequency on mean call spectrum
9	First syllable or initial contact note	Dominant (peak) frequency on mean spectrum of first syllable or initial contact note (if present)
10	Last syllable of the call	Time and frequency at start
11		Max freq = highest frequency of syllable
12		Min freq = lowest frequency of syllable
13		Dominant (peak) frequency on mean spectrum of syllable
14	Penultimate syllable	Time and frequency at start
15		Time and frequency at end
16		Max freq = highest frequency of syllable
17		Min freq = lowest frequency of syllable
18	Antepenultimate syllable	Time and frequency at end
19	Syllable with the highest range of spectral energy	Max freq = highest frequency of syllable
20		Min freq = lowest frequency of syllable
21		Peak frequency on mean spectrum of syllable

All measurements were taken on the fundamental of the call or syllable.

Results and discussion

Call classification

The first three principal component axes obtained with the ten acoustic variables had eigenvalues larger than one, and extracted 85.2% of the variation (42.8, 24.9 and 17.5% respectively). Frequency values showed high positive loading on axis one, call duration and number of elements had high loading on axes two, whereas bandwidth had a high loading on axis three. Most of the 20 call types considered in this analysis showed statistical differences from all other call types in at least one of these three axes (Tab. 3). Likewise, the DFA classified 72.2% of the 205 call recordings in the test sample to their "correct" *a priori* call category (Tab. 3), far exceeding the expected value of 5%. Most recordings that were

Table 3. Results of the discriminant function analysis (DFA) and statistical comparison of the values on principal component (PC) axes one, two, and three for the 20 vocalization types included in the analyses. Numbers in the top row correspond to the vocalization types in the first column. Numbers in the cells indicate the number of calls classified *a priori* as a particular call type that were placed in a given category by the DFA. Cells filled in dark grey show the number of calls classified correctly by the DFA (*i.e.*, match the *a priori* classification). Results of statistical comparison (Tukey HSD tests) of PC axes one, two, and three are shown above the diagonal; vocalizations that differed significantly ($p < 0.05$) on at least one of the three PC axes are unshaded, while those that did not differ on any of the three axes are shaded in light grey. Call abbreviations as in text.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**Total**
1. Sirena	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**5**
2. Babbling	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**6**
3. ARS	0	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**8**
4. Trill	0	0	1	10	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	**12**
5. IWS	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**5**
6. Hip	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	**4**
7. Hiccup	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	**3**
8. Pip	0	0	0	0	0	2	0	4	1	0	0	0	1	0	0	0	0	0	0	0	**8**
9. P-Hiccup	0	0	0	0	0	0	2	0	11	0	0	0	0	0	0	0	0	0	0	0	**13**
10. LDWS	0	0	0	0	1	0	0	0	0	7	2	1	0	0	0	0	0	0	0	0	**11**
11. MWS	0	0	0	0	0	0	0	0	0	0	9	7	0	0	0	0	0	0	0	0	**16**
12. FAWS	0	0	0	0	0	0	0	0	0	0	3	16	0	1	1	3	0	0	0	0	**24**
13. Cough	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0	0	0	**5**
14. Patinado	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	**2**
15. Chihui	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6	5	0	0	0	0	**12**
16. Grgr	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	14	0	0	1	0	**18**
17. Chuck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	**4**
18. ACN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	12	0	0	**14**
19. Grugru	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	**4**
20. CN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	30	**31**
Total	**3**	**7**	**2**	**20**	**6**	**4**	**2**	**6**	**15**	**7**	**14**	**26**	**6**	**2**	**9**	**23**	**5**	**13**	**5**	**30**	**205**

misclassified fell into a call category for which Tukey HSD comparisons made on the principal component scores showed non-significant differences (Tab. 3). Tab. 4 summarizes the information on the mean values for some of the variables used in the analyses for each of the vocalization types.

Description of the vocal repertoire and vocal usage

Contact note. The *contact note* (CN) constitutes the most common vocalization emitted by the capuchins, accounting for more than 90% of the 7.425 calls recorded from the focal animals in natural conditions. It consists of a brief tonal note of relatively low frequency. The short pulse of energy has a humped frequency modulation (inverted U or chevron shape) and usually presents several harmonics, which sometimes contain more energy than the fundamental (Fig. 1.1). None of 30 CNs used in the test sample of the DFA were misclassified as another call type (Tab. 3).

CNs were produced in a range of contexts including foraging, feeding, traveling, and resting, but not during grooming or play, when animals became silent. The mean ± SD CN calling rate when foraging was 3.07 ± 0.22 calls/min ($N = 315$ focal animal samples). Individuals gave CNs more often later in the day than early in the morning, when inter-individual distances were shorter, and when engaged in foraging, while the probability of calling was lower when resting (see Di Bitetti, 2001a). These patterns suggest that the vocalization serves to inform group mates regarding the location of the caller (*e.g.*, to regulate inter-individual distances while foraging, as has been shown for a set of calls described in the repertoire of the weeper capuchin, *Cebus olivaceus*; Robinson, 1982). Additional research would be needed to confirm this.

Table 4. Mean values (± SD) for different acoustic variables of different vocalizations. Call abbreviations as in text.

Call	Call length (sec)	Number of elements	Peak freq. on mean spectrum (kHz)	Maximum frequency (kHz)	Frequency range (kHz)	N
CN	0.03 ± 0.01	1.00 ± 0.00	0.76 ± 0.14	0.77 ± 0.15	0.07 ± 0.05	60
Chihui	0.33 ± 0.11	2.47 ± 0.61	1.12 ± 0.29	1.43 ± 0.22	0.65 ± 0.22	19
Grgr	0.41 ± 0.27	4.41 ± 1.29	1.08 ± 0.29	1.28 ± 0.27	0.57 ± 0.20	46
Patinado	1.40 ± 0.40	7.00 ± 1.87	1.23 ± 0.35	1.46 ± 0.26	0.87 ± 0.17	5
Trill	0.93 ± 0.75	8.80 ± 4.25	3.72 ± 1.17	4.04 ± 0.97	1.72 ± 0.69	40
IWS	1.77 ± 0.37	6.67 ± 1.50	2.24 ± 0.14	3.20 ± 0.62	1.06 ± 0.56	12
LDWS	4.02 ± 0.68	13.15 ± 1.57	1.53 ± 0.38	1.79 ± 0.40	0.46 ± 0.26	13
MWS	2.53 ± 0.82	9.46 ± 3.19	1.69 ± 0.22	1.83 ± 0.18	0.90 ± 0.25	28
FAWS	1.59 ± 0.69	7.08 ± 2.33	1.73 ± 0.34	1.90 ± 0.36	1.06 ± 0.33	52
P-Hiccup	0.12 ± 0.04	2.24 ± 0.64	3.84 ± 0.85	3.96 ± 0.92	3.32 ± 1.22	29
Hip	0.04 ± 0.03	1.25 ± 0.46	3.61 ± 0.56	4.03 ± 0.62	0.44 ± 0.47	8
Hiccup	0.11 ± 0.04	2.00 ± 0.00	4.25 ± 0.25	4.39 ± 0.27	3.68 ± 0.43	5
Chuck	0.05 ± 0.02	1.00 ± 0.00	1.20 ± 0.36	1.62 ± 0.53	1.15 ± 0.28	10
ACN	0.63 ± 0.58	4.20 ± 3.2	0.85 ± 0.11	0.94 ± 0.15	0.21 ± 0.14	25
Cough	0.06 ± 0.02	1.00 ± 0.00	1.45 ± 0.61	2.56 ± 1.05	1.75 ± 1.38	12
Pip	0.03 ± 0.01	1.00 ± 0.00	3.45 ± 1.00	3.49 ± 0.97	0.16 ± 0.17	11
ARS	0.99 ± 0.38	14.00 ± 5.39	4.61 ± 1.34	4.81 ± 1.14	2.11 ± 1.21	5
Grugru	0.44 ± 0.13	6.80 ± 1.93	0.78 ± 1.00	1.10 ± 0.95	0.78 ± 0.92	10
Sirena	0.90 ± 0.23	1.67 ± 0.52	4.29 ± 0.46	5.17 ± 0.29	2.43 ± 1.30	6
Wahwahwah	3.94	24	0.17	1.34	1.16	1
Babbling	0.53 ± 0.29	2.21 ± 1.37	4.24 ± 0.91	5.04 ± 0.64	1.65 ± 0.65	14

Chihui. The chihui is a relatively low-amplitude vocalization produced by foraging or feeding animals, consisting of a CN followed immediately by one to three higher frequency notes (Fig. 1.2) and being somewhat intermediate between a CN and a *grgr* (see below). There were no significant differences between grgrs and chihuis on their values for the three principal component axes, and the DFA misclassified nearly half of the chihuis as grgrs (Tab. 3). However, one possible difference between the two calls is that grgrs seem to be of higher amplitude. In addition, univariate analyses revealed that chihuis have fewer elements ($F_{1,63} = 57.474$, $P < 0.001$) and a longer duration in the longest element of the call ($F_{1,63} = 8.297$, $P = 0.0054$). Thus, although inclusion of a larger number of variables would likely demonstrate differentiation between these call types, they appear to fall along a graded continuum.

Foraging animals produce chihuis when foraging success is apparently high. Focal animals produced chihuis at higher rates when feeding on fruit (0.67 ± 1.11 calls/min, N = 137) than when foraging (0.12 ± 0.50 chihuis per min, N = 373 focal animal samples; Wilcoxon signed ranks test using the normal approximation: $Z = 9.555$, $P < 0.0001$). The probability of producing chihuis was higher when feeding at fruit trees than during other activities (foraging, foraging/feeding, resting or traveling, $G = 85.911$, $df = 4$, $P < 0.001$). Thus, although more specific to the context of feeding than the CN, chihuis are less specific than other food-associated calls (see below), which are produced almost exclusively when feeding at fruit trees. Thus, chihuis are best considered foraging-associated calls.

Grgr. The grgr is produced by animals feeding on fruit or other concentrated resources (Di Bitetti, 2003). It consists of a rapid series of short notes that individually resemble CNs (Fig. 1.3), although the rapid series does not sound like a series of CNs but as a broken sound with its own concave frequency contour. Typically, the frequency of the grgr increases during the first elements but rapidly descends in the final elements such that the typical general contour of the grgr is of an inverted "U" shape tilted to the right. The first element of a grgr is usually structurally similar to (and sounds like) a CN. The last element may have a longer duration and some elements may become noisy, but there is variation within and among individuals. Grgrs are usually given singly or in series of two or three, although longer series occur. They may also constitute the first syllable and sometimes the last one or two syllables of food-associated whistle series (see below).

Patinado. The patinado is an infrequent call produced by individuals feeding on fruit or other concentrated resources, and may be related to high motivation (*e.g.*, high quality foods or hunger level). Patinados are characterized by relatively long syllables showing rapid oscillatory frequency modulations (Fig. 1.4). They may start or end with a grgr and usually contain between 1–3 long elements. However, few recordings of this call were obtained and it may constitute an intermediate form between the grgr and the food-associated whistle series, as the DFA sometimes misclassified calls between these three categories (Tab. 3).

Trills. The trill (Fig. 1.5) consists of a series of brief tones, with the longest element having a mean duration of 0.044 ± 0.054 s. Notes tend to increase in frequency, with the first

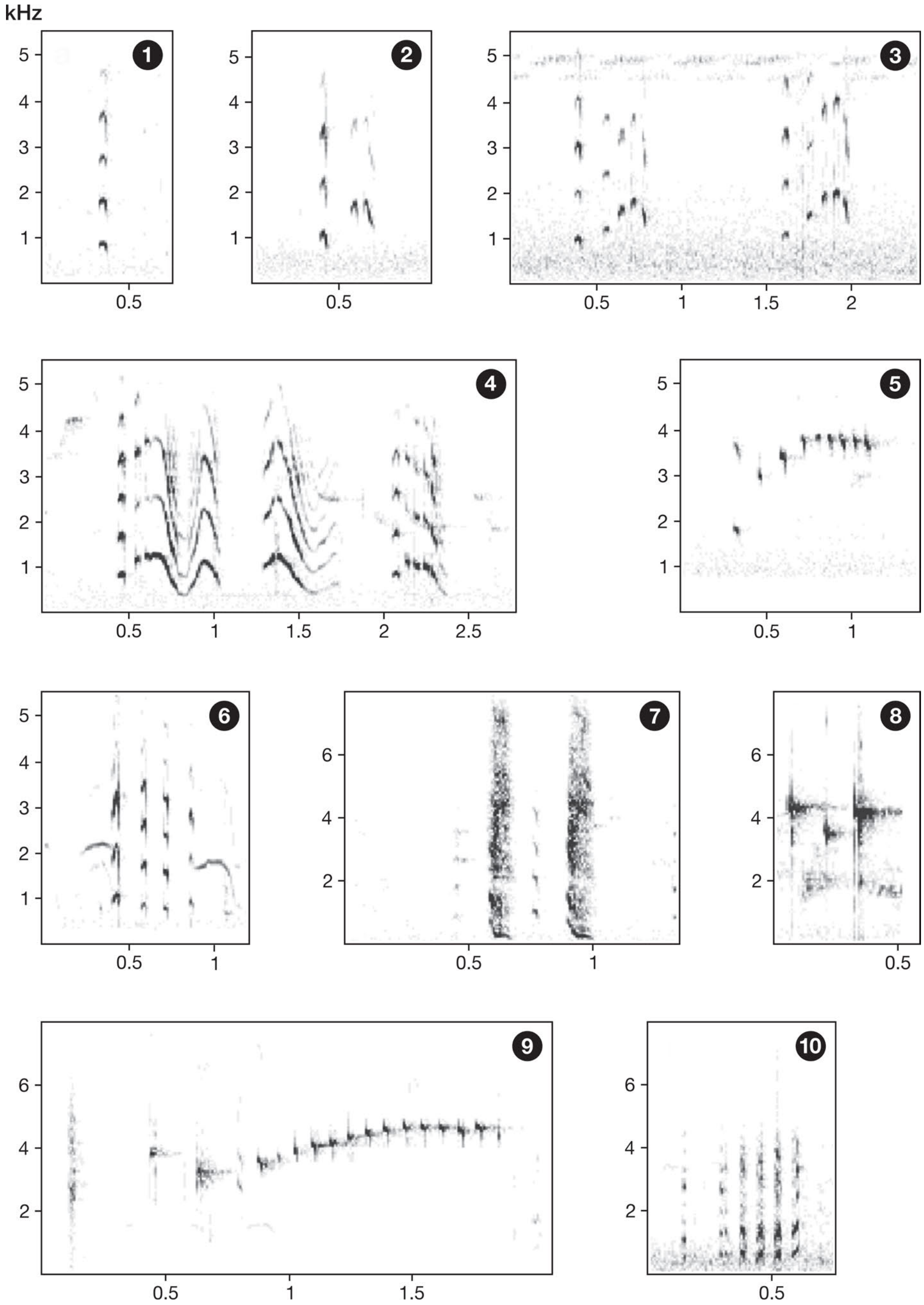

Figure 1. Spectrograms of **1.** contact note (JF); **2.** chihui (JF); **3.** grgr (SaF); **4.** patinado (SaM); **5.** trill (SaM); **6.** aggressive contact note (AM); **7.** cough (AM); **8.** pip (AM); **9.** ke! (first note only) followed by an ascending rapid staccato (AM); **10.** Aggressive grgr (AF). Abbreviations following the call name indicate the age-sex class of the caller (I, infant; J, juvenile; Sa, subadult; A,adult; F, female; M, male; unk, unknown). Calls were digitized with sample frequencies of 11.025 kHz or 16 kHz. Spectrograms were made using a FFT with the following spectrogram parameters: FFT-length = 256, Frame (%) = 100, Hamming window, and 50% overlap. For spectrograms in parts **1** through **6**: frequency resolution = 55 Hz, time resolution = 11.61 ms; for parts **7** through **10**: frequency resolution = 81 Hz, time resolution = 8.0 ms.

note of the trill usually being the lowest and sometimes having a structure similar to that of a contact note with some harmonics but with a fundamental of a higher frequency. The mean frequency at the start of the trill (of the first syllable) is 2.42 ± 1.28 kHz and the mean frequency at the end (of the last syllable) is 3.92 ± 0.99 kHz (N = 40). The trill constitutes less than 1% of all the vocalizations given by juveniles and adults, whereas infants produce trills at high rates (of the 358 trills recorded from 15 different focal individuals, 298 were produced by infants). Adult immigrant males were never observed producing trills.

Capuchins produce trills in at least five different contexts. First, infants produce trills when moving independently. They do so at very high rates, and usually in association with infant whistle series (IWS; see below), when they apparently wanted to be carried or nursed (*i.e.*, the calls cease after being retrieved by their mothers or allomothers or after being nursed). If an infant lags behind the group or is unable to cross a gap, trills are given in association with IWS and squeals, or a call intermediate between a trill and a squeal (a *trill-squeal*). Second, individuals produce trills during mother-offspring interactions (*e.g.*, mothers use the calls to invite their infants to nurse, juveniles to solicit grooming from their mothers). Third, young juveniles give trills when following adult females (not necessarily their mothers) carrying a young infant, in apparent attempts to inspect or handle the infant (see Tiddi *et al.*, 2010). Fourth, juveniles sometimes produce trills in association with a submissive grimace and "open legs show genitalia" display after mild aggression by adults. Fifth, adult females, subadult natal males and old juveniles (> 3 years) seem to use trills to coordinate group movements. This latter function is suggested by two lines of evidence. On the one hand, we never recorded trills from animals feeding on fruit or other concentrated food sources when the group was stationary. Trills were usually given by adult or subadult animals in the front of a foraging or traveling group or on the periphery of a stationary group. Adults and subadults in the vanguard of the group have a higher probability of producing trills than when in other spatial positions (G = 4.334, df = 1, P = 0.0374; expected values were estimated from the amount of time spent in the different positions by the individuals). By contrast, monotonous whistle series are used by animals to coordinate the spatial movements of the group or to change the direction of the group's movement (see section on whistle series). When adults, subadults or old juveniles produce trills during foraging, traveling or resting bouts (feeding bouts were not included because whistle series given while feeding are of the food-associated whistle series type, see below), there is a very high probability that they will also produce monotonous whistle series. The association between these two calls in the focal animal samples was highly significant (*G*-test of independence, G = 24.714, df = 1, P < 0.0001).

Given that trills are produced in different contexts, it is possible that there are different variants of this call type. Are the trills produced by infants to attract their mothers or allomothers different from those used by adults or old juveniles? To answer this question we used the results of the PCA to compare the values for the trills of adults *vs.* infants on the principal component axes, to test if they differ in their acoustic structure. We found individual differences in the values for trills on principal component axes one ($F_{5,34}$ = 19.2420, P < 0.0001), two ($F_{5,34}$ = 15.1944, P < 0.0001), and three ($F_{5,34}$ = 12.5678, P < 0.0001). All-pairs comparisons using the Tukey's HSD test showed that there were no differences between the trills of the two infants on any of the three axes. Similarly, most adult-adult or adult-subadult pairs did not show differences on these three axes. In contrast, most infant-adult or infant-subadult pairs were statistically different on the three axes. Infants had higher values on PC axis one and lower values on PC axes two and three than adult or subadult individuals (ANOVA, comparison of infant *vs.* adult trills; axis one: $F_{1,38} = 48.9195$, $P < 0.0001$; axis two: $F_{1,38} = 64.6375$, $P < 0.0001$; axis three: $F_{1,38} = 56.1005$, $P < 0.0001$). These results suggest that the infant trills can likely be distinguished by listeners from those emitted by older individuals. However, we have some evidence that the trills of infants become more similar to those of older individuals as the infants mature. Based on a random subsample of 163 trills recorded from one infant male on six different occasions between the ages of two and five months, we found that the values of the trills on the first PC axis become smaller and those on axes two and three became higher as the infant matured, converging on the values of the adult trills (Di Bitetti, 2001a). This result suggests that infant and adult trills do not constitute two different and discrete vocal types, but that the infant trill converges on the adult trill as individuals mature.

Whistle series. Whistle series (WS) consist of a series of pure tone syllables that individually sound like short whistles, and are produced in at least five different contexts. First, infant WS (IWS) are given by infants apparently when they want to nurse or be carried, and usually start with a descending note or descending trill (Fig. 2.1). IWS are usually given in conjunction with trills, and stop when the mother or allomother retrieves the infant, suggesting that the call functions to attract caregivers. Infants also produce IWS and trills at high rates when animals gather at dusk at their sleeping sites. Second, food-associated WS (FAWS) are produced, usually together with grgrs, when animals find or consume food that comes in high concentrations, usually fruit, aggregations of insects, or bird nests with eggs or chicks (Fig. 2.2). FAWS typically start with a grgr or chihui-like syllable. Some of the notes of the FAWS may have marked frequency modulation (the patinado mentioned above is probably a whistle series with extreme frequency modulations and only one or a few syllables). Third, capuchins produce very high amplitude long distance WS (LDWS) when lost or when the group splits (Fig. 2.3). LDWS typically have no introduction but usually start with a short and soft whistle. Some of the syllables may be noisy, something that may denote the degree of “urgency” (*e.g.*, for how long the animals have been lost). Whistle series given by lost animals have longer duration and contain more syllables than those given while feeding. The duration of the syllables increases as the call proceeds. Fourth, animals also produce high amplitude WS during aggressive inter-group encounters (IGEWS; Fig. 2.4). These WS typically contain an introductory series of rapidly frequency-modulated and brief notes resembling an ascending rapid staccato (see below). The context of emission of IGEWS suggest that these vocalizations could be aimed at members of neighboring groups, and indeed these calls initiate strong approach responses when played back in proximity to a neighboring group, especially in the context of high-value food resources (Scarry, 2012). However, it is also possible the neighboring groups may merely be eavesdroppers that take advantage of the knowledge that another group is nearby, as the call does not appear to be a typical long distance call with territorial or mate defense functions. Such a function has been argued, for example, for the long distance calls of howler monkeys, titi monkeys and gibbons (Robinson, 1979; Cowlishaw, 1992; Holzmann *et al.*, 2012), in which calls are frequently given even when neighboring groups have not been detected. In capuchins, it may instead serve to communicate with the caller's own group members, possibly to recruit other individuals, especially adult males, to participate in the encounter (see Di Bitetti, 2001b; Scarry, 2013) and/or to promote group cohesion in a situation that frequently leads to lost infants and other individuals. The function of this call is worth further exploration. Finally, individuals

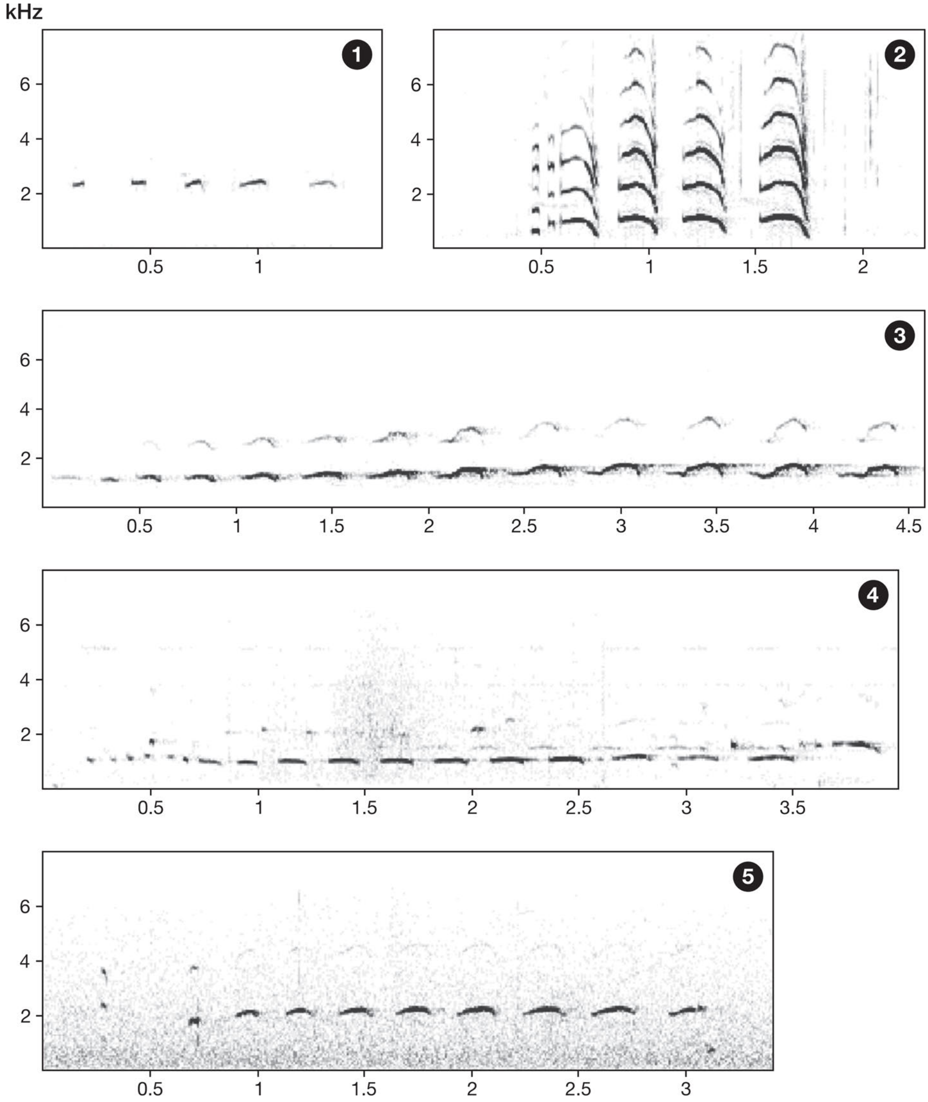

Figure 2. Spectrograms of whistle series (WS): **1.** infant WS (I); **2.** food-associated WS (AF); **3.** long distance WS (AF); **4.** intergroup encounter WS (unk); **5.** monotonous WS (JF). Caller abbreviations as in Fig. 1. All spectrogram parameters as in Fig. 1.7. Faint traces slightly above and out-of-phase in **4** are from different individuals calling from further away from the focal animal.

of all ages (except infants) produce monotonous WS (MWS) when foraging, traveling, or when the group is stationary but not feeding on fruit trees (Fig. 2.5). MWS typically start with a contact note and are of lower amplitude than the LDWS, with little variation among syllables in duration or frequency. Adults and subadults appear to use MWS interspersed with trills to attempt to change the direction of group movement, being far more likely to give MWS when at the edge of a foraging or stationary group than in more central positions (logistic regression using the emission or not of MWS as the response variable and spatial location—edge *vs.* interior position—as the main factor, and sample length as a covariate: Wald $\chi^2 = 6.97$, $P < 0.01$, $df = 1$, $N = 314$ focal samples).

Although the PCA and DFA indicated that WS given in different contexts were not well differentiated based on the 10 acoustic variables considered here, a more detailed analysis based on 21 acoustic variables demonstrated that, although the different WS types grade into one another, there are significant differences in the acoustic structure of WS given in the different contexts with 79% correct classification among IWS, MWS, LDWS, and FAWS (see Di Bitetti, 2001a for details on this analysis). Further, listeners respond with distinct reactions to playbacks of FAWS *vs.* those of MWS or LDWS, suggesting that capuchins perceive the different WS types categorically, and that FAWS specifically are interpreted as indicating the presence of food (Di Bitetti, 2003). The use of such a call to announce the presence of food to others seems somewhat paradoxical given that more dominant listeners will often displace the caller from the food patch once alerted to the food's presence by the call (Di Bitetti and Janson, 2001), although callers tend to limit this cost by varying their latency to call in some situations (Di Bitetti, 2005).

Bark. Barks are alarm calls produced by black-horned capuchins upon detecting a raptor, or, most often, when a large bird superficially resembling a raptor (*e.g.*, vulture or toucan; most likely due to a better-safe-than-sorry strategy when dense vegetation makes correct identification of predators difficult) flies over the group (Fig. 3.1). Experiments using predator decoys suggest that this call is given to both perched and flying raptors (Wheeler, 2010b), although there may be some subtle acoustic differences between calls given in these two contexts. Few good recordings of this alarm call were available for the acoustic analysis, and the call was thus not included in the multivariate statistical analyses. Based on seven recordings from unidentified individuals, the bark is characterized by a single, explosive, high amplitude sound with a duration of 0.06 ± 0.01 sec, a peak amplitude located at 2.90 ± 1.01 kHz, a rapidly descending frequency modulation, and a fading end at 1.51 ± 0.75 kHz. The mean frequency range of the call is 1.41 ± 1.03 kHz. Callers tend to produce just one or two barks and then fall silent. Playbacks of barks elicit raptor-specific reactions, including rapid scans to the sky or rushing toward cover, although the most common response of listeners is to look in the direction of the caller (Wheeler, 2010b).

