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DEER SPECIALIST GROUP NEWS

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Editorial

Dear DSG members,

The last year has been a very challenging one for many of us, personally and professionally. We hope that the pandemic has not hit you and your dear ones too badly and, looking forward, we would like to wish all of you good health and to ask you to stay safe. While 2020 was a difficult year and our lives and work suffered a set-back, we have also learned new ways to communicate and work from a far, ways that will most probably stick even after the pandemic will be long gone.

Most notably among the new methods, is the new SSC Data platform that the Chair of the Species Survival Commission, Dr. Jon Paul Rodriguez, has pushed the new platform is one of the initiatives that will help to enhance and support the SSC network and its alliances and to deliver the IUCN Species Strategic Plan. We are working in this new platform entering the DSG targets for the upcoming 2021-2024 IUCN quadrennium.

Entering the new quadrennium the DSG has set targets to update (re-develop) its website and establish and maintain visibility of the Group and of its mission and goals in various social media platforms. These proposed targets contribute both to the Network and Communicate stages of the Species Conservation Cycle, as we hope that the updated and new website and social media presence would allow us to extend our network of collaborators and promote our goals, work, and achievements for the benefit of deer (including musk-deer and chevrotains). Once those platforms are set, we hope that you will join us in the effort to develop and share content!

More targets included re-assessments of the Red List status of several priority species, write deer conservation translocation guidelines, better incorporate climate change into our plans, write action plans, and more. All of these so we can all continue to work together to improve and reinforce the DSG network, integrate deer biology knowledge, and be more effective in planning and implementing conservation and management work.

But we cannot start a new quadrennium without thanking all of you for your work and contribution during the past quadrennium. We understand that for most of you the DSG work is done voluntarily on top of your day jobs, so your contribution is not taken for granted and we greatly appreciate it. Thank you all! And we would like to give a special thanks to Dr. Sarah Brook, our Old-World Deer Red-List Authority Coordinator, for her enormous contribution, dedication, and hard work during the past few years, which included handling the complete re-assessment of Old-World DSG species. Sarah has decided to step down late in 2020 but she remains a member of the DSG. Thank you very much Sarah!!!

We also wish to acknowledge our supporting agency, DINAMA, the Environmental Minister in Uruguay for its research and contribution to the advancement of scientific knowledge on Neotropical deer species.

Finally, we want to thank all those who contributed to this edition of the newsletter and invite all of you to submit manuscripts for the next issue by sending them to Dr. Patricia Black (black.patricia@gmail.com).

Our best wishes,
Susana and Noam
Susana González and Noam Werner, Co-Chairs, IUCN/SSC Deer Specialist Group.

Individual Identification through lateral spot patterns in the Visayan Spotted Deer, *Rusa alfredi*

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Abstract

The Visayan Spotted Deer (*Rusa alfredi*) is under threat and requires urgent action to rescue its wild populations. Endemic to Negros and Panay, Philippines, *R. alfredi* populations have declined to <1000 mature individuals remaining. The disappearance of the species within protected areas implies a need for adequate assessments of remaining populations. Within a small reintroduced population, there is a need to monitor the fate of each individual. While GPS and telemetry techniques are effective, their use is limited by financial and human resource constraints, leaving remote camera trapping as a low resource alternative. Using photo-ID techniques, we trialed the first study for individual identification on a population of *R. alfredi* reintroduced to a reserve in Negros. Fourteen male *R. alfredi* released into the 300ha reserve were monitored using camera traps beside 6 artificial feeding stations. After 6 weeks, we cross-referenced the images with control images of the same individuals from captivity. Camera trap images were matched to control images by human observers and the software *Hotspotter*. We collected 118 images from 9 deer. The software identified 7 of the individuals in 66 separate images whilst human observers identified 9 of the individuals from 93, while 14 images were not matched by the software or human observers. As assessments begin to re-evaluate the status and size of the *R. alfredi* populations in the wild, photo-ID and assisting software will be intrinsic to population modeling, individual health monitoring and early indicators of local population collapses.

