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DEER SPECIALIST GROUP NEWS

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Editorial

One of our main tasks is to increase communication and dissemination to improve deer biology knowledge. We maintain a List server (iconservacionneotropical@gmail.com) and webpage (http://www.icneotropical.org/home.htm) with a database of current deer research and conservation articles. Our main dissemination channels include the Deer Specialist Group Newsletter, an electronic, peer-reviewed journal, and our list server exclusively devoted to deer biology and conservation. We have appointed Dr. Patricia Black de Decima as the scientific editor and obtained the Newsletter's ISSN in 2014. Also we have updated the author guidelines to assure the uniformity and quality of the articles published.

As a part of the Species Survival Commission one of our main tasks for this period is the Global Mammal Assessment. In consultation with our RLAs and SSC staff, we shortened the Red List Assessment process to DSG-recognized 71 deer species. We are reassessing species who meet the following criteria: a) species that DSG members believe have shown dramatic changes in distribution and numbers over the past five years; b) species where increased knowledge of their populations and distributions might lead to changes in IUCN Red List status; or c) new species as a result of revised taxonomy. We did not reassess species whose status has not changed significantly in the past five years (according to local experts) or for which we have no new information.

Sarah Brook (RLA for Old World deer species) reported that the following species have been re-assessed, submitted to the RLU, and will be available *on line* in June: *Axis axis, Axis calamianensis, Axis kuhlii, Cervus nippon,* Elaphodus cephalophus, *Hydropotes inermis*, *Przwalskium albirostris*, *Rucervus duvaucelli*, *Rucervus eldii*, *Rucervus schomburgki*, *Rusa alfredi*, *Rusa marianna*, *Rusa timorensis*, *Rusa unicolor*, *Moschiola indica*, *Moschiola kathgyre*, *Moschiola meminna*, *Moschus anhuiensis*, *Moschus berezovskii*, *Moschus cupreus*, *Moschus fuscus*, *Moschus leucogaster*, *Moschus moschiferus* and the all 6 *Tragulus* species. The re-assessments for the remaining 26 species are ongoing, and will be completed soon. The striking result is how little additional knowledge on ecology or conservation we have for most species in the 5 years since the last assessment.

Eveline Zanetti (RLA for New World deer species) has focused on species thought to have on dramatic changes in the distribution and numbers over the past five years. We identified three species as first priority for review: *Hippocamelus antisen*sis, *Hippocamelus bisulcus*, and *Mazama nana*. These re-assessments are now complete and we are working on the remaining 14 species.

We wish to thanks to everyone who has assisted Sarah and Eveline with these re-assessments and additional assistance is needed for the remaining 40 species. All re-assessments must be completed by September 2015.

In December 2014 we organized a Deer Symposium during the "*Congreso de Zoología del Uruguay*", attended by participants from Argentina, Brazil and Uruguay. The main symposium topic was *ex situ* conservation, a need we identified because few advances have occurred in Uruguay and the region. It is important that zoos and breeding institutions share information, achievements and standardize management criteria. Other important issue is to record the genealogies based on an identification of individuals. For endangered deer species, such as pampas deer, it is important to maintain a studbook of lineages in captivty. This symposium addressed: (i) identification of the deer species found in zoos of Uruguay and the region, (ii) standardize guidelines for animal management, and (ii) how zoos can contribute knowledge to local, regional and national level to achieve the proper management and conservation of Neotropical deer. We included 11 abstracts from the symposium in this issue. We thank *Comisión Sectorial de Investigación Cientifica* (CSIC-UdelaR-Uruguay), for funding the Neotropical Deer Project.

In December 2014, Bill McShea, Myint Aung and John Linnell assisted the Myanmar government in creating a Conservation Plan for Elds deer. This plan was endorsed by the government in March 2015 after agency review. The first steps of the plan are a national survey to locate remaining populations and increased support and training for the two reserves known to contain the species. The national survey was started in April and should be completed by the end of May 2015. Preliminary results are that no populations outside of protected areas have increased in extent since the last survey in 1998. We also acknowledge Conservation Force (www.conservationforce.org) for providing funding for Eld's deer ecology and conservation projects in Southeast Asia.

In this issue we are including six interesting original articles that update the knowledge of endangered deer and methods for research in the field and laboratory. We want to acknowledge to all who contribute in this edition, also we extent our thanks to all of you for being part of the DSG and we invite to submit articles to the next issue.

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Possible occurrence of *Muntiacus gongshanensis* in Dibang Valley district of Arunachal Pradesh, Northeast India

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Abstract

In January 2013, photographs were taken by the second author of two dead muntjacs in Dibang Valley district, Arunachal Pradesh, Northeast India which appear most likely to be *Muntiacus gongshanensis.* The photographs showed diagnostic feature of a blackish upper tail, absence of prominent tuft on the forehead in the male and the female appears to have small tufts at the side of the forehead which distinguish the species on the basis of tail colour from *M. muntjak sensulato*, and *M. crinifrons* and *M. feae* on the basis of hair tufts. *M. montanus* cannot be completely ruled out, but is highly unlikely on the basis of range, whilst *M. reevesi* has a paler upper tail, *M. atherodes* a dark forehead and neither species has tufting in the female. *M. vuquangensis* differs in tail, antler and tufting characteristics. This leaves animals in the *M. rooseveltorum* group of species, which however are currently poorly understood. Within this group some animals ostensibly lack tufts on the forehead of the male and some have blackish tails, although no male animals with both these features have yet been recorded and some females have tufting and dark tails. This is the second report of this species from India. *M. gongshanensis* has only recently been found in Arunachal Pradesh and in Nagaland. Outside India, *M. gongshanensis* is reported from North Myanmar, China/South East Tibet and West Yunnan.

Resumen

Fueron tomadas, por el segundo autor en enero de 2013, las fotografías de dos de los muntjacs muertos en el distrito Dibang Valley, Arunachal Pradesh, noreste de la India, que podrían tener mayor probabilidad de ser *Muntiacus mangshanensis*. Las fotografías mostraban características diagnósticas cola superior negruzca, ausencia de mechones prominente en la frente en el macho y en la hembra hay pequeños mechones en la parte de la frente los cuales distinguen a las especies sobre la base del color de la cola de *M. muntjak sensulato* y *M. crinifrons* y *M. feae* sobre la base de mechones de pelo. *M. montanus* no se puede descartar por completo, pero es muy poco probable, mientras que *M. reevesi* tiene una cola superior más pálida, *M. atherodes* la frente oscura y ninguna de las especies tiene formación de nudos en la hembra. *M. vuquangensis* difiere en las características de la cola, cornamenta y mechones. Esto deja a los animales en el grupo *M. rooseveltorum* de especies, que son actualmente poco conocidos. Dentro de este grupo algunos animales aparentemente carecen de mechones en la frente de los hombres y algunos tienen cola negruzcas, aunque no hay animales machos aún no se han registrado estas características y algunas hembras tienen formación de nudos y colas oscuras. Este es el segundo informe de esta especie en la India. *M. gongshanensis* ha sido encontrado recientemente en Arunachal

Pradesh y en Nagaland. Fuera de la India, *M. gongshanensis* se informó desde el norte de Myanmar, China / Sureste de Tíbet y el oeste de Yunnan.

Key words: Gongshan muntjac, *Muntiacus gongshanensis,* Arunachal Pradesh, Dibang Valley, Mishmi hills, India

Introduction

There are 12 species of Muntjacs presently in the world according to the IUCN red data list (IUCN 2014), out of which, three are found in India. The most common and widespread is the red muntjac (*Muntiacus muntjak*) (*M. vaginalis* in the IUCN Red List), the other two species have been reported only recently from the region. The Gongshan muntjac, *M. gongshanensis* was reported from the Mishmi hills of Arunachal Pradesh (Choudhury 2003, Choudhury 2009) and the leaf muntjac *M. putaoensis* was reported in 2003 from Namdapha National Park in Arunachal Pradesh (Datta *et al*. 2003).

There is considerable confusion that arose from the application of the name '*M. crinifrons*' in a series of papers in the late 1990s (e.g. Rabinowitz *et al*. 1998, Amato *et al.* (1999) which considered *M. gongshanensis* to be junior synonym of *M. crinifrons*, because no significant differences were detected in a small amount of mitochondrial DNA analyzed; there was no discussion of morphological differences between these two taxa. *M. crinifrons* was reported from Namdapha National Park in Arunachal Pradesh (Datta 2003), but the authors made no mention of *M. gongshanensis*. A new book on field guide to Indian mammals also refers to Gongshan muntjac and Black muntjac as the same species (Menon 2014). The situation has been complicated by the report that muntjacs with 'black' pelage have recently been observed in 2013 from Darjeeling, India, according to a newspaper article (Mukherjee 2013); the article speculates that these are most likely melanistic muntjac, but also could be *M. crinifrons* or *M. gongshanensis*. In fact it is most likely that these Darjeeling animals are melanistic *M. muntjak,* as has been previously reported (Inglis 1952). All muntjacs have varying degrees of orange (/red/yellowish crowns/hind crowns with or without tufting, the main diagnostic external features of *M.*

crinifrons are extensive and long tufting on the forehead (forecrown and crown) of both sexes, with very subdued and often barely noticeable frontal stripes, in combination with large body size and a dark upper tail (Timmins unpub).

The only explicit report of Gongshan muntjac for India is by Choudhury (2009) from Dibang Valley, Lohit and Anjaw district of Arunachal Pradesh. The information is based mainly on skulls and skin samples from local villages in the Mishmi hills. However, all reports from India of black muntjac that do not explicitly discount Gongshan muntjac and or melanistic *M. muntjak* from the identification should be considered as potential Gongshan muntjacs and or melanistic *M. muntjak*.

Material and Methods

We report the probable presence of *Muntiacus gongshanensis* in Dibang Valley district of Arunachal Pradesh and also probably the first photographic evidence of entire body specimens of a male and female. Two animals were found in the possession of a hunter who had just killed them at a site approximately 25 km from Gipulin village N 28^{O} 51' 690, E 95 ^{O} 55' 835 on 13 November 2013 at an altitude of 2060 m. The second author had sighted 5-6 individuals of the same species near Awa River, a day before. Two individuals (male and female) were shot by a local villager on the following day (see images) close to where the animals were sighted earlier. The weight was approximately between 15-20 kg. Hunting wildlife is common in Dibang Valley as in the rest of the state and in Northeast India (Aiyadurai 2011). Muntjacs are mainly hunted for meat, skins are preserved and used as mats; their antlers and tail are used as key chains. Body parts were not preserved and the meat was partially consumed. Later the remaining meat was smoked and carried back to the village. The second author, who happened to see the animals, is not a biologist and the first author was not present to make any measurements or collect and preserve any body parts. Though there is a practice of preserving skulls and

skins as a local practice, not all animals are preserved. It is done only if the animals were shot near the village.

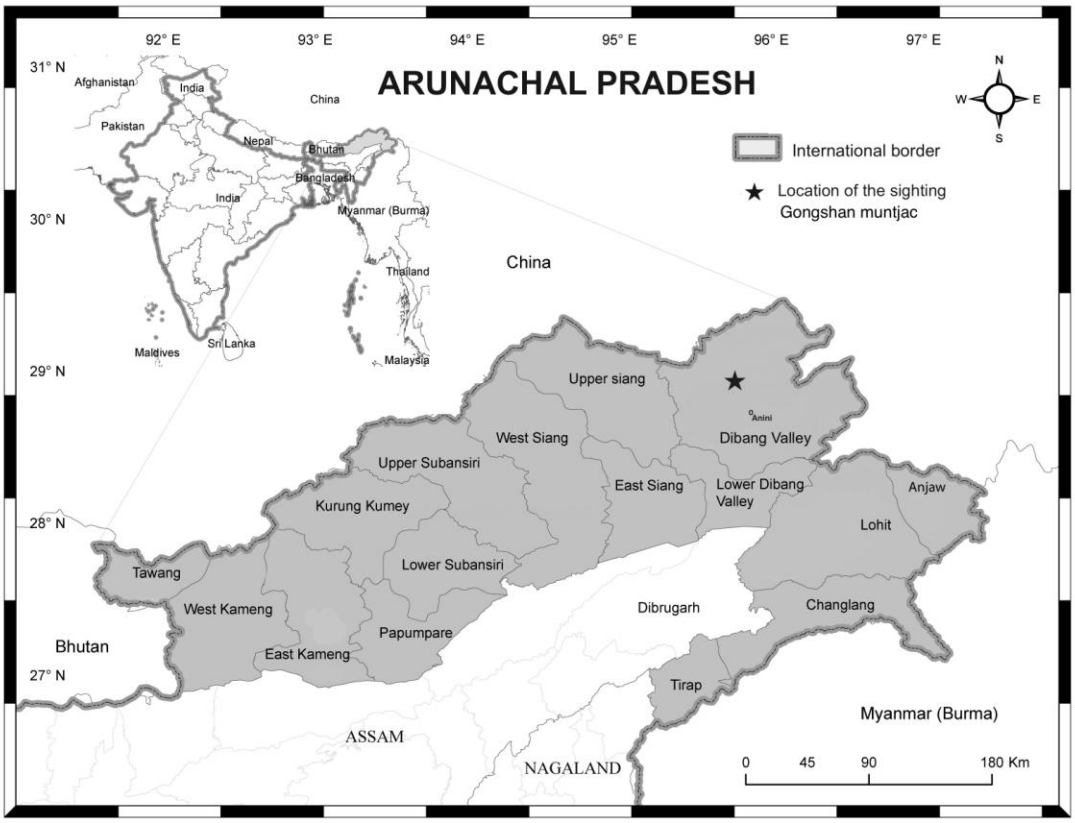

Figure 1 Map of Arunachal Pradesh showing the location of the sighting of possible *M. gongshanensis*.

Study Area

Dibang Valley district is spread over an area of 9129 sq. km and nearly half the district is under state protection in the form of Dibang Wildlife Sanctuary (4149 sq.km) (Fig. 1). This sanctuary was declared in 1998 but there is still resentment among the local Idu Mishmi tribe that the sanctuary was declared without their consensus. The district has the least human density in the country at one person per sq. km and most of the district's terrain is unsuitable for agriculture or other sustenance practice. The remoteness, rugged terrain and low infrastructural development, and lack of road networks have made

field research difficult.

Figure 2. Male with the diagnostic morphological features.

Results

These animals are identified possibly as *M. gongshanensis.* The photographs showed diagnostic feature of a blackish upper tail (Fig. 2), absence of prominent tuft on the forehead in the male and the female appears to only have small tufts at the side of the forehead (Fig. 3). This distinguished the species on the basis of tail colour from *M. muntjak sensulato*, and *M. crinifrons* and *M. feae* on the basis of hair tufts. *M. montanus* cannot be completely ruled out, but is highly unlikely on the basis of range. *M. reevesi* has a paler upper tail, *M. atherodes* a dark forehead and neither species has tufting in the female. *M. vuquangensis* differs in tail, antler and tufting characteristics (Timmins *et al*. 2008). This leaves animals in the *M. rooseveltorum* group of species, which however are currently poorly understood, some animals ostensibly lack tufts on the forehead of the male and some have blackish tails, although no male animals with both these features have yet been recorded and some females have tufting and dark tails (Timmins *pers comm*).

Northern part of Dibang valley bordering South Tibet is known for abundant musk deer *Moschus chryogaster populations* and to contain takin *Budorcas taxicolor*. Due to its proximity to Tibet, the Idu Mishmi tribe, in the past, bartered musk pods, Mishmi teeta *Coptis teeta* and animal skins with Tibetans for pieces of metal, yarns and salt. After the India-China war of 1962, people movement to Tibet was

largely restricted due to militarization of the borders that led to the increase in trade links with Assam, Dimapur and Nepal. The district of Dibang Valley falls under the Eastern Himalayan Biodiversity Hotspot (Myers *et al*. 2000). Forests in the region are classified as temperate broad-leaved forest.

Figure 3. The photographs show diagnostic feature of a blackish tail and absence of prominent tuft on the forehead of male and the female

Local information on the species

Locally *M. gongshanensis* is apparently identified as Manzo-imbu (Idu Mishmi name). The name for *Muntiacus muntjac* appears to be Manzo and the suffix imbu is probably added for *M. gongshanensis* (Manzo-imbu). It is not absolutely clear whether the local people used this term Manzo-imbu consistently for *M. gongshanensis.* Choudhury (2003) in his book on the mammals of Arunachal Pradesh also reported the Idu Mishmi name for Gongshan muntjac as Menjo-Imbu.