Hiccups and hips. A second call type, the hiccup, is given in response to terrestrial predators, including felids and snakes (Wheeler, 2010b). These calls are also given in response to the (apparent) alarm calls of agoutis and some understory birds, especially the Formicariidae *Pyriglena leucoptera*, and to other relatively large (non-predator) animals on the ground (*e.g.*, agoutis and deer). Calls given in the latter contexts are probably best explained as cases in which the caller misconstrued the stimulus to be a potential predator due to poor visibility. Hiccups given in predatory contexts ("P-Hiccups" in Tabs. 3 and 4) are short and loud calls (Tab. 4). The call is typically composed of two elements: a high frequency note that sounds like a "hip!" and sometimes shows some upward frequency modulation, immediately followed by a short broadband noise that sounds like a "ku!" (Fig. 3.2). In some cases the broadband element is very low amplitude and almost inaudible or absent completely. In cases in which the broadband element of the call was absent, we classified it as a separate call type, the hip (Tab. 4, Fig. 3.3), although hiccups and hips likely grade into one another. Hiccups sometimes have more than one (two or even three) high pitch notes before the broadband noise. Individuals of all ages and both sexes produce hiccups, and calls given in response to predator or predator-like stimuli tend to be contagious and given repeatedly for several minutes, often while showing aggression and dropping branches near the predator. Calls given later in these bouts typically seem to be of lower amplitude than the first calls given immediately upon perceiving the predatory stimulus. Playbacks of these calls elicit terrestrial predator-specific responses (look down, run up), although, as with the bark, the most common response is to look towards the caller (Wheeler, 2010b). Receivers sometimes respond to hiccups by producing their own hiccups, and often approach the caller, most likely looking for the eliciting stimulus.

Unlike the bark, which appears to be specific to encounters with raptors, hiccups and hips are commonly produced in non-predator contexts and have been suggested to generally be an indicator of stress in the caller (Boinski *et al.*, 1999; but see Wheeler *et al.*, 2014). Hiccups are a common response to aggression from other individuals, and are also frequently given spontaneously by relatively low-ranking individuals when competing for access to feeding platforms (Wheeler, 2009). Calls given in the latter context appear to be functionally deceptive because they often cause listeners to run off of the platform, allowing subordinates to gain access to food otherwise monopolized by dominants (Di Bitetti and Janson, 2001). Hiccups given in response to aggression are also potentially functionally deceptive; aggressors appear to usually stop chasing the recipient of the aggression when hearing the calls, although further research is needed to confirm this.

Hiccups and hips are also commonly given by animals foraging in a relatively precarious position, for example when they rapidly extend the body to grab a piece of bamboo shoot or an arthropod while hanging from rear limbs and tail, or when on an unstable substrate. Hiccups given in this context seem to be of lower amplitude than most hiccups given in predatory contexts, in response to aggression, or during feeding platform experiments. These low amplitude hiccups rarely elicit any response in listeners, although on rare occasions individuals in the caller's immediate vicinity may become vigilant. In the present analysis, the DFA usually classified the hiccups given during routine foraging ("hiccups" in Tabs. 3 and 4) as a *P-Hiccup* (see Tab. 3). Likewise, a more detailed analysis including 28 acoustic variables by Wheeler and Hammerschmidt (2013) compared hiccups given in response to predators with those given during feeding platform experiments and in response to aggression, finding no significant differences.

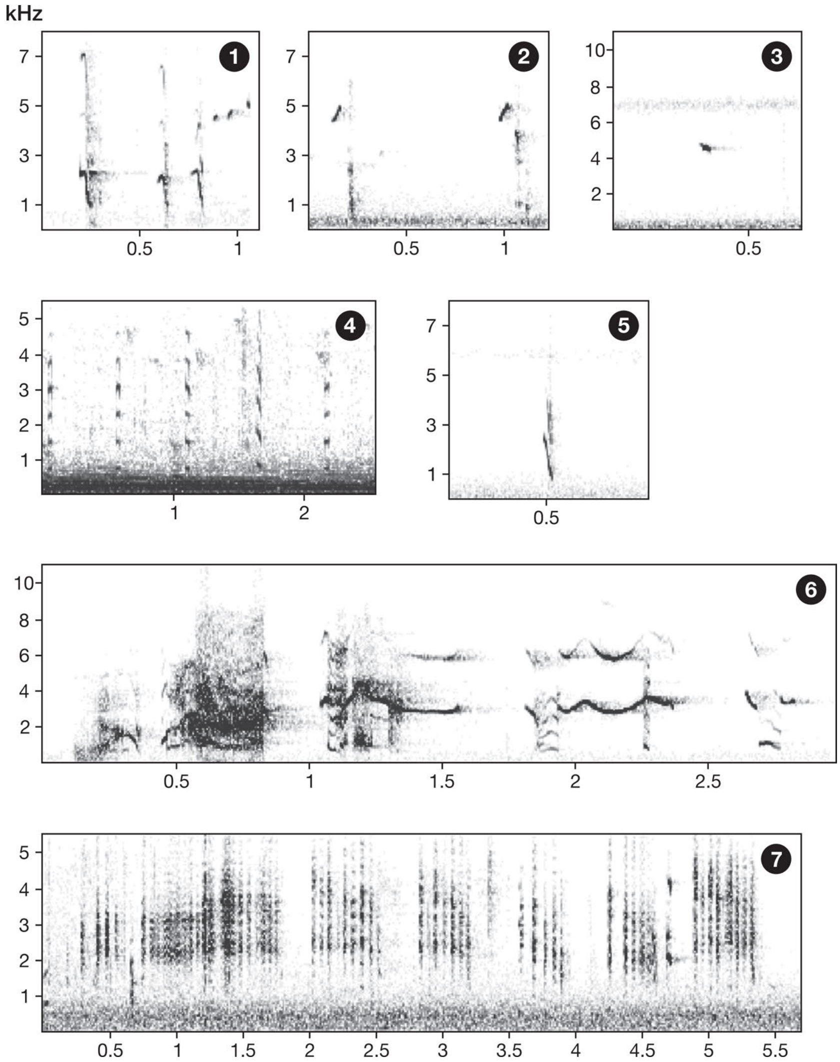

Figure 3. Spectrograms of **1.** bark (unk); **2.** predator-elicited hiccup (AM); **3.** hip (JF); **4.** peeps (AF); **5.** chuck (AM); **6.** screams (first two elements) and squeals (final three elements) (AF); **7.** pulsed scream (JF). Caller abbreviations as in Fig. 1. Spectrogram parameters of parts **1**, **2** and **5** as in Fig. 1.7; parts **4** and **7** as in Fig. 1.1. For illustration purposes vocalizations in parts **3** and **6** were digitized with a sampling rate of 22.050 kHz. Spectrograms were made using a FFT with the following spectrogram parameters: FFT-length = 256, Frame (%) = 100, Hamming window, 50% overlap.

Peeps. A third type of call given in predatory contexts is the peep (Fig. 3.4). Like the hiccup, this call is associated with terrestrial but not aerial predators (Wheeler, 2010b). Peeps are normally given together with hiccups, although call bouts consisting solely of peeps occur, but are a more common response to snakes than to felids (Wheeler, 2010b). Like hiccups, peeps are often associated with mobbing behaviors. In contrast to the capuchin's other predator-associated calls, and indeed the alarm calls described for most species (Owren and Rendall, 2001), the peep is a relatively low amplitude call. The peep resembles the CN, but is given repeatedly at a rate of about 4 calls per second, with bouts lasting a minute or more. Like the aggressive CN (see below), peeps may in fact constitute contact notes given at a relatively high rate, although the rate at which the individual elements are delivered appears to be lower than that of the aggressive CN. However, comparisons of the acoustic structure of the individual elements of peeps and CNs are needed as are comparisons with aggressive CNs.

Chucks. The chuck is a rapidly descending and short call resembling a bark, but with a lower frequency than the aerial predator alarm and with the maximum frequency (1.62 ± 0.53 kHz; N = 10) occurring at the start of the call (Fig. 3.5). Like the bark, the minimum frequency (0.47 ± 0.34 kHz,) occurs at the end of the call. Additional analyses are needed to determine if barks and chucks represent opposite ends of a graded continuum. The call seems to be given in situations of high arousal with some anecdotal observations suggesting that chucks are sometimes given when a neighboring group is detected, as well as in response to perched raptors. More commonly, chucks are given by victims of aggression, often in association with screams and squeals. The chuck is also produced at high rates (> 10 chucks per min for a half hour or more) by adult males, especially the alpha male, when being courted by an estrous female. If the calls do indeed have a regular association with raptors as our limited observations suggest, then such use of chucks may function to distract competitor males or the estrous female, reducing the likelihood of other males mating (Tamura, 1995; Bro-Jørgensen and Pangle, 2010), although more research is needed to test this hypothesis.

Aggressive contact note. Black-horned capuchins give several different vocalizations in association with threat displays. The particular call type that an individual produces depends largely on the intensity of the aggression and the distance between the aggressor and the recipient.

The aggressive contact note (ACN) is the most common call produced during mild aggression directed to a close neighbor (Fig. 1.6). ACNs are usually accompanied by a slap, branch shaking, a fixed stare with a lunge and sometimes an open mouth bared teeth (OMBT) display with the tail curled up over the back like an “S” (*S-tail display*). The target is always within a few meters of the aggressor. Adults (especially the alpha male and female) usually produce these vocalizations when threatening small juveniles and infants.

ACNs sound louder than normal CNs and are usually delivered in a rapid sequence of 1 to 14 notes (Tab. 4) at a rate of one note every 0.15 s, lasting up to 2.1 s. ACNs in apparently more intense aggressions are louder and may include some broadband noise, and there seems to be a transition between ACNs and the cough and between ACNs and aggressive pips and *ke!* vocalizations (see below). All ACNs delivered singly were classified as normal CNs by a DFA; the main difference between the two may thus be the rate in which the calls are delivered, although additional acoustic measures, such as amplitude measurements and noisiness, may also differentiate these call types.

Cough. The cough is a noisy broadband vocalization that sounds like a raspy cough (Fig. 1.7, Tab. 4). It is emitted by aggressors during intermediate to intense aggression, and is usually directed to targets (including human observers) in full view of the aggressor. It is usually produced in a series of two or more calls, sometimes in association with pips or ascending rapid staccatos (see below).

Pips. Pips are very loud and high frequency calls (Fig. 1.8, Tab. 4) that look somewhat like a hip (or first syllable of a hiccup) and were sometimes misclassified as one of these call types. Pips are usually produced by aggressors during very intense aggression. Sometimes, pips are given with an ascending rapid staccato (see below), especially when the target is far (> 10m) and even out of the aggressor's view. The alpha male usually produces pips (with ascending rapid staccatos, ke!s and coughs) when other individuals start a fight far from him. He accompanies these vocalizations with the OMBT display directed towards the conflict, and sometimes rushes in the direction of the fight.

Ke! Aggressive animals sometimes accompany the open mouth bared teeth (OMBT) display with vocalizations which sound like a "ke!" (first note in Fig. 1.9). These vocalizations look intermediate between a cough, pip, and ACN. They are higher pitched than a cough, and more noisy than a pip or a CN.

Ascending rapid staccato. The ascending rapid staccato (ARS) is a series of usually ascending pips delivered at a very high rate (Fig. 1.9). It is usually given by the alpha male and female during high intensity aggression, and is often directed to a distant recipient that is sometimes out of view. The shape of the ascending rapid staccato on a spectrogram resembles that of a trill, and the values for these two calls in the PCA were not significantly different (Tab. 3); likewise, the DFA sometimes misclassified these calls (Tab. 3). The ascending rapid staccato is clearly a higher amplitude call than trills, but this variable was not used in the analysis.

Aggressive grgr. On rare occasions, individuals produce a series of brief and harsh notes that sound like grgrs, but which are accompanied by aggressive displays (*e.g.*, OMBT and S-tail; Fig. 1.10). These calls are usually interspersed with other aggressive vocalizations.

Squeal, scream, and pulsed scream. Victims of aggression produce squeals and screams. Like most primate vocalizations given in response to aggression, these are loud, noisy, and with high variability in duration and frequency modulation, making them rather noxious-sounding (Owren and Rendall, 2001). Squeals are very loud, tonal vocalizations given in response to aggression. They contain rapid and usually oscillatory frequency modulation (Fig. 3.6). They are usually produced in a long series that may last for several seconds. They sometimes become broken and only the descending portion of the oscillations remains.

Screams are very noisy calls with few or no tonal elements (Fig. 3.6). The amount of noise, the loudness, and the duration of screams are apparently related to the intensity of the aggression. Screams and squeals are usually produced together in long bouts in which the first pulses of sound tend to be noisy screams, and the last ones more tonal squeals. The recipient of aggression sometimes produces screams and squeals while looking intermittently to the aggressor and other individuals. Coalitions are frequent in this population (Di Bitetti, 1997), and it is possible that screams or squeals function to recruit allies (*e.g.*, Slocombe and Zuberbühler, 2007).

Pulsed screams are also given in response to aggression and consist of a rapid series of noisy notes (Fig. 3.7). This call is of lower amplitude than normal screams and is usually associated with mild aggression.

Estrous and copulation calls. Black-horned capuchin females advertise sexual proceptivity with gestural and vocal displays (Janson, 1984; Carosi *et al.*, 1999). The vocal display of estrous females consists of at least two different calls. A high frequency, low amplitude call with descending frequency modulation is given continuously when the female follows a male (Fig. 4.1); it sounds like the last portions of calls given during reunion displays (see below), and these two calls may be related. The descending continuous sound sometimes becomes broken and sounds like a repeated series of "piripipi". The second call is a rapid series of short, noisy notes (Fig. 4.2). These short individual notes blend into a single noisy sound that becomes noisier as the display increases in intensity. This noisier estrous call is also produced by females during copulation, and more detailed analyses are needed to determine if calls given during copulation can be reliably distinguished from those given during intense proceptive displays. Before and especially after copulation, the male produces his own display and calls in a duet with the female, although good recordings of this duet were not available for analysis. While there are a number of possible functions of copulation calls, they seem likely to have evolved in the context of strong sperm competition and infanticide risk (Pradhan *et al.*, 2006), both of which appear to be present in this population (Janson *et al.*, 2012).

Grugru and teeth and lip smacking. The grugru is a low amplitude purring sound usually produced by individuals inspecting a newborn infant. It consists of a rapid series of short broadband elements (Fig. 4.3, Tab. 4), with a steady increase in amplitude of the elements. The call contains some tonal structure, with a fundamental at about 160–220 Hz and several (> 10) harmonics. It is usually produced with teeth or lip smacking (as in Fig. 4.3), which consist of a very rhythmic series of teeth smacking made by a rapid opening and closing of the mouth. The grugru and the teeth-lip smacking are usually directed to the newborn's face or to its ano-genital area during close interactions with the infant (usually when the infant is still in its mother's possession). The teeth smacking sound is also produced during sexual interactions, especially by estrous females, and by immature males attempting to court estrous females or during sexual play. Estrous females and other individuals habituated to human observers sometimes produce the teeth smacking sound to observers, usually in association with the "open leg showing genitalia" display and sometimes the "chest rubbing" display typical of sexual invitations (Janson, 1984; Carosi *et al.*, 1999). The grugru and the teeth smacking appear strongly associated with appeasement.

A sound very similar to the grugru is sometimes produced by juveniles during social interactions with adults or other juveniles. In this case the sound is sometimes accompanied by a rapid lateral shaking of the head (like saying "no"). It is not clear if the grugru and the vocalization associated with the NO display are the same vocalization or two different ones.

Wahwahwah. This rare call is given in response to sudden loud and low frequency sounds, most commonly thunder and falling branches. Although we observed only adult and subadult males producing the wahwahwah, juveniles and females in other populations have been reported to produce this call (de Resende *et al.*, 2007). We could not obtain high quality recordings of this intermediate amplitude vocalization, but initial analyses indicate

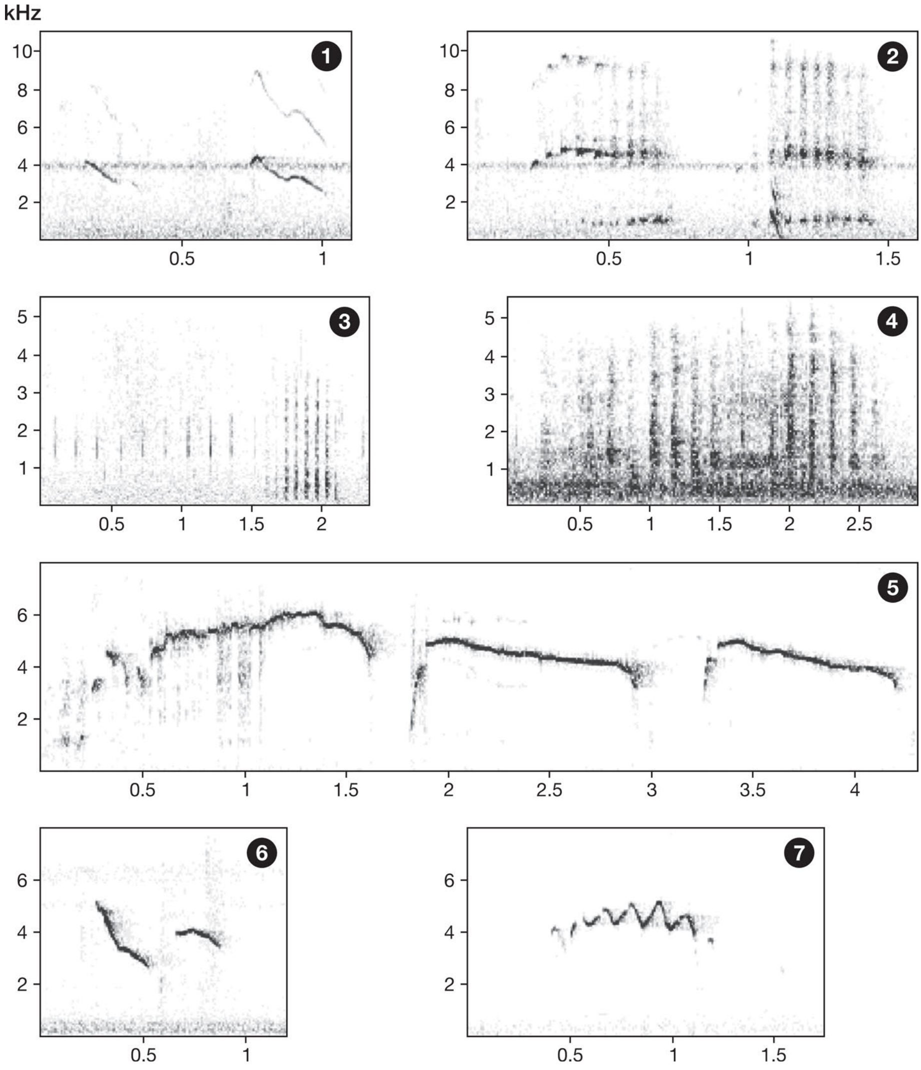

Figure 4. Spectrograms of **1.** piripipi estrous call (AF); **2.** raspy estrous call (AF); **3.** teeth/lip smack (first element) followed by a grugru (second element) (SaM); **4.** wahwahwah (AM); **5.** sirena (AM); **6.** babble (i); **7.** infant squeal (I). Caller abbreviations as in Fig. 1. Spectrogram parameters of parts **1** and **2** are as in Fig. 3.3; parts **3** and **4** as in Fig. 1.1; parts **5** through **7** as in Fig. 1.7.

that the call lasts 2–3 s and consists of a repetitive series of 16–22 broadband elements that sound like a deep "wah" sound and contain some tonal components (Fig. 4.4). Callers usually stop doing the activity they were engaged in and become attentive when producing the wahwahwah.

Sirena. The sirena is probably the loudest vocalization in the black-horned capuchin vocal repertoire (Fig. 4.5). It accompanies reunion displays (Matheson *et al.*, 1996; Lynch Alfaro, 2008), in which animals that have lost contact for several hours meet again, usually after the group was split or an individual was lost. This display usually occurs between the most dominant individuals in the group, usually involving the alpha male and alpha female or the alpha male and other high ranking males (Lynch Alfaro, 2008). The alpha male usually responds to reunion displays of other individuals with aggressive displays and ascending rapid staccatos. We were not able to distinguish which animals vocalized during most of the reunion displays because usually the two animals making the display and other individuals nearby all vocalized. One individual participating in the display usually produced the sirena. The alpha male apparently always produced this vocalization when involved in the display, while the other individual may produce a call similar to the estrous vocalization but of higher amplitude. Other individuals nearby may produce LDWS (or possibly another type of WS).

The sirena is not a very stereotyped vocalization, showing considerable variation. They are relatively long, high amplitude, high pitch calls, usually showing descending, and sometimes oscillating frequency modulation (Tab. 4). They are usually delivered in series of several sirenas. The first of these calls may sound like an ascending rapid staccato. Other sirenas in the series start with a rapidly ascending introductory note followed by a slowly descending (sometimes steady or oscillating) longer sound which may descend abruptly in frequency at the end. The last calls in the series are usually of lower amplitude with oscillating frequency modulation and are sometimes broken (*i.e.*, interspersed with very short periods of silence). The values of the sirena on the principal component axes were not statistically different from ARSs, trills, or IWSs (Tab. 3), which may be the result of either a small sample size, the large amount of acoustic variation that characterizes the call, or the relatively small number of acoustic parameters used to discriminate vocalizations.

Infant babbling. Infants produce a variety of high pitch (2.7–6.1 kHz, N = 14 calls from one infant), ascending or descending pure tone sounds during their first weeks of life. These calls have no consistent pattern and do not clearly resemble any calls of the adult repertoire (Fig. 4.6).

Infant squeals. Young infants produce squeals during stressful situations, usually when they are left behind or apparently want to be carried and had been giving IWS for a while without a response from others. Infant squeals are similar to the high amplitude, narrow band squeals produced by adults, but tend to be more rapidly oscillating and slightly higher in frequency (Fig. 4.7), although statistical comparisons are needed. Infants also produce many calls intermediate between squeals and trills (*trill-squeals*).

General discussion

In general, black-horned capuchins' vocalizations belong to discrete categories rather than to continuous ones (*e.g.*, wahwahwah, grugru). However, some calls used in different contexts (*e.g.*, contact note, peeps, and aggressive contact notes; chihui and grgr; the different whistle series; ascending rapid staccatos and trills) likely comprise groups of graded calls, with calls falling on one part of the continuum having a higher probabilistic association with a particular context and those on the other side being associated with different contexts. For example, although we treated the calls in the whistle series (WS) vocal system as belonging to discrete categories based on the context of their production, and the multivariate and univariate analyses revealed significant differences among the WS given in different contexts, there are some calls that are intermediate between the different WS variants. However, a graded system can be perceived by receivers categorically (Nelson and Marler, 1989; Fischer, 1998), and this indeed seems to be the case with the whistle series (Di Bitetti, 2003).

In addition, in cases in which there are limited or no acoustic differences between calls given in different contexts, the calls may elicit distinct reactions and function in different ways if listeners are able to take contextual cues into account (Rendall *et al.*, 1999; Wheeler and Fischer, 2012). For example, hiccups given in response to predators and those given when feeding on high value food resources elicit similar escape reactions in listeners (Wheeler, 2009; 2010b), although listeners are more likely to ignore the calls given in the latter context (Wheeler, 2010a) apparently because they account for the general behavioral context in which the call is given (Wheeler and Hammerschmidt, 2013). The production of certain call types in combination with other vocal or gestural signals may also affect how listeners interpret or respond to a given vocalization (*e.g.*, Arnold and Zuberbühler, 2012). Listeners may, for example, move in the direction individuals producing trills when these calls are given together with monotonous whistle series in order to facilitate group movement. Alternatively, trills given in association with a “show genital” display may be inferred as signal of that individual's subordinance.

A number of calls appear to function to regulate within-group spacing. The CN is the most commonly produced call with this function and may be equivalent to the *heh* or the *huh* vocalization of wedge-capped capuchins (Robinson, 1982) and the *huh* vocalization of white-faced capuchins (*Cebus capucinus*; Boinski and Campbell, 1996; but see Gros-Louis *et al.*, 2008). Robinson (1982) describes the heh call of wedge-capped capuchins as an aggressive call related to the vocal system used for within-group spacing, producing a repelling effect on recipients, and in this sense may be similar to the aggressive contact notes of black-horned capuchins. Chihuis also appear to function in the regulation of intra-group spacing and in fact may be more functionally similar to the huh/heh calls of white-faced and wedge-capped capuchins (Robinson, 1982; Boinski and Campbell, 1996; Gros-Louis *et al.*, 2008) than is the CN because, although given in many different foraging situations, they are produced at higher rates when the animal is feeding on fruit. The whistle series (WS) also appear to be used to regulate within-group spacing and to provide location or directional cues to other individuals, hypotheses that should be tested with further observations or field experiments. At one extreme of this vocal system, the long distance WS are given when individuals have lost visual and vocal contact with other group mates. White-faced capuchins also have long distance calls emitted in similar contexts (Digweed *et al.*, 2007; Gros-Louis *et al.*, 2008). At shorter ranges, infant WS and monotonous WS are given in contexts where individuals seek close contact or group cohesion. Black and gold howlers (*Alouatta caraya*) have also been described as using a call type (the *moo*) in these contexts (Holzmann, 2012; da Cunha and Byrne, 2013).

The results presented here provide some insight into the ontogeny of vocal production in this species. We found some preliminary evidence that the first vocalizations produced by capuchins are clearly different from the adult call types, and have limited evidence that infant trills develop into the mature form as individuals age. Given that nonhuman primates are not vocal learners, with the gross acoustic structure characterizing adult vocal repertoire being largely innate and unaffected by learning (Hammerschmidt and Fischer, 2008; Seyfarth and Cheney, 2010), these ontogenetic changes are most likely explained by the physical maturation of the vocal apparatus. Further research is needed to know if the finer structure of capuchin calls might be somewhat flexible and modifiable based on experience as has been shown in a number of other primate taxa (*e.g.*, Lemasson *et al.*, 2011).

The large (about 30 different call types) and relatively discrete nature of the black-horned capuchin vocal repertoire is comparable to, but mostly larger than, that of other forest-dwelling New World monkeys (Schott, 1975; Cleveland and Snowdon, 1982). It is similar in richness to that of the white-faced capuchin, with *ca.* 27 different vocalizations (Gros-Louis *et al.*, 2008). The large vocal repertoire of capuchin monkeys is consistent with the hypothesis that the diversity of primate calls is positively correlated with group size and the complexity of social interactions (McComb and Semple, 2005).

Analysis of the vocal repertoire in terms of both acoustic structure and the contexts of occurrence provides a starting point for understanding many aspects of vocal communication, including how callers use these signals to alter the behavior of receivers (Owren and Rendall, 2001); why receivers would respond to such signals at all (Seyfarth *et al.*, 2010); the proximate mechanisms underlying call production (Jürgens, 2009); the ontogeny of call production, usage, and perception (Seyfarth and Cheney, 2010); and the possible use of calls in novel combinations (Arnold and Zuberbühler, 2012). Detailed investigation in the black-horned capuchin vocal repertoire is so far largely limited to the function and meaning (to listeners) of food- and predator-associated calls and the sirena "reunion" call (Di Bitetti, 2003, 2005; Lynch Alfaro, 2008; Wheeler, 2008, 2010b). Additional research in natural contexts as well as using experiments to elicit calls and playbacks to monitor listener responses will provide additional insight into a number of aspects of capuchin vocal communication.