Keywords: Photo-ID, Endemic, Endangered, HotSpotter

Resumen

El sambar manchado de Filipinas (*Rusa alfredi*) es amenazado y requiere acción urgente para rescatar las poblaciones silvestres. Endémico a las islas de Negros y Panay en las Filipinas, las poblaciones han quedado en

<1000 individuos adultos. La desaparición de esta especie dentro de áreas protegidas implica una necesidad de asesorar adecuadamente las poblaciones que quedan. Dentro de una población pequeña reintroducida, hace falta monitorear el destino de cada individuo. Mientras que las técnicas de GPS y telemetría son efectivas, su uso es limitado por restricciones financieras y de recursos humanos, dejando el uso de cámaras trampas remotas como un alternativo de bajo costo. Usando técnicas de foto-identificación, hicimos el primer ensayo de identificación individual en una población de *R. alfredi* reintroducida a una reserva en Negros. Catorce machos de *R. alfredi* fueron monitoreados con cámaras trampas ubicadas al lado de 6 estaciones de alimentación artificial después de estar reintroducidos en la reserva de 300 ha. Después de 6 semanas comparamos las imagenes captadas con imagenes de control de los mismos individuos en cautiverio. Imágenes de las cámaras trampas (118 imágenes de 9 ciervos) fueron comparadas con las controles por observadores humanos y por el software *Hotspotter*. El programa identificó 9 individuos basado en 66 imágenes, mientras que los humanos identificaron 9 individuos basado en 93 imagenes. No se pudieron identificar 14 imágenes ni por el software ni por los observadores. Cuando el estado y tamaño de las poblaciones de *R. alfredi* en silvestría son re-evaludados, identificación por fotos y por software van a ser esenciales para las simulaciones de la población, el monitoreo de la salud de individuos son indicadores tempranos del colapso de poblaciones locales.

Palabras clave: Identificación por fotos, endémico, amenazado HotSpotter

Introduction

Many species have distinct patterns or markings for camouflage or displays of fitness (Endler *et al.* 2018). In many of these cases however, these seemingly random or simple patterns are distinctive to the individual (Hilby *et al.* 2009, Koivuniemi *et al.* 2016). Whether this distinction is visible to the species themselves is unknown, but using these subtle variations in pattern is useful for identifying individuals for research and conservation purposes (Velli *et al.* 2015, Koivuniemi *et al.* 2016).

The Visayan Spotted Deer (*Rusa alfredi*) is endemic to the West Visayan faunal region of the Philippines. The islands of Negros and Panay are home to the last remaining populations, with hunting and habitat loss fragmenting the island populations and driving the species to endangered status (Brook, 2016). *R. alfredi* has predominantly brown fur (from tan to golden brown) with small white or gold spots across its flanks and back. Growing to 90kg and 1.2m at the shoulder *R. alfredi* is the second largest deer in the Philippines after the Philippine Brown Deer (*Rusa marianus*) and the largest mammal in the West Visayan faunal region (Oliver 1994). The species ecological preferences and activity patterns are unknown and remaining wild populations have not been assessed. Given their continued threat from humans, the lack of sightings and knowledge gaps for the species, their conservation outlook is bleak and much is needed to recover the species.

The Talarak Foundation Inc. (herein, TFI) has been captive breeding and managing *R. alfredi* on Negros since 2010. In 2016 TFI took over the facilities and personnel of another Negros based captive breeding organization, Negros Forest and Ecological Foundation Inc. (NFEFI), absorbing a large breeding group of *R. alfredi* which had been active since the mid 1990's. The Talarak Foundation has collectively bred over 119 individuals from two breeding centers with most of the offspring going to satellite breeding centers or zoological institutions around the world, yet others stayed to continue expanding the local captive population. TFI aims to assess the Negros island population of *R. alfredi*, identify the measures that need to be taken to restore or protect remaining populations, and identify suitable habitat in former *R. alfredi* range which can be used for reintroductions.

TFI has launched the first *R. alfredi* reintroduction program at the Danapa Nature Reserve in Bayawan City, Negros island, thus creating a new population of semi-wild deer with access for monitoring and research studies. Within this population, several animals will be monitored through telemetry collars; however, the majority of the animals will be without, leaving camera trapping as an important tool for monitoring the health and growth of this new population. Recognizing individual animals is important to accurately track the success of the reintroduction program, however identifying animals by eye can be time-consuming and inaccurate.

When population sizes are unknown (or expected to grow/vary) and the species are of conservation importance, there is a need to monitor the fate of each individual. While GPS and telemetry techniques are effective, their use is limited by financial and human resource constraints. Conversely, the monitoring of a population of individuals through remote camera traps can be conducted by a single person with less financial investment than telemetry devices. Photo identification using camera traps is a relatively new method used by wildlife researchers to conduct population and ecology studies, where individual identification is necessary but other tagging methods are not feasible because of financial or ethical concerns (Maho *et al.* 2011, Sreekar *et al.* 2013). Photo identification has already been successful with large mammals like tigers and marine species like sea turtles but could still be used in more instances (Hilby *et al.* 2009, Dunbar *et al.* 2014, Carpentier *et al.* 2016). Given the use in photo identification combined with camera trap imagery there are multiple software programs available to conduct individual recognition through camera trap pictures. Here we use the program *HotSpotter* (Crall *et al.* 2013) to test if the spot patterns of *R. alfredi* are distinct enough to discern individuals using camera trap footage taken in a captive and semi-wild setting.