According to the local people, Imbu means large tree groves far from human settlements or cultivable areas (Imbupa in Idu language is a place where there is no cultivation but only dense forests). According to the Idu Mishmi people, Manzo-imbu is a very rare species and not too many are found. While Manzo is reported to be plentiful and found very close to the villages, Manzo-imbu never come close to the village. Local people say that Manzo-imbu is slightly larger than Manzo, which suggests that local names might not be consistently applied to the biological species. Other wildlife found in the region where Gongshan muntjac was found are Takin (*Budorcas taxicolor)*, Asiatic black bear (*Ursus thibetanus)*, clouded leopard (*Neofelis nebulosa*) and tiger (*Panthera tigris).*

Discussion

M. gongshanensis was first described by Ma *et al*. (1990) from Gaoligong and Biluo mountain, Yunnan, north-western China. It is distributed in North Myanmar where camera traps have recorded multiple images of animals that show characteristics consistent with *M. gongshanensis (*Timmins, *et al*. 2008), although genetic analysis of material collected from the same regions as the camera-trap imagery was reported as *M. crinifrons* (Amato *et al*. 1999, Rabinowitz *et al*. 1998). Neither paper considers *M. gongshanensis* and *M. crinifrons* as two different species, nor does either give any indication of morphology that would allow identification as *M. gongshanensis*. The species has been reported from Mishmi hills (Arunachal Pradesh) and Pangsha village (Nagaland) based on skin and skull remains by Choudhury (2009). According to the IUCN (2014), *M. gongshanensis* falls under the category of Data Deficient. There is no mention of this species in India's Wildlife protection Act, as this is a new entrant into the list of Indian mammals with the only record as reported by Choudhury (2009).

The history of confusion makes it challenging to determine the identity of the two specimens reported here. The present finding is the result of an anthropological enquiry into the Idu Mishmi people's perception of nature. The first author had chanced upon the pictures of Muntjac and assistance from deer

specialists, which led to the possible occurrence of *M. gongshanensis* in Dibang Valley. Less accessible frontier regions like Dibang Valley district in Arunachal Pradesh in Northeast India have often hampered academic research for a long time and long-term research in any discipline has only begun. More research, tissue samples for genetic analysis, and morphological measurement of skulls of *M. gongshanensis* from the region are needed to ascertain the current status of the species.

Acknowledgements

We thank the department of Sociology at the National University of Singapore for providing the field grant to carry out AA's fieldwork. Our sincere thanks to Arunachal Pradesh's Forest Department for providing the research permit to work in Dibang Valley. AA is grateful to Rufford Small Grant (UK) and Firebird Foundation for Anthropological Research (US) for financial assistance. We thank Dr. William Duckworth and Robert Timmins for help in identifying the species and for providing valuable comments on this manuscript.

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Indochinese Hog Deer *Axis porcinus annamiticus* on the brink of extinction

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Abstract

The Hog deer *Axis porcinus* has undergone dramatic range-wide declines, largely unnoticed. The species has been extirpated from Lao PDR, Vietnam, and Thailand; it has been reintroduced to a number of protected areas in the latter, some of which require ongoing management, (controlled burning to prevent succession and maintain grassland) and control of livestock in grasslands. Cambodia now holds the only known wild populations of the Indochinese subspecies *Axis porcinus annamiticus*. Surveys conducted in 2013 confirmed five very small populations of *A. p. annamiticus* in Cambodia, all of which are outside protected areas and under heavy pressure from surrounding human populations. The conservation priorities for *A. p. annamiticus* are 1) formalize effective long-term protection at two sites (Andoung Teuk and Kratie) to conserve *A. p. annamiticus* and its habitat, as approved by the government and supported by local communities; 2) eliminate hunting of *A. p. annamiticus* and maintain two stable/growing populations at Andoung Teuk and Kratie, supported by local communities, and; 3) establish a secure and functioning local conservation breeding programme for *A. p. annamiticus* and mitigate the potential effects of small population size on the *A. p. annamiticus* population. These measures need to be implemented as a matter of urgency if extinction of this taxon is to be averted.

Resumen

El ciervo *Axis porcinus* ha sufrido una dramática disminución de su amplio rango de distribución geográfica que ha pasado en gran medida inadvertida. La especie ha sido extirpada de Laos, Vietnam y Tailandia; se ha vuelto a introducir la en un número de áreas protegidas en este último país, algunos de los cuales requieren de la gestión en curso, (quema controlada para evitar la sucesión y mantener las praderas) y el control del ganado en los pastizales. Camboya es donde permanecen ahora las poblaciones silvestres conocidas de la subespecie indochina *Axis porcinus annamiticus*. Las encuestas que hemos realizado en 2013 confirmaron cinco pequeñas poblaciones de *A. p. annamiticus* en Camboya, las cuales están fuera de las áreas protegidas y bajo fuerte presión de las poblaciones circundantes humanas. Las prioridades de conservación de *A. p. annamiticus* son: 1) la formalización de la protección eficaz a largo plazo en dos sitios (Andoung teuk y Kratie) para conservar *A. p. annamiticus* y su hábitat, tal como fue aprobado por el gobierno y con el apoyo de las comunidades locales; 2) eliminar la caza de *A. p. annamiticus* y mantener dos poblaciones estables: a Andoung Teuk y Kratie, con el apoyo de las comunidades locales, y; 3) establecer un programa de cría en cautiverio segura así como mitigar los posibles efectos de pequeño tamaño de la población. Estas medidas deben aplicarse con carácter de urgencia para evitar la extinción de este taxón.

Key words: *Axis porcinus annamiticus*, Cambodia, Hog deer

Introduction

Hog deer *Axis porcinus* (Zimmerman, 1780) is previously occurred over much of lowland Southern and mainland Southeast Asia (Evans 1902). Formerly widespread and common, during the mid and late twentieth century *A. porcinus* underwent rapid range-wide declines as a consequence of hunting and conversion of floodplain grasslands to agriculture (Timmins *et al*. 2012, Wilson & Mittermeier 2011). This led to its listing as Endangered on the IUCN Red List of Threatened species in 2008; prior to this the species had not been categorized as threatened (Timmins *et al*. 2012).

The internal taxonomy of *Axis porcinus* is still under deliberation. Most published checklists and taxonomic authorities classify *A. porcinus* as a polytypic species with two subspecies, namely the nominate occurring in Pakistan, India, Bangladesh, Bhutan, Nepal and Myanmar, and *A. p. annamiticus* in Thailand, Lao PDR, Vietnam, Cambodia and China (e.g. Grubb 2005). There is uncertainty as to where the geographic boundary lies between *A. p. porcinus* and *A. p. annamiticus* (although it is likely to be located in Myanmar or Thailand), and whether the taxa have come into contact in modern times (Maxwell *et al*. 2007). A new taxonomy of ungulates treats *A. annamiticus* as a species level taxon based on morphology, thus rendering *A. porcinus* monotypic (Groves & Grubb 2011). Confirmation of this arrangement, through the use of genetic markers (the morphological basis for it is not made persuasively) is desirable before it is widely accepted. Meijaard & Groves (2004) suggest that *A. porcinus* and the two related taxa typically treated nowadays as full species, i. e., Calamian Deer *A. calamianensis* and Bawean Deer *A. kuhlii*, be grouped under the subgenus *Hyelaphus* to highlight their close relationship. *Axis porcinus* of unknown but probably mixed provenance have been introduced to Australia, Sri Lanka and South Africa (Wilson & Mittermeier 2011).

In South Asia, *A. p. porcinus* is now largely confined to protected areas, many of which are established and managed for the conservation of Great One-horned Rhinoceros *Rhinoceros unicornis* and Tiger *Panthera tigris* (Timmins *et al*. 2012). *Axis porcinus*is is locally abundant in the Nepali *terai* and Indian *duars*: alluvial grasslands of the Himalayan foothills (Johnsingh *et al*.2004, Jnawali *et al*. 2011, Gautam 2013). At least 10,000 *A. porcinus* are thought to inhabit Kaziranga National Park alone (Karanth & Nichols 2000). The geographic overlap between *A. p. porcinus* and charismatic megafauna ensures that there is significant government investment targeting poaching and habitat encroachment in these sites, rendering these populations comparatively secure. Smaller remnant populations remain in Pakistan, Bhutan and possibly Bangladesh (Iqbal *et al* .2013, Arshad *et al* .2013; Timmins *et al*. 2012). *A. p. annamiticus* has almost completely disappeared from its entire range. It is thought to have become extinct in the wild in Thailand, Lao PDR, Vietnam and China by the 1980s (Wilson & Mittermeier 2011). Captive-bred *A. porcinus* has been introduced to several protected areas in Thailand but the origin of these animals is unknown; at least some were reportedly *A. p. annamiticus* captured from the wild in southern Thailand (B. Stewart-Cox pers. comm.), but it is also possible that stock of unknown taxonomic identity were brought into the breeding programme from Myanmar (Humphrey & Bain 1990). The reintroduced population in Phu Khieo Wildlife Sanctuary requires management; including habitat management (prevention of succession and maintenance of grassland through controlled burning) and prevention of competition with livestock in grazing areas (Prasanai *et al*. 2012); and confirmation of the taxonomic status of these populations is an immediate priority. Cambodia now holds the only known wild populations of *A. p. annamiticus* (Maxwell *et al*. 2007, Timmins *et al*. 2012).

The decline of *A. p. annamiticus* in Cambodia occurred more rapidly than for other similar sized mammals owing to its particular habitat requirements. The grasslands to which it is tied are typically targeted for agricultural conversion and housing development, suffer from mismanagement and fall outside the traditional protected area systems that prioritise forest. Habitat structure and intense human

use of grasslands render *A. p. annamiticus* more easily hunted than other ungulates (Timmins & Sechrest 2012). Like other taxa, *A. p. annamiticus* went unrecorded in Cambodia from the 1970's till the end of the civil unrest in the 1990s. Wildlife populations were heavily hunted during and after the Khmer rouge leading to local extirpations, including of *A. p. annamiticus* (Loucks *et al*. 2009). In 2013 only two small populations of *A. porcinus* were known in Cambodia.

A population of *A. p. annamiticus* was re-discovered by WWF on the west bank of the Mekong River *c.* 8 km north of Kratie town in 2006. Following preliminary surveys it was estimated that 50-80 individuals inhabited a potential area of *c.* 1200ha of flooded grasslands (Maxwell *et al*. 2007). Following the rediscovery, WWF initiated a small-scale conservation programme for *A. p. annamiticus* at the site. Under the project, community-based patrols supported by the Forestry Administration and Military Police were initiated, community engagement was conducted in fifteen villages to obtain community support for *A. p. annamiticus* conservation, and a proposal was developed to establish the site as a protected area, owing to its significance for *A. p. annamiticus* (Bezuijen *et al.* 2008). This proposal ultimately failed, and in 2008 WWF ceased conservation activities at the site (G. Ryan *pers. comm.*). A second population of *A. p. annamiticus* was discovered in 2008 by Global Wildlife Conservation (GWC) in the coastal lowlands of southwest Cambodia (Timmins & Sechrest 2012). The animals were found through sign and camera-trapping surveys in an area of highland and marshy grasslands, remnant patches of *Melaleuca* woodland, scrub and agricultural land *c.* 2500 ha in size, located near Andoung Teuk village, south of the Chi Phat stream, close to Botum Sakor National Park. The survey team did not propose a population estimate, but it was thought to be smaller than that around Kratie. Overflights and subsequent analysis of satellite imagery identified other areas of suitable habitat which may hold populations of *A. porcinus;* these sites were recommended for field surveys (Timmins & Sechrest 2012).

In 2013, Fauna & Flora International (FFI) obtained a grant from the Critical Ecosystem Partnership Fund (CEPF) to follow up on the recommendations of previous reports and determine whether *A. p. annamiticus* was still extant in Cambodia, with a view to developing a comprehensive conservation programme for the taxon if its presence was confirmed. Here we present the results of these field surveys and make conservation recommendations for *A. p. annamiticus* based on the outputs of a national workshop.

Methods

Following the recommendations of Timmins & Sechrest (2012), surveys were conducted in twelve locations in Kratie, Koh Kong and Preah Sihanouk Provinces in Cambodia between January and June 2013 (Table 1). Preliminary unstructured interview surveys were conducted with several villagers at each location to ascertain which deer species were likely to be present locally. Wherever possible, hunters or ex-hunters were interviewed, because they aremore likely to have an accurate knowledge of the species that occur in the local area. People who had lived in the area for less than two years were not interviewed. Species reports were checked for validity with verbal descriptions of morphology and ecology, and were sometimes triangulated with identification using photo cards where necessary.

Verbal reports of *A. p. annamiticus* were followed up with rapid field surveys. Signs of *A. p. annamiticus* can usually be relatively easily distinguished from similarly sized sympatric species with some simple prior training and guidelines. Footprints of *A. p. annamiticus* are similar in size to those of Red Muntjac *Muntiacus muntjak* but more similar in shape to Sambar *Rusa unicolor*. Dung pellets of *A.p. annamiticus* are smaller than Sambar (circa or less than 1cm long, and 2cm long respectively), but similar in size and shape to Red Muntjac. Locations of presumed *A. p. annamiticus* tracks and dung and direct observations were recorded using a GPS unit. Footprint width and length were measured for identification purposes. Broad habitat types and any potential threats or disturbance to *A. p. annamiticus*

or its habitat were also recorded at each site, including the presence of grazing animals, people or dogs, snares, fire, and habitat encroachment/conversion. Interviewees were also asked whether hunting of *A. p. annamiticus* still occurred in their area.

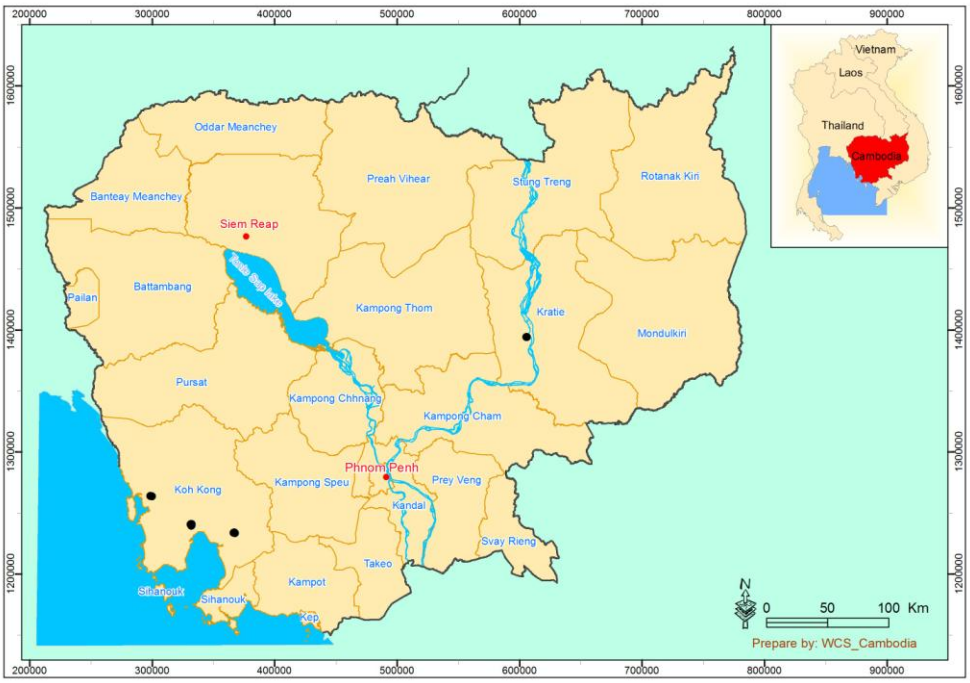

Figure 1. Map showing locations in Cambodia where presence of *Axis porcinus annamiticus* was confirmed in 2013

In October 2013, a planning workshop was held to develop a conservation action plan for *A. p. annamiticus* in Cambodia. Representatives from relevant government departments including the Forestry Administration and Ministry of Environment attended, along with a number of conservation organisations (Fauna and Flora International, WWF, Wildlife Conservation Society, Wildlife Alliance, Conservation International, IUCN SSC, BirdLife International in Indochina) and zoological facilities (San Diego Zoo, Royal Zoological Society of Scotland, Angkor Centre for the Conservation of Biodiversity and the Jerusalem Zoo). In advance of the workshop, Caroline Lees, a representative from the IUCN SSC Conservation Breeding Specialist Group conducted a population viability analysis for Indochinese hog deer to help inform discussion and decisions at the workshop.