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11 SHORT-DISTANCE VOCALIZATIONS OF THE BLACK AND GOLD HOWLER MONKEY (*ALOUATTA CARAYA*) IN THE ATLANTIC FOREST OF ARGENTINA

VOCALIZACIONES DE CORTA DISTANCIA DEL MONO AULLADOR NEGRO-Y-DORADO (*ALOUATTA CARAYA*) EN EL BOSQUE ATLÁNTICO DE ARGENTINA

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Abstract. The vocal behavior of howler monkeys (*Alouatta* spp.) includes long and short-distance vocalizations, the latter of which have seldom been studied. Naturally emitted short distance vocalizations used in intra-group communication of two groups (26 individuals) of black and gold howler monkeys (*Alouatta caraya*) in the Atlantic Forest of Argentina, were recorded from June to November 2007. For each sound-recording, sex and age of the sender and behavioral context (feeding, playing, aggression between group members, isolation, tense moments and infant distress situations) were noted. Four spectrogram measurements (duration, minimum, maximum and peak frequency) were taken from 331 calls using the software *Raven* 1.4 (Cornell University). We recognized (by sound and form) eight different types of short-distance calls: *hu-hu-hu*, *heh*, *bark*, *moo*, *ar*, *yell*, *screech* and *oodle*. Although we did not test functionality, the consistency of some of the contexts in which the signals were recorded shows that some of these vocalizations can function to reestablish contact between lost individuals (moo call), as a warning of a close danger (bark), or during aggressive interactions between group members (screech). This descriptive study sets the stage for future ones designed to test hypotheses about the proximate mechanisms and function of each call to further our knowledge of intra-group communication in howler monkeys.

Resumen. Los monos aulladores (*Alouatta* spp.) poseen un amplio repertorio vocal compuesto por voces de corta y larga distancia. Las voces de larga distancia (aullidos) han sido las vocalizaciones que mas atención han concentrado, siendo el foco de numerosos estudios de comportamiento desde 1930 hasta la actualidad. Por el contrario, las vocalizaciones de corto alcance (utilizadas principalmente en la comunicación entre individuos del mismo grupo) han sido muy poco estudiadas. Los escasos trabajos sobre las voces de corta distancia en los monos aulladores negros-y-dorados (*Alouatta* *caraya*) se han concentrado mayormente en unas pocas voces particulares poniendo a prueba hipótesis acerca de su funcionalidad y potencial significado. Sin embargo, no se ha descrito hasta el momento el repertorio completo de las voces de corta distancia utilizadas en esta especie para la comunicación intra-grupal. En el presente estudio registramos durante un período de seis meses consecutivos (junio 2007 – noviembre 2007) las vocalizaciones de corta distancia emitidas naturalmente por 26 individuos residentes en 2 grupos de monos aulladores negros-y-dorados en el Bosque Atlántico de Argentina. Llevamos a cabo este trabajo en el Parque Provincial El Piñalito, en la provincia de Misiones. Utilizamos una grabadora digital (Hi-Mini disc *Sony*) y un micrófono direccional (*Sennheiser* ME 67/K6) para registrar cada vocalización, describiendo además el sexo y la clase de edad del individuo emisor junto con el comportamiento que realizaba al momento de vocalizar. Registramos vocalizaciones en los siguientes contextos: alimentación, juego, agresión entre individuos del mismo grupo, aislamiento, momentos tensos y situaciones de angustia de infantes. Obtuvimos un total de 15 horas de grabación en las cuales registramos 458 vocalizaciones. Sin embargo, debido a la calidad de algunas grabaciones, solo realizamos espectrogramas de 331 de las mismas. Sobre los espectrogramas tomamos mediciones de cuatro parámetros acústicos básicos: frecuencia mínima, máxima, pico y duración, a través del software *Raven* 1.4 (Universidad de Cornell). Archivamos todos los tipos de grabaciones aquí mencionadas en la Biblioteca de Sonidos Naturales Macaulay (Macaulay Library of Natural Sounds, Universdad de Cornell) por lo que se hallan disponibles abiertamente. Reconocimos (en base al sonido y la forma) ocho tipos diferentes de llamados de corta distancia: *hu-hu-hu*, *heh*, *bark*, *moo*, *ar*, *screech*, *yell* y *oodle*. El *hu-hu-hu* y el *heh* fueron voces fuertemente asociadas al contexto de alimentación. El *bark* estuvo relacionado a situaciones de tensión (cercanía a un potencial peligro) y fue más que nada dirigida a nosotros, los observadores. El *moo* fue emitido mayormente en contextos en donde uno o mas individuos se encontraban aislados (fuera de vista) del resto del grupo, y también fue emitido por infantes en situaciones en las cuales se encontraban separados de sus madres o pedían ayuda para cruzar una abertura en la vegetación. Los *ar* y *yells* estuvieron asociados a situaciones de juego y conflictos leves entre individuos del grupo. Los *screeches* fueron emitidos por individuos en contextos de agresiones leves relacionadas a conflictos de intereses entre los mismos. Por último, la voz de *oodle* la oímos en dos ocasiones y solo la grabamos en una de ellas. Esta voz fue emitida por el macho dominante del grupo cuando había individuos de su mismo grupo emitiendo *yells* y *ar* durante un conflicto entre los mismos. A pesar de no haber testeado hipótesis sobre la funcionalidad de estos ocho tipos diferentes de voces, la consistencia de los contextos de emisión en los que fueron registradas algunas de ellas muestra que las mismas podrían funcionar re-estableciendo el contacto entre individuos aislados (*moo*) o como una advertencia de una situación cercana de peligro (*bark*) o manifestando un estado emocional durante conflictos entre individuos del mismo grupo (*screech*). El presente estudio descriptivo sienta las bases para futuros estudios diseñados para testear hipótesis sobre mecanismos próximos y funciones últimas, ampliando así nuestro conocimiento sobre las voces de corta distancia en monos aulladores.

Introduction

Howler monkeys (*Alouatta* spp.) have been the focus of many studies of ecology, behavior and/or long distance roars. However one interesting aspect of the social behavior of howler monkeys that has been largely neglected is the repertoire of comparatively quiet, short-distance vocalizations used in intra-group communication. Surprisingly few studies about these short distance calls have been carried out in howler monkeys (reviewed in da Cunha *et al.*, 2015), with a single detailed description of the short-distance vocal repertoire in the mantled howler monkey (*A. palliata*; Altman, 1959; Baldwin and Baldwin, 1976; Eisenberg, 1976). The few studies on short distance vocalizations in howler monkeys have focused their attention on particular calls or contexts (*i.e.*, contact calls: Jones, 1998; Jones and Van Cantfort, 2007a, b; da Cunha and Byrne, 2009, 2013; distress calls: da Cunha and Byrne, 2013). Although the concentration of sampling efforts on a particular type of call or context allows deeper insights into ultimate causes and functions, the description of complete vocal repertoires promotes a fuller understanding of how structure is related to function (Gross-Louis *et al.*, 2008).

The objectives of this chapter are to 1) describe the short-distance vocal repertoire of the black and gold howler monkey (*Alouatta caraya*) in a protected area of the Atlantic Forest of Argentina, and 2) set the basis for future comparative studies on the function and structure of short-distance vocal signals in howler monkeys.

Methods

The present study was part of a major study on the feeding ecology and vocal behavior of two howler monkey species (*Alouatta caraya* and *A. guariba clamitans*) living in sintopy in a protected area in the Atlantic Forest of Argentina: El Piñalito Provincial Park, in Misiones Province (Agostini *et al.*, 2010a, b, 2012; Holzmann *et al.*, 2012). During a period of six consecutive months (June to November 2007), IH recorded short distance calls emitted by individuals of two black and gold howler monkey groups (26 individuals).

Behavior sampling and contexts

Each of two howler monkey groups was followed two to three consecutive days per month (35 complete days) from dawn to dusk. Both groups moved mostly in the medium strata of the forest (between 11–20 m, Agostini *et al.*, 2010b), so recordings were generally obtained from these distances. For each recorded signal, behavioral context of emission, age class, and sex of the caller were noted.

Vocal signals were assigned to one of the six following contexts: feeding, playing, aggression between group members, isolation, tense moments and infant distress situations. We defined playing as an interaction between two or more individuals engaged in physical contact, including soft bites and hair pulling, but where individuals did not try to run away (as it happens in aggressive interactions, see below). One of the most characteristic playing behaviors of howler monkeys is when two individuals, hanging by their tails while swinging and facing each other grab each other's face while emitting the calls described below (ar and yells). During play interactions, chases can occur; however, during play, individuals run away but then stop to keep playing. Our concept of play agrees with Altman's (1959) definition of play for *A. palliata*: “The play was highly unstereotyped. It generally involved varied patterns of wrestling, chasing, or manipulation of parts of the body. It was always characterized by a high degree of reciprocal interaction”.

Aggressive interactions involved body contact that was rougher than during play. The aggressive interactions we observed were not severe; we never witnessed a group member seriously injured as result of these interactions. Frequently, a friendly interaction between two playing individuals graded suddenly into a light aggressive one, when one of the individuals hit the other (apparently) too roughly or refused to continue playing and tried to get away. When the friendly interaction ended, chases through the branches started with the chased individual giving kicks and slaps in defense. This light aggression has been described as "rough play" in a previous study of *A. caraya* (da Cunha, 2005).

Even when both howler monkey groups had been well habituated to human presence since 2005, all tense moments in which vocalizations were emitted were related to close encounters with researchers. During traveling, monkeys used some low (5 to 10 m from the ground) branches as bridges; when passing on these branches monkeys emitted threat calls (barks) while staring at the observers.

Acoustic analyses

Recordings were made using a *Sony* MZ-RH1 Hi-MiniDisc Walkman digital recorder and a *Sennheiser* ME67/K6 directional microphone. All recordings were uncompressed 16 bit and 44.1 kHz PCM waveforms (wav). Our recordings are archived at the Macaulay Library of Natural Sounds, Ithaca, NY (*http://macaulaylibrary.org*).

Recorded calls were identified based on visual inspection of spectrograms (call shape) and on their sound. We took spectrogram measurements on good quality recordings with on-screen cursors using *Raven Pro* 1.4 (*http://www.birds.cornell.edu/raven*).We measured four acoustic variables in each vocal signal: minimum frequency (Hz), maximum frequency (Hz), peak frequency (Hz) and duration (s). All measurements were taken using the following spectrogram parameters: Hann window type, window size of 0.0522 s, 50% overlap, and DFT size of 4096 samples.

Statistical analysis

In order to test whether the vocalization types that we distinguished a priori based on visual inspection of spectrograms and sound could be identified as such with acoustic measurements, we used the four acoustic variables measured to perform a Discriminant Function Analysis (DFA) to predict membership to naturally occurring groups of calls. We performed the statistical analysis with *Statistica* 7 (*Statsoft*, Inc.) and we used an α level of 0.05 to reject null hypotheses.

Results

In total, 15 hours of recordings were obtained during sampling of both groups. We recorded 458 calls, 331 of which were of sufficient quality to perform spectrogram measurements.

Based on call shape in spectrograms and on sound, we identified eight different types of calls: 1) *hu-hu-hu*, 2) *heh*, 3) *bark*, 4) *moo*, 5) *ar*, 6) *yell*, 7) *screech*, and 8) *oodle*. The oodle vocalization was the only vocalization not included in the Discriminant Analysis because the quality from the only call recorded was too poor to perform measurements.

The Discriminant Function Analysis signaled that the four acoustic variables (low, high and peak frequency and duration) created a function that explained a significant amount of the variation between the seven types of calls included in the analysis (Wilk's Lambda = 0.03, F (24, 1121) = 76.19, p = 0.001). This function classified 77.9% of the calls to the correct group. Standardized canonical discriminant coefficients signaled duration (0.61), high frequency (0.55) and peak frequency (0.39) as the most important variables in the resulting discriminant function and low frequency (0.13) as the least important

Table 1. Features and context of emission of calls identified in the vocal repertoire of *A. caraya*. **HF**, High Frequency; **LF**, Low Frequency; **PF**, Peak Frequency and **D**, Duration. **IDS:** infant distress situations.

Call	Context	Recorded	Measured	HF (Hz) (± SD)	LF (Hz) (± SD)	PF (Hz) (± SD)	D (s) (± SD)
Hu-hu-hu	Feeding	137	17	603.25 (± 148.4)	122 (± 39.6)	285.6 (± 118.3)	0.5 (± 0.3)
Heh	Feeding	42	12	1467.1 (± 1262)	168.3 (± 90.7)	325.6 (± 163.4)	0.1
Bark	Tension	18 (series)	66 barks from 12 series	677.7 (± 239.2)	96.1 (± 21.3)	210.2 (± 66)	0.1
Moo	Isolation/aggression/IDS	42	18	2340.6 (± 1356.9	144.2 (± 44.9)	416.2 (± 163.1)	1.4 (± 0.3)
Ar	Play/Aggression	96	96	4216.3 (± 1718.5)	843.1 (± 687.4)	1657.7 (± 911.4)	0.8 (± 0.7)
Yell	Play/Aggression	85	85	12779.7 (± 6444.1)	645.1 (± 324.1)	2589.3 (± 849.4)	0.8 (± 0.3)
Screech	Aggression/IDS	37	37	2921 (± 1411.3)	144.5 (± 27)	691.6 (± 392.1)	1.5 (± 0.5)
Oodle	Aggression	1	–	–	–	–	–

Vocal repertoire of *Alouatta caraya*

In this section we describe the context and spectrographic characterization of all eight types of calls distinguished in this study. All the recorded vocalization types described here are available for listening: *http://macaulaylibrary.org/*, and we provide a link to examples of all vocal types in the corresponding account.

Hu-hu-hu call. We recorded 137 hu-hu-hu calls of which 126 (91.9%) were given by individuals during feeding bouts while eating (Tab. 1). The vocalizing animal was out of view in the remaining 11 cases. We recorded this call in 15 different individuals belonging to all age classes and sexes (with the exception of infants). As an anecdotal case, on one occasion we heard individuals emitting the hu-hu-hu call after the reunion with one of its group members (the dominant male) that was isolated for a couple of hours after an aggressive encounter with another group. This call has a pulsed structure (five to seven pulses) and is given by the animal with the mouth closed (Fig. 1; to listen: *http://macaulaylibrary.org/audio/177781/play*).

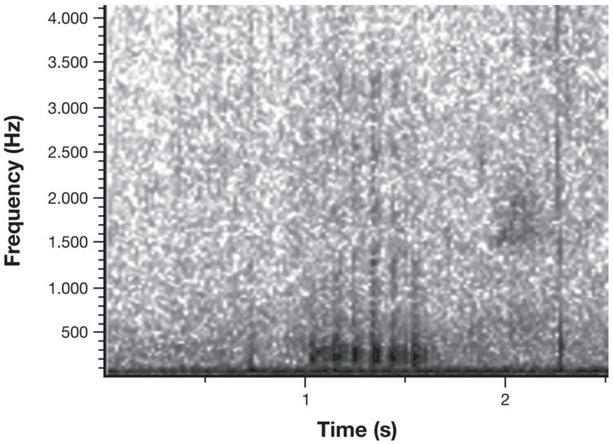

Figure 1. Spectrogram of a hu-hu-hu call.

Heh. We recorded 42 heh calls, 39 (92.8%) of which were given by individuals during feeding bouts while eating; the vocalizing animal was out of view in the remaining 3 cases (Tab. 1). Heh calls were recorded in eight different individuals from all age classes and sexes (with the exception of infants). This soft call is given with the mouth closed and the sound resembles a quick and sharp "M" or "eh" (Fig. 2; to listen: *http://macaulaylibrary.org/audio/177777/play*).

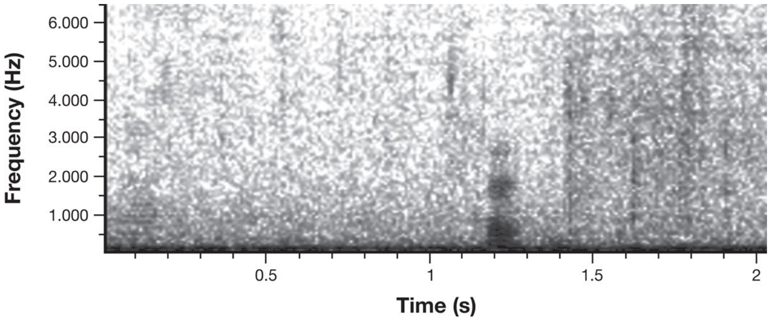

Figure 2. Spectrogram of a heh call.

Barks. This call was always given in series and no isolated barks were recorded. We recorded 18 bark series (Tab. 1). The mean duration of the barking series recorded was extremely variable: 67.8 s (range: 1 to 173, *SD* = 50.9) with an average of 62.2 (range: 2 to 217, *SD* = 60.4) barks per series. We measured 66 barks from 12 series. We recorded this call in nine different individuals belonging to all age classes and sexes (with the exception of infants). In all cases where this vocalization was recorded, individuals were traveling at low height (5–10 m from the ground) emitting this vocalization while staring at the observers. Sometimes the emission of this call was accompanied by branch shaking. This call is given with the mouth closed (Fig. 3; to listen to this: *http://macaulaylibrary.org/audio/177783/play*).

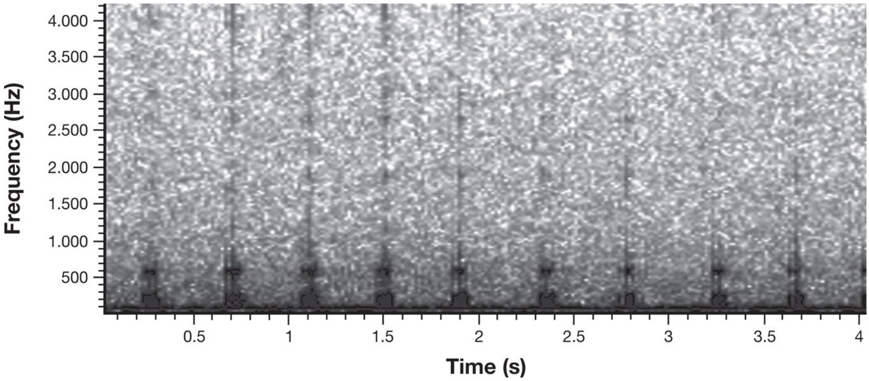

Figure 3. Spectrogram of a bark series.

Moo call. We measured 18 moo calls (Tab. 1); 10 (55%) of them were produced on different occasions by isolated individuals out of visual contact with the rest of the group members. Five (27%) moo calls were emitted by infants during two "infant distress situations" (moving in search of her/his mother or trying to cross a gap in the vegetation). On one occasion we recorded two moo calls during an aggression between group members: a juvenile male approached his mother, who was breastfeeding a small infant, which caused her to hit the juvenile with her hands and to grab him by the hair; after this, the juvenile moved away and emitted the two moo calls. Also we recorded one moo call during play (emitted by a sub-adult male playing with a juvenile). In all contexts this call was given with the mouth open forming an "o" with the lips and the sound of this call resembles the mooing of cows (Fig. 4.1; to listen: *http://macaulaylibrary.org/audio/177850/play*).

Moo calls can sometimes be preceded by a strong inspiration (Fig. 4.2; to listen: *http://macaulaylibrary.org/audio/177801/play*).

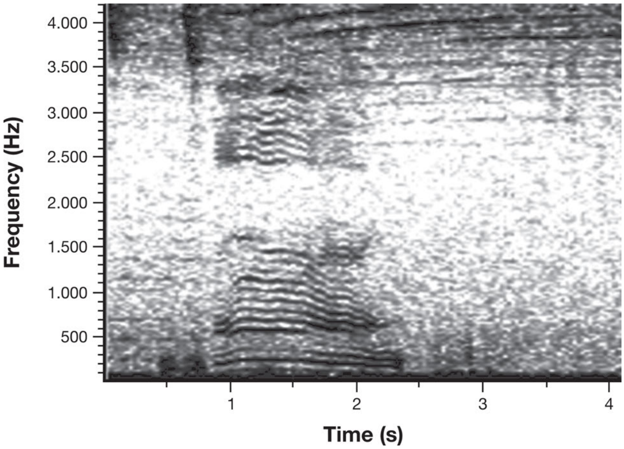

Figure 4.1. Spectrogram of a moo call.

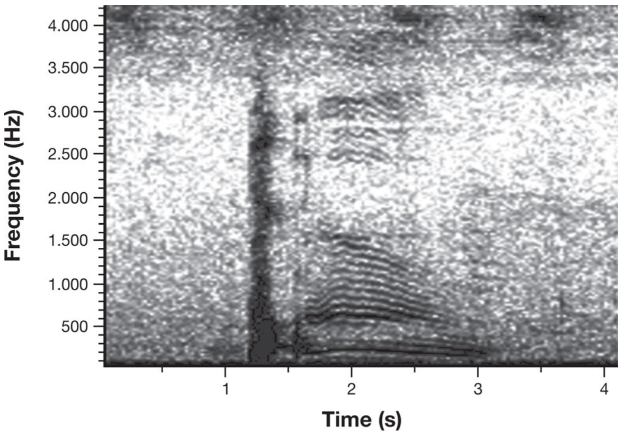

Figure 4.2. Spectrogram of a moo call preceded by a strong inspiration.

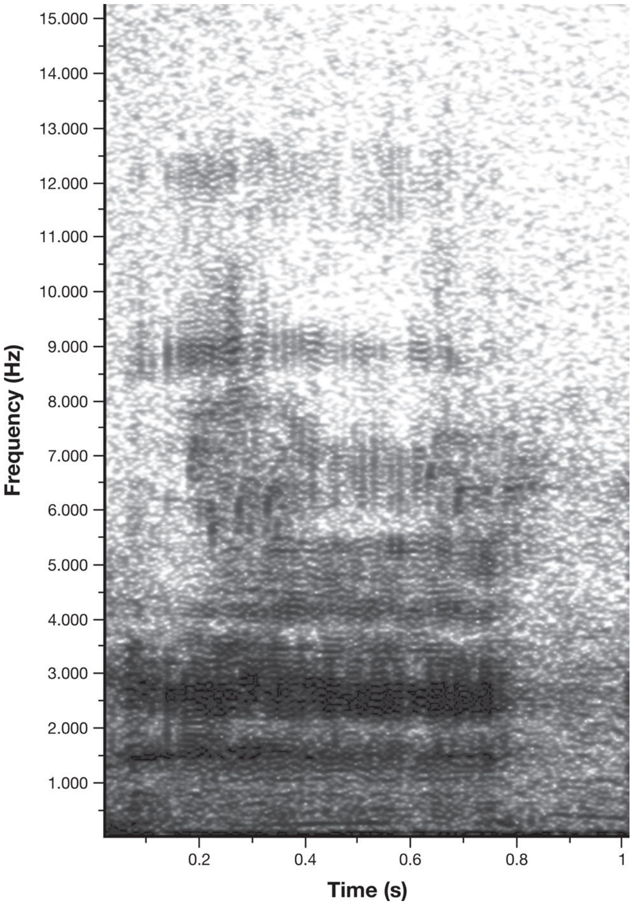

Figure 5. Spectrogram of an ar vocalization.

Ar. We recorded 96 ar calls in two different behavioral contexts: playful interactions and light aggressions between group members; 42 (43.5%) were during 15 play interactions, and 54 (56.2%) during 17 aggressive ones. We recorded this call in 18 different individuals representing all age classes and sexes (Tab. 1). This vocalization is given with the mouth open (Fig. 5; to listen: *http://macaulaylibrary.org/audio/177792/play*. This recording shows how a playful interaction between two members of one group graded into a light aggression when one of the individuals refused to continue playing).

Yells. We recorded 85 yells mostly during two different contexts: playful interactions and light aggressions between group members; nine yells (10.5%) were during eight play interactions, and 68 yells (80%) during 18 aggressive ones (Tab. 1) in 17 different individuals from all age classes and sex. This modulated and sharp vocalization is composed of a series of harmonics and contrary to the rest of the calls used in intra group communication in this species, yells can be very loud and the amplitude of the calls seem to be highly related to the motivational state of the vocalizing animal. We also recorded eight yells emitted on one single occasion by an infant female trying to be carried by her mother while traveling (Fig. 6; to listen: *http://macaulaylibrary.org/audio/177792/play*).

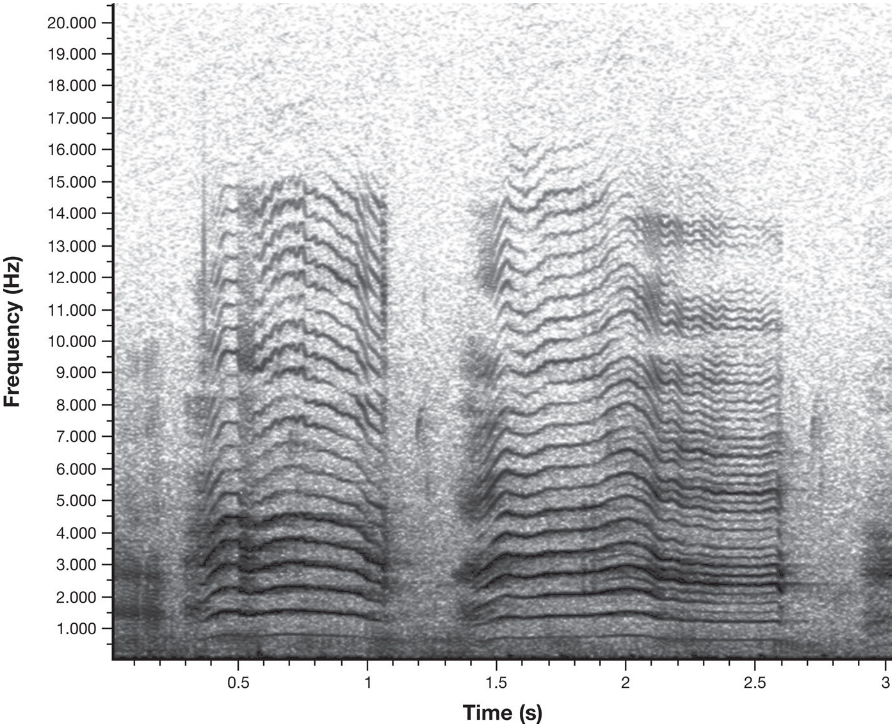

Figure 6. Spectrogram of a yell.

Screeches. We recorded 37 screeches; 22 (59%) were emitted during three aggressive interactions between three different individuals (juveniles) and 15 (40%) were emitted by two infants during a distress situation (Tab. 1). (Fig. 7; to listen: *http://macaulaylibrary.org/audio/177790/play*).

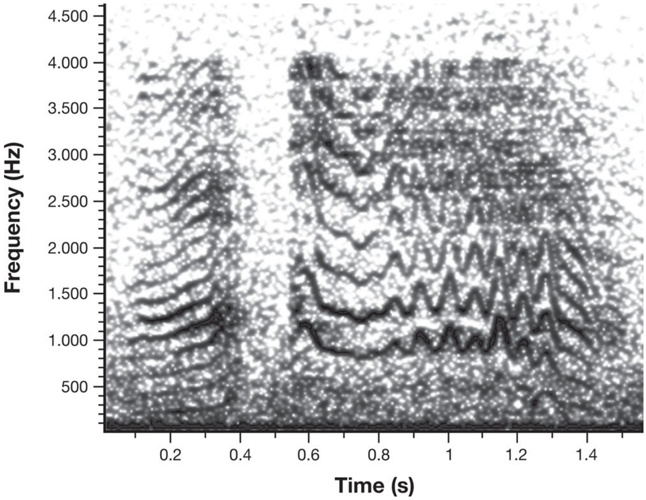

Figure 7. Spectrogram of a screech.

Oodle. We heard this vocalization on two different occasions, and recorded it only once. The quality of the recording was too poor to draw a spectrogram or to perform measurements. On both occasions the vocalization was emitted by the dominant male from the group, during an aggressive interaction between other individuals from its group. The male emitted the call while moving his head up and down (to listen: *http://macaulaylibrary.org/audio/177812/play*).

Discussion

During six consecutive months we recorded eight different types of short-distance vocalizations emitted in six different contexts by 26 individuals from two different groups of black and gold howler monkeys (*Alouatta caraya*) in the Atlantic Forest of Argentina.