Materials and Methods

We photographed each individual in captivity creating a profile with pictures of the right and left flank spot patterns. These control images were used to identify animals within camera trap footage by human observers and the software *HotSpotter*, with *HotSpotter* also adding confirmed images of known individuals for comparison of subsequent camera trap footage.

We tested *HotSpotter* for its suitability for pattern recognition within our trial group, an *in situ* reintroduced group of 14 male *R. alfredi.,* Each had their control photos taken inside soft-release enclosures in the Danapa Nature Reserve. The Danapa Nature Reserve is a 300ha protected and fenced site owned by the Bayawan City government but donated to the Talarak Foundation Inc. for use in conservation activities. Within the reserve we placed 6 camera traps (Cuddeback) alongside artificial feeding stations, producing the images to record the deer across the 6 week recording period. *HotSpotter* works to assist human observers in identifying individuals which match with the reference images or already confirmed images of the same individual. The software relies on a large database of pictures for more accurate matching, with greater variation in confirmed images enabling the algorithm to adjust for alternate angles or limited views. Following the *HotSpotter* manual and previous studies by Park *et al.* (2019) and Nipko *et al.* (2020), we uploaded the entire image database, starting with the known reference images for each individual, before each image in the database had a "chip" placed on all suitable individuals within each picture (Park *et al.* 2019, Nipko *et al.* 2020). The chip is a rectangular section of the individual with which you aim to match against other chips from other images (particularly the reference images) in the database. We placed the chip along a clearly visible area of the individual's spot pattern between the shoulder and the rump. After the chips were placed (the most time consuming part of the process) we ran queries against each of the images which returned with match successes. The top 6 ranking images within the database which matched with the image queried were displayed, often identifying the reference and those that have already been confirmed as that individual. We continued this process for all images with chips. The ranking provides a scoring figure based on the similarity of the queried image against each matched image, the higher numbers suggesting a greater similarity of spot placement and interspot distance. These similarity scores were variable and ranged from 200-300 for low matched images to 3,000-7,000 for images of the same individual at different angles and 500,000 for images of the same individual unmoved.

In addition to using the *HotSpotter* software we had three human observers inspect each camera trap image and try to identify the individuals within each photo using the control pictures as reference. The primary and secondary suspected individual along with the confidence of the identification was recorded and averaged between the three observers.

Results

We placed camera traps at 6 feeding stations between July 15 and August 28. Over 6 weeks we recorded 90 viable pictures with 118 images of *R. alfredi* where one flank of the individual was clearly seen. Within those 118 images the *HotSpotter* software identified a known individual on 66 occasions (with a proportion of 0.56 positive matches), whereas the human observers identified individuals on 93 occasions (with a proportion of 0.79 positive matches). There were 48 images where the software and the human observers agreed upon the identification of individuals, 8 occasions where there were differences in identification and 10 occasions where *HotSpotter* identified an individual where the human observers did not. There were a total of 14 images in which neither *HotSpotter* nor the human observers could

Figure 1. The reference image (left) and an observer identified camera trap image (right) of “Beast” which are only recognisable by the human observers.

identify the individual in shot and 38 times when the human observers identified an individual which could not be identified through the software.

However, certain individual deer accounted for most discrepancies between human and software identification. One individual named Beast (Figure 1) has a particularly distinct pattern with very faint and sparse brown spots which were not picked up with the software. Beast appeared in 35 out of the 38 images, with another deer Ethan also appearing in 1 of those 38 images, where human observers identified him in the frame but the software did not. Removing these two individuals alone would increase the proportion of matching to 0.80, with 66 successful matches from 82 images. There were 7 remaining individuals identified by both parties, where we see a similar level of confidence and "similarity score" in the identification of individuals from the human observers and software.

Discussion

The classification of success using *HotSpotter* as a photo-ID tool for *R. alfredi* is mixed. It successfully matched 7 out of 9 individuals across numerous pictures (of various image qualities), in agreement with our human observers 59% of the time, with only 10% of decisions split between the software and humans. It also managed to identify individuals in 12% of the images which could not be identified by the human observers (although some of these identifications were low scoring and potentially erroneous matches). In contrast, the software was unable to match 2 individuals to any of their chips (constituting 36 images) which were unanimously identified by our human observers with a relatively high level of confidence (77% average confidence in identification). These individuals are known to have particularly faint spots and a sparse pattern, which despite being clear in their reference images, are harder to make out on the camera trap pictures (Figure 1).