Table 1. *Axis porcinus annamiticus* survey locations, results and estimated population status in 2013

Site	Location	Survey dates	Observers	Methods	Results	*A. p. annamiticus* status and population estimate
Botum Sakor National Park (Ministry of Environment), Koh Kong Province	11.128607 103.435191	21-22 January 2013	SMB, PC, CN	I	No credible local reports	Probably extirpated
Andoung Teuk, south of Chi Phat stream (BotumSakorNational Park and unprotected agricultural land), Koh Kong Province	11.197825 103.453644	24 January 2013	SMB, PC, CN	I; F	Credible local reports, no sign observed	Probably present Perhaps <20 individuals
Andoung Teuk, north of Chi Phat stream (state land under Forestry Administration), Koh Kong Province	11.222915 103.462056	23January 2013 27-30 May 2013	SMB, PC, CN CN, JH	I; F F	Tracks, dung Sighting	Present Perhaps <100 individuals
Chhloung District (state land, Forestry Administration), Kratie Province	12.1615135 105.973758	5-6 February 2013 12-16 June 2013	SMB, PC, CN CN, JH	I; F F	Tracks, dung	Present Perhaps <100 individuals
Dumbei and Preaek Prasab Districts, (State land, Forestry Administration)Kratie Province	12.164120 106.014430	7 February 2013	SMB, PC, CN	I		Probably extirpated
Dong Peng, Sre Ambel (State land under Forestry Administration, and agricultural land), Koh Kong Province	11.162966 103.777398	21February 2013 & 6 March 2013	SMB, PC, CN	I; F	Tracks, dung	Present Perhaps a few tens of individuals
Chi Phat (State land under Forestry Administration and agricultural land), Koh Kong Province	11.341429 103.543681	22February 2013	SMB, PC, CN	I	No credible local reports	Probably extirpated
Kampong Seila (State land, Forestry Administration), Koh Kong Province	11.096358 103.932231	5March 2013	CN	I	No credible local reports	Probably extirpated
Vicinity of Highway 4 and 48 Kampong Seila to Sre Ambel (State land and agricultural land), Koh Kong Province	11.062440 103.804790	5 March 2013	CN	I	No credible local reports	Probably extirpated
Coast southwest of Sre Ambel (near Chrouy Svay) (State land and agricultural land), Koh Kong Province	11.004272 103.671446	6 March 2013	CN	I	No credible local reports	Probably extirpated
Trapeang Rung, (Protected Forest, Forestry Administration) Koh Kong Province	11.439473 103.277737	27 March 2013	CN	I	No credible local reports	Probably extirpated
Koh Kong Knong, Koh Kong Province (Economic Land Concession)	11.428068 103.155278	28 Mar 2013	CN	Interview; field	Tracks, dung	Present Perhaps <10 individuals

NB: SMB = Sarah Brook, PC = Phan Channa, CN = ChanthaNasak, JH = Jeremy Holden, I = Interview survey, F = Field survey

Population size has been very crudely estimated here on the basis of preliminary surveys only and refers to total estimated population size. Further surveys need to be undertaken to clarify the actual size of populations to better inform conservation efforts.

Results

Axis porcinus annamiticus tracks and dung were found at four sites; including the Kratie site (Figure 1, Annex 1). At one of the new sites (Andoung Teuk, north of the Chi Phat stream) two *A. p. annamiticus* fawns were observed between 27th and 30th May 2013 by CN and Jeremy Holden. In addition, credible reports of *A. p. annamiticus* were received from south of the Chi Phat steam near Andoung Teuk Village, where the taxon had been reported previously (Timmins & Sechrest 2012). However, *A. p. annamiticus* signs were not observed there during the field survey. The five locations where *A. p. annamiticus* were recorded or reported are largely or wholly outside existing protected areas.

Site accounts

In Kratie Province, the general area occupied by *A. p. annamiticus* is under the jurisdiction of the Forestry Administration, as part of the Permanent Forest Estate although it lacks formal protected status. Maxwell *et al*. (2007) estimated the total area of flooded grassland at 12 km^2 but the extent of area occupied by hog deer is still unknown. A 30,000 ha Forest Restoration and Extension Station has been designated by the Ministry of Agriculture, Forestry and Fisheries, over much of the suitable habitat for *A. p. annamiticus.* This area was designated for the purposes of scientific research, forest restoration and community development, although it is not currently actively managed (S. Masoomi pers. comm).The area is a matrix of grassland, shrubland and agricultural land with some small patches of gallery forest, and is seasonally inundated during May to October. The dominant grasses include *Imperata cylindrica* and *Sorghum propinquum*. Scattered trees in the area include *Mitragyna* spp., *Buteamono sperma, Barringtonia acutangula, Lagerstroemia floribunda, Hymenocardia wallichii* (Maxwell *et al.* 2007). From these preliminary field surveys and anecdotal reports of local people, it seems likely that a significant population of *A. p. annamiticus* still occurs in this area, possibly the largest remaining in Cambodia. Hunting was reported to occur; the meat is typically consumed locally and crop depradation

by *A. p. annamiticus* was also reported. Agricultural encroachment of remaining grassland appears to be a problem.

The Andoung Teuk area south of the Chi Phat stream is just outside Botum Sakor National Park. The area is a complex mosaic of habitat types influenced to some degree by human activity and livestock. These include upland and marshy grasslands, short thick scrub to taller dense tree stands, agriculture including rice paddies and watermelon, small plantations and remnant patches of *Melaleuca* woodland (Timmins & Sechrest 2012). Preliminary field surveys did not find any signs of *A. p. annamiticus* but credible local reports suggest that they were present at least in 2011, probably in very small numbers and perhaps only seasonally (coinciding with crop seasons). Crop depredation (particularly watermelon) by *A. p. annamiticus* apparently occurs in this area from May to July. Villagers reported that there was only one male left in this area, although this has not been verified. This area is rapidly being converted to plantation agriculture, for rice, cashew, cassava and rubber. Given the low population size and ongoing threats, long-term persistence of *A. p. annamiticus* seems unlikely.

The Andoung Teuk site north of the Chi Phat stream is also under the jurisdiction of the Forestry Administration as part of the Permanent Forest Estate, although it is not a protected area. This site is less intensively used by local people than the area south of the river, consisting of more extensive marshy grasslands, including tall sedge marsh, interspersed with patches of open *Melaleuca* woodlands (Timmins & Sechrest 2012).Signs of domestic cattle and water buffalo were found, and in January 2013 it had been relatively recently burned. Habitat in this area has largely been protected as a consequence of the joint Forestry Administration, Military Police and Wildlife Alliance law enforcement programme. Joint patrolling teams operate from 6 ranger stations situated in the Southern Cardamom Mountains to prevent illegal activities within protected areas and state forest, including development of settlements, agricultural encroachment, illegal logging, hunting and wildlife trade. This site is bordered by the river

on its western edge and by a 10,000 ha sugarcane plantation on its northern edge. The Dong Peng Multiple Use Area, under the jurisdiction of the Ministry of the Environment extends southeast from this area along the coastline. Interview surveys were conducted in the Multiple Use Area but no reports of *A. p. annamiticus* were obtained. The site is therefore limited in size (circa 1500ha) and the population relatively isolated from other areas of potentially suitable habitat. However, given the taxon's rarity, this site is likely to hold a relatively significant population of *A. p. annamiticus* (several tens perhaps) and represents one of the best opportunities for in-situ protection of the taxon. Hunting by people from nearby villages and possibly also by workers residing within the sugarcane concession occurs. We received a report of at least one *A. p. annamiticus* that was hunted at the site shortly after the survey was conducted.

The Koh Kong Knong site is within the Ministry of Environment's Botum Sakor National Park, but this particular area is now a privately managed economic land concession of 60,200 ha under The Green Rich Company (Open Development Cambodia 2014). The area of previously suitable habitat was approximately 2500 ha in size but the land was being cleared at around the time of the survey and planted with acacia. Local villagers reported that a very small number (three to five estimated) of *A. p. annamiticus* occur in the strip of grassland and secondary growth along the river. These individuals are likely to be under a very high risk of poaching and the taxon has no long-term future at the site.

The area where signs of *A. p. annamiticus* were found in Dong Peng Commune, Sre Ambel, is a mosaic of agricultural land, grassland and secondary woodland. It is just outside the boundary of the Dong Peng Multiple Use Area to the southeast of Andoung Teuk. Villagers reported that *A. p. annamiticus* typically occurs in their locality from May to July only, moving elsewhere in the dry season because there is no fresh water. Villagers reported killing three or four *A. p. annamiticus* here in 2012 as a result of crop depredation. It is thought that there is only a small population of *A. p. annamiticus* remaining here (a few

tens of individuals perhaps) but it is possible given the extent of the habitat matrix in this area (circa 7000 ha), that a slightly larger population exists, although hunting pressure is undoubtedly considerable.

In all of the other areas visited by this survey, interviewees reported that *A. p. annamiticus* has not occurred there since before the Khmer rouge (1970s/1980s), some were aware of its existence in Andoung Teuk and Sre Ambel.

Threats

Although habitat loss has pushed *A. p. annamiticus* into a few small, isolated fragments of habitat, it is hunting that is likely to cause the loss of the last few populations. People, and signs of human presence such as domestic cattle and buffalo, were recorded at all of the sites where *A. p. annamiticus* occur. Hunting of *A. p. annamiticus* was reported from all five locations where it persists. It is probable that even when people are not actively hunting they (or their dogs) will take fawns opportunistically. Moreover, during the wet season *A. p. annamiticus* is concentrated in small areas of higher ground, making it extremely vulnerable to hunting. All sites appeared to be burned at least in part every year, although this is potentially essential for maintaining grassland. Anthropogenic pressures appeared to be less intense at Andoung Teuk north of the Chi Phat stream, although boundary markers to claim land were evident in some places, and perhaps at the Kratie site. Encroachment was apparent in the Kratie and Sre Ambel sites. Flooding poses a risk of extinction to all remaining *A. p. annamiticus* populations, which have limited opportunity to disperse to suitable habitat owing to the increase in plantation agriculture.

Lastly, given the small size and lack of connectivity between extant *A. p. annamiticus* populations, it is possible that they already, or may in the future, suffer from reduced population viability as a result of inbreeding (Lees *pers comm*).

Discussion

This survey has confirmed that *A. p. annamiticus* persists in Cambodia in at least five locations, three of which are sites where the taxon had not been previously recorded. These are the only known wild populations of this taxon in the world. It is possible but perhaps not likely that small populations may still be found elsewhere in Cambodia. However, any as-yet undiscovered populations are likely to be extremely small and rapidly declining. It is not known to which subspecies the populations in Myanmar and Thailand belong; determining this is a high priority for the taxon.

All confirmed locations for *A. p. annamiticus* are small, isolated, outside the existing protected area system, and are under considerable pressure from surrounding human populations. Although we were not able to estimate population size of *A. p. annamiticus* with any degree of accuracy from this preliminary survey, it is evidently lower than the populations of other deer in Indochina and declining more rapidly. If *A. p. annamiticus* were evaluated using IUCN Red List criteria, then it would qualify as Critically Endangered under criterion C1, with perhaps less than 250 mature individuals estimated (assuming, in the absence of taxon-specific real information, that the number of mature individuals is about two-thirds of the total population size), and an estimated continuing decline of at least 25% within one generation (taken as 7 years; Pacifici *et al*. 2013). We suggest that this scenario is likely given that the current populations are largely unprotected and outside existing protected areas. The taxon could also qualify for Critically Endangered under additional criteria (C2ai – no subpopulation estimated to contain more than 50 mature individuals).The results of the population viability analysis suggest that remaining populations have a high likelihood of extinction within the next 14-40 years if hunting and the effects of small population size and fragmentation are not controlled.

Classified as an "endangered species" in Cambodia, *A. p. annamiticus* is protected by law and hunting is not permitted. However, in practice law enforcement efforts are typically severely under-resourced, especially outside protected areas, and as such *A. p. annamiticus* is receiving little conservation attention. Small deer (such as *Muntiacus muntjak*) are typically regarded as "common species" by law enforcement agencies and even NGOs (these groups may not be aware of or know how to identify *A. p. annamiticus*); as such subsistence hunting is generally not considered a conservation or legal issue. An international wildlife conservation NGO project with the Ministry of Environment at Botum Sakor National Park was not aware of Hog Deer presence until the surveys by GWC, exemplifying this species' low profile.

If a wild population of *A. p. annamiticus* is to be preserved, targeted conservation interventions are required as a matter of urgency. To this end the following objectives were agreed and detailed actions developed under each of these objectives during the October 2013 national *A. p. annamiticus* workshop: 1) formalize effective long-term protection at Andoung Teuk and Kratie to conserve *A. p. annamiticus* and its habitat, approved by government and supported by local communities; 2) eliminate hunting of *A. p. annamiticus* and maintain two stable/growing populations at Andoung Teuk and Kratie, supported by local communities, and; 3) establish a secure and functioning local conservation breeding programme for *A. p. annamiticus* and mitigate the potential effects of small population size on the *A. p. annamiticus* population. The Ministry of Agriculture, Forestry and Fisheries have not yet approved this action plan and no conservation organisation is following up to ensure it is done. Moreover, without the technical and financial support of conservation organisations the plan is unlikely to be implemented, except by WWF at the Kratie site.

At the site in Kratie Province, WWF is seeking to re-start previous work to collaborate with the Forestry Administration and engage local communities in the protection of the local *A. p. annamiticus* population,

as outlined in the action plan. The site has relatively recently been classified as a Forest Restoration and Extension Station, which could, with appropriate technical support, provide a strong framework for community-led conservation focused management. In the absence of appropriate technical support it is naïve to expect management of this site to be sensitive to the conservation needs of *A. p. annamiticus.* Furthermore, the conservation of only one population in a restricted area may not be sufficient to prevent extinction. Lees (*pers comm*) suggest that a carrying capacity of at least 300 individuals is likely to be required for populations of circa 50 individuals, and if those populations are not able to grow, the restoration of connectivity between populations or the input of unrelated individuals from elsewhere may be required to reduce the effects of inbreeding. Increasing female breeding rates and decreasing female mortality are key to rapidly increasing abundance (Lees *pers comm*).

At the Andoung Teuk site no conservation efforts targeted towards *A. p. annamiticus* are currently being implemented nor are they planned for the near future, other than education and outreach conducted by Wildlife Alliance in local communities. Unfortunately, although this is likely the second largest population of *A. p. annamiticus*, and one of perhaps two or three populations of the taxon with more than a handful of individuals, it is falling between the gaps in terms of the geographic and thematic focus of existing government and NGO-led conservation programmes. This scenario is typical of species that inhabit grasslands, in part because the habitat itself is typically viewed as of lower priority than forest or wetland habitats (Parr *et al*. 2014).

The third objective of the action plan recommends that a conservation breeding programme should be established. There are no *A. p. annamiticus* known in captivity, although the nominate form is represented in a number of collections, and it remains possible that large numbers of the taxon are captive and released in Thailand; it is perhaps implausible that these would be pure-bred. In order to establish a captive population it is recommended to begin by capturing the remaining individuals from

the two to three smallest populations (Koh Kong Knong, Andoung Teuk south of the Chi Phat stream and perhaps Sre Ambel). Populations of 20 or less may not be capable of recovering solely through protection from hunting and habitat loss (Lees *pers comm*). Although the Forestry Administration's Phnom Tamao Wildlife Rescue Centre and Zoological Park are supportive of establishing and managing a conservation breeding programme for *A. p. annamiticus* (and previously kept two *A. p. annamiticus* individuals) there is no individual or organisation in Cambodia with the time and financial resources to lead this effort. Consequently, it is feared that these small populations will disappear before any action is taken, thus significantly reducing the chance that a captive breeding population can be established and increasing the risk of extinction for *A. p. annamiticus*.

Conclusions

The future does not look bright for *A. p. annamiticus.* Already lost from the rest of its range, it is now on the verge of extinction in Cambodia. We applaud WWF for taking the initiative to re-engage in assisting the Forestry Administration in the management of the Kratie *A. p. annamiticus* site. However we caution that unless conservation interventions specifically focus on *A. p. annamiticus* it might yet be lost. Nonetheless, even under a best-case scenario of *A. p. annamiticus* persisting at one site (Kratie) in the medium term, in the longer term extinction remains a big risk. It is imperative that all possible efforts are made to ensure that *A. p. annamiticus* does not go the same way as that taxon with which it shares a trinomial as well as a similar range, and possibly (historically at least) habitat preferences; namely, the Javan Rhinoceros *Rhinoceros sondaicus annamiticus.* That taxon was confirmed extinct in 2010 as a result of centuries of habitat loss and hunting, compounded by failures of governance and prioritization (Brook *et al.* 2012, Brook *et al*. 2014).