Vocalizations emitted by *A. caraya* during feeding contexts were the hu-hu-hu and the heh calls. The hu-hu-hu was recorded in previous studies in the same context (Calegaro-Marques and Bicca-Marques, 1997; Orué, 2005). The consistency of the context in which this call was recorded strongly indicates that this call is related to food and feeding activity. However, future studies including playback and field experiments (see Di Bitetti, 2003; 2005) will be necessary to back up our results and to test some hypotheses in relation to the possible function of this vocalization. It would be interesting to explore if this call is related to "happy" or relaxed moments (based on the hearing this call after the group reunited with the dominant male) and function as an indicator of the emotional state of the vocalizing individual. The heh calls recorded here in association with feeding bouts were not described in previous studies in howler monkeys. This call was extremely subtle and very difficult to hear in the field; the use of headphones in the field was an important aid for its detection. Heh calls can be interpreted as a type of *contact call*. Contact calls can be defined as vocalizations that are given in the vicinity and often visual presence of other group members and in many contexts during the daily activities from a group (Robinson, 1982; Di Bitetti and Wheeler, this volume). Some of the functions attributed to contact calls are regulation of use of space within group members (Robinson, 1982), attraction of other group members to a feeding source (Izawa, 1979) and/or reciprocal individual localization (Gautier and Gautier, 1977). The heh call recorded here could be considered to function in reciprocal individual localization during feeding bouts. Our perception in the field was that during feeding bouts, when an individual emitted this heh call, members of the same group which were also feeding seemed to answer to each other with more of these heh calls. This idea should be tested with solid data in future studies that test for the existence of a call-and-answer system in contact calls in howler monkeys (da Cunha and Byrne, 2009).

As in other monkey species, calls of *A. caraya* that are emitted when members are separated but within visual contact (like contact calls) can often grade into calls uttered when individuals are isolated from or loose visual contact with other group members (Robinson, 1982). Individuals that lost visual contact with their group were recorded giving mostly moo calls. According to our observations individuals that lost contact with the rest of the group start giving moo calls and then alternate the moo calls with moo calls preceded by a strong inspiration (Fig. 4.2). In this context, the gradation from moo to moo with inspiration could have a strong motivational basis and be related to the level of distress of the lost animal. The moo call has been recorded in previous studies in *A. caraya* (Calegaro Marques and Bicca Marques, 1997; Orué, 2005; da Cunha, 2005; da Cunha and Byrne, 2009; 2013). During this study we did not test the functionality of this call but in a previous study in Brazil it was suggested that the moo call is a vocalization conforming to the Reunite hypothesis (da Cunha and Byrne 2009; 2013): the emission of moo calls was higher when the study group was traveling (when the probability of getting lost increases), the group diameter was bigger than usual (with monkeys dispersed and probably out of view) and, group spread decreased after the emission of moo calls. We also recorded moo calls that were given by infants during infant distress situations. Previous studies recorded moos emitted during infant distress situations under different names too ("choros": Calegaro-Marques and Bicca-Marques, 1997; "moo call": da Cunha, 2005; da Cunha and Byrne, 2013; "lamentos" and "llantos": Orué, 2005). The data we presented here strengthen the results from a previous study with the black and gold howler monkey in Brazil; moo calls can function as a contact call for isolated individuals or as a distress call depending on the age of the caller (da Cunha and Byrne, 2013). Infants, early juveniles, and adult females of *A. palliata* gave *caws* and *wrah-ha* calls when out of contact with their troop (Baldwin and Baldwin, 1976). Caws graded into wrah-ha calls in *A. palliata* as moo graded into moo preceded by inspiration in *A. caraya*. Given the similarity in context, grading and structure we suggest that these are homologous short-distance vocalizations. A broad band contact call structurally different to the moo call described here but with a very similar context of emission described in *A. palliata* (Jones, 1998), was not found by us to have an equivalent in *A. caraya*.

The bark series, emitted during contexts of stress or tension, were previously described in this species (Orué, 2005). In this study, tension moments were only related to close encounters with observers, and no predator was observed during our study. In two other howler species, *A. paliatta* (Baldwin and Baldwin, 1976; "woofs") and *A. guariba clamitans* (Oliveira, 2002; Holzmann, 2012; "latidos"), bark series were given by adult males and females when observers were too close. A possible bark ("faltering vocalization"; no acoustic description provided) was uttered by the oldest male mantled howler during an encounter with a group of tayras (*Eira barbara*) (Asencio and Gómez-Marín, 2002). Encounters of howler monkeys with predators, as described in the literature, usually elicit long distance vocalizations (roars) but no soft calls (Eason, 1989; Peres, 1990). Future studies shall elucidate whether bark series really function as an anti-predator signal by informing the predator that it has been discovered or by alerting other troop members that a predator is nearby, as it has been observed in capuchins (*Sapajus nigritus*, Wheeler, 2010; Di Bitetti and Wheeler, this volume) and diana monkeys (*Cercopithecus diana diana*) (Zuberbhüler *et al.*, 1997). Bark series were also emitted in a different context by adult females (mothers of dependent infants) while moving, when followed by their own whining infant (*i.e.*, giving moo calls). Baldwin and Baldwin (1976) also described that adult male mantled howler monkeys emitted barks or "gentle woofs" to infants that were uttering caw calls (homologous to moo calls in *A. caraya*, see above) and were separated from their mothers. Future studies will shed light on this call and its relationship with interactions between adults and infants.

During aggressive interactions and play we recorded *ar* and yells. Yells and ar have been previously recorded in this species in similar contexts (Orué, 2005). Another study carried out in *A. caraya* also recorded vocalizations that were shared between aggressive and playful interactions named *stress screams* (Calegaro-Marques and Bicca-Marques, 1997). In a couple of captive mantled howler monkeys, Eisenberg (1976) also refers to a playful interaction call *grappling* in which individuals mock agonistic patterns, and he mentioned two types of calls: the *uh-uh* and the *growls*, but based on his description and the spectrograms, these vocalizations are not structurally similar to ar or yells. Screech calls occurring during aggressive interaction between group members were also recorded previously by da Cunha (2005) in *A. caraya*. He described this call within the *screech complex* associated with conflicts of interest between individuals of the same group.

The oodle call was given by an adult male to juveniles that were engaged in a light aggressive interaction. It seems that in this context, this call can function as a "call to order" and to try to put end to the aggressive and noisy interaction between his group members. The oodle vocalization seems to be homologous to the oodle described by Baldwin and Baldwin (1976) in *A. palliata* at the end of a roar or as a spontaneous call during day activities. During our study period we also heard the oodle vocalization as part of roaring sessions, at the end of long distance roars. Another study with *A. caraya* in Brazil mentioned an oodle described as a soft vocalization also emitted by the alpha male but that could be use as an "acoustic beacon" for individuals who lost visual contact with the members of its group (da Cunha and Byrne, 2009). The oodle also seems to agree with the description given by Wang and Milton (2003) of a call given during ritualized greetings between two males in *A. palliata*. The variety of behavioral contexts in which this vocalization was heard in the different howler species and study sites is confusing. More studies on the acoustic structure and the context of emission of these calls are necessary to clarify its evolutionary origin and function.

Our understanding of howler monkey short-range communication is still cursory. Eighty years have passed since Carpenter's (1934) founder study on the vocal behavior of howler monkeys. Ever since, the vast majority of studies focused on the long distance roars (Altmann, 1959; Baldwin and Baldwin, 1976; Sekulic, 1982a, b; Whitehead, 1987; 1989; Chiarello, 1995; Kitchen, 2004; Kitchen, 2006; da Cunha and Byrne, 2006; da Cunha and Jalles-Filho, 2007; Oliveira, 2002; Holzmann *et al.*, 2012; Hopkins, 2013; Van Belle *et al.*, 2013; 2014; Van Belle 2015; Briseño-Jaramillo *et al.*, 2015), with few trying to understand the usage and description of soft calls use in intra-group communication (Altman, 1959; Baldwin and Baldwin, 1976; Eisenberg, 1976; Calegaro-Marques and Bicca-Marques, 1997; Jones, 1998; da Cunha, 2005; Holzmann, 2012). Inaccessibility to recordings made by researchers working with short-distance calls and usage of divergent phonetical names for calls have resulted in difficulties in the interpretation of the characteristics of presumed homologous calls in howler monkeys. In order to construct a solid body of evidence that can be used to unambiguously describe and sort signals under consistent behavioral contexts (a pre-requisite to hypothesize on the ultimate function of the calls) it is fundamental that researchers include spectrograms in their publications and make their recordings and accompanying metadata accessible at scientific repositories. Finally, comparative studies should benefit from both, increased availability of recordings and more detailed spectrogram measurements that take into account the specific acoustic structure of howler calls (*i.e.*, formant dispersion, aggregate entropy, refined frequency grid measurements). More detailed description of each call can help clarify whether calls given in different behavioral contexts are actually the same or different calls.

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Part IV

CONSERVATION THREATS

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12 | *BERTIELLA* SP. INFECTION PATTERNS IN BLACK AND GOLD HOWLER MONKEYS ACROSS THEIR DISTRIBUTION

PATRONES DE INFECCIÓN POR *BERTIELLA* SP. EN MONOS AULLADORES NEGROS Y DORADOS A LO LARGO DE SU DISTRIBUCIÓN

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Abstract. Bertielosis is a zoonotic disease caused by infection with the cestode *Bertiella mucronata*. The definitive hosts of *B. mucronata* are non-human primates. In this study, we explore the relationship between *Alouatta caraya* and *Bertiella mucronata*. *Alouatta caraya* is the only species of the *Alouatta* genus infected by *B. mucronata* across its distribution. In our study site, we intensively sampled numerous howler monkeys. *B. mucronata* prevalence was high, independent of the habitat type or season. In a meta-analysis comparing patterns of infection across sites we observed no differences in prevalence due to sex or age. High prevalence of *B. mucronata* at all sites suggests that black and gold howler monkeys may serve as a viable reservoir for this tapeworm. These results highlight the need for future research into the epidemiology, cross-species transmission ecology, and clinical consequences of *B. mucronata* and other infectious agents not only in humans and livestock, but also in wild animals that coexist in the same environments.

Resumen. La bertielosis es una zoonosis producida por el parásito *Bertiella* sp. *Bertiella* sp. son parásitos de primates no-humanos que se encuentran tanto en simios como en monos de África, de Asia y de las Américas. En Sudamérica encontramos a *Bertiella mucronata*, cuyos hospedadores definitivos son efectivamente los primates. Se ha reportado anecdóticamente una asociación particular entre *B. mucronata* y los monos aulladores negros y dorados (*Alouatta caraya*), por lo cual decidimos explorar el patrón de infección de *B. mucronata* en diversas poblaciones de *A. caraya* a lo largo de su distribución. Además de sitios de trabajo propios, incluimos en este estudio una detallada búsqueda bibliográfica sobre las infecciones de *Bertiella* en poblaciones silvestres de *Alouatta* en Sudamérica. Nos propusimos evaluar cuantitativamente (cuando fue posible) y cualitativamente la variación de los patrones de infección por *Bertiella* en sitios del noreste Argentino y del este de Paraguay con diversos grados de impacto antropogénico, esto último basándonos en que las alteraciones antropogénicas sobre hábitats naturales tienen la capacidad de alterar la susceptibilidad a infecciones parasitarias y así también de modificar los riesgos de infección en poblaciones de primates silvestres. Se colectaron muestras fecales de individuos de *A. caraya* a lo largo de cinco sitios en Argentina y tres sitios en Paraguay con diferente grado de perturbación (remoto, urbano y rural), en forma estacional. Las áreas con colecta más intensiva fueron la Isla del Cerrito (N = 124–140 individuos remotos; N = 10 individuos urbanos) en la provincia del Chaco (27° 18' S, 58° 38' O), Argentina, y áreas de la Estación Biológica Corrientes y Parque provincial San Cayetano (N = 88–134 individuos rurales; N = 10–14 individuos urbanos) en la provincia de Corrientes (27° 33' S, 58° 40' O), Argentina. Se incluyen también en este análisis otros muestreos estacionales en Campo Las Lomas (CLL), provincia de Corrientes (27º 23' S, 58º 22' O), y la ciudad de Ayolas y la Reserva Isla Yacyretá, Departamento de Misiones (27º 25' S, 56º 47' O), Paraguay. La prevalencia de *Bertiella* sp. varió entre el 55% y cerca del 90% a través de los sitios y estaciones del año. En general no se encontraron diferencias significativas en niveles de prevalencia cuando se compararon machos y hembras e individuos maduros e inmaduros. El alto valor de prevalencia de *B. mucronata* en todos los sitios sugiere que los monos aulladores negros y dorados pueden servir como reservorio viable para esta tenia. Aunque reconocemos que nuestros datos pueden ser más completos, incluyendo un mayor número de poblaciones de aulladores y otros años de estudios repetidos, nuestros análisis sugieren que no hay diferencia cuando se consideran individuos de diferentes categorías sexo-etarias; y, por otro lado, las alteraciones antropogénicas del hábitat parecerían no alterar los patrones de infección por *Bertiella*, aunque hay una tendencia a una ligera disminución de las prevalencias en áreas más antropizadas. Cuando integramos estudios de literatura, se mantiene el mismo patrón; por lo tanto, sugerimos que la infección de *Bertiella* en niveles moderados, como se suele encontrar, parecería no tener un impacto en el estado de salud de los aulladores. La presencia de *Bertiella* en varias poblaciones a lo largo su distribución demostraría un largo período de coexistencia, asociado potencialmente con una gran capacidad de resistencia al parásito, sumado a una baja patogenicidad del mismo. Por otro lado, planteamos que estos parásitos podrían ofrecer ciertos beneficios a los aulladores: 1) pueden ocupar un nicho en los aulladores, con lo que impiden que otros parásitos similares, pero con mayor patogenicidad, colonicen su organismo (*A. caraya* es la especie que habita los boques más marginales en comparación con otras especies del mismo género), y 2) pueden estimular inmunidad contra otros cestodos similares, pero con mayor patogenicidad. Nuestros resultados resaltan la necesidad de establecer una línea de investigación en epidemiología, ecología de infección entre especies, las consecuencias clínicas de las infecciones por *B. mucronata* y otros agentes infecciosos, no solo en humanos y ganado, sino también en animales silvestres que coexisten en un mismo ambiente.

Introduction

Primates are particularly vulnerable to parasitic infections because many species live in cohesive social groups that facilitate their parasite transmission (Freeland, 1983; Stoner, 1996; Stoner *et al.*, 2006; Nunn and Altizer, 2006). Response to parasitism is expected to vary among primate species according to differences in ecology, behavior, and life history (Nunn and Altizer, 2006; Gillespie *et al.*, 2008), and there is also evidence for anthropogenic alteration of habitats affecting primate-parasite dynamics (Gillespie *et al.*, 2005a, b;

Gillespie and Chapman, 2006; Nunn and Altizer, 2006; Zommers *et al.*, 2013; see Kowalewski and Gillespie, 2009 and Martínez-Mota *et al.*, 2015 for a complete review of parasites in wild howlers). One of the dominant causes of wild primate population declines is deforestation of tropical and subtropical forests (Brown and Zunino, 1994; Gillespie *et al.*, 2004; Chapman *et al.*, 2005a, b; Gillespie and Chapman, 2008). Likewise, human population growth, changes in agricultural practices and the extraction of natural resources, as well as ecotourism, result in more contact between humans and wildlife that may contribute to the propagation of diseases (Stuart and Strier, 1995; Stoner, 2005; Vitazkova, 2009). However, it is known, that many parasite species may act as commensals with non-human primates, inflicting no apparent harm to their hosts (Howells *et al.*, 2011). For example, although *Giardia* sp. prevalence is high in different populations of black and gold howler monkeys in northern Argentina, there is a lack of association between infection and acute gastrointestinal symptoms (*i.e.*, diarrhea)or apparent clinical disease signs (Kowalewski *et al.*, 2011). Thus, despite the association of *Giardia* with clinical disease in humans in developed economies, there is mounting evidence that it may be commensal in rural settings where exposure may be common and occur at an early age. Howells *et al.* (2011) suggested that some protozoa present at high prevalence in chimpanzees served as an indicator of healthy gastrointestinal function and that they could be used as a proxy of health in chimpanzee populations. Numerous threats to the conservation of primate populations and their habitats stress the urgency of developing rapid and noninvasive proxies to evaluate the health status of populations. In this regard, it is of great importance to find commensal parasites of easy identification that may act as health status indicators of the individuals exposed to stressful situations (Howells *et al.*, 2011). Thus, patterns of infection with gastrointestinal parasites can provide an excellent proxy for the overall health of a population.

Howler monkeys are known to be the host of several types of parasites in the wild (see reviews in Kowalewski and Gillespie, 2009; Martínez-Mota *et al.*, 2015). *Alouatta caraya* is one of the 12 species of extant howlers (Cortes-Ortiz *et al.*, 2015). This species inhabits marginal habitats as most howlers do (Garber *et al.*, 2015), but it also occupies the southernmost distribution of the genus, reaching subtropical and temperate forests in northern Argentina. Although categorized as least concern by the IUCN (2008), *A. caraya* was categorized as vulnerable in Argentina (SAREM, 2012), due to several factors that include landscape change, introduction of monocultures and artificial forestations, pet trading and epidemics of yellow fever (Oklander *et al.*, 2010; Holzmann *et al.*,2010). Similar to other howler monkeys, black and gold howlers are frugivorous-folivorous (their diet can include a substantial proportion of leaves—especially in winter and in semi deciduous forests (Zunino, 1989; Bravo and Sallenave 2003; Kowalewski, 2007; Agostini *et al.*, 2012), they are arboreal and live in social groups that include multimale-multifemale, unimale and age-graded groups (Kowalewski and Zunino, 2004; Zunino *et al.*, 2007; Oklander *et al.*, 2010).

Members of the genus *Bertiella* are parasites of non-human primates, present in apes and Old World primates as well as in New World primates. *Bertiella mucronata* belongs to the family Anoplocephalidae, and are parasites that require oribatid mites, important members of the leaves and soil fauna, as intermediate hosts (Souza Júnior *et al.*, 2012). *Bertiella mucronata* is acquired through the ingestion of oribatid mites (Stunkard, 1940). Most human infections are by *B. studeri* (a member of the genus primarily occurring in Africa) and *B. mucronata*, which has been reported to infect children with some contact or association with non-human primates (Furtado *et al.*, 2012). New World primates were reported to present *B. mucronata*, including *Callicebus oenanthe* in Peru (Gómez-Puerta *et al.*, 2009), *Cebus* sp. and *Callithrix* sp. (Cabrera, 1939; Dunn, 1963; Denegri, 1985). *Alouatta caraya* has been reported to be the definitive host for *Bertiella mucronata* (Boero, 1967) and has not been reported in other howler species, except for members of *A. guariba* located in areas where they share forests with *A. caraya* (see Tab. 1). Indeed, local people in northern Argentina even call this parasite the "carayá taenia". Due to the particular relationship between *B. mucronata* and *A. caraya*, and its importance as a helminthic zoonosis (Denegri and Perez Serrano, 1997; Paçô *et al.*, 2003), we investigated the pattern of infection of *Bertiella mucronata* in several populations of *Alouatta caraya* across their distribution. We have also included a literature review of *Bertiella* sp. infections in wild howlers in South America.

In this study, we quantitatively (when possible) and qualitatively explore the variation of *Bertiella* sp. patterns of infection at sites with different degrees of habitat alteration. Following previous publications, we consider that anthropogenic alterations have the capacity to alter susceptibility to parasite infection and infection risk in resident primate populations (Gillespie *et al.*, 2005a; Gillespie and Chapman, 2006; 2008).

Methods

Literature revision

We reviewed all published articles, book chapters, and unpublished dissertations available through *Primatelit*, *Google Academics*, and *The Global Mammal Parasite Database* up to September 2014 that focused on the relationship of *Bertiella* sp. and *Alouatta caraya* and *A. guariba*, as these species are found living in sympatry in northern Argentina and southern Brazil (Cortes-Ortiz *et al.*, 2014). The results of this review are shown in Table 1 (*A. caraya*) and Table 2 (*A. guariba*), along with data that we collected from several sites in northern Argentina and Paraguay. Table 1 provides the following data: study site, year of sample collection, type of habitat, type of forest, season of collection, number of samples collected, number of individuals sampled, number of groups sampled, prevalence (measured as the proportion of individuals infected with *B. mucronata* over the total number of individuals sampled), type of analysis used to identify *B. mucronata*, and the referenced literature. We categorized forest as either fragmented, continuous, or urban, based on the definition provided in Marsh (2003). As such, fragments are defined as small (1–10 ha), medium (10–100 ha), and large (100–1,000 ha). Due to our limited database, we considered any forest with less than 1,000 ha as fragmented and forests over 1,000 ha as continuous. We considered urban forest to be those within villages or associated with weekend houses. Habitats were classified as one of three types: 1) remote: site almost or totally isolated from humans (we included sites without nearby human settlements, but we cannot exclude the possibility of humans going to the forest for hunting or other subsistence activities); 2) rural: close to rural population or fishing camps or regularly visited by people (we considered rural sites when human settlements were located nearby, assuming that interaction between humans and the forest was maintained on a regular basis, through for example, selective logging, shade for cattle, trails, and hunting); and 3) village: close to dense human populations, and intensely visited by tourists or local people—*i.e.*, non-protected areas, certain natural reserves, or national parks. Although we understand the limitation of such a comparison, we will assume that any natural variation in forest structure and composition among sites (within this region) is minimal relative to large-scale anthropogenic change in influencing the diversity of parasites present in howlers. When data were available we separated prevalence into males and females and mature and immature individuals (Tab. 3).

Study sites

Our samples were collected at five sites in northern Argentina and three sites in eastern Paraguay (Fig. 1). The study sites differ in type of habitat and forest (Tab. 1). The sites included are in northern Argentina: Isla Brasilera (IB), Chaco Province (27° 18' S, 58° 38' W); Estación Biológica Corrientes – Parque Provincial San Cayetano (EBCo, Biological Field Station Corrientes), Corrientes Province (27° 33' S, 58° 40' W); Cerrito (C), Chaco Province (27° 15' S, 58° 36' W); San Cayetano (SC), Corrientes Province (27° 34' W, 58° 43' W); and Campo Las Lomas (CLL) Corrientes Province (27º 23' S, 58º 22' W). In Paraguay, our study site was Ayolas City (A) and a reserve at Isla Yacyretá (IY) – Department of Misiones (27º 25' S, 56º 47' W).

Sample collection and laboratory analyses

Fecal samples were collected opportunistically from known individuals immediately after defecation and examined macroscopically for tapeworm proglottids. Samples were stored individually in 20 ml vials in 10% buffered formalin. Tubes were shaken to mix the sample with the formalin solution. Each tube was labeled with date of collection, observer, location, and animal identifier. Samples from IB, SC, EC, EBCo, A, and IY were analyzed by fecal flotation using a saturated solution of $NaNO_3$ and fecal sedimentation were performed on each sample to isolate helminth eggs, larvae, and protozoa cysts (Gillespie, 2006). Slides (18 mm × 18 mm) prepared according to each method (one from flotation and 2 from sedimentation) were examined with compound microscope after the addition of a drop of Lugol's iodine. Parasites were identified on the basis of egg coloration, shape, contents, and size. Each slide was scanned thoroughly under both 10 × and 40 × objective lenses to confirm presence or absence of helminth eggs. Representatives of each parasite species recovered per sample were measured at 400 × to the nearest 0.1 µm with an ocular micrometer, and unknown and representative parasite species were photographed. Samples from CCL were collected in the same fashion, but techniques of concentration by sedimentation (Ritchie) and by flotation (Willis) were used (Becerril Flores and Romero Cabello, 2004).

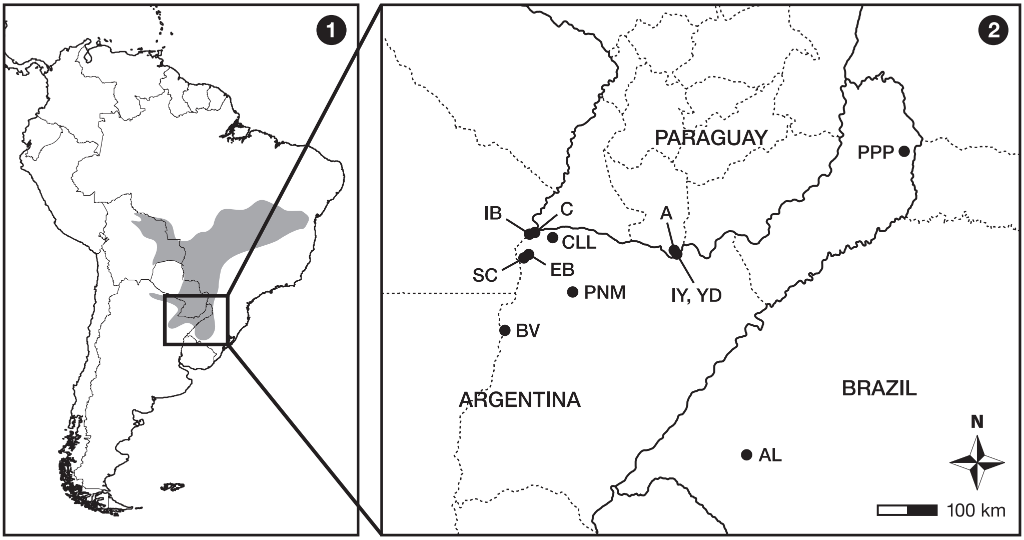

Figure1. Location of the study sites and all the reviewed sites in Table 1 and Table 2. **1.** Geographical distribution of *Alouatta caraya* in South America. **2.** Sites considered in this study in reference to *A. caraya* distribution.

Statistical analysis

Meta-analysis of published studies was challenging due to low sample size; however, when possible, we used G tests and Mann Whitney U tests to explore patterns of infection between males and females and mature and immature individuals (Sokal and Rohlf, 1995). To improve sample size, results from fecal analyses and post-mortem examinations were pooled. Making comparisons with studies conducted at different scales proved to be difficult. Despite these challenges, the results indicate the direction of important trends in the relationship between patterns of parasitism and certain environmental factors. All tests are with alpha set at 0.05.

Results

Our previously unpublished data provided a robust database for examining *Bertiella* infection patterns affected by seasonality and inter-habitat variability. During three seasons (spring, SP; summer, SU, and fall, F) several groups were visited and at least three samples per individual were collected. IB was visited during the three seasons, and we sampled 135 individuals in SP, 124 in SU, and 140 in F. *Bertiella* prevalence was 86%, 50%, and 90%, respectively. At the rural site, EBCo, we monitored 94 individuals in SP, 134 in SU, and 88 in F and recorded a prevalence of 86%, 45%, and 91%. Finally, at the village site we sampled 20 individuals in SP, 34 in SU, and 34 in F and noted a *Bertiella* prevalence of 80%, 68% and 10%, respectively. Thus, the prevalence of *B. mucronata* was always high, independent of season or site (Prevalence RE = 75% ± 22; RU = 74% ± 25; VI = 53 ± 37; Tab.1).

Table1. Characterization of study sites where samples of *Alouatta caraya* were analyzed and *Bertiella mucronata* prevalence was estimated.

Study Site	**Year**	**Habitat**	**Forest**	**Season**	**# samples**	**# ind**	**# groups**	**P (%)**	**Lab**	**Source**
Isla Brasilera (IB), Chaco, ARG | 27° 18' S, 58° 38' W	2008 / 09	RE	C	SP	405	135	22	86	1	This study
				SU	372	124	25	50	1	This study
				FA	420	140	22	90	1	This study
	2000	RE	C	–	29	29	–	21	2	Santa Cruz *et al.*, 2000a
	2000	RE	C	–	14	14	–	+	3	Santa Cruz *et al.*, 2000a
EBCo, Corrientes, ARG | 27° 30' S, 58° 41' W		RU	F	SP	279	93	28	86	1	This study
				SU	402	134	28	45	1	This study
				FA	264	88	20	91	1	This study
EBCo, Corrientes, ARG | 27° 30' S, 58° 41' W	1975	RU	F	–	51	51	–	11.7	3	Coppo *et al.*, 1979
San Cayetano, Corrientes, ARG | 27° 30' S, 58° 41' W	2000	RU	F	–	21	21	–	+	3	Santa Cruz *et al.*, 2000a
Yacyretá dam (YD), Corrientes, ARG | 27° 28' S, 56° 44' W	2000	RE	F	–	9	9	–	+	3	Santa Cruz *et al.*, 2000a
San Cayetano (SC), Corrientes, ARG | 27° 34' S, 58° 43' W		VI	U	SP	60	20	6	80	1	This study
plus El Cerrito (EC), Chaco, ARG | 27° 18' S, 58° 38' W				SU	102	34	3	68	1	This study
				FA	102	34	8	10	1	This study
Co. Las Lomas (CLL), Corrientes, ARG | 27° 23' S, 58° 22' W		RU	F	Across 1 yr	50	20–26	2	12	1	Milozzi *et al.*, 2012
Bella Vista (BV), Corrientes, ARG | 28° 30' S, 59° 03' W	1966	RE	C	–	84	84	–	7	3	Pope 1966
PN Mburucuyá (PNM), Corrientes, ARG | 27° 58' S, 58° 08' W	2010	RE	F	SU–WI	57	31	4	68	1	Violi 2012
PP Piñalito (PPP), Misiones, ARG | 26° 30' S, 53° 50' W	2010	RE	C	SP	20	6–7	1	40	1	Kowaleswski, unp. data
Ayolas (A), Misiones, PAR | 27° 23' S, 56° 49' W	2012	VI	U	Across 1 yr	19		2	26	1	This study
Isla Yacyretá (IY), Misiones, PAR | 27° 25' S, 56° 47' W	2012	RU	F	Across 1 yr	29		2	24	1	This study
		RE	F	Across 1 yr	25		2	8	1	This study
Alegrete (AL), Rio Grande do Sul, BRA | 29° 47' S, 55° 47' W	2005 / 06	VI	U	Dec–Jul	88	2	1	70	4	De Oliveira *et al.*, 2011
Alegrete (AL), Rio Grande do Sul, BRA | 29° 37' S, 56° 17' W	2012	RU	F	Feb–Jul	473	10	1	50–91	4	Souza Jesus 2013

Habitat type: RE, remote; **RU**, rural; **VI**, village. **Type of forest: C**, continuous; **F**, fragmented; **U**, urban. **Season: SU**, summer; **SP**, spring; **WI**, winter; **F**, Fall. **Lab: 1**, Sodium nitrate / Detergent; **2**, Modified Sheather / Ritchie; **3**, Post-mortem inspection; **4**, Willis. A study by Santa Cruz *et al.* (2000a) did not inform prevalence but only presence or absence of the parasite.