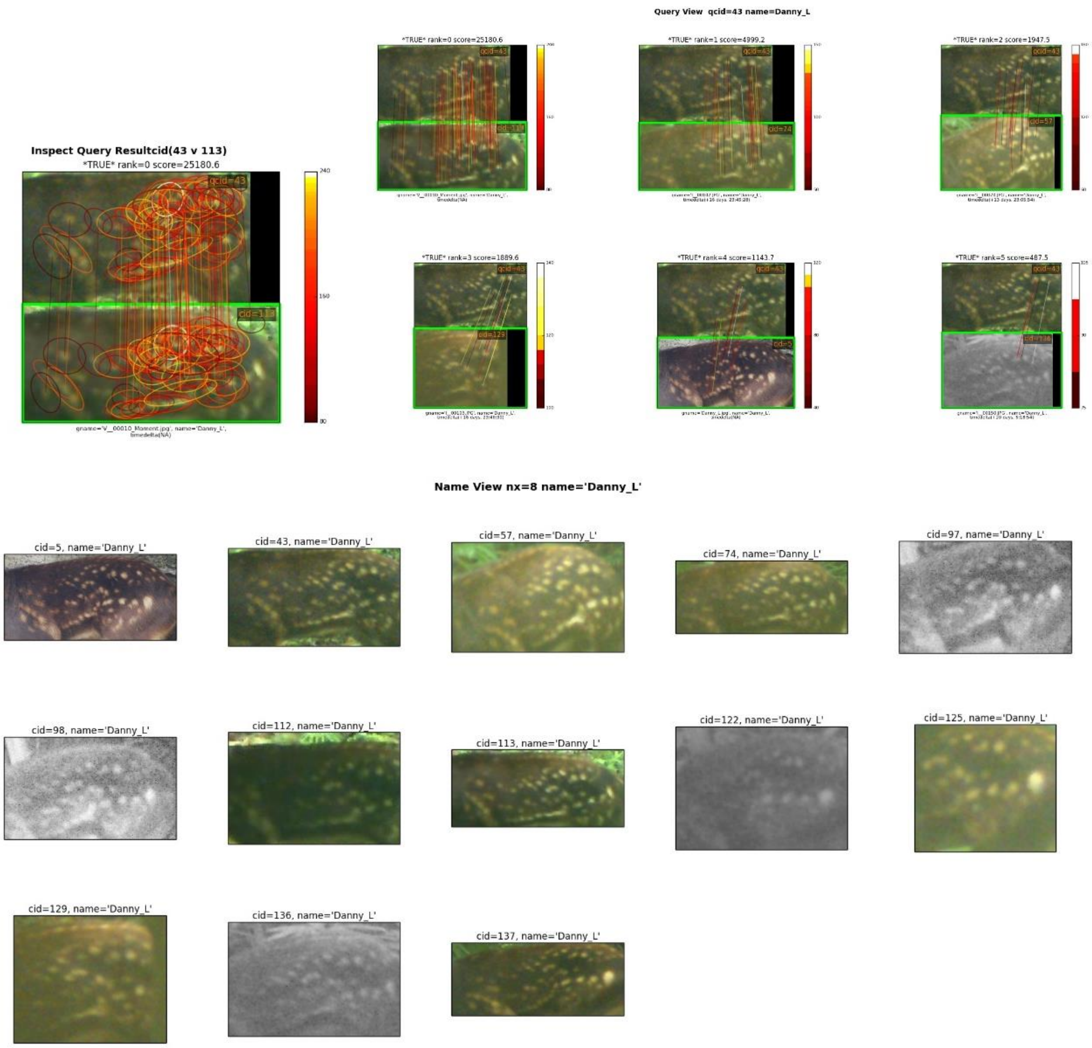

Figure 2. The matching process for an image paired with "Danny" (top left and right). The matching process creates clusters and central points (top left) from which to identify and measure the space between in alternate images. High similarity images contain paired clusters and points with fewer points being paired between images as the image quality decreases or angle of the individual's body changes (top right). There are multiple different angles and image qualities observed in the camera trap footage improving later identification through varied reference imagery (bottom).

We also saw a severe decline in the software scoring and matching ability as the quality of the image declined. The 6 camera traps being used were split between infra-red and low infra-red leading to many of the nocturnal images having poor clarity past 2m distance from the camera. We also placed the cameras at the start of the rainy season and the appearance of rain or high humidity interfered with the ability to accurately see the spot patterns on some deer. The distance and angle of the deer to the camera plays a large part in the scoring of the software matching algorithm. Images where the deer is close and perpendicular to the camera scored highly and are easily comparable to the reference images and one another; however if the animal is bent or moving away/toward the camera at an angle, there is only a partial pattern available and the ratios between spots become skewed (Figure 2). In some cases this led to mismatches or no matches, but in most cases these were matched with low similarity scores.