A small window of opportunity briefly remains to increase the chances of survival for *A. p. annamiticus*, by in-situ conservation of the two largest wild populations and gathering doomed individuals into a

captive breeding programme. In the short term the captive population would provide a safety net to mitigate against potential failure of in-situ conservation efforts, and could be used to re-stock wild populations or re-introduce the taxon to well protected grassland sites when the opportunity arose. As such, we suggest that international zoological institutions with an interest in supporting field conservation could play a significant role in preventing the extinction of *A. p. annamiticus*, by providing technical and financial support to the Cambodian government to establish and maintain a national *A. p. annamiticus* conservation breeding and reintroduction programme that is complementary to in-situ conservation. However, there is hesitancy amongst zoological institutions in Europe to engage in safeguarding the Cambodian populations until the taxonomic status of the captive herds in Thailand has been determined (J. W. Duckworth pers. comm.). If these captive herds are predominantly of the Indochinese subspecies then the taxon is perceived as being relatively safe and the money for an emergency programme in Cambodia could be better spent elsewhere. However, if this is not the case, Cambodia offers the only opportunity to conserve this taxon. Therefore, the unresolved taxonomy of the Thai captive herds is a considerable impediment to the conservation of *A. p. annamiticus*.

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Activity patterns of white-tailed deer in the Tehuacán-Cuicatlán Biosphere Reserve, Puebla-Oaxaca, Mexico

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Abstract

Activity patterns are related to the way in which the basic needs of individuals of a species are met. The white-tailed deer *Odocoileus virginianus* is considered a crepuscular species; however its activity patterns can vary with sex, age, reproductive physiology, the quantity and availability of forage, and other factors. Little is known about the activity patterns of deer in tropical dry forest. The objective of this study was to document deer activity, both daily and by reproductive season for four sites in the Tehuacán-Cuicatlán Biosphere Reserve (TCBR) in Puebla-Oaxaca, Mexico. Thirty-six camera traps were set up from September 2011 to January 2013. With a total sampling effort of 11,204 trap-days, 798 independent records were obtained. White-tailed deer were most active from 0900 – 1159 h, and least active from 2400 – 0259 h, and there were no significant differences between sites. Activity was highest during the mating season and lowest during gestation. Our results allow us to understand which factors affect the activity level of white-tailed deer in this type of tropical habitat, and can help improve management strategies in the TCBR.

Resumen

Los patrones de actividad están relacionados con la satisfacción de las necesidades básicas de los individuos de una especie. El venado cola blanca *Odocoileus virginianus* se considera una especie crepuscular, sin embargo sus patrones pueden variar de acuerdo al sexo, la edad, su fisiología reproductiva, la calidad y disponibilidad de alimento, y otros factores. Se conoce poco acerca de los patrones de actividad de los venados en bosques tropicales secos. El objetivo de este trabajo fue determinar la actividad diaria y por época reproductiva en cuatro localidades de la Reserva de la Biosfera Tehuacán-Cuicatlán (RBTC), Puebla-Oaxaca, México. Se colocaron 36 cámaras trampa de septiembre 2011 a enero 2013. Con un esfuerzo total de muestreo de 11,204 días-trampa, se obtuvieron 798 registros independientes. El venado cola blanca presentó mayor actividad de 09:00-11:59 h y menor de 24:00-02:59 h y no se encontraron diferencias significativas entre localidades. Fue más activo durante la época de apareamiento y menos durante la de gestación. Los resultados obtenidos nos permiten comprender cuáles son los factores que influyen en la actividad del venado cola blanca en este tipo de hábitat tropical, lo cual puede contribuir en las estrategias de manejo en la RBTC.

Keywords: *Odocoileus virginianus*, camera trap, activity, reproduction

Introduction

The activity patterns of animals are related to their need to meet their basic requirements for food, movement, social interaction and rest (Beier & McCullough 1990). These patterns can vary as a function of characteristics intrinsic to the animal such as sex, age, physiological state, and as a function of external factors such as forage availability and habitat quality (Marchinton & Hirth 1984, Beier & McCullough 1990). White-tailed deer (*Odocoileus virginianus*) are known to carry out the majority of activities in the crepuscular hours (sunrise and sunset) when climate conditions are favorable and energy loss owing to cold temperatures can be avoided, as can water loss resulting from high temperatures (Beier & McCullough 1990, Galindo-Leal & Weber 1998, Gallina *et al*. 2005). There are also studies that suggest that activity peaks in this species are related to the seasonality of resource availability (Sánchez-Rojas 1995). That is, during the rainy season when food availability increases, deer spend more time searching for and selecting food, and are thus active throughout the day; whereas during the dry season when food and water availability is very low, the deer are more active during the crepuscular hours (Mandujano & Gallina 1995, Sánchez-Rojas *et al*. 1997). There are reports that where water availability is not a limiting factor, the hours of activity between the two seasons (dry and rainy) do not vary, and crepuscular activity is much less pronounced (González 2003). Both females and males are most active during the rut, as they must find a mate to breed with. They are less active in the post-reproductive period, which coincides with the dry season when temperatures are higher and the quality and quantity of food decreases (Holzenbein & Schewede 1989, Corona 1999). This is considered a behavioral strategy for saving energy when environmental conditions are not favorable (Gallina & Bello 2010).

The Tehuacán-Cuicatlán Biosphere Reserve is located in the Mexican states of Puebla and Oaxaca, and it is only legal to hunt white-tailed deer populations inside the Management Units for the Conservation of Wildlife (UMAs: *Unidades de Manejo y Aprovechamiento de Vida Silvestre*). Recently, population

density (Ramos-Robles *et al.* 2013) and habitat description studies (Barrera-Salazar 2012) have been carried out to evaluate the status of this species' populations, its habitat use and to determine whether the populations can withstand the pressure of hunting. However, neither the activity pattern of these deer nor the factors that might affect their patterns are known. The objective of this study was to determine the activity patterns of these white-tailed deer in order to obtain useful information for the local inhabitants and to incorporate the resulting strategies into conservation proposals and management plans.

Study area

The Tehuacán-Cuicatlán Biosphere Reserve (TCBR) covers 490,186 ha. It is located in the southeastern part of the state of Puebla and the northeastern part of the state of Oaxaca in Mexico (17°32'24.00"-18°52'55.20" N and 96°59'24.00"- 97°48'43.20" W; Téllez-Valdés *et al*. 2010). This reserve is considered to be of great importance owing to its high floristic diversity and the number of endemic species, with a predominantly xerophyte flora (Arriaga *et al*. 2000). The study was carried out at four sites in the TCBR, in the La Cañada, Oaxaca region: San Gabriel Casa Blanca, Los Cues, San Pedro Chicozapotes and Cuicatlán (Figure 1). The type climate is Bs semi-arid, annual mean temperature varies from 16 to 30 °C, the rainy season is from June to September and annual precipitation from 400 to 500 mm. The vegetation types are tropical dry forest, semi- arid shrubland and thorn forest.

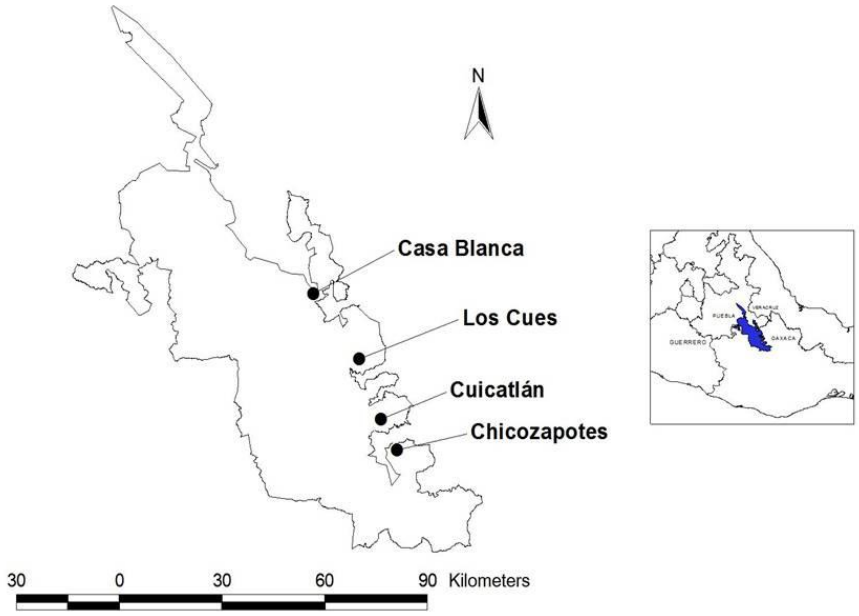

Figure 1. Location of the four study sites in the Tehuacán-Cuicatlán Biosphere Reserve, Puebla-Oaxaca, Mexico.

Methods

To record deer activity, digital camera traps with motion detectors were used (PrimosTruthCam 35® and MoultrieGameSpy D-55IR®). In Casa Blanca and Chicozapotes, ten cameras were set up from February 2012 to January 2013, and in Cuicatlán and Los Cues there were eight cameras from July 2012 to January 2013. The cameras were fixed to trees or stakes, approximately 20 to 30 cm above the ground and with at least 500 linear m between them. Cameras were programmed to function 24 h a day, and take a series of three photos at 10 to 15 second intervals. On each photo, the date, time and phase of the moon were automatically recorded (Figure 2).

Figure 2 Photographic records of the white-tailed deer in the TCBR. The bar at the bottom of each photo indicates the phase of the moon, temperature, date (month/day/year) and the time (24 h format).

Sampling effort was obtained by multiplying the number of cameras by the total number of days they were active. Activity pattern was determined by the number of independent records per hour. A record was considered independent when a given camera took one or more photographs of a deer an hour or more apart. To determine daily activity peaks, the records were grouped into three-hour intervals because of the low number of records obtained at two locations. Activity by reproductive season was divided into three stages of equal length: the rut (November to February), gestation (March to June), and fawning (July to October).

A two-way ANOVA was used to detect any differences in activity between sites and time interval, with a *post hoc* Holm-Sidak test to identify where the differences occurred. To determine if there were any differences between seasons, a Kruskall Wallis test and a *post hoc* Tukey were used (only for Casa Blanca and Chicozapotes). In Cuicatlán and Los Cues there were only records for two seasons (rut and fawning) so a Mann-Whitney test was used to detect any significant differences.

Results

Total sampling effort was 11,204 trap-days, as follows: 4356 for Casa Blanca, 3994 for Chicozapotes, 1504 for Cuicatlán, and 1350 for Los Cues. A total of 798 independent records of deer that were photographed were obtained: 466 for Casa Blanca, 83 for Chicozapotes, 27 for Cuicatlán and 222 for Los Cues.

Daily activity

In Casa Blanca, Chicozapotes and Cuicatlán the peak activity time for the deer was in the morning from 0900 to 1159 h (112, 15 and 8 independent records respectively), unlike Los Cues where it was from 0600 to 0859 h (50 independent records). In the afternoon in Casa Blanca and Los Cues there was another peak in activity from 1500 to 1759 h (89 independent records), and in Chicozapotes from 1800 to 2059 h (14 independent records). The least active times for the deer were 0300 to 0559 h (19 independent records) in Casa Blanca, 2100 to 2359 h (11 independent records) in Los Cues, and 1500 to 1759 h in Cuicatlán and Chicozapotes (1 and 4 independent records respectively). There was a significant difference in activity level between the 0900 - 1159 h interval and the 2400 - 0259 h interval ($F= 3.804$, $df= 7$, $p= 0.008$), indicating that they are the time intervals of the greatest and least deer activity, respectively, although there were no significant differences between sites ($F= 0.307$, $df= 3$, $p= 0.993$) (Figure 3).

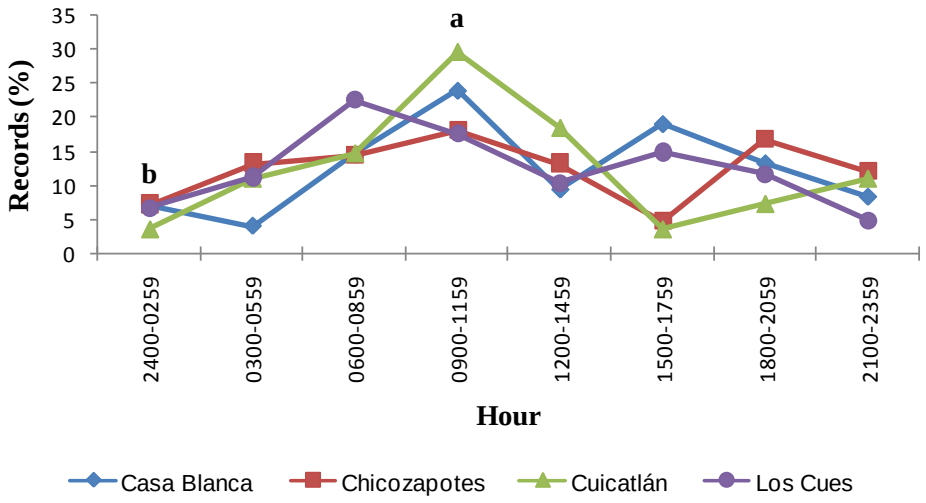

Figure 3. Activity pattern of the white-tailed deer at four sites in the TCBR (percent records at three hour intervals). The letters a and b indicate significant differences between intervals.

Activity by season

In Casa Blanca and Chicozapotes activity was greatest during the rut, followed by the fawning season and was lowest during gestation. In Casa Blanca, activity during the rut was significantly greater than during gestation (H= 8.79, df= 2, P= 0.01); with no significant differences between the other seasons. In Chicozapotes there were no significant differences between seasons (H=1.940, df= 2, P= 0.38) (Figure 4). In Cuicatlán, activity was greater during the rut than during the fawning season. In Los Cues activity was greater during the fawning than during gestation (Figure 4). However, there were no significant differences between seasons in either of the two sites (Cuicatlán: U= 32, P= 0.96, Los Cues: U=32, P= 0.96).

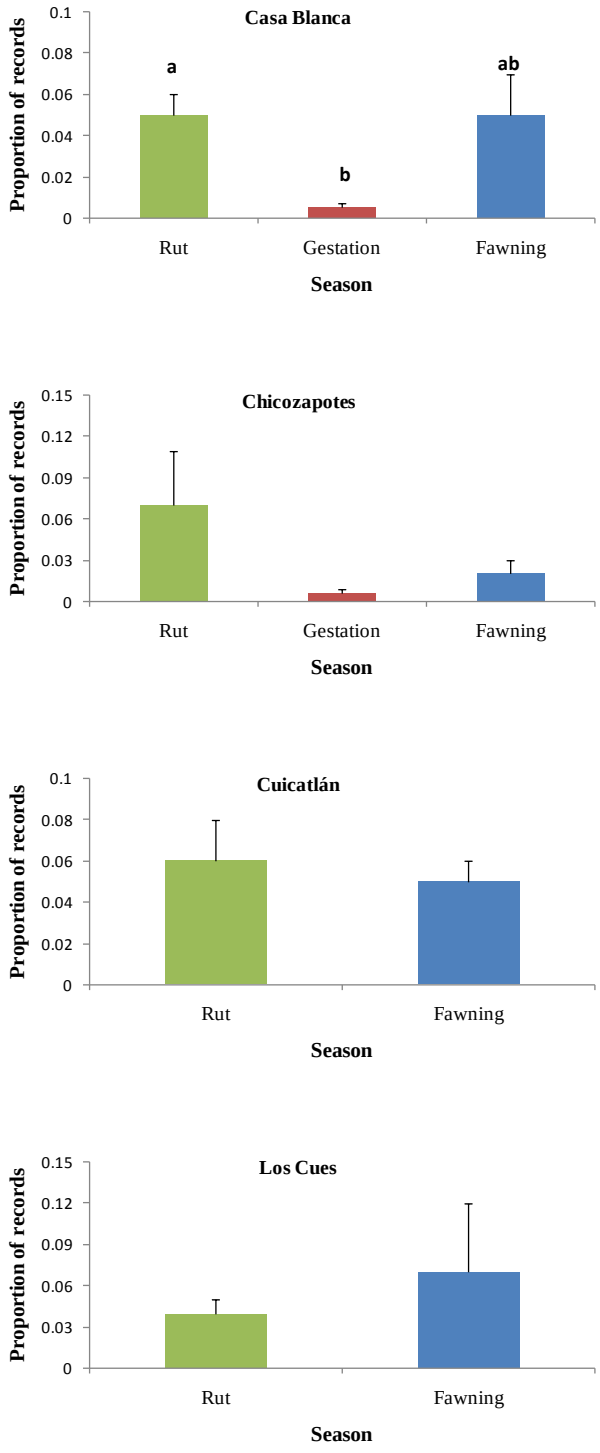

Figure 4. Activity by season (mean proportion of records ± standard error) for the white-tailed deer. Bars with different letters (a and b) differ statistically.

Discussion

The results indicate that the daily activity pattern of the white-tailed deer at the four sites of the TCBR is diurnal, with more activity from 0900 to 1159 h, and less from 2400 to 0259 h. In contrast to reports from other studies (Beier & McCullough 1990, Galindo-Leal & Weber 1998, Gallina *et al.* 2005), activity was low during the crepuscular hours. This may be a result of food and water availability in the region; according to Sánchez-Rojas *et al.* (1997) and González (2003) when availability increases or is not limited, the deer are active throughout the day. The four study sites are characterized by marked seasonality, however throughout the year there are plant species available that meet the deer's need for water when permanent bodies of water are absent (Ramos-Robles *et al*. 2013). This likely allows the deer to continue their activities outside of the crepuscular hours. The times of least activity by the deer are similar to those reported in other studies (Sánchez-Rojas *et al*. 1997, Gallina *et al*. 2005).