In our review, we found two studies that included samples collected from IB, almost 10 years earlier (Tab. 1). Although sampling effort was lower (43 individuals, 1 sample per individual), *B. mucronata* was reported to be infecting black and gold howlers. We also found two studies that were conducted at EBCo, from 2000, in which 21 individuals were sampled, and although *B. mucronata* was found, prevalence was not provided. In 1975, 51 individuals were analyzed post-mortem in this area and *B. mucronata* was found in at least six individuals. Another study conducted during one year on two groups in CLL, a rural site similar to EBCo and located approximately 40 km NE away, reported a prevalence of 12% (only 20–26 individuals were sampled, with a total of 50 fecal samples collected by CM). In a different study conducted at the Parque Nacional Mburucuyá, approximately 80 km southeast from EBCo, Violi (2014) studied four groups (31 individuals) from which a total of 50 fecal samples were collected. In this study, a prevalence of 68% was found. A study conducted in Bella Vista (120 km southwest from EBCo) by Pope (1966), reported that 7% of the 84 individuals killed were infected with *B. mucronata*. Two sites were sampled in Paraguay approximately 175 km E from EBCo, and 74 samples from 74 individuals belonging to 29 groups were collected during a year, resulting in a prevalence of 8–26% (samples collected by JT). Two studies conducted separately in Brazil reported a prevalence of 70% and 50–91%. Both studies were concentrated on 1 group (see Tab.1).

We provide data of two sites with *A. guariba* (Tab. 2). At both sites, *A. guariba* lives in contact with *A. caraya*. A study from Brazil reported a prevalence of 43% based on seven post-mortem *A. guariba* individuals coming from different sites on the Itajaí-Açu River Valley region, which included Blumenau, Ascurra, Indaial and Pomero towns in Brazil (see Souza Júnior *et al.*, 2012 for details). At a different site where *A. caraya* live in sympatry with *A. guariba*, we estimated a prevalence of 26% after analyzing 40 samples from 15 individuals (Tab.2).

Table 2. Characterization of study sites where samples of *Alouatta guariba* were analyzed and *Bertiella mucronata* prevalence was estimated (see Table 1 for details).

Study Site	Year	Habitat	Forest	Season	# samples	# ind	# groups	P (%)	Lab	Source
PP Piñalito (PPP), Misiones, ARG | 26° 30' S, 53° 50' W	2010	RE	C	SP	40	7–8	1	26	1	Kowalewski, unp. data
Municipalities, Santa Catarina, BRA	2005–2006	RU	F	–	7	7	7	43	4	Souza Júnior *et al.* 2012

Data in Table 1 denotes that we still need intense sampling on several individuals to understand the dynamics of *B. mucronata* and *A. caraya*. Although sample-size and variation in inter-study design limited the strength of our analyses, some patterns of importance emerged. Even though prevalence differed across sites and seasons, *B. mucronata* was present at all sites where *A. caraya* samples were analyzed. Where sampling was intense, *B. mucronata* exhibited moderate to high prevalence in black and gold howler monkeys—reaching almost 95% of individuals sampled. Interestingly, *B. mucronata* prevalence did not differ relative to habitat type ($\chi2 = 113.6$, $p > 0.05$; MK unpublished data). *Bertiella mucronata* prevalence did not differ between males and females (Mann-Whitney U test, $U = 13$, $N_{1,2} = 6$, $p > 0.05$; Tab. 3; Fig. 2), nor between immature and mature individuals (G test, $Gadj = 1.5$; $df = 3$, $p > 0.05$; Tab. 3; Fig. 3). Table 2 includes the first reports of *Bertiella* infection in *A. guariba* at both sites where *A. caraya* is present.

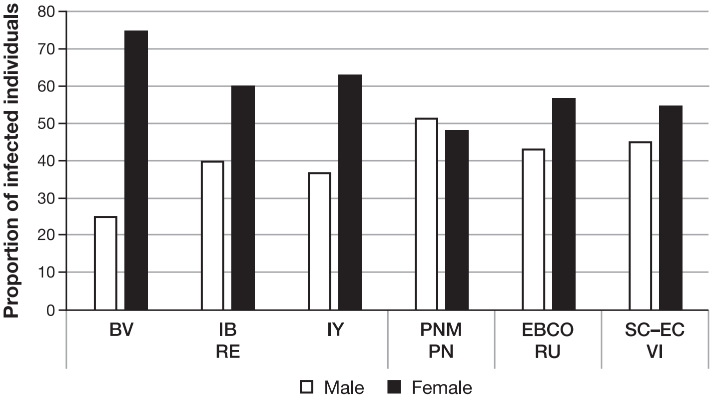

Figure 2. Proportion of infected male and female individuals of *A. caraya* with *Bertiella mucronata* (type of habitat: **RE**, remote; **RU**, rural; **VI**, village; **PN**, national park; **BV**, Bella Vista; **IB**, Isla Brasilera; **IY**, Isla Yacyretá; **PNM**, Mburucuyá National Park; **EBCo**, Estación Biológica Corrientes; **SC–EC**, San Cayetano and El Cerrito).

Table 3. Prevalence of *Bertiella mucronata* in males and females, and in mature and immature individuals of *Alouatta caraya*.

Study Site		**Sex category**		**Age category**	
		Male	**Female**	**Mature**	**Immature**
Bella Vista (BV), Corrientes, ARG 28° 30' S, 59° 03' W (Pope, 1966)	Individuals	51	33	–	–
	Prevalence (%)	4	12	–	–
PN Mburucuyá (PNM), Corrientes, ARG 27° 58' S, 58° 08' W (Violi, 2014)[1]	Individuals	7	7	14	11
	Prevalence (%)	16	15	18	2
EBCo, Corrientes, ARG 27° 30' S, 58° 41' W (this study). Fall, 2008	Individuals	39	49	62	27
	Prevalence (%)	35	46	26	55
Isla Brasilera (IB), Chaco, ARG 27° 18' S, 58° 38' W (this study). Fall, 2008	Individuals	56	84	90	49
	Prevalence (%)	50	76	42	84
San Cayetano – El Cerrito towns (this study). Fall, 2008	Individuals	15	19	26	8
	Prevalence (%)	14	17	8	28
Isla Yacyretá (IY), Misiones, PAR 27° 25' S, 56° 47' W (this study)	Individuals	29	25	40	15
	Prevalence (%)	14	24	15	27

[1] Number of individuals is informed but number of samples per individual is not informed. See Table 1 for details.

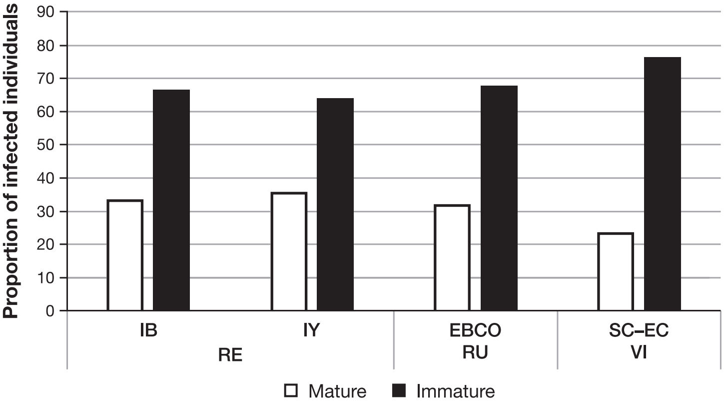

Figure 3. Proportion of infected mature and immature individuals of *A. caraya* with *Bertiella mucronata* (type of habitat: **RE**, remote; **RU**, rural; **VI**, village; **IB**, Isla Brasilera; **IY**, Isla Yacyretá; **EBCo**, Estación Biológica Corrientes; **SC–EC**, San Cayetano and El Cerrito).

Discussion

Bertiella mucronata is ubiquitous across the distribution of black and gold howlers in Argentina, Paraguay, and Southern Brazil (we did not find any data from Bolivia). This information is supported by investigations that have screened fecal samples or inspected digestive systems post-mortem. The wide distribution of this parasite is unique among the parasites hosted by black and gold howlers (see review Kowalewski and Gillespie, 2009, and Martínez-Mota *et al.*, 2014). Pope (1966) suggested that the higher incidence of *B. mucronata* in older howler monkeys substantiates the acquisition of this tapeworm as a function of time and a folivorous diet. Although we recognize that our database is limited, our meta-analysis suggests that there are no significant differences in prevalence between males and females or mature and immature individuals. In addition, anthropogenic disturbance does not appear to alter patterns of prevalence with the parasite. These results are in accordance to what Young *et al.* (2013) suggested about conservation of biodiversity being detached from any protection of primates from parasitic infection.

In her study of 1996 in northern Argentina, Pope euthanized 84 individuals and explored their digestive tracts (Tab. 1). She found that five of the six *B. mucronata* infections were in mature animals, the sixth was in a late juvenile, and no infections occurred among the younger animals (N = 12). Dunn (1963) mentions the relationship between copious leaf-eating and tapeworms such as *B. mucronata* that have free-living oribatid mites as vectors roaming on leaves. The higher incidence of these tapeworms in older howler monkeys from one population substantiates the acquisition of this tapeworm as a function of time and a leaf diet for this particular group of animals (see trend in Tab. 3). In a previous study, Santa Cruz *et al.* (2000a) conducted a study at three different sites, two of which were also sampled in this study (IB and EBCo, see Methods). They sampled 44 animals: 14 from IB, 21 from EBCo, and 9 from a site 200 km East from EBCo on the Paraná River – YF (animals rescued from a dam project) and analyzed fecal samples using a modified Richtie's method for flotation and simple sedimentation (Santa-Cruz *et al.*, 2000b). They found at

IB: *Bertiella mucronata* (prevalence of 7.14%), and at the YF site *B. mucronata*, *Strongyloides* sp. and Oxiuridae (prevalence 44.4%).

When studying a population intensely over time, we found that *B. mucronata* prevalence was high across the black and gold howler population distribution. Moreover, it does not seem that *Bertiella* infection at moderate levels has an impact on the health status of the black and gold howlers. In addition, *Bertiella* presence in howlers across multiple populations suggests a long period of coexistence, potentially associated with greater resistance to the parasite and reduced pathogenicity of the latter (Garnick, 1992; Souza Jesus, 2013). They may provide two types of benefits to the howlers: 1) they may occupy a niche in howlers that hinder other pathogenic parasites from colonizing (*A. caraya* is the species that occupy the most marginal forest in the genus distribution), and 2) they may stimulate immunity to similar but pathogenic cestodes.

The genus *Bertiella* (Stilles and Hassall, 1902) includes a large number of species that parasitize several mammals, including humans, in Africa, Asia, Australia, and the Americas (see Denegri *et al.*, 1998). The zoonotic infection of humans occurs in a similar way (by accidental ingestion of the intermediate host infected with cysticercoids) principally in areas in which the population co-exists with non-human primates (Denegri and Perez-Serrano, 1997; Denegri *et al.*, 1998; Paçô *et al.*, 2003). However, human cases of *Bertiella* infection are rare and data on these are scarce (Denegri 1997; Denegri and Perez-Serrano, 1997; Furtado *et al.*, 2012). With so few human cases of bertiellosis known, the clinical aspect of this disease is difficult to define. Cases are often asymptomatic; however, some patients have experienced abdominal discomfort, anorexia, vomiting, or loose or fatty stools (Furtado *et al.*, 2012). Due to the fact that *B. mucronata* infections are zoonotic, we need to pay attention to this parasite infection in areas of interface with humans where howlers show high prevalence, as, for example, in urban forest and village sites (Tab.1). High prevalence of *B. mucronata* at all sites suggests that black and gold howler monkeys may serve as a viable reservoir for this tapeworm. These results highlight the need for future research into the epidemiology, cross-species transmission ecology, and clinical consequences of *B. mucronata* and other infectious agents not only in humans and livestock, but also in wild animals coexisting in same environments.

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13 THE BROWN HOWLER MONKEY (*ALOUATTA GUARIBA CLAMITANS*) IN ARGENTINA: CURRENT STATUS, THREATS AND CONSERVATION CHALLENGES

EL MONO AULLADOR MARRÓN (*ALOUATTA GUARIBA CLAMITANS*) EN ARGENTINA: ESTADO ACTUAL, AMENAZAS Y DESAFÍOS PARA LA CONSERVACIÓN

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Abstract. The brown howler monkey (*Alouatta guariba clamitans*) is an endemic species of the Atlantic Forest of Brazil and Argentina. It persists at extremely low densities over an area of < 10.000 km² in the central-eastern portion of Misiones Province, and its presence has been confirmed in five small, strictly-protected areas. The species is classified as Critically Endangered for Argentina. The objectives of this contribution are to review our knowledge on brown howler's status, analyze the main threats and challenges that affect the species in Misiones and recommend some necessary steps for the development of a conservation strategy for this population. Most of our knowledge on the brown howler in Argentina comes from a study conducted at Parque Provincial El Piñalito between 2005 and 2007. Following the 2008–2009 yellow fever outbreak, brown howlers have been decimated throughout the region. Brown howlers have been affected by progressive and severe habitat loss in Misiones. In addition, as for all members of the genus, this species is particularly susceptible to epidemic diseases such as yellow fever. Thus, recurrent yellow fever epidemics coupled with the high susceptibility of howlers to this disease may be the most important threat to the persistence of this small population in Argentina. Finally, these two threats, habitat loss and yellow fever, are further compounded by a lack of awareness among governmental, non-governmental institutions and general public. A workshop held in 2013 on brown howlers in Argentina that used Population Viability Analysis as a tool indicates yellow fever as the main threat to this brown howler population persistence. The threat analysis, focused on the dynamics of yellow fever outbreaks and their impact on howler populations, led to the identification of gaps in knowledge that helped prioritize objectives and actions for the development of a Species Conservation Strategy in Argentina.

Resumen. El mono aullador marrón (*Alouatta guariba clamitans*) es una especie endémica del Bosque Atlántico de Brasil y Argentina. Este primate persiste en densidades extremadamente bajas en un área de menos de 10.000 km² en la porción centro-oriental de la provincia de Misiones y su presencia ha sido confirmada en cinco pequeñas áreas estrictamente protegidas. La especie ha sido clasificada como En Peligro Crítico para Argentina. Los objetivos de esta contribución son revisar los conocimientos sobre el status de los aulladores marrones, analizar las principales amenazas que afectan a la especie en Misiones y recomendar algunos pasos necesarios para el desarrollo de una estrategia de conservación para esta población. Aunque la sistemática del género *Alouatta*, y en particular de *A. guariba*, no está todavía bien definida, hay información disponible sobre comportamiento y ecología de algunas poblaciones de monos aulladores marrones en fragmentos de selva en Brasil. Para Argentina, donde todavía permanece un gran remanente de hábitat, existen pocos registros de presencia de la especie y las densidades poblacionales son muy bajas. La mayor parte de nuestros conocimientos sobre el aullador marrón en Argentina viene de un estudio llevado a cabo en el Parque Provincial El Piñalito entre 2005 y 2007. En este sitio, el aullador marrón coexistía con su congénero, el aullador negro y dorado (*Alouatta caraya*), con el cual compartía gran parte de su nicho trófico y espacio-temporal. En 2008–2009 un brote de fiebre amarilla diezmó a los aulladores marrones en toda su área de distribución en Argentina y sudeste de Brasil. Dadas la condiciones precarias de esta especie en Misiones, debidas a su escasa densidad y reducida distribución, es probable que este último brote de fiebre amarilla haya llevado al aullador marrón al borde de la extinción en Argentina. Los resultados de dos relevamientos sucesivos en la época posterior al brote han confirmado esta sospecha. Mientras en 2008 todavía se obtuvieron registros de la presencia de la especie en algunas áreas protegidas y no protegidas, ya en el 2010 no se obtuvo ninguna evidencia directa de la presencia del aullador marrón en Misiones, y las entrevistas a pobladores locales plantearon un escenario poco alentador para la conservación de la especie. Con el fin de establecer las prioridades de conservación para el aullador marrón y su hábitat en la Argentina, es crítica una evaluación de la situación actual de la población de dicha especie y las principales amenazas a las que se enfrenta. Este paso es necesario para desarrollar e implementar planes de manejo y conservación eficaces que aumenten la probabilidad de supervivencia de esta población en el mediano-largo plazo. Para avanzar en esta dirección, en marzo de 2013 realizamos el primer taller sobre conservación de aulladores marrones en Argentina. A través de la facilitación aportada por miembros de la IUCN/SSC's Conservation Breeding Specialist Group (CBSG), desarrollamos unos modelos de viabilidad poblacional, utilizando los softwares *Vortex* y *Outbreak*. Estas herramientas nos permitieron explorar cómo distintos parámetros biológicos y demográficos de los aulladores marrones, así como varios factores relacionados a la epidemiología de la fiebre amarilla, pueden afectar la probabilidad de extinción de esta especie en Argentina. El aullador marrón ha sido afectado por una pérdida progresiva y severa de hábitat a través de Misiones, siendo el Bosque Atlántico uno de los biomas más amenazados a nivel mundial. Además, como todos los miembros de su género, esta especie es particularmente susceptible a enfermedades epidémicas como la fiebre amarilla. Esta enfermedad, causada por un Flavivirus transmitido por mosquitos, afecta principalmente a primates humanos y no-humanos. Pero los aulladores, comparados con otros grupos de primates, desarrollan muy rápidamente la enfermedad y mueren en un breve lapso de tiempo. De esta forma, los aulladores actúan como «centinelas epidemiológicos» para la salud pública: sus mortandades masivas alertan en forma temprana a la población humana sobre la presencia del virus en una determinada zona. La discusión entre especialistas y los resultados de

los modelos elaborados durante el taller confirmaron que epidemias recurrentes de fiebre amarilla, junto con una elevada susceptibilidad de los aulladores a esta enfermedad, constituyen actualmente la amenaza más importante para la persistencia de esta pequeña población en Argentina. La mortandad por fiebre amarilla, sumada a la pérdida de hábitat, son agravadas por la falta de conciencia entre las instituciones gubernamentales, las ONG y la opinión pública. En el taller, el análisis enfocado en las dinámicas de la fiebre amarilla y su impacto en las poblaciones de aulladores ha llevado a la identificación de lagunas de conocimiento, permitiendo priorizar objetivos y acciones para el desarrollo de una Estrategia de Conservación para la Especie en Argentina. Algunas de estas acciones están enfocadas en los aulladores (p.ej., estimación de densidad y distribución actual y potencial de la especie en Misiones; implementación de un sistema de vigilancia, monitoreo y estudios de salud de las sub-poblaciones remanentes), mientras que otras abarcan temas relacionados a la fiebre amarilla y sus vectores (p.ej., captura e identificación de especies de mosquitos potenciales vectores del virus de la fiebre amarilla; intentos de aislamiento del virus de la fiebre amarilla de mosquitos adultos y larvas y de otros potenciales hospedadores o reservorios de la fiebre amarilla). Finalmente, mantener una población de aulladores saludable no es solamente una cuestión de conservación de la especie y de la biodiversidad, sino que también es importante para la salud pública. Debido a su rol de centinelas que alertan de manera temprana sobre la presencia de epidemias de fiebre amarilla, la presencia de aulladores en la selva puede contribuir a salvar muchas vidas humanas.

Introduction

Two species of howler monkeys live in Argentina: the black and gold howler (*Alouatta caraya*) and the brown howler (*Alouatta guariba clamitans*). The black and gold howler monkey inhabits the more humid portion of the Chaco region of Argentina (Rumiz, 1990; Brown and Zunino, 1994), and extends east to the Atlantic Forest of Misiones (Di Bitetti, 2003).

The brown howler monkey (*Alouatta guariba*; Fig. 1) is an endemic primate species of the Atlantic Forest, ranging from the Brazilian states of Bahia and Espirito Santo in the north to Rio Grande do Sul and the Argentinian province of Misiones in the south (Kinzey, 1982) (Fig. 2.1). Brown howlers have been recently re-classified globally from Near Threatened to Least Concern by the IUCN due to the presence of the species in most of the extant conservation units of the Atlantic Forest in Brazil. However, the population trend is still "decreasing" and the future of this species is quite uncertain, since the spatial extent of Brazilian Atlantic Forest is dramatically reduced and fragmented, and the species is threatened by hunting and epidemic diseases such as yellow fever (Mendes *et al.*, 2008). Furthermore, the species is listed on CITES Appendix II (CITES, 2013).

In Argentina, the brown howler (*Alouatta guariba clamitans*) occupies an area of < 10.000 km² concentrated in the central-eastern highland region of Misiones (Di Bitetti *et al.*, 1994; Rylands *et al.*, 1996; Di Bitetti, 2003) (Fig. 2.2). This species has been re-classified from "endangered" to "critically endangered" (Agostini *et al.*, 2012a) by the Argentinian Society for the Study of Mammals (SAREM) due to its small size, restricted distribution, and the suspected dramatic effects of the last yellow fever outbreak. Further, in Misiones it has been declared a Provincial Natural Monument by a provincial law (Ley XVI–56 [previously Ley #3455]).

Figure 1. Adult male and female (left), and adult male (right) brown howler monkey (*Alouatta guariba clamitans*) in Parque Nacional El Piñalito. Photos by Ilaria Agostini.

In Argentina, well established long-term studies have been carried out and are still ongoing on black and gold howler monkeys (*e.g.*, Zunino and Kowalewski, 2008; Oklander *et al.*, 2010) as well as black horned capuchin monkeys (*Sapajus nigritus*, *e.g.*, Di Bitetti, 2005; Janson *et al.*, 2012) and Azara's night monkeys (*Aotus azarae*, *e.g.*, Fernández-Duque, 2011; Fernández-Duque and Huck, 2013). In contrast, after the hooded capuchin monkey (*Sapajus cay*), which inhabits the Yungas region in northwestern Argentina, the brown howler monkey can be considered the second most neglected primate species in this country. Little information is available for this population and most of it comes from a three-year long study carried out in Parque Provincial El Piñalito (Agostini *et al.*, 2008; 2010 a, b; 2012b; Holzmann *et al.*, 2012) and two subsequent population surveys conducted in Misiones (Agostini *et al.*, 2015; Holzmann *et al.*, 2015). The aim of this chapter is to review the status of the Argentinian brown howler population, evaluate its main threats and the major challenges faced in its conservation efforts, and finally propose some recommendations.

Brown howler monkey nomenclature and systematics

Taxonomy of the brown howler monkey is somewhat confusing and not totally defined. Commonly, the species is named *Alouatta guariba* or using the synonym *Alouatta fusca.* Rylands and Brandon-Jones (1998) and Gregorin (2006) discussed the validity of these two alternative names for brown howler monkeys. While the first two authors indicated that *guariba* (Humboldt, 1812) is the correct name, the second author sustained that *fusca* (Geoffroy, 1812) is the correct one. Since Humboldt's nomenclature anticipated Geoffroy's by two months, Rylands and Brandon-Jones (1998) consider *guariba* as a senior synonym of *fusca.* From Ihering (1914) onward, brown howler monkeys have been separated into two different subspecies: the northern (*A. guariba guariba* = *A. fusca fusca*), and the southern brown howler (*A. guariba clamitans* = *A. fusca clamitans*). The northern subspecies is restricted to the Brazilian states of Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro. The southern subspecies occurs throughout the Atlantic coast from Rio de Janeiro and Minas Gerais, through São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul in Brazil and Misiones Province in Argentina (Rylands *et al.*, 2000; Groves, 2001). The distributional limit of the two subspecies is somewhat confused, but probably corresponds to the Jequitinhonha River, in Minas Gerais (Rylands *et al.*, 1996). Gregorin (2006), studying cranial and hyoid apparatus morphology, as well as differences in patterns of pelage color and karyotypes, proposed that the two brown howler subspecies should be considered two full species (*i.e.*, northern: *A. fusca*; southern: *A. clamitans*). Finally, even within southern brown howlers, Harris *et al.* (2005) found considerable genetic differences between populations in southern Brazil—from Rio de Janeiro on the one hand and Santa Catarina on the other. This discovery has led these authors to argue that further research may result in the recognition of three species. Although the debate is still open, we opt for maintaining the subspecific classification of Rylands *et al.* (2000) and Groves (2001; 2005) for the northern and southern brown howler until the taxonomy becomes well-defined.

Brown howlers' biological, behavioral and ecological traits

The brown howler monkey is an arboreal, diurnal, and relatively large-bodied primate (average adult female = 4.55 kg, average adult male = 6.18 kg; reviewed by Di Fiore and Campbell, 2007). Adult individuals are sexually dimorphic in body size (*i.e.*, males are larger than females); besides adults of the southern subspecies (*A. guariba clamitans*) present sexual dichromatism: males are bright red-orange, while females are darker brown (Gregorin, 2006).

This species is generally polygynous (Jones, 2004), and groups, which average 5.8–7.0 individuals (reviewed by Di Fiore and Campbell, 2007), are typically uni-male (Mendes, 1989; Ingberman *et al.*, 2009). There is some evidence that, as demonstrated for other howler species, brown howler individuals of both sexes can emigrate from the natal group when they reach sexual maturity (Miranda and Passos, 2005). Females give birth at any time of the year and inter-birth intervals average 22.8 months (Strier *et al.*, 2001).

Brown howlers rely mainly on a folivorous diet, but also eat considerable amounts of fruits and flowers when available (Mendes, 1989; Chiarello, 1994; Agostini *et al.*, 2010a; see Chaves and Bicca-Marques, 2013 for a review). The diet based on leaves, which are generally low in energy, may affect the entire ecology and behavior of brown howlers. In fact, brown howlers are characterized by small home ranges, short day ranges and reduced time of activity compared to other primate species of similar size but different trophic categories (Mendes, 1989; Chiarello 1993; Agostini *et al.*, 2010b; see Di Fiore and Campbell, 2007 for a review).