The use of photo-ID technology for conservation purposes is on the rise with multiple species already being tracked using photo-ID software (Arzoumanian *et al.* 2005, Bolger *et al.* 2012, Sreekar *et al.* 2013, Nipko *et al.* 2020). In the Cervinae subfamily there are currently 4 Threatened deer species including; *R. alfredi, Hyelaphus porcinus*, *Dama mesopotamica* and *Rucervus eldii,* and 3 further species listed as Least Concern (*Axis axis*, *Cervus nippon* and *Dama dama*), which have spotted coats (IUCN (2012). Regardless of the threatened status, any population monitoring program for spotted (or otherwise patterned) deer and antelopes could benefit hugely from the successful implementation of Photo-ID programs. Conservation initiatives and research studies are underway for some of the other species; however, as yet there is no literature indicating the use of photo-ID techniques in them. The use of photo-ID software in conservation largely revolves around population monitoring. By identifying which individuals are still present within a population, we can further map the population's size, growth/reduction, sex dynamics and age ratio using population models (Gamble *et al.* 2008, Morrison *et al.* 2011, Gore *et al*. 2016). We can even use these images and the individual identification to monitor individual health, body condition and movement patterns, helping conservation initiatives with early warnings of impending population crashes (Sreekar *et al.* 2013, Velli *et al.* 2015, Carpentier *et al.* 2016, Endler *et al.* 2018).

The use of *HotSpotter* as a photo-ID software has shown mixed results, largely positive in our opinion, but it should also be noted that its matching quality is supplemented by a high user friendliness when compared to the manual method used otherwise (Nipko *et al.* 2020). The simplicity and ease of use for the software, requiring only 4 actions to import the images and run the matching process, leads to *HotSpotter'* being a useful tool in reducing the time required to identify individuals. Despite the similar results to human observers in individual matching and acknowledging the failure to identify two individuals across all of their images, the matching process for the software took approximately 2 hours from database upload to chip placement and matching confirmation. In comparison the average time for the human observers to match the camera trap images against the reference images was 6 hours. As a tool for assisting the identification of individuals from camera trap footage it provides benefits to the alternative human observer method. A greater number of images for each individual to match against improves the algorithm comparing images and the likelihood of successful matches in suboptimal images (Crall *et al.* 2013, Park *et al.* 2019, Nipko *et al.* 2020). When compared to alternative population monitoring options, including telemetry devices or drone surveillance, this software's free download, easy access and adaptability to medium-low quality camera trap images also encourage its use. *HotSpotter* and the use of photo-ID techniques are already important tools in population monitoring activities for many species of conservation concern and have the potential to be used in many more. Even without easily recognizable spot/line patterns this software has shown effectiveness in some species through use on facial markings and body morphometrics (Osterrieder *et al.* 2015, Gore *et al.* 2016, Park *et al.* 2019).

The use of photo-ID techniques to identify individuals of *R. alfredi* here was overall considered a success. Given the limited quantity and quality of images to build reference from, the ability for the *HotSpotter* software and human observers to match a combined 81% of individuals from spot patterns could become crucial in monitoring populations of this species. With an unknown population size in the wild and less than 300 individuals in captivity internationally, there is an urgent need for action to keep this species from extinction. The reintroduction efforts from the Talarak Foundation at the Danapa Nature Reserve highlight the first attempt to boost the wild populations of the species on the island of Negros, however it does not address the lack of data on remaining wild populations. The supporting evidence from this pilot study and learning from further study on this reintroduced population could help to develop a method for the effective assessment of the remaining populations in Negros and Panay islands. For the first time population estimates can be measured from sites where wild *R. alfredi* can be easily observed, allowing for conservation initiatives to be enacted if/when deemed necessary for the population survival. Moving forward with *HotSpotter* there does need to be an improvement in camera trap picture quality and the placement should take into account the preference for images where either flank is in full perpendicular view from the lens in order to improve the matching ability of the software and reduce the need for human interference. However, we are impressed with *HotSpotter* given the limited image database and poor image quality, and we intend to test it further.

With multiple other photo-ID softwares in use and alternative methods for monitoring populations such as hair traps, live traps, telemetry devices and drone surveillance, there are various ways in which technology is assisting with population monitoring (Maho *et al.* 2011, Wilson *et al.* 2011, Velli *et al.* 2015, Silva *et al.* 2018). However each of these alternate techniques and the aforementioned other softwares also suffer from drawbacks and require some level of human interaction (which could lead to human bias/error) or are not appropriate for the species, given financial, ecological or biological restrictions. Selecting whether photo-ID is a suitable method for population monitoring within a species and which software to use is dependent largely on the species biology and habitat use. We intend to explore this further with *R. alfredi*, collecting more images from higher resolution camera traps across a greater number of individuals, habitats and weather, before comparing several photo-ID softwares to see which provides the best solution for user friendliness, matching success and adaptability for real-world use.