In Casa Blanca, deer were significantly more active during the rut than during gestation. In Chicozapotes, though there were no significant differences between seasons, a decrease in activity was observed during gestation, as found in other studies (Holzenbein & Schewede 1989, Rosas Alvarado 1990, Corona 1999), suggesting that there is greater activity during the rut because the deer are searching for a mate. During gestation however, which coincides with the dry season, the does limit their movement before giving birth (Sánchez-Rojas *et al.* 1997). Forage availability decreases, as does the deer's activity, which is a strategy to save energy when conditions are less favorable (Gallina & Bello 2010).

In Cuicatlán activity was greater during the rut than during the fawning season. In contrast to Cuicatlán, at Los Cues activity was greater during fawning than during the rut. However, there were no significant differences between seasons in either of the two sites. Activity during the rut and the fawning season are likely similar because the latter coincides with the rainy season, when more food is available and deer

spend more time selecting better quality food (Bello *et al.* 2004). Better quality food is required because the does are lactating and males start growing antlers for the next breeding season.

The results obtained throughout this study indicate that the activity pattern of the white-tailed deer in the study sites is diurnal, with a peak in activity from 0900 to 1159 h, and the greatest level of activity during the rut (November to February). As such, these sites are considered to have suitable habitat conditions that allow the deer to be active during the day. It also implies that conservation activities carried out by the local communities have decreased the pressure of poaching on deer. This information is useful for setting up management strategies in the Management Units for the Conservation of Wildlife (UMAs) including, for example, setting the hours when hunting is allowed and establishing regional closed season periods.

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Social interactions and latrine use in a group of brown brocket deer (*Mazama gouazoubira,* Fischer 1814) in a large fenced area

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Abstract

The brown brocket deer, *Mazama gouazoubira*, is a species widely distributed in the central part of South America. It is basically diurnal and solitary. The aim of this study was to characterize social interactions, times of diurnal activity, and analyze latrine use in a population reared in semi-captivity. The study was conducted in a fenced pasture area (8332.28 m^2) in REHM, Tucuman, Argentina. Observations were made on 4 adults (2 males, 2 females), 1 sub-adult male and 1fawn. Activity was recorded with *ad libitum* and focal animal methods. A higher frequency of observations was recorded between 14 -20hs.. Social interactions (allogrooming, play) were most common among probably related deer, while pre-courtship behavior (sniffing hindquarters) occurred with several males and one female. Fifty three latrines were identified and characterized. Differences between frequencies of use, category of latrine and individuals were not significant. Home ranges overlapped extensively among the presumably related deer, and each deer used several latrines within its own home range. Latrines were used by more than one individual per day at the pasture´s west end, the access point with the rest of the reserve. The resident deer probably indicate their occupation by scent marking at this point.

Resumen

La corzuela parda, *Mazama gouazoubira* es una especie ampliamente distribuida en la zona central de América del Sur. Posee hábitos diurnos y solitarios. El objetivo del estudio fue caracterizar las interacciones sociales, actividad diurna y analizar el uso de letrinas de corzuelas, en una población criada en semi-cautiverio. El área de estudio consistió en un cercado de pastizal (8332.28 m^2) en la REHM, Tucumán, Argentina. Se observaron 4 adultos (2 hembras, 2 machos), 1 macho sub-adulto y 1 cría. La actividad se registró con los métodos *ad libitum* y animal focal. La mayor frecuencia de observaciones fue entre las 14 -20hs. Las interacciones sociales (aloaseo y juego) fueron más comunes entre ciervos probablemente emparentados. Un comportamiento pre-cortejo (olfateo de cuartos traseros) fue observado entre algunos machos y una hembra. Se identificaron y caracterizaron 53 letrinas. Las diferencias entre frecuencia de uso, tipo de letrina e individuos no fueron significativas. Las áreas de acción se interponían ampliamente entre las corzuelas que probablemente estén relacionadas, y cada una usó varias letrinas dentro de su propia área de acción. Las letrinas fueron usadas extensamente, algunas por más de un individuo en el mismo día en el sector oeste del pastizal, el cual es un punto de acceso al resto de la reserva. Las corzuelas residentes probablemente indican su ocupación del área por marcas olorosas en este punto.

Keywords: *Mazama gouazoubira*, Activity patterns, Latrines, Scent marking

Introduction

The genus *Mazama* includes 10 species of American deer (IUCN 2014). The brown brocket deer, *Mazama gouazoubira* (Figure 1) is a medium-sized deer (35 - 70 cm high, weighing 8-25 kg). Its distribution runs from southern Brazil to central Argentina, with longitudinal limits being Uruguay and eastern Bolivia (Black-Decima *et al*, 2010). Its current protection status is Least Concern–LC (IUCN 2014). Brown brocket deer are usually solitary or found in pairs. Both sexes show territorial behavior, which involves scent marking and territorial defense (Canevari & Vaccaro 2007, Juliá 2002, Black-Decima 2000). Males are polygynous, and females are philopatric, establishing adjacent or overlapping territories with their daughters (Black-Decima 2000). Brown brocket deer are predominantly diurnal (camera trap studies: Maffei *et al*. 2002; Rivero *et al* 2005).

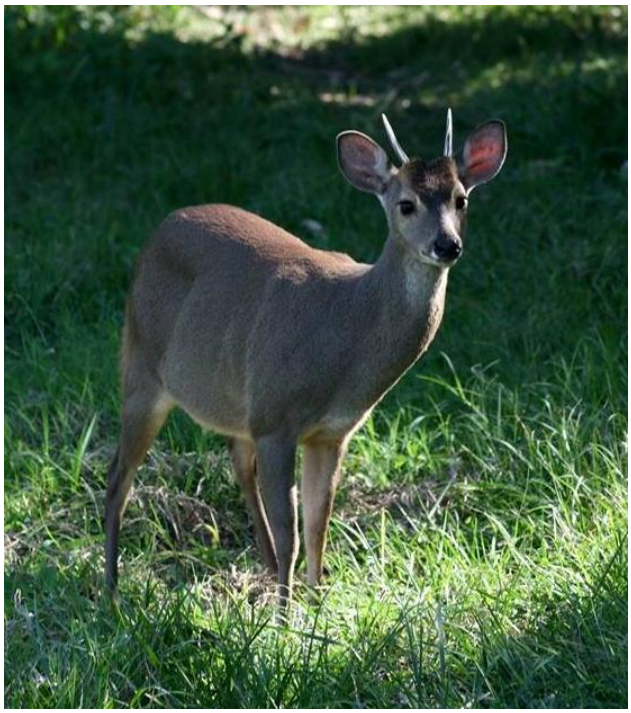


Figure 1: Male Brown Brocket deer in the study area (grassland) in the REHM. (Photograph by Eduardo Vargas)

Chemical communication is important in mammals, especially those that are solitary, whose scent marks persist in the absence of the owner. Scent marking is indicative of territoriality when done by the same individual and in a specific area (Walther *et al.* 1983). According to Gosling's theory of scent matching, scent marks are placed where intruders are likely to find them, around the border in small territories and centrally and on trails, in large territories (Gosling 1982; Gosling & Roberts 2001). Intruders can then identify the territory owner by matching the scent of the marks with the owner when they find him. Brown brocket deer form and use many small latrines, in addition to marking with cutaneous glands (Black-Decima 2000; Ajmat *et al.* 2004; Rivero *et al.* 2004). Latrine location and use has been studied in several ungulates, principally bovids. Latrines function in territoriality in oribi (*Ourebia ourebi*), where males mark territorial borders (Brashares & Arcese 1999) and serve in intersexual communication in bushbuck (*Tragelaphus scriptus*), where females first use latrines and males then counter-mark (Wronski *et al*. 2006). Black-Decima (2000) suggested that males mark the borders of their territories by urinating and defecating in latrines and by marking trees and bushes, while females mark their core areas, principally with latrines. The brocket latrines may be part of a communication network in which these solitary animals communicate their occupation and ownership of an area and perhaps their sexual status, since they regularly mark their latrines and counter-mark in response to dung of presumed invaders (Black-Decima & Santana 2011).

The objective of this study was to characterize social interactions in brown brocket deer; to analyze the location and use of latrines by identified individuals and to determine the principal times of diurnal activity. Due to their cryptic habits such a study could not be done in the wild. We hypothesized that latrines would be more frequently located near landmarks and that there would be more and larger latrines in the preferred forest and edge habitats.

Material y Methods

The study was conducted in an open fenced pasture area (8,332.28 m^2), which is part of the Horco Molle Experimental Reserve (REHM) (Busnelli *et al .pers comm*), Tucumán, Argentina. The REHM consists of 14 ha of fenced secondary environment in Yungas (grassland, high and low secondary forest); in which the following species are free: brown brocket deer (*Mazama gouazoubira*), greater rhea (*Rhea americana*), collared peccary (*Pecari tajacu*) and capybara (*Hydrochoerus hydrochaeris*) (Zaia, 2004). The deer population consists of approximately 14 individuals. Observations focused on 4 adults (2 females and 2 males), 1 sub-adult male and 1 newborn fawn (15 days-6 months), which were the individuals inhabiting the study area. The exact relations between these deer are unknown, as most animals are not identified or marked, but, probably the adult female (FM), mother of the fawn (Nb), the other adult female (F) and sub-adult male (JM) are related (FM is probably the mother of all). The other 2 adult males (M and DM) are probably not closely related. Access to the rest of the reserve where the other deer have their home ranges was not permitted. Individuals were identified by external body characteristics such as size, color and form of antlers.

Daily activity was recorded with *ad libitum* and focal animal methods from two observation points outside the fenced grassland area. Observations were made in summer - autumn (January 31 - June 4, 2013) from 8:00hs to 20:00hs, in 2 shifts: morning (8:00hs -12:00hs) and afternoon (14:00hs -20:00hs) (effort sampling was similar in each time band). Observations were confined to these times since these deer are primarily diurnal and equipment for night viewing was not available.

The observed behaviors were classified into 2 categories: Individual (Grooming, Latrine use, Urination, Object exploration, Pawing ground, Rubbing forehead against branch) and Interaction (Allogrooming, Sniffing hindquarters, Nursing, Jump -jumping in circles or parallel to another deer-, Following -one deer walking beside or behind another-, Alarm -an individual runs perpendicular away from another-,

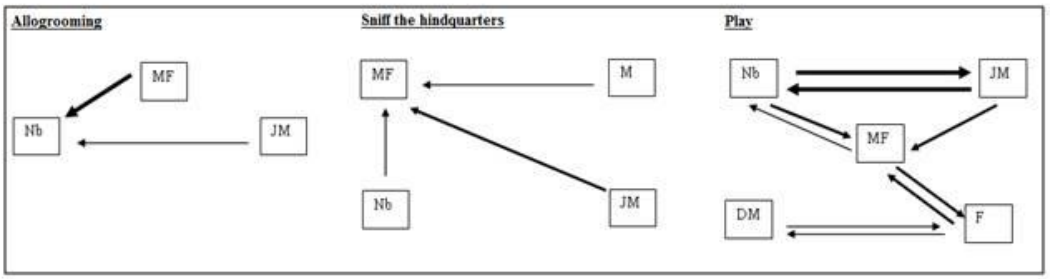
Play -both individuals jumping in parallel or in circles-, Run -2 deer running in parallel-, Visual and olfactory contact -2 individuals face to face look, then approach mutually and sniff without contact- and Naso-nasal contact) . Behaviors of solitary animals bedded, walking or foraging were not recorded. A distance of 3m between individuals was considered a group. The locations of the focal animals were also recorded and used to draw approximate home ranges.

In the study area the latrines were numbered, classified according to habitat, located with GPS and measured. The date, time and animal that used each latrine were recorded. Dung piles which were less than 1m apart were considered a single unit. The distance between latrines was measured within a 5m radius; and their distribution was plotted on a map. The latrines were classified as _1- "Forest" latrines located under the canopy of a tree or bush > 1.5mts high (shaded), 2- "Edge" latrines located on the edge of the canopy of a tree or shrub height > 1.5mts (occasionally shaded), 3- "Grassland" latrines located "in the open" (under the sun).

The characteristics of the latrines were analyzed using InfoStat® 2.0 free version 2013. An ANOVA was applied with a completely randomized design; and Tukey multiple comparisons were made; the type of latrine was used as the dependent variable and size as the classification variable. A nonparametric ANOVA (Kruskal-Wallis) was also performed.

Results

A total of 67 observations of behavioral patterns were obtained, 31were individual and 36 interactions. We observed 26 episodes of interaction between 2 brocket deer; and 10 between brocket deer and rhea. Nb was involved in 90% of the interactions and FM in 80%. The principal interactions (Allogrooming, Sniff hindquarters, Play -including Jump) and the individuals involved are shown in Figure. 2. In Allogrooming, Nb was the only recipient and FM the principal donor. FM was the object of Sniffing



Fi

Figure 2: - Interaction patterns, frequencies and individuals involved. The width of each arrow indicates the frequency of action by each individual**. References**: **FM** Adult female with fawn, **F** Female without fawn, **Nb** Newborn fawn, **JM** Sub-adult male, **DM** Adult male**.**

Hindquarters by 2 males and Nb, while play was observed between most of the individuals. Twenty five groups were observed; most were of 2 individuals with a maximum of 4. The diurnal activity of the deer in the grassland was higher in the periods of 11-12hs, 17-18hs, 18-19hs and 19-20hs (Figure 3). The polynomial trend lines show strong diurnal activity in the females; while the activity of the young increased as the day progressed, with more afternoon activity. Adult males showed less morning and early afternoon, and more late afternoon activity in the pasture.

In the study area we identified and classified 53 latrines but only observed use of 28 latrines and one urination site outside of a latrine. The location of the latrines and the deer that used them are shown in Fig. 4. Eleven latrines were seen to be used by more than one individual and of these, 5 on the same day. The latrines used by 2 deer on the same day were located at the western end of the pasture, where it is open to the rest of the reserve and all deer enter and leave. The 5 communication events involved counter-marking of FM by the males JM and MD (2 cases), one male counter-marking another and FM marking twice in the same latrine., The home ranges (approximate 90% minimum convex polygons)

drawn from the locations where the deer were observed are also shown in Figure. 4. FM utilized the entire pasture as home range and also marked the entire area. F and JM had more limited home ranges,

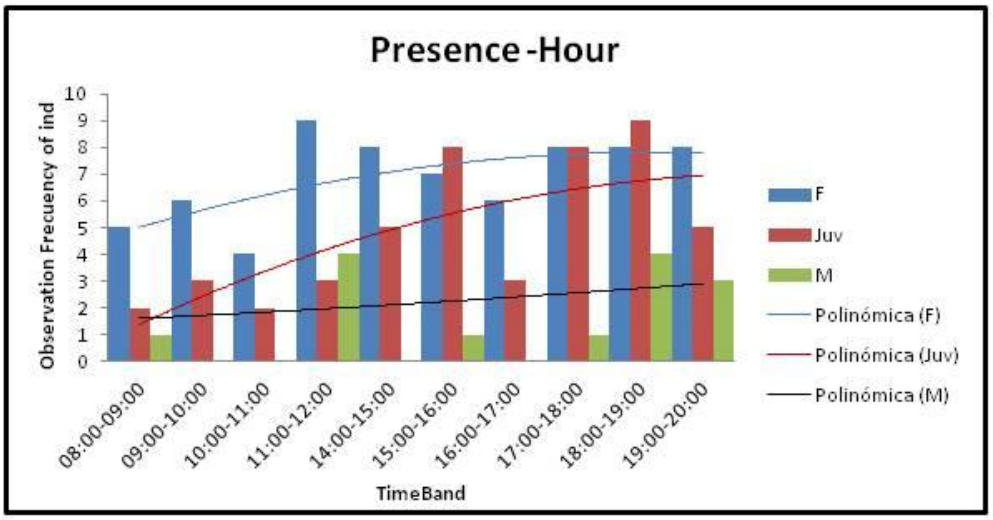


Figure 3: Graph times brown brocket deer activity. The colored bars represent the frequency of observations of the 3 groups of deer (**F** females, **M** males and **Juv** juveniles, sub –adult and newborn) at the respective times. The polynomic trend lines for the same data are superimposed.

and their home ranges overlapped completely. JM marked only within his home range and close to several borders, while F also marked only within the home range and more centrally. The other males (DM, M) did not have recognized home ranges in the pasture (few observations) but marked at the western end and entered to mark latrines close to the eastern border.