Brown howlers, as with other members of the genus *Alouatta*, perform powerful long-distance roars (da Cunha and Jalles-Filho, 2007; Holzmann *et al.*, 2012). These loud calls are most often initiated and led by adult males, which may be joined by the rest of the group to form a chorus (Holzmann *et al.*, 2012). Most evidence seems to support a role

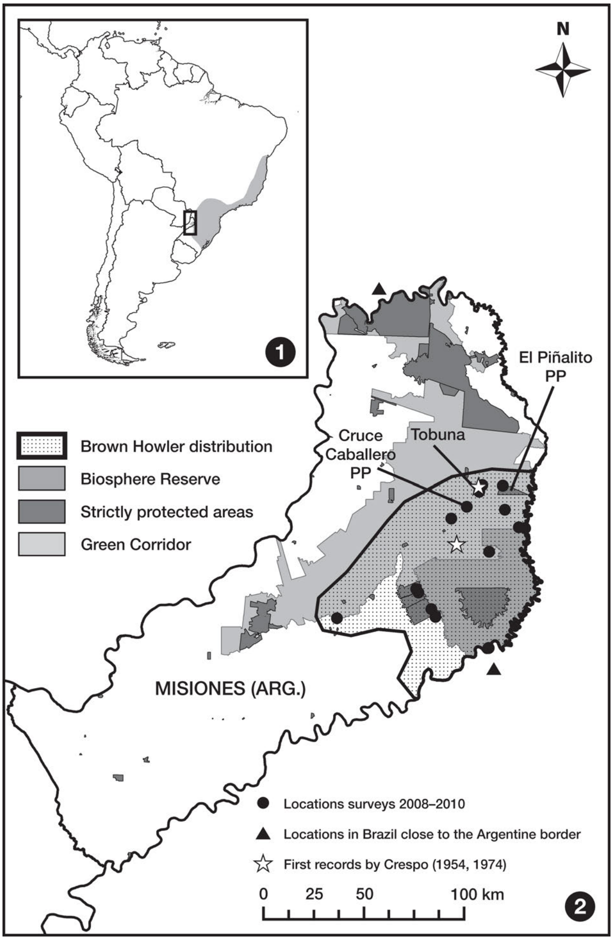

Figure 2. Distribution map of brown howler monkeys on a global (**2.1**) and a local scale within Argentina (**2.2**). Different markers indicate records of species presence collected by other authors as well as by the authors of this chapter (see the legend).

in intergroup communication (Chiarello, 1995; Holzmann *et al.*, 2012). Finally, potential predators of brown howlers are jaguars (*Panthera onca*), pumas (*Puma concolor*), ocelots (*Leopardus pardalis*) and eagles (*e.g.*, black hawk-eagle, *Spizaetus tyrannus*) (Miranda *et al.*, 2005, 2006).

Records for Argentina and nearby areas of Brazil

The first records of brown howler monkey for Argentina were collected by Crespo (1954; 1974), who found this species in two localities of the department of San Pedro in Misiones Province (Fig. 2.2). The vegetation in this area is rather peculiar compared to the rest of the province, because it is a transition area with mixed Upper Paraná Atlantic Forest and Araucaria Moist Forest (Di Bitetti *et al.*, 2003). Some authors have suggested a close association between this primate species and Paraná pines (*Araucaria angustifolia*; Crespo; 1954; Cordeiro da Silva, 1981; Jardim and Oliveira, 2000). However, this hypothesis has no support, given that the brown howler and this tree species do not share the same distribution in most of the Atlantic Forest (Di Bitetti, 2003).

The past few attempts to revise all record locations to build a distribution map for brown howlers in Misiones (Massoia *et al.*, 2006; Chebez, 2008) made clear the difficulty in tracking and distinguishing reliable records from others that come from more anecdotal data or speculative guesses. Discarding the uncertain records (*i.e.*, considering only direct observations of living individuals), before 2005 the presence of the species was confirmed only for two protected areas: Parque Provincial (PP) Cruce Caballero (Di Bitetti *et al.*, 1994) and PP El Piñalito (Di Bitetti, 2003; see Massoia *et al.*, 2006 for a review) (Fig. 2.2).

On the Brazilian side, the brown howler has been reported in areas contiguous to the border with Argentina (Fig. 2.2). In particular, the species has been recorded for the state of Paraná in the Parque Nacional do Iguaçu, very close to the Argentinian border, during surveys conducted between 1990 and 1994 (Crawshaw, 1995), and later re-confirmed in 2006 (M. Xavier Da Silva, pers. comm.). It is noticeable that brown howlers have not been recorded in the adjacent Parque Nacional Iguazú of Argentina or in northern Misiones. Another record of the species' presence has been recently confirmed for the Parque Estadual Turvo in Rio Grande do Sul (Fialho, 2007) (Fig. 2.2). In these cases, the species has been also recorded on the contiguous Argentinian side (Holzmann *et al.*, 2015).

Why so few?

Despite the paucity of data on its past distribution, it is clear that, during the last 60 years, brown howlers were found at low densities in Argentina, as suggested by the extreme difficulty in encountering them (Crespo, 1982; Di Bitetti *et al.*, 1994). Thus, the most intriguing question is: why is this species so scarce in Misiones, given that a large remnant of suitable habitat still persists in this region?

One of the possible reasons is the high susceptibility of howlers (genus *Alouatta*) to yellow fever (Strode, 1951). In 1966, three brown howler individuals were reported dead in Misiones during a yellow fever epidemic that also caused at least 53 human cases in

Misiones and Corrientes provinces (Crespo, 1974). At that time, however, there were no records of its presence other than the one in Tobuna (Fig. 2.2) provided by Crespo (1954) when the species was first recorded in Argentina. Thus, it is impossible to know if that epidemic had decimated what was a relatively abundant population or if brown howlers were simply historically scarce throughout Misiones.

Another potential factor that could explain the low brown howler densities is that the Upper Paraná and the Araucaria moist Atlantic Forest may represent a sub-optimal habitat with a low carrying capacity (*i.e.*, an "edge of niche" situation, Braunisch *et al.*, 2008), given that these eco-regions cover the southern margins of brown howlers' global distribution. However, apparently there is not a clear relationship between brown howler density and vegetation types within the Atlantic Forest (interior semi-deciduous subtropical forests *vs.* coastal dense evergreen forests; data reviewed in Chiarello and de Melo, 2001; Ingberman *et al.*, 2009).

The presence of the congener black and gold howler monkey in Misiones may be a further point to take into account to explain the low abundance of brown howlers. While the brown howler is thought to prefer mature forests within its range, the black and gold howler generally inhabits more fragmented and disturbed forests and has a high potential of colonization of new patches in Chaco region. Therefore, it has been argued that the degradation of Misiones forest may ultimately prove advantageous to the black and gold howler at the expense of the brown howler in this area of coexistence (Di Bitetti, 2003). However, even black and gold howlers are known to live at low densities in this region (Zunino *et al.*, 2001), so it seems unlikely that the presence of this congener species is the reason for the low density of brown howlers.

Finally, it is worthwhile to question whether low densities are actually a negative and abnormal condition for brown howlers in general. During the three-years study at PP El Piñalito (see below), we calculated a population density based on the "block method" outlined in Struhsaker (1981) and Chapman *et al.* (1988). This method is based on dividing the number of individuals within a group by the home range size, taking into account the degree of home range overlap with adjacent groups. We estimated a density of 10 ind/km², which is relatively low compared to other studies (data reviewed in Ingberman *et al.*, 2009, Fig. 3). However, as pointed out by Ingberman *et al.* (2009), many of the sites where brown howlers have been recorded at high densities are indeed small forest fragments, while sites where the species is present at lower densities are commonly large forest reserves (Chiarello and Galetti, 1994). Thus, perhaps even though low densities may indicate some ecological constraints such as quality, phenology and productivity of digestible foliage during periods of resource scarcity (Peres, 1997) or predation (Chiarello and de Melo, 2001), this may be rather a natural and probably healthy condition. Large remnants of habitat with relatively intact ensembles of predators and ecosystem functions may support smaller but perhaps healthier populations of brown howlers that are stable in the long term. By contrast, small fragments, while supporting high densities of howlers, may not be a suitable habitat for these monkeys within a few years or decades due to increased costs of dispersal for individuals, decreased population genetic variability and health status.

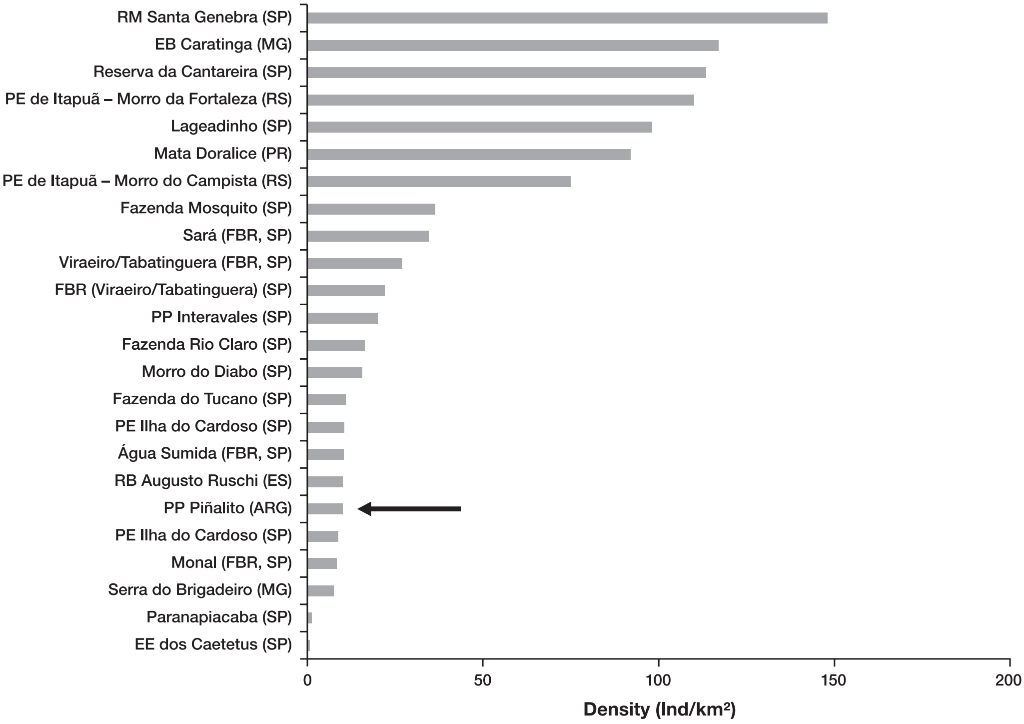

Figure 3. Histogram illustrating different sites in Brazil (reviewed in Ingberman *et al.*, 2009) and one in Argentina (Parque Nacional El Piñalito, indicated by an arrow) for which estimates of brown howler population densities are available.

The first behavioral and ecological study (2005–2007)

Early in 2005, prompted by the huge gap in knowledge about this elusive species inhabiting the forests of Misiones, we started a study on brown howler monkeys in PP El Piñalito (26° 30' S, 53° 50' W; Fig. 2.2). This 3,796-ha strictly protected area within the Atlantic Forest of Misiones is characterized by a humid subtropical climate with marked seasonality in temperature and day length, but not in rainfall (Crespo, 1982). At the relative high altitude of El Piñalito (*ca.* 750 m.a.s.l.), the predominant vegetation is a mixed forest dominated by the Paraná pine (Brown and Zunino, 1994). However, most of the forest in the park had suffered relatively recent timber exploitation and also hosted 92 ha of old (> 30 yr) monospecific plantations of exotic pines *Pinus elliottii* (Pinaceae) and native Paraná pines.

El Piñalito was chosen not only because of the confirmed presence of brown howlers, but also because there was a previous report of sympatry with its congener, the black and gold howler monkey (Baldovino and Benesovsky, pers. comm. in Di Bitetti, 2003), which provided the opportunity to conduct the first comparative study of two howler species living syntopically. Results of preliminary transect surveys inside the park in 2005 confirmed that the two species were actually coexisting on a local scale (*i.e.*, groups of different species highly overlapping in their home ranges and sometimes observed < 50 m apart), but at relatively low densities. Although the number of sightings during the first year census surveys was not enough to provide a reliable density estimate, encounter rates in PP El Piñalito were lower (brown howlers: 0.29 / 10 km; black and gold howlers: 0.4 / 10 km) than those in other sites where the species were censused in Brazil and Argentina (brown howlers: median 1.2 / 10 km, range: 0.25–2.2, reviewed by Ingberman *et al.*, 2009; black and gold howlers: 0.75 / 10 km, Arditi and Placci, 1990). Later, we were able to estimate the population density from the data on home ranges of the groups studied in the park (see above).

Our study helped clarify several interesting issues about niche differentiation and interspecific competition. Our main findings indicated that both howler species share considerable trophic, spatial and temporal niche overlap and could potentially compete for resources (Agostini *et al.*, 2010a, b; 2012b). Also, we reported direct evidence of hybridization between the two species occurring within the area of sympatry (Agostini *et al.*, 2008), and tested several hypotheses about the adaptive function of the howlers' roaring behavior using sympatry as a novel scenario (Holzmann *et al.*, 2012).

The yellow fever epidemic (2008–2009)

This promising research was abruptly interrupted in January 2008, when we discovered that all study groups had died during a yellow fever outbreak that lasted until 2009 and dramatically decimated howlers throughout their southern distribution (Bicca-Marques and Freitas, 2010; Holzmann *et al.*, 2010; Moreno *et al.*, 2011; Almeida *et al.*, 2012). Although estimates of the real impact of this epidemic on the already small brown howler population could not be assessed, we know that the outbreak was particularly strong due to the number of howler corpses found in Argentina (n = 59, Holzmann *et al.*, 2010) and nearby areas of Brazil (n = 2013, Almeida *et al.*, 2012). Further, data obtained by the surveillance system of Rio Grande do Sul State Secretariat of Health during the same epidemic (between 2008 and 2009) indicate extremely high rates of expansion of the yellow fever virus: around 100 km/month (Almeida *et al.*, 2012), much faster than recorded in any other documented burst (Elton, 1952).

Given the precarious conditions of the brown howler in Misiones, due to reduced distribution and low density, this yellow fever epidemic is thought to have brought the population to the verge of extinction. To assess the current situation, we conducted surveys of the brown howler throughout Misiones Province during and after this severe outbreak.

Surveys to define current population status (2008–2010)

From March 2008 to November 2009, I.H. carried out 12 field surveys visiting 35 localities (n = 30 within protected areas, n = 5 within non protected areas) throughout the entire province of Misiones, and focusing on presence-absence of both howler monkey species (*A. guariba clamitans* and *A. caraya*). Each survey in each site involved at least two full days of walking on preexisting trails. Additional records were gathered through interviews with park rangers, colleagues and local people familiar with both study species. This survey added seven new localities for brown howlers in Misiones and confirmed another area of syntopy between the brown howler monkey and the black and gold howler monkey (Holzmann *et al.*, 2015).

Between June and September 2010, I.A. and a field assistant surveyed 31 selected transects (average length ± SD: 2.6 ± 0.8 km; range: 1.8–4.8) for a total length of 151.3 km. Transects showed heterogeneity in their level of anthropogenic disturbance or protection since they were situated in both protected and non-protected areas (n = 12 within protected areas; n = 19 within non-protected areas) within San Pedro and part of Guaraní departments, encompassing the whole range of brown howlers in Argentina, and including six of the seven new localities of species presence found in the first survey (Fig. 2.2). Besides the transect, I.A. conducted 55 semi-structured interviews with local farmers, loggers, Guarani indigenous inhabitants and park rangers about the presence/absence of each howler species (*A. caraya* and *A. guariba clamitans*) before and after the yellow fever outbreaks, in 33 specific areas distributed throughout our survey region (Agostini *et al.*, 2015).

During the surveys we did not find any direct or indirect evidence of the presence of brown howlers or black and gold howlers in the study area. In a pre-yellow fever epidemic scenario, the chances of zero encounters over 151.3 km would have been extremely low (< 0.01), assuming that the densities of howlers had been comparable between PP El Piñalito and other surveyed areas. This suggests that the abundance of both howler populations has heavily dropped following the recent yellow fever outbreaks.

Moreover, results from the interviews showed that the probability of disappearing from a site after the outbreak was significantly higher for howler monkeys than for sympatric capuchins (44% *vs.* 5%), which confirms the hypothesis of a greater susceptibility of howler monkeys to yellow fever compared to capuchin monkeys (Kumm and Laemmert, 1950; Strode, 1951).

The lack of direct records of presence of brown howlers during this latter intensive survey especially focused on this species drove us to the further step: if brown howlers are in such a critical situation, what are the relative effects of the factors we identified as potential threats? We have to acquire knowledge about the major threats to this small population if we want to take effective actions to protect the species.

Major threats and challenges

Habitat loss

The Atlantic Forest of South America is one of the most endangered rainforests on Earth, with only 7–12% of its original coverage still persisting (Galindo-Leal and Gusmão Câmara, 2003; Ribeiro *et al.*, 2009). It has been included in the list of Global Ecoregions by WWF (2013) and among the eight "hottest" of the biodiversity hotspots analyzed by Conservation International (Myers *et al.*, 2000; Mittermeier *et al.*, 2005). Despite the high degree of fragmentation, the Atlantic Forest is still one of the most diverse ecosystems on Earth (it contains 7% of the world's species) and it is characterized by a high level of endemism (*e.g.*, 40% of plant species and 42% of terrestrial vertebrate species are endemic; Myers *et al.*, 2000).

The Upper Paraná Atlantic Forest is the largest (471,204 km²) of 15 ecoregions included in the Atlantic Forest ecoregions-complex (Di Bitetti *et al.*, 2003). It extends from the Serra do Mar in Brazil, to east Paraguay and Misiones Province in Argentina. This ecoregion holds the largest forest remnants that maintain original ensembles of great vertebrates such as large predators (jaguars, pumas, ocelots, harpy and crested eagles) and large herbivores (tapirs, brocket deers, peccaries). In Argentina the Upper Paraná Atlantic Forest is also known as Misiones Forest or *Selva Paranaense*, and is recognized as an area of relatively high endemism and diversity. The province of Misiones retains the most continuous and least disturbed portions of Upper Paraná Atlantic Forest (Giraudo *et al.*, 2003). This large and relatively unfragmented forest area has a crucial role to play in the conservation of the ecoregion because it offers real opportunities to preserve extensive (resilient) areas, maintain populations of top predators (*e.g.*, jaguars), create corridors and reserves, and promote sustainable development in non-protected areas (Giraudo *et al.*, 2003). However, forests, still covering approximately 50% of Misiones, are being cleared by large companies that replace native trees with pine monocultures (10%), large-scale agriculture (13%) and areas of subsistence agriculture often mixed with small pastures and shrubland (12%) (Izquierdo *et al.*, 2008). This landscape modification may eventually put some of the endemic species at risk of extinction. This could be the case for the brown howler monkey.

Progressive deforestation may threaten brown howlers, not only by reducing resource availability, but also by enhancing their susceptibility to hunting pressure. In Misiones, for example, the several Mbyá indigenous communities practice subsistence hunting in some parts of the province (Sanchez and Giraudo, 2003). Although the impact of the Mbyá community on brown howlers in Misiones is unknown and probably minor, in areas with long-standing human settlements, hunting has apparently resulted in local extinction of primates, along with many other mammals and birds (Di Bitetti, 2003). Another potential threat is from the pet trade. Although brown howlers are not usually found as pets in the local illegal market (while young black and gold howlers and capuchins are, probably because they are more common), there are a few reports of local inhabitants of Misiones who admittedly have kept brown howler infants as pets in their farms (Agostini, unpublished data). Finally, as humans encroach on the howler's habitat, epidemics of diseases and parasites associated with humans and domestic animals, such as yellow fever and botflies (*Dermatobia* sp.) larvae infection, also pose a grave threat to howlers because they are especially susceptible to these pathogens (Collias and Southwick, 1952; Milton, 1982; Di Bitetti, 2003).

Yellow fever

The data obtained by interviews during the 2010 survey in Misiones helped to highlight the critical situation of both howler populations and the threat posed by yellow fever to this small brown howler population (Agostini *et al.*, 2015).

Yellow fever is a zoonotic infection provoked by a Flavivirus (Flaviviridae), a virus which is endemic to northern South America and sub-saharian Africa, where it causes sporadic outbreaks and epidemics (Vasconcelos *et al.*, 2001). In South America, only the sylvan cycle is recognized and transmission occurs when an infected mosquito bites human or non-human primates (Monath, 2001; Vasconcelos *et al.*, 2001). The yellow fever virus' main vectors in South America are diurnally active mosquitoes that breed in tree holes in the forest canopy (*Haemagogus* spp. and *Sabethes* spp.; Monath, 2001; Vasconcelos *et al.*, 2001). Monkeys are the main hosts and the source of amplification of the virus, because a viremic monkey can infect several mosquitoes (Vasconcelos *et al.*, 2001). People can be sporadically exposed to infected mosquitoes when they come into this cycle during occupational or recreational activities in the forest (Monath, 2001). While humans and non-human primates other than howlers are usually more resistant (Strode, 1951), howler monkeys are extremely susceptible to yellow fever, quickly developing the disease and dying (Strode, 1951). In this way, howlers act as "sentinels" for public health: their mass mortalities warn human population about the presence of the disease (Butcher, 1991).

On the basis of outbreak frequency and severity, two distinct areas can be acknowledged: endemic areas, where the virus is always circulating (*e.g.*, Amazon and pre-Amazon regions), and disease-free areas, in which yellow fever never occurs (*e.g.*, coastal regions of Brazil). The area between these two zones, sometimes called "transition area", can be affected by periodic expansions in epizootic activity (Vasconcelos, 2003). In the transition area, yellow fever usually occurs as outbreaks every 7 to 10 years, alternating with periods with lower numbers of cases (Monath, 1988). Since 2000, yellow fever seems to have been progressively expanding its range, transcending its usual boundaries (Moreno and Barata, 2012). Misiones Province, in Argentina, lies at the edge between a transition area and a disease-free area. Here the outbreaks may be more spaced in time than in typical transition areas. Climatic changes as well as invasive human activities such as deforestation for cattle ranching or wood trade, flooding of extensive areas for dam construction, are all factors that can favor yellow fever outbreaks (Vasconcelos *et al.*, 2001). Thus, current changes in climate and land use that are taking place throughout Misiones may be the basis for the progressive advance of the yellow fever transition area's boundaries in this region.

In the next few years, we could witness a new yellow fever epidemic in Misiones Province when brown howler monkeys may have not had the opportunity to recover from the population crash caused by the disease in 2008–2009. Although at low population densities, the probability of infection may significantly decrease, but the threat of local extinction for brown howlers is still high; the next yellow fever outbreak may drive the already small population into an extinction vortex due to stochastic factors. This would not only be a tragic regional loss, but a matter of concern for the preservation of human public health. In fact, in a forest depleted of howler sentinels, the upcoming spread of a yellow fever epidemic in the future may take longer to notice and cause lots of human life losses before an effective response is in place (*e.g.*, through vaccination campaigns).

Lack of public awareness

Even though yellow fever and habitat loss are the main direct threats to brown howler conservation, they are compounded by a lack of awareness and knowledge of the critical situation of this population among governmental, non-governmental institutions and the general public. To progress in raising each of these different levels of awareness we have to develop different strategies, from boosting dialogue with policy makers, to collaborations among NGOs and developing education programs to increase the general public interest and commitment to species conservation. The latter level, *i.e.*, raising awareness of the general public, may be the most complex task, requiring a deep understanding of public attitudes, and at the same time the most pivotal in affecting the success or failure of conservation efforts (Jacobson and McDuff, 1998).

As we stated at the beginning of this chapter, the brown howler monkey can be considered the most neglected primate species in Argentina, together with the hooded capuchin monkey. This holds true also at a more local level within Misiones Province. In fact, although this species is protected as a natural monument within the province, there have been no real efforts from institutions to improve our knowledge about this small population and foster its preservation. National and provincial environmental institutions, as well as NGOs, generally ignore the situation of brown howlers in this region. Following this lack of institutional commitment, no education program has ever been tackled to increase awareness among local inhabitants, who consequently do not feel committed to the conservation of a species that has no immediate economic value for them (for example, as a tourist attraction). Still worse, sometimes people do not even know that this species inhabits Argentina. Thus, if we want to conserve brown howlers it is clear that actions must be directed at filling this vacuum.

Towards the development of a species conservation strategy

In March 2013 we organized the first Brown Howler Monkey Conservation Workshop in Misiones in order to establish conservation priorities and initiate the development of a conservation plan for the species in Argentina (Agostini *et al.*, 2014). This originated from our awareness that “something has to be done” before the brown howler monkey disappears from Misiones. During this event, a group of nine experts in different fields (primate ecology, eco-epidemiology, mosquito ecology and virology) dedicated themselves to gathering, systematizing and discussing all available data and information on brown howlers and yellow fever in the Atlantic Forest. The workshop was facilitated by members of the IUCN / SSC's Conservation Breeding Specialist Group (CBSG), who also provided expertise in the development and use of quantitative risk assessment models used in data assembly and analysis. We developed population viability models using *Vortex* and *Outbreak* software packages. These tools allowed us to explore how several biological and demographic parameters of brown howlers, as well as factors related to yellow fever epidemiology, affect the probability of species extinction. The discussion among specialists and analysis of model results confirmed yellow fever as the main threat to brown howler population persistence in Argentina. Our threat analysis, focused on the dynamics of yellow fever outbreaks and their impact on howler populations, led to the identification of gaps in knowledge that helped prioritize objectives and actions for the development of a Species Conservation Strategy (IUCN/SSC, 2008) in Argentina.

Recommendations and conclusions

One of the results of the workshop was a list of actions to address the most important current gaps of knowledge, focusing on yellow fever (YF) as the main threat (Agostini *et al.*, 2014).

Action 1. Implement a regular surveillance program for alerting local authorities about suspected YF outbreaks in monkeys and/or people.
Action 2. Estimate the population distribution and abundance of brown howler monkeys in Misiones.
Action 3. Conduct health studies of brown howler monkeys in Misiones to evaluate and compare physiological stress, innate and acquired immunity, hematology, etc., across different sub-populations especially before and after YF outbreaks.
Action 4. Isolate YF virus from mosquito adults and larvae.
Action 5. Conduct a thorough literature and archive review to enhance our understanding of the interactions (environmental and anthropogenic) involved in the maintenance and dynamics of YF outbreaks in South America.
Action 6. Capture adult mosquitoes in areas inhabited by howlers to identify YF vectors and monitor presence of the virus.
Action 7. Define the potential distribution of brown howlers in Argentina.
Action 8. Attempt to isolate or detect the YF virus in suspected vertebrate hosts using virological assays, cell cultures and molecular techniques.
Action 9. Conduct a systematic review of the YF virus virulence from different strains in different vertebrate hosts in Misiones and Brazil.
Action 10. Understand the factors that define carrying capacity for brown howler monkeys and their habitat requirements (limiting factors, food, threats).

Improving knowledge on these issues is vital for understanding and therefore effectively preserving and managing the small brown howler population and its habitat and for reducing the impact of yellow fever outbreaks in Argentina. This, together with the urgent need to tackle actions aimed at raising awareness of institutions, NGOs and general public, will help us develop an effective Species Conservation Strategy for this brown howler population.

Ultimately, maintaining a healthy population of brown howlers is not only a matter of species and biodiversity conservation but also an important issue for public health. Due to their role as sentinels giving early warning of a potentially serious epidemic disease such as yellow fever, howlers' presence in the forests may save many human lives.

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14 AZARA'S OWL MONKEY IN THE HUMID CHACO: PRIMATOLOGICAL LONG-TERM STUDIES IN ARGENTINA

LOS MONOS MIRIKINÁ DEL CHACO HÚMEDO: 20 AÑOS DE PRIMATOLOGÍA EN ARGENTINA

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Abstract. The Owl Monkey Project started in 1996 as a multi-disciplinary program on the Azara's owl monkey of the Argentinian Chaco. The main goals of the project have been to investigate the evolution of the monogamous mating system and parental care of this species. The project has expanded and, for many years, we have also been exploring the potential relationship between demography, the spatial and temporal distribution of food resources, and the monogamous social organization of the species. Additionally, since 2007, we expanded our studies to include the examination of groups that inhabit two different natural habitat types in the humid Chaco of Formosa Province. In this chapter, we use data from 20 years of study, to elucidate factors underlying the demographic structure of different owl monkey groups inhabiting different types of habitats. The study was conducted in the Estancia Guaycolec (a private 25,000-ha cattle ranch) and in Río Pilcomayo National Park (a 52,000-ha protected area). In each study area, two sub-sets of owl monkey groups could be identified: those within the gallery forests (continuous habitat), and groups in forest patches. Our results confirm that the estimated densities for the private ranch are higher than in the National Park. In contrast, group size, birth rates and age structure were similar between sites. Group sizes, birth rates, and specific densities were larger for gallery forests than for forest patches at both study sites. Our studies contribute to the understanding of the evolution of social monogamy and male care, and also provides information on the demography and habitat use of a species that has been declared a Natural Monument in the Province of Formosa.