Acknowledgments

We would like to acknowledge our partners supporting the conservation work with *R. alfredi* both in the captive and wild conservation initiatives. Particularly Wildlife Reserves Singapore, Ocean Park Conservation Fund Hong Kong, Bristol Zoo Gardens, Synchronicity Earth and ZGAP. We would like to extend a special thank you to the local government of Bayawan City, who are the owners of the Danapa Nature Reserve land, primary funders for the reintroduction of *R. alfredi* and are proactive in bringing about a new future for these species and other endemic Negros wildlife. We want to include Chester Zoo with our sincerest thanks for their loan of camera traps which made it possible to carry out this study and explore the populations of *R. alfredi* in the wild. Finally our thanks go to the people involved in this project, including the expert advice from Noam Werner, Jeff Holland and other deer/ungulate specialists, and the ground force inside the reserve for maintaining it and keeping these animals safe during this initial period of the reintroduction.

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The status of Calamian Deer, *Axis calamianensis*, on Calauit Island, Palawan, Philippines, and its implications for conservation

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Abstract

Calauit Island Game Preserve and Wildlife Sanctuary was long regarded as the global stronghold of the Calamian Deer *Axis calamianensis*. However, the return of former residents, which were displaced under the presidency of Marcos during the establishment of the protected area, and the additional influx of settlers of unrecorded origin, has put the management of the reserve in limbo. As a consequence, a recent decline of Calamian Deer was suspected. We conducted a distance sampling survey on Calauit and estimate 348 individuals (CI 95%: 138 to 878) to be present on the island. These low numbers in conjunction with misinterpreted survey results from the mid-1990s suggest that the decline of Calamian Deer on Calauit was more severe than previously reported. Since conservation measures for the species throughout the present range have not yet resulted in measurable recovery, we suggest the establishment of a viable captive population within Palawan and eventual reintroduction within the prehistorical range (including the main island of Palawan). We have undertaken the first steps towards this objective, but are hampered by legal and logistical constraints.

Keywords: Calamian Islands, conservation breeding, distance sampling, protected area

Resumen

Por largo tiempo, *Calauit Game Preserve and Wildlife Sanctuary* era considerado como el bastión global del Ciervo Calamian, *Axis calamianensis.* Pero el regreso de los residentes desplazados por la presidencia de Marcos, cuando el área protegida fue establecida, así como la llegada de nuevos inmigrantes de origen desconocida, han perjudicado el manejo de la reserva. Como consecuencia, se suponía una disminución de los Ciervos Calamianos. Hicimos un relevamiento empleando el método de muestreos por distancia (Distance Sampling) y estimamos que hay 348 individuos (CI 95%: 138 - 878) en la isla de Calauit. Estos números bajos, en conjunto con un relevamiento del medio de los 1990s, sugieren que el decaimiento del Ciervo Caluit fue más severo que fue registrado anteriormente. Dado que las medidas de conservación para la especie en todo su área de distribución no han resultado en una recuperación medible, sugerimos establecer una población captiva viable dentro de Palawan y la reintroducción eventual a la distribución prehistórica (incluyendo la isla principal de Palawan).

Hemos hecho los primeros pasos hacia este objetivo, pero tenemos dificultades dado las restricciones legales y logísticas.

Palabras clave: Islas Calamian, reproducción en cautiverio, muestreos por distancia, área protegida

Introduction

The Calamian Deer, *Axis calamianensis*, is restricted to the Calamian group of islands within the Greater Palawan faunal region of the Philippines, with records from Busuanga, Culion and Calauit. The species was also reported from three smaller satellite islands (Bacbac, Marily and Tambon) according to secondary information in 1992 and extinct by that time from Capare, Apo, Uson, Maglambay North Malbinchilao and Dicabaaito Islands (Oliver & Villamor 1992). Calamian Deer is currently listed as “Endangered” (Widmann & Lastica 2015), but in need of reassessment. Fossils found in the northern and central portion of the main island of Palawan indicate that it had once a wider distribution, where it was common until the early Holocene and got extinct from the main island within the past 6,000 years (Piper *et al.* 2011).