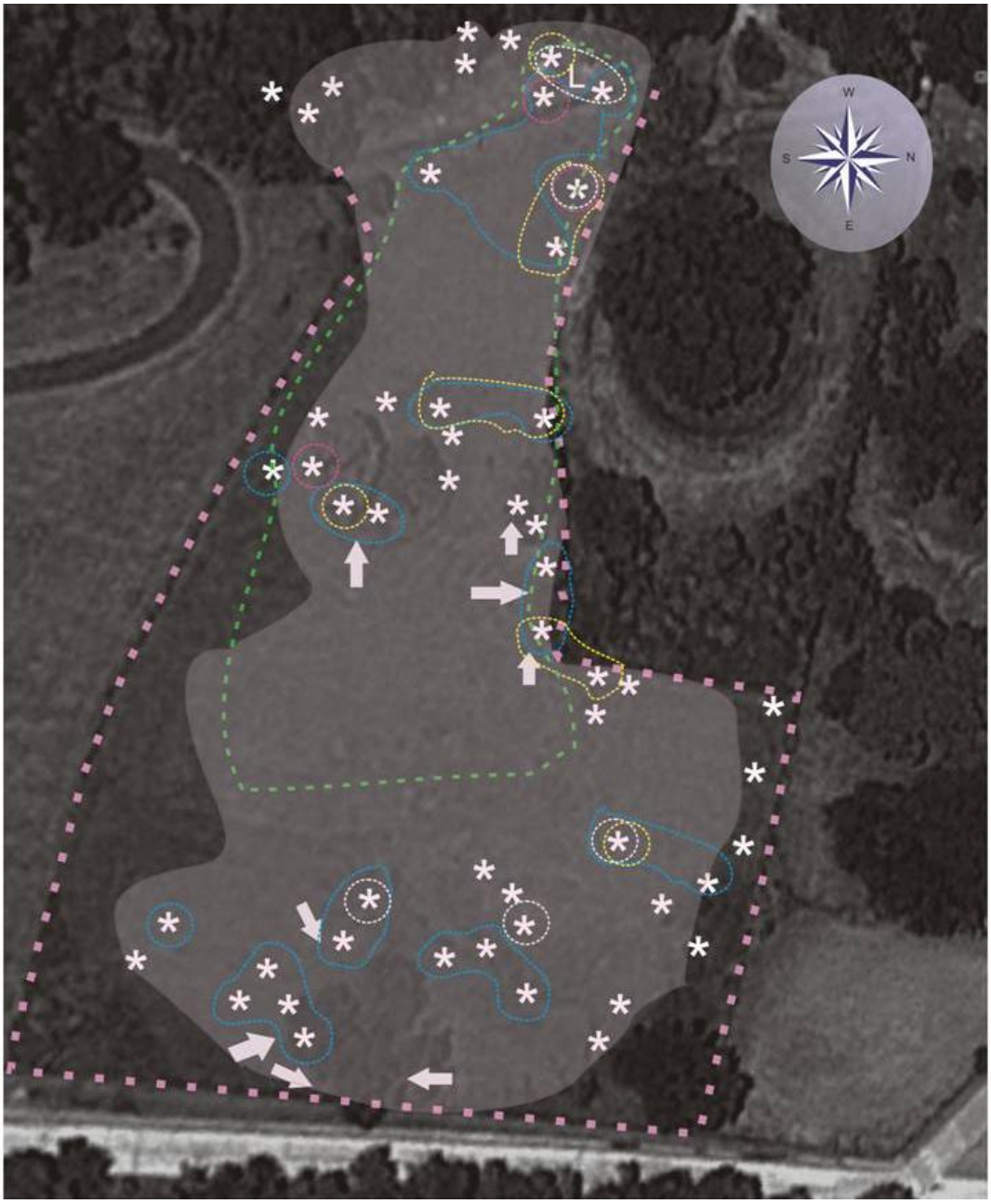


Figure 4: Details of the distribution of latrines in the study area. On the map latrines are indicated with (*), the arrows point marking sites, the pink dotted line demarcates the study area, the green dotted line defines the overlapped home range of **F**, **MJ**, **MD,** the shaded area defines the home range of **FM**. Latrines marked with blue were used by **FM** and **Nb**, with yellow, by **JM**, with red, by **F** and with white, by **DM.**

The latrines (53) in different habitats were classified as "Forest" 10, "Border" 17 and "Grasslands" 26. Their size decreased with increasing sun exposure (Table 1). The ANOVA testing differences in sizes of latrines showed no significant differences (F = 1.17, p = 0.32; the Tukey and Kruskal-Wallis were also not significant (F = 2.5; p = 0.28). The greatest distances between latrines were in "Grassland" (325, 62 ± 42, 01); and the highest frequencies of Use of latrines were observed between 15- 19hs. The Correlation Coefficient (Pearson and Spearman) calculated for the variables Time band and Frequency of use of latrines was also not significant.

Table 1 - Descriptive measures of the size of latrines (mean ± standard error).

CATEGORY	(X ± ES) cm	N
Forest	87,10 ± 15,67	10
Edge	77,47 ± 10,97	17
Grassland	64,84 ± 6,90	25

Discussion

Allogrooming was not reciprocal and Nb was the only animal to receive allogrooming in this study, similar to observations by Black –Décima (1997) and Tessaro (2005) (Figure. 2). Aggressive behavior was not observed in any individual. The only courtship behavior seen in the males was sniffing of hindquarters, presumably because the females were not in estrus during the observation periods. Play behavior (Figure. 2) was seen in several deer, usually initiated by Nb. Most interactions occurred among the deer that are presumably related (FM, Nb, JM, F) and suggest that social behaviors occur among kin in usually solitary brocket deer. There was also essentially complete overlap of the home ranges of these presumably related deer with FM and Nb having larger home ranges that encompass those of JM and F.

The interactions between F and DM could be courtship related. Juliá (2002) noted an increase in sociability among brockets in REHM; but not the absence of aggressive interactions.

We observed substantial diurnal activity in the focal deer. Tessaro (2005) found, in captivity, greater activity toward evening and night (18-20hs and 3-6hs) and less activity during morning and afternoon (9-16hs). In the Chaco, in the dry season Maffei *et al*. (2002) and Periago & Leynaud (2009), found higher percentages of photographic records in the morning, and afternoon with lower values in the evening. Rivero *et al*. (2005), also in the Chaco, observed increased activity between 5:00-10:00 am. The most active individual in this study was FM, followed by Nb. Robbins (1993) postulates that females with fawns require extra energy as mothers, as does Juliá (2002); this necessity can lead them to choose more exposed or potentially dangerous habitats such as grassland.

The study of latrines is a widely used, noninvasive method for studying small and cryptic species, since it provides information for estimating population size, habitat use and diet, and to the greater ease of detecting feces than the animals (Wronski & Plath 2010). The formation of latrines has been reported for 9 deer species, mainly small, tropical forms (Black-Decima & Santana 2011). The lack of statistical significance in the analysis of latrine placement and size does not indicate the existence or absence of relations between frequency-time-use of latrines, which could be due to small sample sizes and the low N in the study area. Most of the recognized latrines were associated with a landmark in the landscape, as also noted by Wronski & Plath (2010) and Wronski *et al*. (2013) in gazelles.

The uses of the latrines by these deer provide further evidence that they serve as a communication network (MacGregor 2005). While we cannot distinguish between central and peripheral marking strategies in males and females without more complete home range data, the distribution of latrines and marking events show that they were extensively used in marking The communication events (where 2 deer used the same latrine in succession) were located at the western end of the pasture where the deer

enter and leave and thus would be especially important in announcing presence or occupation of the area. It is interesting to note that the tree area at the eastern end of the pasture was an important part of the home range of FM and Nb, as the birthplace and hiding area for Nb, FM did not mark this area in the first month after the birth of Nb but thereafter marked it extensively. This may have been an anti-predator strategy to prevent detection of Nb in the vulnerable period after birth. The adult males (M, DM) that entered marked at the entrance point (west end) and near the central bedding area of FM and Nb at the eastern end. The pasture may have been a peripheral part of their home ranges or they may have invaded looking for females.

With respect to *ex situ* conservation, we can note that the brown brocket deer lives and breeds well in the REHM, which offers an opportunity to study their poorly known biology in a natural and controlled environment.

Acknowledgments

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Population structure and genetic variability of the gray-brocket deer (*Mazama gouazoubira*; Mammalia: Cervidae) in Uruguayan populations

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Abstract

The gray brocket deer (*Mazama gouazoubira*) is a Neotropical deer with a wide distribution range in the southern part of the Amazonian basin. The aim of this study was to analyze the genetic variability and population structure of 50 individuals of this species from Uruguayan populations, using mitochondrial DNA as molecular marker. We designed a protocol for amplification with Real Time PCR and subsequent sequencing of a fragment of 159 bp of the *Dloop* region. We found high levels of genetic variability and determined 24 haplotypes. We analyzed the patterns of population structure using AMOVA and determined four different groups within the country. These preliminary results allow us to identify Management Units (MU) for conservation, and contribute to the understanding of how historical environmental changes affected the distribution of genetic diversity for this species.

Resumen

El guazubirá (*Mazama gouazoubira*) es un ciervo Neotropical con una amplia distribución en el sur de Sudamérica, que habita principalmente en bosques nativos y mixtos. El objetivo de este estudio fue caracterizar genéticamente 50 individuos de esta especie pertenecientes a poblaciones uruguayas, con el fin de analizar la estructura poblacional y variabilidad genética de estas poblaciones, utilizando el ADN mitocondrial como marcador molecular. Se diseñó un protocolo para amplificación por PCR en tiempo real y posterior secuenciación de un fragmento de 159 bp de la región *Dloop*. La especie mostró un alto polimorfismo para este fragmento, encontrándose 24 haplotipos. Analizamos los patrones de estructuración genética utilizando AMOVA, y determinamos cuatro grupos diferenciados. Estos resultados nos permiten identificar unidades genéticas de manejo para la conservación, y contribuir a la comprensión de cómo los cambios ambientales históricos afectaron la distribución de la diversidad genética en esta especie.

Keywords: Genetic variability, mitochondrial DNA, *Mazama gouazoubira*, phylogeography.

Introduction

The gray-brocket deer (*Mazama gouazoubira*) is a small to medium sized deer, with head and body length of approximately 882 – 1060 mm and shoulder height of 500 – 650 mm (Rossi 2000, Figure. 1). It

has a wide distribution range in South America, south of the Amazon basin and east of the pre Andean regions (Black-Décima *et al.* 2010). This deer lives mainly in native and mixed forest, but it is also found in secondary woodlands and, at times, open grasslands. This species is a generalist, and its diet can be adapted to the characteristics of the environment, varying the percentage of leaves, plant species and fruits, adopting frugivory as a nutritional support. This ability could explain the amplitude of its geographic range and the diversity of habitats that it occupies (Pinder & Leeuwenberg 1997, Black–Décima *et al*. 2010). There are no studies in Uruguay about its exact distribution patterns and abundance, but its occurrence is known in 10 of the 19 departments of the country (González & Elizondo 2010, González & Martinez 2010). According to the International Union for Conservation of Nature (IUCN), populations are generally declining due to hunting pressure and habitat loss (Black-Décima & Vogliotti 2008).

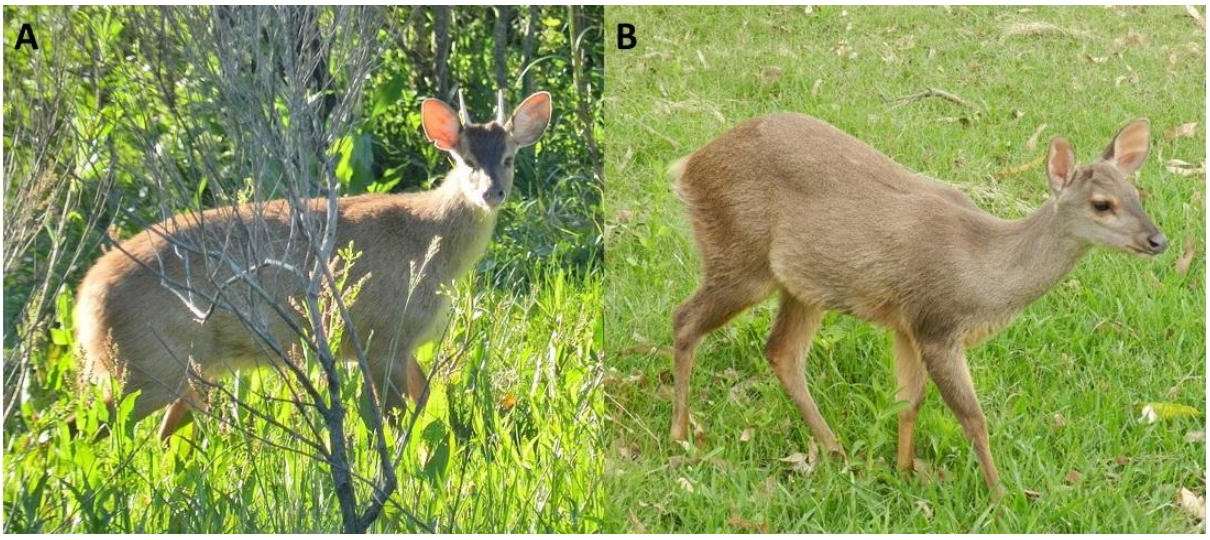

Figure.1 Male (A) and Female (B) individuals of *Mazama gouazoubira*

Designing conservation strategies for a species requires understanding its evolutionary and demographic history. It is crucial to reveal the presence of demographic partitions that could influence gene flow patterns and result in a hierarchical distribution of the genetic diversity among populations throughout the geographic range of the species (Moritz 1995, Crandall *et al.* 2000). Moritz (1999) proposed that two

types of conservation genetic units should be recognized: i) Evolutionarily Significant Units (ESUs) were defined as historically isolated and independently evolving sets of populations, without regard to the current distribution of phenotypic variation, and ii) Management Units (MUs), can be applied to identify strategies for managed species. Defining management units will ensure that the genetic heritage and evolutionary potential of the species are recognized and protected (Black-Décima & Vogliotti 2008). The aim of this study was to analyze the genetic variability and the population structure of representative individuals of gray-brocket deer in Uruguay, in order to better understand the conservation status of the species in the country.

Material and Methods

We analyzed 50 samples collected between 1993 and 2014 in 6 Uruguayan departments (Tacuarembó n= 8; Rivera n=10; Lavalleja n=19; Rocha n=3; Maldonado n=5 y Cerro Largo n=5). These samples correspond to feces of individuals from wild populations (n = 26), hair (n = 5) and blood (n = 7) of individuals in captivity of known origin in nature, and bones (n = 12) of individuals deposited in the collection of the National Museum of Natural History (MNHN) or found in the field. Extraction of genomic DNA from tissue, blood and bones was performed following the protocol of Medrano *et al*. (1990) and the DNeasy® Qiagen commercial kit (QIAGEN, Hilden, Germany) was used for feces, following the manufacturer's protocol. We designed an amplification protocol for Real-Time PCR and posterior sequencing of a 159 bp fragment of the *Dloop* region using primers designed for deer: DLFHB 5`GCGGCATGGTAATTAAGCTC 3` and DLRHB 3`GCATGGGGCATATAATGTAATG 5` (Gonzalez *et al*. 2012). PCR was performed in a total volume of 25 µl containing 12.5 µl of SensiFast (Bioline), 0.4 µM each primer (1 µl), approximately 60 ng of DNA and ultrapure water, according to the following conditions: denaturation at 95 ° C for 3 minutes followed by 35 cycles of 95 ° C for 5 seconds, 56 ° C for 10 seconds and 72 ° C for 20 seconds. The Real Time PCR technique allows the visualization of the amplification process as it takes place, avoiding the agarose gel electrophoresis to confirm the

success of the reaction. PCR products were purified and sequenced by the Sanger method on an automatic sequencer ABI3130 (Applied Biosystems) at the Institute Pasteur of Montevideo .Sequences were aligned and edited using MEGA 6.0 software (Tamura *et al*. 2013). Using BLAST (Basic Local Alignment Search Tool) utility, we compared the sequences obtained with the nucleotide database available in the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/) to verify that they indeed belonged to the species of study. Diversity indices such as haplotype and nucleotide diversities and the number of polymorphic sites were calculated using the software DnaSPv. 5.0 (Librado & Rozas 2009). The haplotype network was constructed using the statistical parsimony algorithm TCS (Clement *et al*. 2002), implemented in the software PopArt. To analyze the population structure in a geographic context, a hierarchical analysis of molecular variance (AMOVA) was performed with the software Arlequin v. 3.5.1.3. (Excoffier & Lischer 2010). We calculated the θ - statistics, which are analogous to Wright's F statistics. The significance of these F-statistic analogs was evaluated by 1000 random permutations of sequences among populations. We experimented with various groupings of populations, those suggested by the population trees, and those suggested by the geographic location of samples. The groupings that maximized values of ΦCT, and were significantly different from random distributions of individuals, were assumed to be the most probable geographic subdivisions. We followed Wright's guidelines to interpret our ΦST values, where a value of 0–0.05 indicates little differentiation, 0.05–0.15 moderate differentiation, 0.15–0.25 great differentiation, and > 0.25 very great differentiation (Wright 1978).