Resumen. En el año 1996 comenzó el Proyecto Mirikiná, un programa de investigación multidisciplinario con monos del género *Aotus*. Los mirikiná, nombre vulgar que reciben en el Chaco argentino, tienen una distribución restringida solo en las provincias de Chaco y Formosa. Este proyecto, el más largo con el género y con animales en estado silvestre, ya lleva 20 años de investigaciones, las cuales comenzaron en el Chaco húmedo de la provincia de Formosa y actualmente se han extendido a diferentes países donde el género se distribuye. Los primates de este género son de tamaño pequeño y presentan características que los hacen únicos: son nocturnos a lo largo de su distribución (catemerales en el Chaco húmedo formoseño) y socialmente monógamos. Dos características dentro de su historia de vida que los hace interesantes, desde el punto de vista teórico, para examinar hipótesis relacionadas con su tan particular sistema social. Es por ello, que el objetivo a largo plazo del proyecto es comprender cuáles son los mecanismos que mantienen la monogamia social y la importancia del cuidado parental en esta especie. Este programa de investigación, que comenzó con estudios básicos de ecología de campo, actualmente lleva 20 años de toma de datos, lo que ha permitido que se amplíen sus líneas de investigación, que hayan surgido colaboraciones entre instituciones e investigadores científicos de muchas partes del mundo, sobre todo de Latinoamérica. En la primera parte de este capítulo se presentan los principales resultados obtenidos dentro de ramas como la etología, la ecología poblacional, la fisiología reproductiva, la genética molecular y la conservación de esta especie. Los estudios etológicos permitieron examinar hipótesis relacionadas al cuidado parental y a cómo la inversión en el cuidado de la cría está relacionada justamente con la evolución y el mantenimiento de la monogamia en esta especie. Los estudios demográficos han incluido estimaciones de densidad y relaciones entre distribución espacial y temporal de recursos alimenticios. Los estudios hormonales están orientados a entender cómo los mecanismos fisiológicos de los mirikinás mantienen esta estructura grupal tan característica en los *Aotus* del Chaco argentino. En relación con esto, los análisis genéticos obtenidos de muestras de pelos, sangre y tejidos han permitido obtener resultados interesantes con respecto a la diversidad de mtDNA y de loci de microsatélites. Finalmente damos un panorama del estado de conservación de la especie en Argentina y sobre todo en el Chaco húmedo formoseño. Desde el 2007, una de las líneas de investigación estuvo centrada en estudiar demográficamente grupos de mirikinás que habitan dos tipos de hábitats naturales, con características espaciales diferentes, en el Chaco húmedo de la provincia de Formosa. El estudio se llevó a cabo en la Estancia Guaycolec (una estancia ganadera y privada de 25.000 ha) y en el Parque Nacional Río Pilcomayo (un área natural protegida de 52.000 ha). En cada área de estudio, dos sub-conjuntos de grupos de mirikinás pudieron ser identificados: dentro de las selvas en galería (hábitat continuo), y grupos sociales en «parches» o islas de bosques, así como dos niveles potencialmente diferentes de perturbación (es decir un parque nacional *vs.* un establecimiento ganadero). Nuestros resultados confirman que las densidades estimadas para la estancia privada son más altos que en el Parque Nacional. En contraste, el tamaño de los grupos, las tasas de natalidad y la estructura de edad fueron similares entre los sitios y también entre diferentes tipos de hábitat en ambos sitios. El tamaño del grupo, las tasas de natalidad y densidades específicas fueron más altas en selvas en galería que en isletas de bosques para ambos sitios de estudio. Nuestros estudios a largo plazo contribuyen a la comprensión de la evolución de la monogamia social y el cuidado paternal, y también a la demografía y el uso del hábitat de una especie que ha sido declarada Monumento Natural en la Provincia de Formosa.

Introduction

General aspects of the genus *Aotus*

Individuals of the genus *Aotus* are characterized by two distinctive traits: they are the only anthropoid primates that are nocturnal and they are socially monogamous. *Aotus* (Illiger, 1811) is the only nocturnal primate genus in Central and South America. It is distributed from Panama to the northeast of Argentina, and from the lowlands of the Andes to the Atlantic coast (Wright, 1984). Eleven species are currently recognized (Fig. 1): *Aotus lemurinus*, *A. zonalis*, *A. griseimembra*, *A. jorgehernandezi*, *A. brumbacki*, *A. trivirgatus*, *A. vociferans*, *A. miconax*, *A. nancymaae*, *A. nigriceps*, and *A. azarae* (Rylands and Mittermeier, 2009; Rylands and Russell, 2009; Menezes *et al.*, 2010). The species *A. azarae* includes the three subspecies *A. a. boliviensis*, *A. a. infulatus* and *A. a. azarae* (Groves, 2005; Ruíz-García *et al.*, 2011). The distribution of *A. a. azarae* (Azara's owl monkey) ranges from parts of Bolivia and Paraguay to northern Argentina. In Argentina, the subspecies is found in the provinces of Chaco and Formosa (Rathbun and Gache, 1980; Mudry de Pargament *et al.*, 1984; Zunino *et al.*, 1985; Fernández-Duque *et al.*, 2001), where owl monkeys are locally known as *mirikinás*.

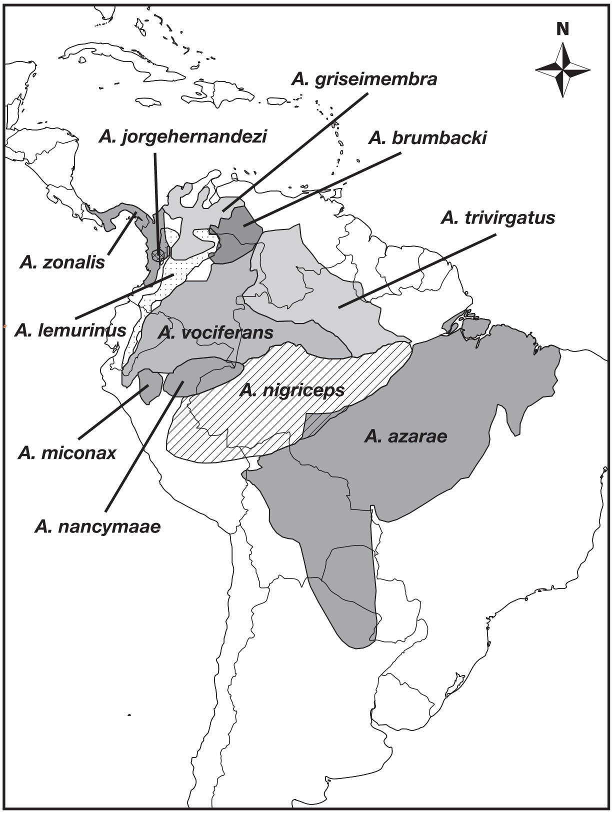

Figure 1. Geographical distribution of the genus *Aotus* in Central and South America (map from de Menezes *et al.*, 2010).

Natural history of Azara's owl monkeys

Unlike other owl monkey taxa, the subspecies *A. a. azarae* of the Gran Chaco region is cathemeral (Wright, 1989; Arditi, 1992; Fernández-Duque and Erkert, 2006; Fernández-Duque *et al.*, 2010); in other words, it can be active during the night, as well as during the day (Tattersall, 1987). Social groups are small, consisting of an adult pair, one infant, and up to three juveniles or subadults (Huck *et al.*, 2011). Traditionally, those groups were assumed to be family groups in which the male and the female formed a reproductive pair during their entire life, or a prolonged period of life until the death of one partner. However, nearly 5–11% of the individuals in a population studied since 1996 in the Guaycolec Ranch of Formosa Province live as solitary floaters, and this holds for both males and females (Huck and Fernández-Duque, 2017). Generally, such floaters are subadults that have dispersed from their natal groups, or adults that have been replaced from their social group by an intruder (territories are between 4 and 10 ha large, and overlap with the borders of neighboring groups; Wartmann *et al.*, 2014). Such replacements occur approximately once every three years in the population, but reproducing pairs remain stable for an average of nine years (Fernández-Duque and Huck, 2013).

The reproductive pair reproduces once a year, with a median inter-birth interval of 370 days (Fernández-Duque *et al.*, 2002). The single offspring is born after a gestation period of 120–130 days (Fernández-Duque *et al.*, 2011). The adult male in the group is the main carrier of the infant (Fig. 2) and takes care of it beginning on the second week of the infant's life (Rotundo *et al.*, 2002; Huck and Fernández-Duque, 2012a). Births are seasonal, concentrated between October and December (Fernández-Duque *et al.*, 2002). Subadults of both sexes disperse when they are between two and four years old (Huck and Fernández-Duque, 2012b). Although dispersal events can be observed throughout the year, they tend to concentrate around the birth season (Fernández-Duque and Huntington, 2002; Fernández-Duque, 2009). Owl monkeys do not show any conspicuous sexual dimorphism (Fernández-Duque, 2011).

Figure 2. Adult male Azara's owl monkey carrying an infant. Photo: Víctor Dávalos / Proyecto Mirikiná, Formosa, Argentina.

20 years of studying Azara's owl monkey in Argentina

The Owl Monkey Project, a multi-disciplinary program on the Azara's owl monkey of the Argentinian Chaco, started in 1996. One of the main goals of the project has been to investigate the evolution of social relationships, the monogamous mating system, and parental care characteristic of the subspecies. In particular, we have been interested in examining the roles that males and females have in the maintenance of a monogamy. Many of the studies have the goal of examining owl monkeys as an approach to understanding the evolution of human behavior, for example with respect to pair bonding and biparental care. Growing from that initial main goal, the project has expanded and spawned studies on behavior, demography, population biology, genetics, endocrinology and conservation. These have been conducted in collaboration with numerous colleagues from provincial, national and international institutions (*http://owlmonkeyproject.wordpress.com/about/*).

Behavioral Ecology. Studies on the subspecies' behavioral ecology allow us to examine different hypotheses that propose explanations for the evolution and maintenance of monogamy, and the intense male care of offspring that is characteristic of the genus. It has been suggested that in monogamous species, given the relatively high costs to a female of raising and caring for offspring, males may care directly for infants or provide some kind of indirect services to females. The male-care hypothesis predicts that males are more likely to provide infant care when paternity certainty is high (Sheldon, 2002; Kokko and Jennions, 2008). Moreover, it is predicted that in the absence of male care, for example due to replacements, the development and survival of infants may be affected, for example by reducing survival or lowering dispersal age (Emlen, 1995, 1997). To evaluate this hypothesis, we have collected behavioral data during the course of the last sixteen years on male-infant interactions before and after paternal replacements; whether the infant was independent or not, and the position of the infant on the individual transporting it (Rotundo *et al.*, 2005; Huck *et al.*, 2011). We have also examined infant survivorship and its possible correlation with the intensity of such care (Huck and Fernández-Duque, 2012a). We found that the male present in the group when an infant is conceived is the genetic father of the infant (Huck *et al.*, 2014). However, when the male adult is replaced (Fernández-Duque and Huck, 2013), the new male still provides intensive care to the infant (*i.e.*, 67% of the time the new resident male transported the (unrelated) infant, compared to 80% of the time by the original male resident; Fernández-Duque et al. 2008; Fernández-Duque *et al.*, 2009). Additionally, infant survival was not directly affected by adult replacements (Huck and Fernández-Duque, 2012b). Our evidence suggests that female owl monkeys may not be capable or willing to invest more in their current offspring, at least not if an adult male is present (Huck and Fernández-Duque, 2012a; Huck *et al.*, 2014). On one occasion, a female with a dependent infant (younger than a week old) was replaced by a female intruder, which offered a natural experiment to evaluate if a female is capable of caring for infants in the absence of a male. The infant did not survive, suggesting that infant survival may be dependent on male care (Huck and Fernández-Duque, 2012a).

Demography and population biology. In Argentina, the first reported survey of this subspecies was conducted in the provinces of Formosa and Chaco (Rathbun and Gache, 1980). Following that first evaluation, different authors estimated population densities in different forest types along the subspecies distribution (Zunino *et al.*, 1985; Arditti and Placci, 1990; Brown and Zunino, 1994; Fernández-Duque *et al.*, 2001). In all cases, the densities were estimated to be higher in the eastern than in the western portions of the Province of Formosa (Tab. 1).

Table 1. Group and individual densities, and group sizes for *Aotus a. azarae* in different habitat types in Argentina (**na**, no data available; **c**, census; **be**, behavior and ecology study).

References	**Group density (groups/km²)**	**Individual density (ind/km²)**	**Mean group size**	**No. of observed groups**	**Habitat types**	**Type of study**	**Location**
Rathbun and Gache (1980)	10.0	29.0	2.9	25	Various habitat types	c	Formosa Province (Argentina)
Zunino *et al.* (1985)	14	32.3	na	na	Gallery forest	c	Guaycolec Ranch (Formosa Province, Argentina)
Zunino *et al.* (1985)	5.4	12.7	2.3	12	Various habitat types	c	Formosa Province (Argentina)
Arditi and Placci (1990)	8	25	3.3	47	Gallery forest	c	Guaycolec Ranch (Formosa Province, Argentina)
Fernández-Duque *et al.* (2001)	16	64	4	11	Gallery forest	be	Guaycolec Ranch (Formosa Province, Argentina)

Over the years, we have been able to explore the potential relationship between demographic parameters, population biology, the spatial and temporal distribution of food resources and the social organization of the species (van der Heide *et al.*, 2012; Fernández-Duque and van der Heide, 2013; Fernández-Duque, 2016). Specifically, we have examined the hypothesis that socially monogamous owl monkey females are spatially distributed in a manner that allows them to maximize their reproductive success, given the distribution and availability of resources. Under this hypothesis, we predict that the reproductive histories of groups and their subsequent demographic characteristic will be related in some manner to the potential and actual access to resources, which in turn is directly influenced by temporal and spatial resource availability. In this theoretical context, we first predicted that there would be no marked differences among home ranges in the spatial distribution and abundance of food resources. Second, we predicted that there would be a relatively even distribution of food in space that prevents the formation of multi-female groups and leads to socially monogamous ones. Third, if home ranges were similar in quality, we predicted that the number of offspring produced in each territory over a 10-year period should not differ significantly. This prediction was formulated under the assumption that the number of offspring produced is related to the nutritional status of a female, and ultimately food availability. Fourth, if territories had similar amounts of resources, we expected that they should support similar numbers of individuals, which would be reflected in similar group sizes. Fifth, assuming that the age of dispersal from their natal groups would be partially influenced by intra-group competition for resources (Fernández-Duque, 2009), we predicted that the ages at dispersal would not be very different between similar territories.

In terms of territory quality, our prior studies show that owl monkey territories differ in size, species evenness, stem abundance and density, total basal area, and food species' stem abundance (van der Heide *et al.*, 2012). Yet, despite those differences, we found no marked differences among parameters expected to be associated with territory quality, including group size, birth rate, age at natal dispersal, and infant mortality (van der Heide *et al.*, 2012). Thus, our prior studies have found no strong relationship between potential territory quality and long-term demography when considering the territory as a whole and all of the foods available throughout the year (van der Heide *et al.*, 2012). Instead, our data suggest that owl monkeys occupy territories that provide similar amounts of reliable dry season foods within the core areas. Although access to these core areas may allow them to overcome severe dry season, our findings underscore the difficulties of understanding the potential causal relationships between ecological factors and demographic and life-history parameters (Fernández-Duque and van der Heide, 2013; Fernández-Duque, 2016).

Reproductive endocrinology. One of the goals of the project is to understand why male owl monkeys mate in a monogamous relationship, presumably foregoing other reproductive opportunities and often investing heavily in the care of offspring they cannot be certain to have sired. Most information about the hormonal mechanisms regulating biparental care and monogamous social systems has been restricted to studies from captive animals (Dixson, 1982; Mendoza and Mason, 1986; Dixson, 1994; Valeggia *et al.*, 1999). In titi monkeys (*Callicebus moloch*), for example, the father was shown to be the infant's primary attachment figure, based on raised plasma cortisol levels in the infant, while both adults experienced increased cortisol levels when separated from each other (Mendoza and Mason, 1986). To evaluate the hypothesis that social monogamy may arise if the temporal distribution of female reproductive cycles limits the mating potential of males, we have analyzed fecal samples collected from wild adult females. Our limited preliminary analyses have shown that female owl monkeys in the Argentinian Chaco have conceptive cycles with an average length of 22 ± 3 days, and with a profile that is similar to other monogamous Neotropical primates such as *Callicebus*; namely that they show the simultaneous rise of estrone-1-glucuronide and pregnenadiol-3-glucuronide during the luteal phase (Fernández-Duque *et al.*, 2011). Apart from the strong birth seasonality peaking at the beginning of October (Fernández-Duque *et al.*, 2002), we do not yet have sufficient data to verify the synchronicity of females' receptive periods.

Molecular genetics. We have conducted numerous studies to examine the genetic structure of the owl monkey population in Formosa. During 14 years,we collected high-quality DNA samples (hair, ear punches, skin biopsies) from 167 owl monkeys in 15 social groups in Argentina. Initial studies investigated the diversity of mtDNA and microsatellite loci of wild animals in Formosa Province in comparison to three other species of *Aotus* (Babb *et al.*, 2011a, b). Results indicated a mean heterozygosity of 0.44 (0.40–0.48) across 13 microsatellite loci that were polymorphic for the wild population (Babb *et al.*, 2011a, b). These microsatellite loci exhibited sufficient allelic variation to enable statistical estimates of kinship to be made between any two individuals in our wild population of owl monkeys (Babb *et al.*, 2011a, b). From that, paternity analyses have shown that owl monkeys of Argentina are the only intensively sampled primate species for which 0% extra-pair paternity has been reported (Huck *et al.*, 2014). Furthermore, molecular examinations of the arginine vasopressin V1a receptor gene (AVPR1A) and the prolactin receptor gene have provided a necessary foundation to begin developing studies investigating some of the genetic mechanisms possibly underlying the behavioral repertoire of the species (Babb *et al.*, 2010, 2011b, 2013).

Conservation in Argentina. In July 2012 the species was declared a Natural Monument in the province of Formosa (Law #1582). The total distribution area of Azara's owl monkeys in Argentina was estimated to be 39,000 km². Of this, the suitable habitat is 156 km² in the Chaco Province and 654 km² in Formosa Province (Rathbun and Gache, 1980; Zunino *et al.*, 1985; Brown and Zunino, 1994). However, these areas were estimated more than 20 years ago, based on maps and satellite images. Recently we found that Azara's owl monkeys were recorded in Vaca Perdida, in the western part of the province, and Campos *et al.* (2004) also found the species in the Dry Chaco of Paraguay. These two findings extend the distribution of the subspecies to longitude 61° (western limit), nearly 200 km further west. These new records, taken together with the continuous encroachment of agricultural areas into natural forests, justify a re-evaluation of the distribution, habitat, and area of available habitat for the subspecies in Argentina. New density estimates, along with a refined territorial evaluation, suggest that the existing owl monkey habitat in the province may host an estimated 18,000 individuals (Juárez, 2012).

Studying the health of wild populations is fundamental for the protection of animals, especially in fragmentary areas, in order to detect potential threats from recent environmental changes. These changes, induced by humans, can generate stress and reduce these wild animals' resistance to naturally occurring pathogens, putting them at health risks that may not have existed before. In order to evaluate the health status of the owl monkeys at our study site, field veterinarians regularly take blood samples, fecal samples and samples of external parasites. Some preliminary analyses of blood samples using PCR–RFLP and sequencing detected DNA of a subgenus of the *Leishmania* (*Viannia*) parasite in four individuals (Acardi *et al.*, 2013). Additional eco-epidemiological and parasitological studies are necessary to confirm this finding.

Demography of Azara's owl monkeys in two different habitat types: gallery forest and forest patches in Formosa Province

Evaluations of the relationships between the demography and the habitat have identified two factors that notably impact primate populations: the floristic and general habitat structure where the primates live, and the anthropogenic impact on this habitat or the individuals of a population (Struhsaker, 1981; Branch, 1983; Wallace *et al.*, 1998; Cowlishaw and Dunbar, 2000; Chapman and Peres, 2001; Struhsaker, 2008; Pyritz *et al.*, 2010).

Generally, the highest quality habitats for primates are those with a larger diversity and density of tree species (Dunbar, 1988; Marsh, 2003), likely due to primates' ecological requirements. Their ability to take advantage of different habitat types makes forest environments complex systems that can support a large number of individuals. The relationship between primate density and floristic structure is less clear (Chapman *et al.*, 2002). Furthermore, the existence of other variables could affect primate density, such as the presence or absence of predators, or behavioral variables such as intra-specific or inter-specific resource competition (Struhsaker, 2008).

The comparative approach offers a useful tool to examine the relationships between habitat types and anthropogenic impact on the one hand, and the demography of a species on the other. Previous comparisons between different species and genera emphasized the limitations faced by primates with specialized diets, where they may have to live in areas that suffer from spatial reductions and, therefore, from a reduction in their preferred foods (Mills *et al.*,1993; Marsh, 2003). Studies that examine differences and similarities between populations of the same species have been rare, since long-term demographic studies have been nearly exclusively limited to a single population (*i.e.*, *Papio cynocephalus*: Altmann *et al.*, 1985 or *Alouatta seniculus*: Rudran and Fernández-Duque, 2003), or to a limited number of groups or communities (*i.e.*, *Pan troglodytes*: Wrangham and Ross, 2008). When the studies are limited to a single population, it is impossible to distinguish between demographic characteristics that are a consequence of normal ecological parameters in the study population, and the potentially disturbing impacts of human activities.

Habitat types of owl monkeys

Aotus species occupy a large variety of environments (Wright, 1989). In different regions of Central- and South America they can be found at altitudes between 200 and 2,056 m above sea level (Aquino and Encarnación, 1986; Aquino *et al.*, 1990; De Sousa e Silva Jr. and Nunes, 1995; Wallace *et al.*, 2000; Castaño and Cardona, 2005; Fernández-Duque, 2011). Although the genus is widely present in tropical areas, studies on how these primates utilize their habitat in Peru (Warner, 2002), Venezuela (Castaño and Cardona, 2005) and Paraguay (Campos *et al.*, 2004) suggest that they do not only use primary forests, but also secondary ones and even some forests that have suffered disturbances.

At the extreme southern end of their continental distribution, in the humid Chaco of Argentina, Azara's owl monkeys inhabit different habitat types within the landscape matrix (Maturo, 2009). In particular, these are gallery forests and naturally fragmented forest "islands" or patches, that show important differences, for example with respect to the floristic composition, fruit availability, proportion of leaves, and phenophases (Placci, 1995; Giménez, 2004). Therefore, the populations of Azara's owl monkeys from the humid Chaco offer ample opportunities for comparative studies on groups that inhabit qualitatively different habitats units within the same macro environment and with different levels of anthropogenic influence. This allows examination of the behavioral plasticity of groups that live in different habitat types and their response to different spatial factors. Therefore, the aim of this research is to understand how different social groups in the humid Chaco of Formosa Province are demographically structured in different habitat types (continuous *vs.* forest patches), and how the possible differences and similarities in group structures are related to spatial factors.

Since 2007, we have been comparing wild social groups of Azara's owl monkeys in different habitats. Our study areas were the Estancia Guaycolec (EG), a private 25,000 ha cattle ranch, and the 52,000 ha protected Río Pilcomayo National Park (PNRP). In each study area, two sub-sets of owl monkey groups could be identified: social groups within the gallery forests (continuous habitat) (EG: n = 14; PNRP: n = 20), and groups in forest patches (EG: n = 16; PNRP: n = 14), as well as two potentially different levels of disturbance (*i.e.*, national park *vs.* cattle ranch). During all birth seasons between October 2006 and January 2011, we collected information on group size, estimated age of individuals, and infant presence from the social groups in EG and PNRP. Additionally, we measured the spatial distances between forest patches and gallery forests.

To locate groups in the control area, we walked through the forest at dawn and at dusk when owl monkeys were consistently active. Usually, we first detected the monkeys from the noises they made while moving, then took advantage of ambient light at dawn and dusk to count individuals and assess relative body size. For our study, we used a method not previously used for *Aotus*. Our method combines previous knowledge of the subspecies, the use of playbacks, and tools of classical field methods used to study diurnal primates (Setchell and Curtis, 2003), in order to minimize errors and optimize sample size. When unsuccessful in locating them, we sometimes played hoot calls (Moynihan, 1964; Wright, 1989), a low-pitch, relatively loud vocalization that is extremely effective in attracting individuals (Depeine *et al.*, 2008).

Territory mapping is one of the most reliable methods used for density estimations of Neotropical primates (Rudran, 1979; Janson and Terborgh, 1985; Ojasti, 2000). We stayed with each group for as long as they remained active in the morning, usually a few hours, or until it was too dark to reliably see at dusk. While following them, we recorded their positions with a GPS. The strict territoriality of owl monkeys and the relatively small overlap that exists among territories make it possible to recognize different groups via a combination of spatial data and group composition data (Wright, 1984, 1989; Fernández-Duque *et al.*, 2008). It is extremely difficult to sex owl monkeys in the field, as there is no obvious sexual dimorphism in body size or fur patterns, and the testes are relatively small (Dixson *et al.*, 1980). We were able to sex some individuals when the female was lactating and the nipples were prominent, or if we were close enough to observe the testes. Otherwise, we assumed that there was one adult male and one adult female in each group. To classify individuals in age categories we used a combination of demographic, behavioral, and morphometric data. We estimated age based on: 1) width and length of the dark stain produced by the subcaudal gland secretion, 2) relative body size, 3) presence of prominent nipples, and 4) behavioral patterns. Adults were the largest individuals in the group, and also had visually conspicuous subcaudal secretions on the ventral side of their tail (Dixson *et al.*, 1980). We also classified an individual as adult if we observed it nursing or transporting an infant because infant carrying by non-adults is extremely infrequent (Fernández-Duque *et al.*, 2008). We classified individuals as juveniles if they were smaller than the adults in the group and showed some staining of the ventral side of their tail due to perianal secretions. Finally, we classified individuals as infants when they were the smallest individuals in the group, had no stains on the tail, and were carried by an adult when moving between trees. To compare the age structure in both areas we limited our analyses to three categories: infants, juveniles, and adults.

We compared group sizes in the both areas with a Mann-Whitney U test for two independent samples.

Results and discussion

The results of our investigations confirm that the estimated individual densities (*i.e.*, total number of individuals per ha) for the private ranch (EG) are higher than those for the National Park (PNRP). In contrast, the size and range of groups, birth rates and age were similar between sites (Tab. 1). This heterogeneity in density of the subspecies had already been mentioned in various studies conducted in the Formosa Province (Rathbun and Gache, 1980; Zunino *et al.*, 1985; Brown and Zunino, 1994), and these new results confirm the conclusions by these authors, even though different methods were employed (Tab. 2).

Table 2. Demographic parameters of social groups in two habitat types: gallery forest and forest patches in Guaycolec Ranch (EG) and Parque Nacional Pilcomayo (PNRP), Argentina.

Variables	**Guaycolec Ranch**		**Pilcomayo National Park**	
	Gallery forest	**Forest patches**	**Gallery forest**	**Forest patches**
Size of groups (mean ± S.D.)	3.5 ± 0.1	3.0 ± 0.3	3.6 ± 0.2	3.0 ± 0.2
Adults (%)	58.0	66.0	55.5	66.0
Juveniles (%)	26.0	20.0	28.0	19.0
Infants (%)	16.0	13.0	16.4	16.0
Individual density (ind/km²)	45.2	13.1	29.8	7.6
Group density (groups/km²)	13.1	4.6	7.9	2.4
Birth rate (%)	54.2	40.0	52.5	48.0

The individual density of Azara's owl monkeys in gallery forests in the EG were high compared to the estimates that exist for the subspecies *A. a. azarae* (33.2 ind/km²; Svensson, 2008). The situation in PNRP is somewhat different, with a slightly lower individual density than the overall mean (Tab. 1). Svensson (2008) compared different study methods that had been used for calculations of density estimates for different *Aotus* species and found that transect censuses over estimated group densities. By incorporating a new, efficient methodological tool (playbacks), we have implemented a field schedule that takes the lunar cycle, birth seasonality, and territoriality of these primates into account in order to estimate individual densities. These densities are high, even when compared to other species like *A. nancymaae* (31.8 ±18.6 ind/km²; Aquino and Encarnación, 1986; Maldonado Rodríguez, 2011) and *A. nigriceps* (38 ind/km²; Wright, 1989).

The results confirm that group sizes, birth rates, and individual and group densities were larger for gallery forests than for forest patches at both study sites (Tab. 2). We found similarities in birth rates between different habitat types in PNRP, and in age structure at both study sites. These results coincide with various studies that suggest that in environments of lower quality, like forest patches, the population demography notably changes (*e.g.*, the lower number of species of primates in patches, low density, lower birth rates, high mortality rates; Janson and Chapman, 1999; Di Fiore *et al.*, 2006; De Moura, 2007; Pyritz *et al.*, 2010). The results of the density estimation in forest patches (rather than gallery forest) at both study sites show that they are lower than the mean value from all other studies on that species (22.4 ±18.6; Svensson, 2008) and are similar to densities observed for *Aotus zonalis* in remaining forest fragments of Panama (18.4 ind/km²; Svensson, 2010).