Calauit Island was long considered to be the stronghold for the species within its small range (OLIVER 1993; Wemmer 1998). The island was declared as Calauit Game Preserve and Wildlife Sanctuary (CGPWR) in 1976 under presidency Marcos. Thirty individuals of deer were introduced to supplement an existing small population, along with eight species of African ungulates (Oliver 1993). The human population of the island was resettled, but since the end of the Marcos presidency, successfully managed to reclaim parts of the island, along with informal settlers of other origins. Initially the deer population thrived on Calauit (Wemmer 1998), since poaching and habitat alteration on the island remained low until at least the early 1990s (Oliver & Villamor 1992). However, in recent years declines were reported, making updated population surveys necessary to assess the conservation status and to better inform the conservation management of the species within its range, and on Calauit in particular.

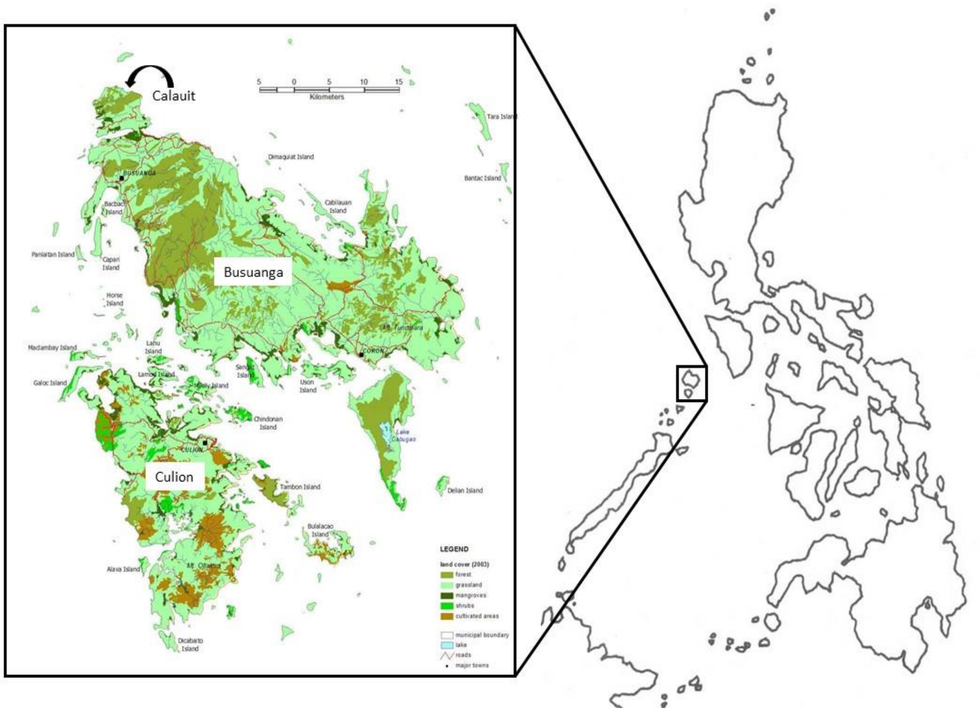

Figure 1. Location of the Calamian group of islands within the Philippines (right) and of Calauit Island within the Calamianes (left).

Material and Methods

Study Area

Calauit is a small landbridge island with an area of ca. 37 km^2, situated on the northeastern tip of the Sundashelf within the Calamianes group of islands, Palawan, Philippines. The relief is undulating with the highest elevation reaching ca. 214 m a.s.l. The vegetation consists of extensive grassland (mostly *Imperata cylindrica*, *Chrysopogon aciculatus*, *Cyrtoccoccum* sp.), interspersed with semi-deciduous woodland patches (*Vitex* sp., *Antidesma ghaessembilla*, *Sterculia foetida*, *Ficus* spp., *Oroxylum indicum*, *Garcinia* sp., *Glochidion* sp., *Canarium* cf. *asperum*). A broad band of woodland stretches along a hill range from the central southwestern to the northeastern portion of the island. Low-energy tidal areas are covered by mangroves, particularly along the southern and western coastline. A narrow mangrove strip patch also connects Calauit to the adjacent larger island of Busuanga. Some small villages were established along the shoreline, mostly in locations where original settlements were situated before the relocation in the 1970s. New cultivated areas are increasingly established close to settlements, but occasionally also in the interior of the island, with mostly rain-fed rice being planted during the rainy season. Some parcels of land were fenced off, commonly using bamboo and barbed wire.

Woodland is increasingly cut down for timber which is used in house construction and boat-building. Portions of the island, where forest was cleared and burned repeatedly, are covered by dense stands of bamboo (*Schizostachyum* sp.). Some open areas are dominated by tall weeds, especially *Chromolaena odorata*, indicating overgrazing in some parts of the more open vegetation. The headquarters of the protected area are situated in the southwestern portion of the island, comprising a complex of low buildings and animal enclosures.