Results and Discussion

We found 23 polymorphic sites (14.7%) and a total of 24 mutations between sequences. Nucleotide diversity (π) was 0.04886, with 5.6 average differences between pairs of sequences. We found 24 haplotypes and haplotype diversity (H) was 0.940 (± 0.016). The haplotype network showed a star-like structure, indicating a population explosion in the recent past, which is also supported by the high

diversity and excess of unique or low frequency haplotypes. Moderate population differentiation between the North and South of the country was observed, with Rocha and Cerro Largo haplotypes scattered on the network. However, they appeared to be more related to the Northern haplotypes (Figure 2).

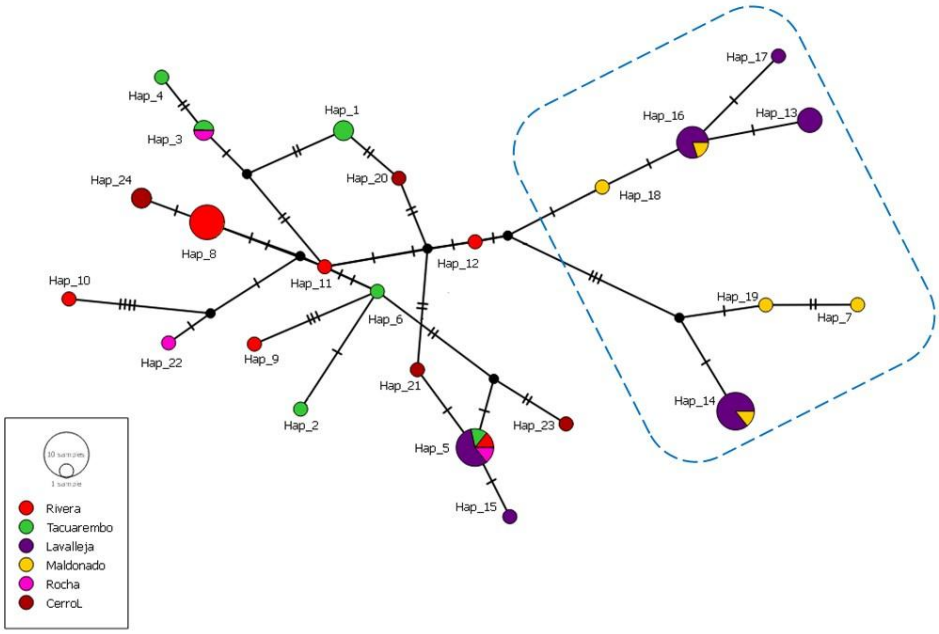

Figure 2. TCS Haplotype network. The Southern clade is shown within dotted lines. Different colors correspond to the different sampling localities. The mutations are shown as lines in the branches. Taken from PopArt (http://popart.otago.ac.nz).

We identified two genetic groups in the North of the country, one in the South (that was the most differentiated) and another that comprises all the samples from the East (Fig. 3). This grouping presented a ΦST of 0.163 ($p \leq 0.001$), meaning moderate to high differentiation. As noted in the network, the haplotypes of Maldonado and Lavalleja (Southern group) form a very distinct group from the rest. These locations should be considered as a single population for the purposes of management. It is important to note that we had only 8 individuals from the East of the country (Cerro Largo and Rocha) so it is necessary to focus sampling efforts in this region, to confirm the existence of a single genetic unit in this

area. Furthermore, we have no samples from the western half of the country. Despite being considered an abundant species in the country, the status of the species has not been evaluated in depth and is not adequately known. It would be important to assess the presence of the species particularly in the southwestern half of the territory, since it is currently unknown. The average number of migrants between groups was two migrants per generation.

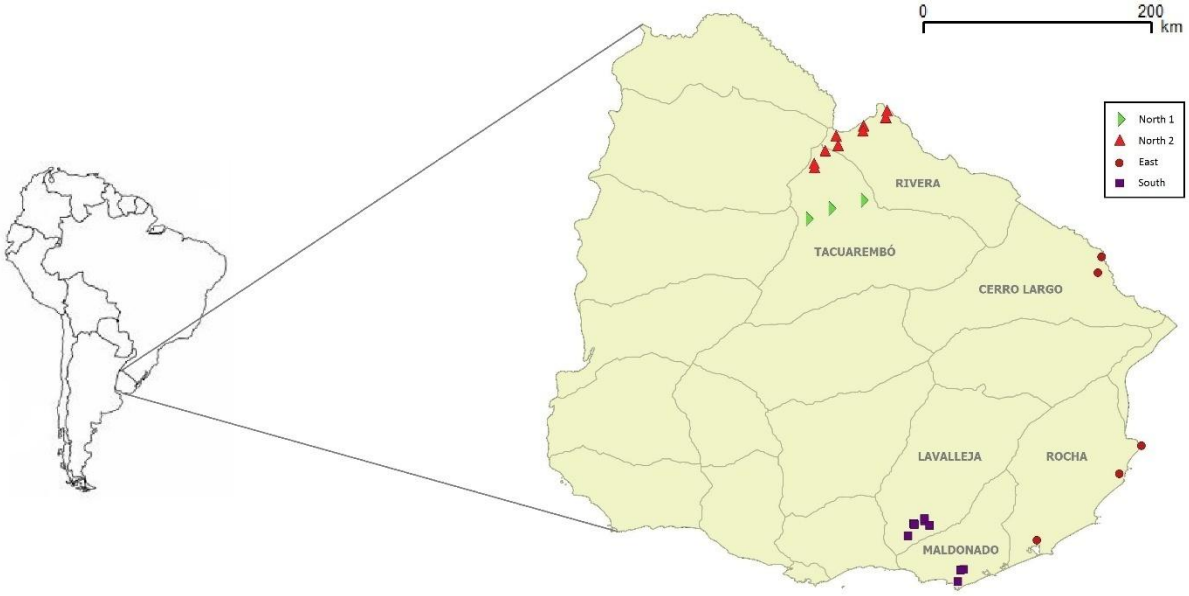

Figure 3. Uruguayan map showing four discrete groups detected with AMOVA: two Northern groups, a Southern group and an Eastern group.

We found high values of haplotype diversity in Uruguay for this species, in comparison to other Neotropical deer species, such as the marsh deer (Márquez *et al.* 2006) or the red brocket deer (Abril *et al*. 2010). Genetic diversity was similar to that found for the pampas deer (González *et al*. 1998), but in this case, we used a smaller fragment (only 159 bp) as a molecular marker, representing a hypervariable region for this species.

We report a moderate degree of population structure in our country, indicating the existence of fragmented populations. Considering the small sampling area and the absence of large geographical barriers within the territory, this fragmentation could be due to anthropogenic causes. More extensive sampling efforts and large-scale data are needed to fully understand the spatial pattern of genetic diversity and the demographic history of this species, as well as applying additional genetic tools such as microsatellite markers and next generation sequencing techniques.

Our results allow us to identify genetic units for conservation, useful in the development of management plans, and contributing to the understanding of how historical environmental changes affected the distribution of genetic diversity for this species.

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Captive Management of the Chilean Pudu (Pudu pudu Molina 1782)

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Abstract

The Southern Pudu has long been classified as Vulnerable by Red List™, however some successful breeding programs are ongoing for this species. Since the introduction of the International Studbook and the European conservation breeding program for *Pudu pudu,* a stable population with more than 100 individuals has been kept at zoos in Europe, North and South America. Not a lot is known about this small deer and we will update soma data on the captive management of this species, including general husbandry, diet and reproduction. The new data is mostly very similar to that published by Schürer and Sliwa (2002) apart from the average life-span which has increased over the years.

Resumen

El Pudu del Sur durante mucho tiempo ha sido clasificada como Vulnerable por la *Red List*™*,* sin embargo existen varios programas de cría en cautiverio exitosos para esta especie. Desde la introducción del Registro Internacional Genealógico y el programa europeo de reproducción para la conservación de Pudu Pudu, una población estable con más de 100 personas se ha mantenido en los parques zoológicos de Europa, Norteamérica y Sudamérica. No mucho se sabe sobre este pequeño ciervo y vamos a actualizar los datos de soma en el manejo en cautiverio de esta especie, incluyendo en general la cría, la alimentación y la reproducción. Los nuevos datos es sobre todo muy similar a la publicada por Schürer y Sliwa (2002), aparte de la duración de la vida media que se ha incrementado en los últimos años.

Keywords: *Pudu pudu,* captive management, zoos, International Studbook, reproduction, diet, general husbandry

Introduction

Since the description of the Southern Pudu *(Pudu pudu)* (Figure 1) by Molina in 1782 little has been published on the captive management of this species. Schürer & Sliwa (2002) published an article on Southern Pudu in Zoological Gardens that will serve as basis for this publication. Moreover, according to their explanation as to which scientific name for the Southern Pudu - *Pudu puda* or *Pudu pudu* – is correct, we will stick to the latter in this article.

Figure 1. Adult male Pudu (© A. Sliwa).

The establishment of the International Studbook (ISB) for the Southern Pudu in 1969 and the European conservation breeding program (EEP) set an important milestone for the successful breeding of this species in zoos. Both programs are currently coordinated by André Stadler at the Wuppertal Zoo. Since the 1970s the captive population has been increasing constantly and since 1990 the number of individuals kept in zoos has been above 100. Since the beginning to the latest edition of the ISB for *Pudu pudu* in 2013, 1159 individuals have been registered (Stadler & Aurich 2013).

According to the IUCN, the Southern Pudu is considered to be Vulnerable and has had this status since 1996 (Jiménez & Ramilo 2008). Despite the problems of maintaining a captive population of South American deer, breeding programs in zoos for the Southern Pudu have been quite successful over the years (Schürer & Sliwa 2002). There are currently 148 individuals (71 males, 76 females, 1

undetermined) kept in 44 ISIS-registered institutions in Europe, North and South America (ZIMS 2014). The largest *ex situ* population is kept at the Wuppertal Zoo with 21 individuals (10 males, 11 females). Another breeding program led by the Universidad de Concepción in Chile had plans to reintroduce individuals into three National Parks (Jiménez & Ramilo 2008). Unfortunately this program was shut down due to a highly inbred population (Zordan, *pers. comm*.). There are, however, sperm banks held at the Chile National Zoo and Buenos Aires Zoo and other successful breeding centers in Chile which do not yet participate in the International studbook (Zordan, Black, *pers. comm*.).

Schürer and Sliwa (2002) primarily discussed the reproduction of *Pudu pudu* by means of the International Studbook over a time period of 34 years (1969-2000). Here, we will update that data as of November 2014 and provide further information and advice on general husbandry and diet.

General Husbandry / Diet

In captivity in Central Europe, Pudus need frost-free stables. If they are provided with a dry shelter, they can be kept outdoors all year long. A pair of pudus should be provided with a sufficient indoor stall with a hard floor of abrasive material to keep hooves trimmed. Dust-free wood chips proved to be better as bedding material than straw. The outdoor enclosure for a pair of pudus should have an area of at least 30 m² with grassy or sandy ground; muddy ground has to be avoided under all circumstances. Cover by overhanging shrubs and shelter against excessive solar radiation is necessary. During very hot weather pudus must have access to cooler stables. A male can be kept with up to 3 females, larger groups are not very stable. Bachelor groups with a maximum of four males have worked out as well (Schürer 1992).
Pudus need easily digestible, highly concentrated plant-food. In the wild *Pudu pudu* mainly browses on young leaves and sprouts of trees, shrubs and forbs, fruits and flowers, as those parts of plants are the most nutritious (Jiménez 2010). At the Wuppertal Zoo the animals get fresh browse as often as possible: ash (*Fraxinus spp.*), oak (*Quercus spp.*), hazelnut (*Corylus avellana*), willow (*Salix spp.*), and others,

mostly in summer, and blackberry (*Morus nigra*) in winter. The free-choice foliage is supplemented with rolled oats, pellets, dried mountain ash berries, and chopped / sliced fruits and vegetables such as apples, carrots, beets, and endives. A saltlick needs to be provided. A healthy adult female pudu should weigh around 12 kg and a male approximately 14 kg.

Birth Weight and Sex Ratio

From January 01, 2001 to the present, 490 (250 males, 219 females and 21 undetermined) fawns have been born worldwide in ZIMS-registered institutions. One hundred and ten (96 males, 40 females, and 1 undetermined) fawns have been born at the Wuppertal Zoo. The mean birth weight of 41 recorded births was 865 ± 148.2 g. Excluding those that did not survive 30 days, the mean weight was 880.42 ± 120.2 g (N=26; Min: 620 g, Max: 1300 g). This value does not differ much from that stated by Schürer and Sliwa (2002) (890.89 ± 89 g, N=55; Min: 630 g, Max: 1200 g). Males were somewhat heavier (909.6 ± 129 g, N=17) than females (825.3 ± 86.8 g, N=9) in contrast to Schürers and Sliwas' (2002) data, where female and male weights were very similar (896 ± 73 g, N=28 and 884 ± 104 g, N=27; respectively). Any fawns born under 550 g did not survive.

According to Schürer and Sliwa (2002) the sex ratio is almost even, with slightly more males being born. In our data 51 % of all newborns were male, 44.7 % were female and 4.3 % were not sexed. At the Wuppertal Zoo the sex ratio of newborns is less balanced, with 62.7 % being males and 36.3 % females (1 % was undetermined).

Mortality Rate

Of 110 newborn Pudus at the Zoological Garden Wuppertal since 2001, 70 individuals survived at least 30 days. This equals a mortality rate of 36.4 % during the first month. Schürer and Sliwa (2002) stated a 30 day mortality rate of 26 %.

Gestation Time

Only a few numbers are known for gestation time. Hick (1967/1968) stated a gestation time of 203 and 204 days, Vanoli (1967) published data for 207 and 223 days and Reyes (1988) 197 to 210 days. Unfortunately, we cannot provide any further information at that point.

Sexual Maturity

Fertile matings between less than one year old males and females are possible. Genetically undesirable father-daughter and mother-son-matings can occur even during the first year of life. Therefore, male fawns should be separated from their mother and the breeding group promptly and at the latest in their second year of life. Female fawns are usually tolerated by their mother for a long time. The reproductive life-span lasts from the 1st to the 14th year, with one offspring per female per year.

Seasonality of Births

We can only confirm all data presented by Schürer and Sliwa (2002). Reproduction is clearly seasonal and births usually occur during spring and summer which coincides with the months of November and December in South America and April to July in Europe and North America. The majority of all births in the northern hemisphere take place in May with 36 %, followed by 15 % in June, 12 % in April and 9 % in July. Births have been recorded in other months as well (Figure 2).

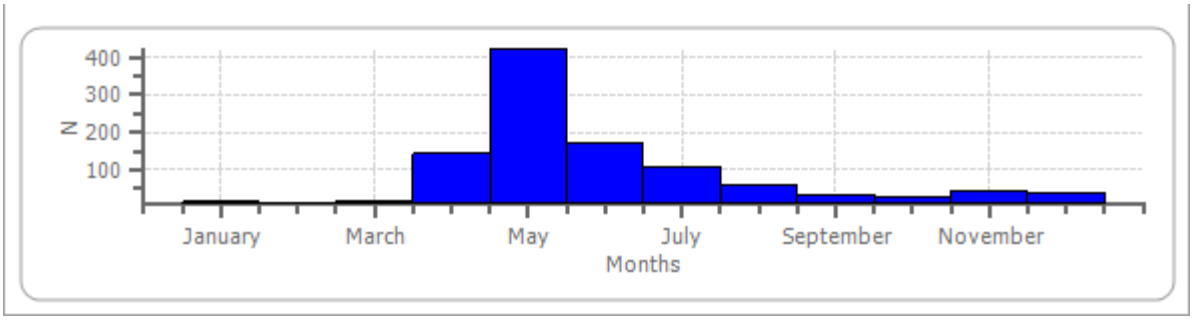

Figure 2. Seasonality of births in Europe, North and South America.

Life Expectancy in Captivity

Life expectancy and average life-span of Pudus in captivity did increase over the years. Frädrich (1975) indicated a maximum life-span of around 9 years. Schürer and Sliwa (2002) reported the oldest female Pudu at 17.4 years and the oldest male at 15.8 years. No older individuals have been recorded since. While Schürer and Sliwa (2002) found an average age of 4.35 years for females and 4.44 years for males (excluding all fawns that died within 30 days) the average is now 4.80 years for females and 5.51 years for males.