The quantitative evidence for smaller group sizes in forest patches raises numerous questions. It has been suggested that food availability is lower in forest patches (with low availability of fruits and leaves in both the dry and humid season, Giménez, 2004) compared to gallery forests. This, in turn, could result in higher mortality rates in forest patches than in gallery forests (Estrada and Coates-Estrada, 1996; Marsh, 2003). Nevertheless, it was observed that the range of group sizes was not very different compared to groups in gallery forests: in all cases group sizes ranged between 2–5 individuals, but the mean number of individuals was lower in the forest patches (Tab. 2). These smaller group sizes could be a reflection of the health status of the animals, and might also be related to the lower birth rates that we observed for groups in the forest patches in EG. This needs further study in order to understand the population dynamics of groups in forest patches. It is of paramount importance to evaluate to what degree mortality, emigration rates, and the health status of the animals influence the demographic structure of the owl monkeys in such habitat types, since these areas are more affected by agriculture and cattle farming than the gallery forests.

Before beginning this study, a clear association had been expected between the presence or absence of Azara's owl monkeys in forest patches and certain spatial factors that had originally been judged to be important (*e.g.*, vegetation structure, degree of isolation, and the surface of the island in ha). We studied 73 forests patches in the two areas (EG = 38 and PNRP = 35 forest patches). In EG, 53% of the patches were smaller than 4 ha (n = 20), while in the PNRP, 46% were that small (n =16). A logistic model predicted a 90% probability of finding monkeys in forests patches with an area of 11.4 ha, and with a 50% probability if the area was between 5 and 6 ha. At least once, we found owl monkeys in one island with an area of only 0.86 ha. Our results confirmed that although monkeys can be found in patches smaller (range = 0.1–3.9 ha; Juárez, 2012) than the smallest territory size recorded for groups in gallery forests (4–10 ha; van der Heide *et al.*, 2012; Wartmann *et al.*, 2014), the proportion of such patches actually containing owl monkey groups was low.

The fact that Azara's owl monkeys can be found in forest patches smaller than the normal territory sizes of groups living in gallery forests suggests a certain plasticity on the part of the sub-species to inhabit spaces of a relatively inferior quality such as the natural patches of shrubs or *algarrobales*, that have a higher number of species such as *Prosopis*, *Acacia caven* or *Geoffroea decorticans* than other forest patches (Juárez, 2012). The only species for which there is information available on the use of forest patches are *Aotus lemurinus* in Venezuela (Castaño and Cardona, 2005), and *A. azarae* in Bolivia (Wallace *et al.*, 1998), where they were found to inhabit anthropogenically fragmented patches of variable sizes (1.5 to ≥ 2000 ha). Group sizes for these two species were similar, with 2–5 individuals. Although the genus *Aotus* has a wide geographical distribution that includes areas of Amazonian forests undergoing the process of fragmentation (De Carvalho Jr., 2003), there is little information on basic aspects of their population ecology in the context of fragmented habitats. Defler (2003) has reported some plasticity in *A. lemurinus* individuals who can live in forests with a certain degree of alteration. Svensson (2008) also mentions this potential plasticity given her observations of *A. zonalis* in primary and secondary forests. Castaño and Cardona (2005) confirm this for different types of landscape matrices including forest fragments immersed in cattle farming land, surrounded by grazing land and coffee plantations, deforested areas, and forest contaminated with trash. These authors have preliminarily reported movements of individuals between these small forest fragments (Castaño and Cardona, 2005).

The movement between forest patches is an important factor affecting the survival of these animals. A local example of such movements between forest patches was documented by accident: an Azara's owl monkey was hit by a tractor while traversing the savanna between forest patches in the PNRP (pers. comm. Matías Carpinetto, Director of the PNRP). Another example for *A. zonalis* is cited by Castaño *et al.* (2010), with the direct observation of animals walking on electric power lines and through isolated trees in order to move from one patch to the next.

Although these are anecdotes, they exemplify that two species of the same genus with widely different geographic ranges (*A. zonalis* and *A. azarae*) have the ability to survive in disturbed and fragmented environments. The subspecies clearly shows the capacity to colonize places distant from continuous habitats, as shown in this study. In EG, the distances between forest patches and gallery forests ranged from 20 and 3,200 m; in PNRP this range was 3,100–12,500 m. Our logistic model suggests that the distance to gallery forests or to continuous humid Chaco forest had no apparent influence on the presence or absence of monkeys in the forest patches ($B_{\text{gallery forest}} = -0.001$; S.E. = 0.000; χ^2 Wald = 2.3; $p = 0.123$). This raises the question of how owl monkeys use the matrix in fragmented landscapes. Dense palm trees, although they are not characterized as a habitat for Azara's owl monkeys, could be used as sleeping sites, additional resources and as bridges between inhabitable environment—functioning as corridors or "stepping trees" between forest patches. Such dense palm trees grow naturally in the humid Chaco, and eventually their density increases in anthropogenically affected environments (*e.g.*, through the use of fire, Neiff *et al.*, 2004). It is necessary to continue our studies in this environment to understand the variables that can influence the habitat use of Azara's owl monkey in the humid Chaco.

Conclusions

Our 20-year long study on the owl monkeys of the Argentinian humid Chaco does not only continue to contribute to the understanding of the behavior and the ecology of this species, as well as the evolution of social monogamy and male care, but it also provides important information on the demography and habitat use of a species that has recently been declared a Natural Monument in Formosa Province.

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15 | CURRENT PERSPECTIVES ON WILD PRIMATE RESEARCH IN ARGENTINA

PERSPECTIVAS ACTUALES EN LA INVESTIGACIÓN DE PRIMATES SILVESTRES EN ARGENTINA

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Recent history of primatology in Argentina

Since the end of the 1970s, Argentina has had a leading role in primatology studies in Latin America, generating knowledge for various species in disciplines such as ecology, physiology and genetics (Mudry, 1980; Mudry and de Salum, 1981; Rumiz *et al.*, 1986; Zunino *et al.*, 1986; Brown and Zunino, 1990). Some of the forerunners of this discipline in Argentina are A.D. Brown, M. Mudry and G. Zunino, who have formed several research groups and established long-term study sites that have served to develop doctoral theses and ongoing research projects for students—local and foreign—and researchers (Tab. 1) (Oklander *et al.*, 2011).

During the early 2000s, new names were added to the discipline in Argentina through a substantial increase in the number of researchers and doctoral fellows that specialized in the study of wild primates. This occurred through substantial increases of governmental or university contracts and through the repatriation of Argentinian researchers working outside the country (mainly through a government program named *Raíces*). Together these resulted in an expansion in range of subjects and species studied (Agostini *et al.*, 2010, 2014; Kowalewski and Garber 2010; Nieves *et al.*, 2011, this volume; Holzmann *et al.*, 2012, 2015; Fernandez *et al.*, 2013; Perez *et al.*, 2013; Steinberg *et al.*, 2014; Tujague *et al.*, in press), including new studies that focused on population genetics, endocrinology, parasites and the effects of habitat fragmentation (Oklander *et al.*, 2010, 2014; Kowalewski *et al.*, 2011, Raño *et al.*, 2013; Pavé *et al.*, 2015). In 2005 a new site was established in Central Misiones near the town of San Pedro in Parque Provincial El Piñalito to study *Alouatta caraya* and *A. guariba clamitans* living in sympatry (Agostini *et al.,* 2010); however, a major outbreak of yellow fever that occurred in the province of Misiones during 2007–2008 decimated the majority of populations for these two species. In particular *A. g. clamitans*, which due to its low natural density in the area was severely affected across its entire distribution including the disappearance of all groups that were being studied (Holzmann *et al.*, 2010; Agostini *et al.*, 2014, 2015). Brown started a series of studies on *Sapajus paraguayanus* (Brown and Colillas, 1984; Brown *et al.*, 1986; Brown, 1989) that could have served as the basis for the development of field studies similar to those developed in other primates present in the country; however, to date no one has continued these studies even though it has been more than 20 years since the last published field work on the species.

With the exception of the El Piñalito site in Misiones that was abandoned after the yellow fever outbreak, almost all of the researchers are incorporated into very few long term study sites managed and supported by governmental institutions and private funds. This suggests the need to create and support long-term study sites in Argentina not only to incorporate new study populations but also to conserve non-human primates in Argentina.

In Argentina there are two field sites that concentrate their studies on *Alouatta caraya*: San Cayetano in Corrientes Province (27° 30' S, 58° 41' W), and Isla Brasilera in Chaco Province (27° 18' S, 58° 38' W), managed by the Estación Biológica Corrientes depending on the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (CONICET). There is one field site that has focused the majority of the studies on *Aotus azarae*, located in Estancia Guaycolec and the surrounding Formosa (25° 58' S, 58° 11' W), managed by Proyecto Mirikiná (Fundación Eco and Yale University). There is also one field site that focused studies on *Sapajus nigritus* at the Parque Nacional Iguazú (25° 40' S, 54° 30' W) in Misiones, managed by the Instituto de Biología Subtropical (CONICET), the Asociación Civil Centro de Investigaciones del Bosque Atlántico (CeIBA) and the Centro de Investigaciones Ecológicas Subtropicales (CIES). All of these sites have more than 20 years of continuous data on the studied species (Di Bitetti and Janson 2001; Kowalewski and Zunino, 2004; Zunino *et al.*, 2007; Oklander *et al.*, 2010; Janson *et al.*, 2012; Pavé *et al.*, 2012; Fernández-Duque and Huck, 2013; Fernández-Duque and Van der Heide, 2013).

Since 2005, as a consequence of these new research programs and new doctoral students dedicated to primatology, several novel spaces of debate were generated for the discipline inside and outside of Argentina. For example, since 2005 the Sociedad Argentina para el Estudio de los Mamíferos (SAREM) has organized diverse symposiums on primatology and since 2009 the Asociación de Antropología Biológica Argentina (AABA) has organized symposiums and discussion tables on primatology. In addition, Argentinian primatology began occupying an important place in Brazilian, Colombian and Mexican primatology congresses and has held elected positions in the Sociedad Latinoamericana de Primatología (SLAPRIM) since its start in 2012.

After 2010, there was a reversal to this growing trend of researchers and students dedicated to primatology, which may follow different reasons including a growing trend in Latin America of concentrating money in more applied research instead of basic science. Another reason could be a plateau in the number of professional researchers with a consequent decrease in the number of students interested or with the real opportunity to pursue doctoral projects in the discipline. In accordance with this trend, the study of wild primates began to include new areas of research, such as issues related to public health and parasite studies, which after some epidemiological events gained notoriety and allowed the funds to be secured so that basic studies of behavior could continue and new studies be developed. As mentioned earlier, due to the lack of senior researchers and the yellow fever outbreak there are no current studies on two out the five species of non-human primates that inhabit Argentina, *Sapajus paraguayanus* and *Alouatta guariba clamitans*. There is also a lack of long-term studies for *Alouatta caraya* and *Aotus azarae* in protected areas (*e.g.*, national parks) and for *Sapajus nigritus* in non-protected areas (*e.g.*, interphase human-wildlife, rural and urban areas).

It is noteworthy that more than 90% of the publications on wild primates in Argentina come from three sites that have generated—and continue to generate—extremely valuable information about Argentinian primates: the Estación Biológica Corrientes, the Estancia Guaycolec and the site in the Parque Nacional Iguazú (Oklander *et al.*, 2011). Nevertheless, there are some new some study sites that currently have students developing Ph.D. studies or sampling primate populations: Parque Nacional Chaco in Chaco Province (26° 47' S, 59° 37' W), San Alonso in Corrientes (28° 18' S, 57° 27' W), Yacutinga in Misiones (25° 34' S, 54° 04' W), and Karadya in Misiones (25° 52' S, 53° 58' W). Hopefully, these sites will join the long-term study sites for wild primate populations.

Two research groups concentrate their studies on the genetics and molecular analysis of wild primates in Argentina: the GIBE (Grupo de Investigación en Biología Evolutiva, FCEyN, UBA) and the SHDG (Servicio de Huellas Digitales Genéticas, FFyB, UBA). There are also studies developed outside the country by local and foreign researchers that include genetic studies on *Aotus azarae* (Huck *et al.*, 2014) and endocrine studies on *Alouatta caraya* (Raño *et al.*, 2013), *Aotus azarae* (Fernández-Duque *et al.*, 2011) and *Sapajus nigritus* (Wheeler *et al.*, 2013).

We can highlight the recent creation of APrimA (Asociación Primatológica Argentina) in 2009, which provides legal and institutional framework to primatology in Argentina and fosters the fluid exchange between researchers. The creation of the APrimA serves to organize research on primates in a consensual way, including avoiding the overlap of studies and themes and identifying those species, issues and areas that lack ongoing research projects. APrimA is currently represented in international organizations related to primatology (SLAPRIM and IPS), providing for the first time a formal position for Argentina in the debate about international policy directed at the study of primates worldwide.

Perspectives on conservation of primates in Argentina

While contributions and studies are numerous, they are concentrated in a few places mainly in the provinces of Corrientes and Chaco (EBCo), Formosa (Guaycolec) and Misiones (PN Iguazú – IBS). These areas are associated with the establishment of long-term study sites and the presence of researchers or doctoral fellows. Most of the scientific contributions that are made in Argentina are generated by research groups that follow the guidelines of government agencies, so most are biased towards those disciplines or subjects that can be analyzed in doctoral studies and have higher probability of publication in scientific journals. This includes several lines of research, mainly those related to basic knowledge on real and current distribution of the species, number of individuals in different populations and the major threats to long-term survival. Currently we have data of these parameters on only a few populations of two (*A. caraya* and *S. nigritus*) of the five species of primates

Table 1. Doctoral Theses produced in Argentina in the last 10 years (some of them are still in progress).

Species	Research group	Author	University	Advisor–Co-Advisor	Year completed	Title
Alouatta caraya	EBCo	Oklander, L.I.	UBA	Zunino – Corach	2007	Social structure and kinship in wild populations of black and gold howler monkeys in northeastern Argentina
Alouatta caraya	EBCo	Kowalewski, M.	UIUC	Garber	2007	Patterns of affiliation and co-operation in howler monkeys: an alternative model to explain social organization in non-human primates
Alouatta caraya	EBCo	Pavé, R.	UNL	Kowalewski	2014	Parent-offspring conflict in two populations of wild black and gold howler monkeys
Alouatta caraya	EBCo	Fernandez, V.	UBA	Kowalewski	2014	Nutritional ecology in black and gold howler monkeys at the southern limit of their distribution
Alouatta caraya	EBCo	Raño, M.	UBA	Kowalewski – Valeggia	2016	Female reproductive strategies in black and gold howler monkeys
Alouatta caraya	EBCo	Perez Rueda, A.	UNNE	Kowalewski	Current	Effects of habitat modification on behavioral and physiological patterns in black and gold howler monkeys
Alouatta caraya	EBCo	Ramirez- Orjuela, C.	UNNE	Kowalewski	Current	Seasonal variation in foraging strategies in black and gold howler monkeys living in a natural fragmented forest in northeastern Argentina
Alouatta caraya	EBCo	Gennuso, S.	UNNE	Kowalewski – Valeggia	Current	Behavioral and physiological trajectories in juvenile black and gold howler monkeys
Alouatta caraya	EBCo	Wollnik, M.	UStirling	Buchanan – Smith	Current	The communicative role of pelage colouration in an all-body sexually dichromatic primate: the black-and-gold howler
Alouatta caraya	UNAM	Sanchez-Fernandez, C.	UNAM	Kowalewski – Badano	Current	Characterization of genetic markers of the immune system in non-human primates (Platyrrhini) of Argentina
Alouatta caraya, A. guariba clamitans	IBS	Agostini, I.	UniRoma	Boitani – Visalberghi	2009	Ecology and behavior of two howler monkey species *(Alouatta guariba clamitans* and *Alouatta caraya)* living in sympatry in northeastern Argentina
Alouatta caraya, A. guariba clamitans	IBS	Holzmann, I.	UNLP	Di Bitetti – Merino	2011	Potential geographic distribution and vocal behavior in *Alouatta guariba clamitans* and *Alouatta caraya*

Table 1. (Continued).

Species	Research group	Author	University	Advisor–Co-Advisor	Year completed	Title
Aotus azarae	CECOALPMirikina	Juarez, C.	UNT	Fernández-Duque	2012	Demography and life history of Mirikina (*Aotus azarae*) in the Humid Chaco from Formosa
Aotus azarae	CECOALPMirikina	Corley, M.	UPenn	Fernández-Duque	Current	Leaving home: genetic, endocrine, and behavioral correlates of dispersal in monogamous owl monkeys (*Aotus azarae)* of Argentina
Sapajus nigritus	IBS	Tujague, M.P.	UNLP	Lahitte – Janson	2013	Within-group spatial behavior and memory in cai monkeys: similarities between human and non-human primates
Sapajus nigritus	IBS	Baldovino, M.C.	UNC	Di Bitetti – Brown	2010	Infant development in (*Cebus nigritus*): motor ability and allomaternal behavior ontogeny
Alouatta caraya	IBS	Brividoro, M.	UNLP	Oklander	Current	Sleeping behavior and sleeping site selection patterns in black and gold howler monkeys
Sapajus nigritus	IBS	Pfoh, R.	UNC	Agostini – Di Bitetti	Current	Adaptive function of grooming in social contexts in *Cebus apella nigritus*: an experimental approach
Sapajus nigritus	PCai	Green, A.	UM	Janson	2014	Consequences of color vision variation on performance and fitness in capuchin monkeys
Sapajus nigritus	PCai	Wheeler, B.	SUNYSB	Koenig – Janson	2009	An experimental analysis of alarm calling behavior in Wild Tufted Capuchin Monkeys (*Cebus apella nigritus*)
Sapajus nigritus	PCai	Tiddi, B.	LJMU	Aureli – Schino	2010	Behavioural Interchanges among Wild Tufted Capuchin Monkeys *(Cebus apella nigritus*)
Sapajus nigritus	PCai	Clara, J. Scarry's	SUNY SB	Janson – Koenig	2012	The functions and consequences of intergroup aggression among argentine tufted capuchin monkeys (*Cebus apella* [*Sapajus*] *nigritus*)
Cebus [*Sapajus*] sp. & multiple species	GIBE	Nieves, M.	UBA	Mudry – Mühlmann	2007	Heterochromatin and chromosomic evolution in Neotropical primates
Alouatta & multiple species	GIBE	Steiberg, E.	UBA	Mudry	2011	Sexual determination in Neotropical primates: the howler monkey case
Alouatta caraya	GIBE	Bruno, G.	UBA	Mudry	2011	Genetic and ethological characteristics of *Alouatta caraya* in forests outside their natural distribution

Table 1. (Continued).

Species	Research group	Author	University	Advisor–Co-Advisor	Year completed	Title
Alouatta caraya	GIBE	Milozzi, C.	UBA	Mudry – Navone	Current	*Alouatta caraya* and endoparasites in wild and semicaptive populations at the southern limit of their distribution: a potential model of application and transference
Cebus [*Sapajus*] sp. & multiple species	GIBE	Fantini, L.	UBA	Nieves	2015	Chromosomic evolution and divergence in *Cebus* and *Ateles* from a cytogenetic perspective
Platyrrhines	MELAD	Aristide, L.	UNLP	Perez – Tejedor	Current	Diversification of platyrrhine primates along their evolutionary history: an analysis based on geometric morphometrics and comparative methods
Suborder Anthropoidea	MELAD	Rocati, G.	UNLP	Perez	Current	Processes of craniofacial diversification and convergence in New and Old World Monkeys (Infraorders Platyrrhini and Catarrhini)
Patagonian Platyrrhini	LAPA	Novo, N.	UNC	Tejedor	2015	Systematics and evolution of the New World monkeys (Platyrrhini, Primates): Understanding the Patagonian Miocene diversity in the Neotropical radiation context

EBCo	Estación Biológica Corrientes CONICET, Corrientes, Argentina
IBS	Instituto de Biología Subtropical CONICET, Misiones, Argentina
UNC	Universidad Nacional de Córdoba, Córdoba, Argentina
UBA	Universidad Nacional de Buenos Aires, CABA, Argentina
UNLP	Universidad Nacional de La Plata, La Plata, Argentina
UNNE	Universidad Nacional del Nordeste, Corrientes, Argentina
UNL	Universidad Nacional del Litoral, Santa Fe, Argentina
UNT	Universidad Nacional de Tucumán, Tucumán, Argentina
UNCom	Universidad Nacional del Comahue, Bariloche, Argentina
CECOAL	Centro de Ecología del Litoral CONICET, Corrientes, Argentina
PMirikina	Proyecto Mirikiná, Fundacion Eco, Formosa, Argentina
PCai	Proyecto Caí, PN Iguazú, Misiones, Argentina
GIBE	Grupo de Investigación en Biología Evolutiva, CABA, Argentina
UIUC	University of Illinois at Urbana, IL, USA
SUNY SB	State University of New York at Stony Brook, NY, USA
UM	University of Montana, MT, USA
UPenn	University of Pennsylvania, PN, USA
LJMU	Liverpool John Moores University, Liverpool, UK
UStirling	University of Stirling, Scotland
UniRoma	Università "La Sapienza" di Roma, Italy
MELAD	Morphological Evolution Lab - Anthropology Division, La Plata, Argentina
LAPA	Laboratorio de Paleontología, Unidad de Investigación en Geología y Paleontología, Centro Nacional Patagónico (CENPAT–CONICET), Chubut, Argentina

inhabiting the country and there are no studies trying to estimate them. The continuous decline of forest environments these primates inhabit in Argentina forces us to deal with the problems of their long-term survival and the coordination of scientific studies on demographic, behavioral and disease patterns with environmental organizations, provincial governments and the national government.

During recent years, some initiatives to this end have been carried out, such as the first workshop for the conservation of the red howler (*Alouatta guariba clamitans*) where a group of researchers from different disciplines developed guidelines to ensure their long-term survival in Argentina (Agostini *et al.*, 2014). As a result of this workshop, funds were secured to organize field surveys that could identify population remnants in Misiones, estimate population parameters and identify key areas for its conservation. Some preliminary results showed that the species has survived in low densities in small areas of the province but disappeared in much of their former home range. These field trips done by a team of researchers and park rangers are the only effort dedicated to knowing the actual distribution and population density of some of the primates that inhabit the country and were managed and organized by an independent group with very little support from the local government (with the exception of the Administración de Parques Nacionales – Delegación Regional Noreste). In addition to this and other specific initiatives, we can highlight as a transcendent fact that a group of researchers and personnel from the Secretaría Nacional de Ambiente y Desarrollo Sustentable just finished writing the first draft of a National Plan for the Conservation of Primates in Argentina. The importance of this plan lies in its support from the Comisión Nacional Asesora para la conservación y utilización sostenible de la Diversidad Biológica (CoNaDiBio) giving an institutional framework and a formal commitment by the government. The Plan sets specific goals and objectives to follow that are intended to ensure the survival of the five primates that inhabit the country, specifically:

- Establish priority actions for the conservation of the five primates inhabiting Argentina.
- Ensure the conservation of the primates of Argentina including their habitats, including the genetic and demographic viability of different subpopulations, their interconnection and their distribution throughout the ecoregions and sub-ecoregions of Argentina.
- Identify and implement priority conservation areas within the ranges of Argentinian primates.
- Incorporate priority areas for primate conservation into management for protection, conservation and restoration of lands.
- Develop strategies focused on securing funds to ensure the objectives detailed in this Action Plan.
- Develop strategies to allow the implementation of inter-institutional collaborations, including academic institutions, civil sector organizations, governmental agencies, the private sector and the society in general.

Although the emergence of this draft is a big step towards the conservation of primates in Argentina, it has yet to be completed or discussed formally, which severely questions its validation and implementation since it has been three years since the first meetings dedicated to this Action Plan.

Regarding the current situation of the Argentinian primates, the lack of knowledge on population status for several species and sites in the northeast and northwest makes it difficult to assign actual threat categories to many of the species that inhabit our forests. A potential method of estimating species dynamics and viability over time could be done through the extrapolation of data obtained through previously studied populations and identified survival threats. With the exception of the *mirikiná* (*Aotus azarae*), in the recent categorization of the Argentinian mammals (Agostini *et al.*, 2012) all primate species have maintained or increased their extinction risk. *Alouatta caraya* has increased from Near Threatened (NT) to Vulnerable (V) and *Alouatta guariba* from Endangered (E) to Critically Endangered (CR). This fact clearly reflects the dramatic increase in the transformation of natural environments over the past decade in areas inhabited by these species and in the particular cases of *Alouatta guariba,* the simultaneous effect of deforestation and yellow fever outbreaks. The new categorization of *Sapajus paraguayanus* within the Data Deficient (DD) category reflects the actual lack of knowledge on this species in the country.

After the adoption of the "minimum budgets" law that attempts to organize habitat transformation at a provincial scale, a decline in the annual rate of deforestation was recorded; however, it currently appears to be increasing (UMSEF 2012, 2014).

All primates inhabiting the country have protected populations within national or provincial reserves; however, the area covered by these reserves represents only 1% of the total estimated area occupied by these species. The recent creation of the Parque Nacional El Impenetrable adds some 100,000 ha of protected territory at the national level. In this park, populations of *Alouatta caraya* and *Aotus azarae* have been recorded. A tool with enormous potential to ensure the conservation of Argentinian primates and encourage new research at innovative locations is the numerous private areas dedicated to the conservation of native forests and tourism within the ranges of these primates. The Red Argentina de Reservas Naturales Privadas, an organization that contains the vast majority of private reserves in the country, was created in 2014 and should be included as a key player within the National Plan for the conservation of primates (*http://reservasprivadas.org.ar/*).

The development of software with the ability to identify potential areas that could be inhabited by primates could facilitate the identification of new sampling areas according to the objectives of the National Plan for the conservation of primates. An excellent example of the use of these tools was implemented in 2011 in Mexico through an agreement between the Mexican National Commission for the knowledge and use of biodiversity (CONABIO) and the Mexican Association of Primatology, A.C. (AMP–AC) researchers, NGOs and other participants who evaluated all the available data on Mexican primates and identified the priority areas for their conservation using specific software to analyze these data (ConsNet).

Argentina has a very similar scenario compared with Mexico. There is a lack of knowledge on population values and occupation areas at the regional scale but we have national agencies (CoNaDiBio) and private or academic organizations (APrimA) with the ability to generate equally valuable results. Studies on genetic variation of different populations (*e.g.*, Oklander *et al.,* this volume) are also important to identify priority areas for the conservation of primates (*i.e.*, Evolutionary Significant Units ESUs and Managment Units MUs). Unfortunately, such analyses were only performed on two of the five primate species (data on *A. guariba* are unpublished).

In summary, in order to promote the conservation of primates in Argentina it is important to do the following:

1. Sustain the long-term sites that already exist. The research conducted in them provides unique and important data that allow us to develop management and conservation strategies for the different primate species;
2. Finish and implement the National Plan for the conservation of primates in Argentina;
3. Promote the establishment of study sites for *S. paraguayanus*, *A. guariba clamitans*, and *A. caraya* in protected areas plus *S. nigritus* in unprotected areas;
4. Develop studies on genetic variability of several populations of each species. These studies will allow us to define priority areas for establishing conservation units applicable to all primates in Argentina.

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PRIMATOLOGY IN ARGENTINA

This is the first book dedicated in its entirety to current primatological studies in Argentina. After more than 40 years of continuous work, we have put together a volume that represents most of the research groups studying Argentinean wild and captive primates. We have also included a summary of the primatological research in Argentina from its beginning until the present. This book appears within social and academic contexts related to the establishment and expansion of the *Asociación de Primatología Argentina* (APrimA) as well as the *Sociedad Latinoamericana de Primatología* (SLAPRIM), and in parallel with similar volumes edited in Brazil, Peru, Mexico, Colombia, and Venezuela. We hope that this volume will serve to engage students in this discipline, and will provide valuable information in order to build a National Plan for Primate Conservation in Argentina, and consequently, preserve the habitats of our cousins, the non-human primates.

Martín M. Kowalewski and **Luciana I. Oklander**
EDITORS

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