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Ex situ deer conservation Symposium

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In December 2014 we organized a Deer Symposium during the "*Congreso de Zoología del Uruguay*". We had participants from Argentina, Brazil and Uruguay. The main symposium topic was devoted to *ex situ* conservation that we identified as needing to improve because few advances have occurred in Uruguay and the surrounding regions. It is important that zoos and breeding institutions share the information, standardize management criteria and achievement. Another important issue is to record the genealogies based on an effective identification of individuals. For endangered species such as pampas deer it is necessary to maintain an accurate studbook. This symposium addressed: (i) identification of the species found in zoos of Uruguay and the region, (ii) standardization guidelines for stock management, and (iii) how this knowledge can contribute at local, regional and national levels to achieve proper management and conservation of Neotropical deer species.

Captive Breeding Genetic Management in Neotropical deer: principles and perspectives

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Maintaining Neotropical deer in captivity is a challenging and difficult process, especially because of the deer´s high reactivity to stressors that can cause high mortality. However, many zoos and breeding centers in South America keep a number of deer species. In this respect, it is important to connect these groups genetically, or these centers cannot be considered to be involved with conservation "*ex situ*". Studbooks should be implemented for all species, especially the endangered ones, in order to understand the genetic structure of the population and prevent the loss of genetic diversity and inbreeding. One of the main problems of captive populations is the adaptation to captivity of the animals, due to an involuntary selection, changing allele frequencies and making the animals less able to return to nature in further generations. Therefore, the principle of equal contribution of founders should be used in

breeding, preventing the action of selection and consequently preserving allele frequencies closer to the originally existing wildlife populations. This principle also makes it possible to avoid inbreeding, which causes the expression of deleterious alleles and high neonatal mortality. Despite the great importance of the genetic control of captive populations, the most serious problems they face are the management errors caused by inadequate facilities and dietary errors, leading to the decline of captive populations of most of the Neotropical species. The aim of this presentation is to discuss the applicability of genetic management for captive populations of deer in Uruguay and the effect of other captive management practices in maintaining healthy populations from the clinical and genetic points of view.

Captive Breeding Cervidae Survey in Uruguay

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Zoological institutions play an important role in species conservation, investigation, ex situ and genetic resources conservation, and also an educational role. A species inventory provides for a more efficient management of zoological institutions. The objective was to inventory all the cervids found in Zoological and captive breeding institutions in Uruguay. We visited 21institutions located in all Departments from Uruguay, with the exception of Lavalleja. In total, 633 animals were recorded, which belonged to 5 species: two native [gray brocket deer (*Mazama gouazoubira*) and pampas deer (*Ozotoceros bezoarticus)*] and three introduced species [red deer *(Cervus elaphus),* axis deer, *(Axis axis*) and fallow deer (*Dama dama*)]. With the exception of the Artigas zoo, all institutions possessed at least one deer species. The most abundant species was the axis deer with a total of 288 animals (45%), distributed in 12 institutions, in which one institution concentrates 70 % of the animals. Red deer and fallow deer were represented by 28 (4%) and 233 (37%) individuals respectively. We recorded the following native species, 75 (12%) pampas deer and 9 (1.5%) gray brocket deer individuals. The pampas deer was the only local and internationally endangered deer species represented in zoos. However, it was only present in five institutions [Pan de Azúcar (n=60), Zoo de Salto (n=7), Villa Dolores (n=4), M'Bopicua (n=3), and Reserva Tálice (n=1)], and all population

founder individuals belong to the same original population. Although cervids were highly represented at Uruguayan zoos, native and endangered deer species were a minor proportion, suggesting that species selection at these institutions was not based on local conservation or educational goals.

Social rank and pampas deer *(Ozotoceros bezoarticus*) females time budget

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The pampas deer (*Ozotorecos bezoarticus*, Linnaeus, 1758) is an endangered species, classified in CITES Appendix 1. As a number of ruminants present differences in their time budgets according to the individuals' social rank, our aim was to describe the time budget pattern of pampas deer females, and to determinate whether it differs according to their social rank. The study was performed at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (Maldonado, Uruguay), with 26 females distributed in 6 different paddocks, each consisting of one stag and 3-6 hinds and fawns. The Dominance Index (DI) for each female was calculated by recording agonistic behavior during feeding with ration. Females were considered as high-ranked (HR) when their DI was ≥ 0.5 and low-ranked (LR) when their DI was <0.5. The behavior of each female was recorded using instantaneous scan sampling every 15 minutes. We recorded data from each female 35 times per day, and repeated the procedure for 3 days for every female. Except for run, there were significant differences according to the time of day for all other activities ($P \leq 0.03$). Females were observed 34.6 ± 7.2% (mean ± SEM) times bedded, 58.4 ± 7.5%, standing, 6.2 ± 3.6% walking, and 0.2 ± 0.7% of the recordings running. Grazing was the most frequent feeding activity (40.5 ± 7.0%); it was more frequent during the early morning and late afternoon. Females spent 8.6 ± 3.5% of the day eating ration, 1.9 ± 2.3% browsing, and 4.2 ± 3.2% ruminating. The pattern of lying down and walking during the day differed according to the hinds' social rank ($P=0.03$ and $P=0.05$, respectively). HR females were observed lying down more frequently (37.7 ± 1.9 vs 31.6 ± 1.9%) and walking less frequently (5.1 ± 0.8 vs 7.3 ± 0.8%, respectively) than LR females. We concluded that pampas deer females present a well-defined diurnal activity rhythm, with a peak of activity early in the morning and late in the afternoon, slightly influenced by females' social rank, where HR females lay down more and walked for less time than LR ones.

Agonistic strategies of high and low-social ranked adult male pampas deer (*Ozontoceros bezoarticus*)

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The pampas deer has a hierarchical structure established and maintained by agonistic behaviors, which can be physical or not. The agonistic strategies may vary according to the social rank and the age of the animal. Our objective was to compare the use of agonistic behavioral units between high (HR) and low (LR) ranked pampas deer males. The study was performed in the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar, during the breeding season (February) for two consecutive years with 4 groups of males (n=25). Each animal was considered an independent experimental unit. The animals received ration for dairy cows and had free access to water and native pastures. Behavior was recorded while animals ate ration, based on an ethogram of previously defined agonistic behaviors between males, that included 7 behavioral units: Present Antlers (PA); Glare (GL); Chase (CH), Presence (PR); Antler Locking; Fight (FI) and Attack (AT). A total of 1247 interactions were recorded during 31.6 h, and a dominance index (DI) was calculated for each male (males displaced/total males, range: 0 – 1). Those males that had a DI > 0.7 (n = 8) were considered as HR, and those with a DI < 0.33 (n = 9) as LR individuals. HR males used more GL (17.7 ± 3.0 % vs 7.2 ± 2.9 %, p= 0.046) and CH (11.4 ± 3.4 % vs 3.9 ± 23 %, p= 0.046) than the LR ones; and tended to use more AT (1.5 ± 0.8 % vs 0.2 ± 0.2 %, p= 0.083), without significant differences in the other behaviors. In conclusion, although most agonistic units were used with similar frequencies, HR individuals used more non physical behaviors.

Agonistic behavior studies of semi-captive pampas deer (*Ozotoceros bezoarticus; Cetartiodactyla Cervidae*)

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Various aspects of agonistic behavior of pampas deer males and females from the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (Piriápolis, Uruguay) have been studied for several years. As a result, seven agonistic behaviors used by males (Presence, Glare, Present Antlers , Chase, Kick, Lock antlers, and Fight) and six performed by females (Presence, Glare, Directing head, Chase, Kick, and Box) were described. Males are dominant over females, mainly by using non-energetically costly behaviors. Females are easily subordinated by males by non-physical agonistic behaviors, avoiding physical injuries. Females use energetically costly behaviors more frequently than males. Reproductive seasonality of agonistic behavior was studied, which more frequently occurs in autumn. Otherwise, agonistic strategies of male and females differ according to social rank. High ranked males use non physical agonistic behaviors more frequently than low ranked males. On the contrary, high ranked females used more energetically costly behaviors than low ranked females, probably because they displaced individuals of closer social rank. Males displaced high ranked females more frequently than low ranked ones, and used different behaviors: mainly using "Presence" with high ranked females and "Kick" with low ranked females. Also, low ranked females tended to be farther away and more easily dominated by males than high ranked females. In conclusion, numerous advances in the knowledge of agonistic behavior of this species were made, which was favored by the possibility of animal identification, as well as other specific features of captivity.

Capture and anesthesia in pampas deer (*Ozotoceros bezoarticus*).

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The Pampas deer (*Ozotoceros bezoarticus*) is a native deer species which is currently endangered. Knowledge and application of different capture and anesthetic methods could contribute to improving management of the species under captive and wild conditions. The aim of this study was to determine the effect of successive captures and general anesthesia (GA) on different response parameters of pampas deer males. The study was conducted at the Estación de Cría de Fauna Autóctona del Cerro Pan de Azúcar (ECFA; Maldonado, Uruguay) where a pampas deer semi-captive population exists with approximately 80 animals. A total of 107 captures and GA were performed over a period of three years. A group of 28 males was used. After capture, and with animals under anesthesia, the following parameters were analyzed: heart rate (HR), pulse rate (PR), respiratory rate (RR), partial oxygen saturation (SpO2) and rectal temperature (RT). Also, blood samples were extracted and creatine kinase (CK) and cortisol concentrations were determined. Capture repetition and GA did not affect the response of the parameters studied, showing only possible effects related to time or season in which the study was performed. It can be concluded that the method of capture and GA used in this study can be safely used for managing males of this species, as they are not detrimental to the health of animals.

Influence of age and time of antler growth on histological characteristics of velvet in pampas deer *Ozotoceros bezoarticus (Mammalia: Cervidae)*

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Male deer species show annual antler cycles. During the non-breeding season antlers are cast and thereafter grow enveloped by skin (velvet). When antlers complete their growth, the velvet is shed and the antlers remain clean throughout the breeding season. In pampas deer, the antlers´ size and weight increase annually until the age of 6 years. The aim of this study was to determine the influence of age and time since commencement of antler growth on the histological characteristics of epidermis and dermis from the velvet of male pampas deer. The study was done at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (Piriápolis). The animals, 6 adult and 5 young males, were anaesthetized 31-81 days after casting the previous antlers, during the non-breeding season. Velvet samples were taken from the third tine, distal end, immediately proximal to the soft, cartilaginous tip. Samples were histologically processed and fixed in paraformaldehyde. Sections were cut perpendicular to the longitudinal axis of the antler, and stained with haematoxylin-eosin. Total epidermal thickness, thickness of the corneum, intermediate and basal stratum of the velvet epidermis, total dermis thickness, superficial and deep dermis thickness, and number of keratinocyte layers were determined. A multiple regression analysis was performed considering as independent variables the age and the number of days since antler casting of each deer, and the quantitative histological data as dependent variables. Age, but not time of antler growth influenced positively the thickness of the corneum stratum (R^2= 0.52; P= 0.05). Neither age nor time of antler growth influenced the other variables measured. The thickness of the corneum stratum increased with age; however the other histological characteristics studied were not influenced either by age or time of antler growth.

First marsh deer (*Blastocerus dichotomus*) record in Canelones –Uruguay

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The marsh deer (*Blastocerus dichotomus*) is the largest South American deer (height of 1000 to 1200 mm). It is a deer species found in wetlands and marshes and is threatened by the advance of agricultural activities, channeling and damming of waterways. In the Brazilian Pantanal large populations currently exist, but in Paraguay and Argentina the species is seriously threatened, while in Uruguay it can be considered extinct. The last record was in Rocha Department in *Bañados los Indios* where a specimen was caught in 1959. Later, in 1991, there is another record of a specimen caught near the town of *Villa Soriano* in the Department of Soriano. Recently, near the town of *Pueblo Aznares* in the department of Canelones, a skull of an adult male marsh deer was found buried on the banks of the Arroyo Solis. The skull is complete and the estimated age is about 100 years. Bone samples were collected in order to isolate DNA, amplify it with mitochondrial markers and compare the variability of Uruguay with Argentina, Bolivia and Brazil. This record is relevant because it was possible to recover genetic material and a complete skull which will enable us to assess the diversity that marsh deer had in Uruguay

Pampas deer (*Ozotoceros bezoarticus*) conservation: development of a bank of cryopreserved semen

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In several studies we have characterized the basic pampas deer spermiogram, including the determination of the volume, concentration and total number of spermatozoa in the ejaculate, quality and percentage of motile sperm, percentage of normal sperm, acrosome and membrane integrity of the sperm. In addition, the morphometric descriptors of sperm heads were described, observing the existence of three sperm sub-populations that have different morphometric characteristics. There is a positive relationship between these parameters and semen characteristics, and the parameters are highly repeatable in the same males. The ejaculates showed great heterogeneity in both seminal and morphometric parameters between males and collections. We have also compared the effectiveness of different commercial and lab-made extenders to cryopreserve sperm samples collected during the breeding and non-breeding seasons. Their effects were evaluated during refrigeration, freezing and thawing in the basic spermiogram and sperm DNA integrity. Overall, these samples are preserved in the first sperm bank of the species, which currently has cryopreserved sperm from more than 20 animals, including animals which died several years ago. This work provides a basis for developing reproductive biotechnologies to preserve the species. The results will enable new studies about freezing and thawing protocols, including testing fertility, both *in vitro* and *in vivo*.

Analysis of vocalizations in Neotropical deer species

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Males of most species of *Cervus*, *Dama dama*, *Rangifer*, and others produce striking, species-specific vocalizations during the rut. However, the vocalizations of Neotropical deer species have not been studied, although most species produce some type of vocalization. Vocalizations mentioned are short, low intensity and difficult to record except in captivity. The objectives of this study were to record and analyze acoustic parameters of the vocalizations of various Neotropical species, especially *Mazama* species, in order to see whether there are differences that can be used to identify species or act as a reproductive barrier at the time of courtship. All recordings were done at the NUPECCE in Jaboticabal, SP, Brazil and in the REHM, Tucumán, Argentina. We recorded deer from the following species: *Mazama americana* (5 males, 2 females, 1 fawn), *M. gouazoubira* (3 males, 2 females, 1 fawn), *M. nemorivaga* (2 males), *M. nana* (1 male), *Odocoileus virginianus* (1 male) and *Hippocamellus antisensis* (1 male). Deer of all species recorded produce short duration calls (bleats) during courtship (males) or when calling or caring for fawns (females). Mean durations and mean, minimum and maximum fundamental frequencies were different for males of all species. The statistical analysis (Linear and non-linear hierarchical models) for 3 species showed that *M. nemorivaga* durations were significantly longer than for *M. gouazoubira* and that *M. americana* frequencies were significantly higher than for *M. gouazoubira*. There were significant differences among all individuals. These data suggest that courtship vocalizations of males of Neotropical species can serve as a guide to distinguish species and individuals and perhaps they are used by females to select males for mating.

Genetic characterization of *Mazama bororo* (Mammalia: Cervidae) using mitochondrial markers

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There is no literature about basic aspects of natural history, such as variation of morphological patterns, historical and current geographical distribution for the Small red brocket deer, *Mazama bororo* Duarte 1996. Apparently this species is restricted to the coastal region of the Atlantic Rainforest which is a biome that has faced strong anthropic pressures. In Brazil, this is the most threatened species of deer in the country and it is classified as "Vulnerable" on the Red List of the IUCN. The aim of this research was to characterize genetically *M. bororo* using mitochondrial markers (D loop and Cytochrome b) to analyze intraspecific variability of the species. We used nine samples from three different locations in Brazil (Barra do Turvo SP, Criadouro Onça Pintada, PR and Centro de Triagem de Animais Silvestres, CETAS, PR) that were stored in the NUPECCE (Núcleo de Pesquisa e Conservação de Cervídeos) cell bank, located in Jaboticabal, Sao Paulo, Brazil. We used one universal primer D loop to amplify a fragment of 690 pb and two primers for the region of cytochrome b to amplify a total region of 1140 pb. From the first primer we analyzed a fragment of 506 pb and we found an impressive haplotype diversity of 0.88. From the cytochrome b marker we analyzed a fragment of 967 pb and we found a haplotype diversity of 0.41. Thus, the two fragments were concatenated and from nine samples we found six distinct haplotypes indicating a large genetic variability of the specimens analyzed. Mitochondrial markers (D loop and Cytochrome b) proved to be very informative and appropriate for studies to determine the genetic variability at the intra-specific level. Our results indicated high haplotype diversity for*M. bororo.* We recommend a study with larger samples to assess whether there are phylogeographic patterns in this species. That research also would contribute to our knowledge about the natural history of *M.bororo* and as a tool to public policies development for the preservation of Brazilian fauna.

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