Study Methods

We conducted distance sampling surveys using line transects on Calauit Island, Palawan, Philippines to estimate population densities of Calamian Deer (see details in Buckland *et al.* 2001). Surveys were undertaken on two different occasions, in April 2016 within the dry season, and June 2019 within the start of the rainy season. Transects were placed randomly covering the entire island, however excluding settlements and their immediate vicinities for security reasons. Since it turned out that deer were extremely unevenly distributed on the island, with most of the records coming from ca. two square kilometers surrounding the park headquarters, we excluded clusters of six and more individuals from the analysis to avoid overrepresentation of samples from this small area. We truncated records of more than 100m to account for the more open vegetation in the headquarter area, compared to most of the rest of the island. Deer were recorded by measuring distances of animals individually from the transect line with a range finder and estimating angles in reference to the line to the nearest 22.5 degrees with the help of an analog compass. Analysis was done using the software package Distance 7.3 with standard settings.

We also noted presence or absence and estimated frequency of indirect signs, particularly hoof prints and fecal pellets, as markers of presence. However, we did not count these, since these records require a different kind of visual focus, due to their size and position on the ground, and we feared overlooking actual deer while trying to record both.

Results

We covered a total of 58.6 km on the island in 116 transects. All deer records (51 observations after truncation) occurred within two square kilometers around the park headquarters. We also did not come across any deer on the way to, or back from, the transect start and end points in areas outside the headquarter vicinity.

A half-normal key function provided the best fit for our data set. We estimated a deer density of 9.4 individuals/km^2 (SE 4.65) for Calauit, which translates to 348 individuals on the island (SE 171.89) with a confidence interval (95%): 138 to 878.

Indirect signs of Calamian Deer outside of the vicinity of the park headquarters were found sparsely at the woodland ecotone, particularly in burned areas, although the latter could be an effect of better detectability. Prints and fecal pellets were already old during the dry season survey, and fresh ones were only found in the rainy season in these locations. Old and fresh indirect signs were found near the headquarter area year-round and at higher density by at least two orders of magnitude, compared to the remaining parts of the island.

Figure 2. Calamian Deer near headquarters of Calauit Game Preserve and Wildlife Sanctuary (Photo: Peter Widmann, KFI).

Discussion

A population estimate from the mid-1970s put the total number of Calamian Deer at a maximum of 900 individuals for the complete range, but it is likely to be much lower (Grimwood 1976). The remnant deer population on Calauit (augmented by 30 individuals in 1976), steadily increased until ca. 550 individuals were estimated to be present on the island in 1992, based on headcounts conducted by the staff of CGPWR (Oliver & Villamor 1992).

The only population estimate of Calamian Deer in the last century, based on a Hayne's line transect count, was conducted on Calauit in 1994 (Orig & Rosell 1994). However, not the whole island was covered in this survey, as was implied in the IUCN Deer action plan (Wemmer 1998), but only three sectors, each covering ca. 740 ha. The population estimate for these three sectors (out of five sectors covering the whole island) was 1,122.75 (892.25-1,363.25) individuals. Assuming that the chosen transects were representative for the entire island, this would suggest that by the mid-1990s, there might have been around 1,870 Calamian Deer on Calauit, way more than the headcounts suggested any time before or after.

One of the authors of this study (PW) visited Calauit Island in 1996 and found Calamian Deer to be common within and outside of the vicinity of the park headquarters, giving the impression that deer density was higher overall and the population more equally distributed over the island. Whereas the headcount, which is still done monthly up until now on Calauit, may yield consistent results for some of the larger African Ungulates, at least for those which remain in open habitats for most of the time, it is unlikely to yield reliable results for deer.

The decline of Calamian Deer from Calauit in the past 25 years or so therefore was likely much steeper than previously suggested. Although the island is not representative for the rest of the range of the species, it allows for a glimpse on how quickly the deer population can deteriorate when exposed to threats similar to those it has faced for decades elsewhere on Busuanga and Culion.

We have not yet been able to come up with population estimates for deer outside of Calauit, due to scarcity of direct observations during transect surveys. However, an occupancy survey conducted within the entire range of the species suggests that only a fraction of suitable habitat is currently utilized by the species (Katala Foundation, Inc., unpubl. data). A population density for Calamian Deer for the entire range, based on a survey in 2018 was given with 0.15±71.01 per hectare (PCSDS 2018). It seems, however, that direct observations and indirect contacts (hoof prints, fecal pellets) were lumped in the data set, and no description was provided on how the counts of fecal pellets (deposition and decomposition rates) or hoof prints (age, number within one track, etc.) were calibrated. In combination with habitat modeling it was estimated that 5,868 individuals remain in the global range of the species (PCSDS 2018). However, given the limitations described above for the distance sampling, the global population estimate appears too optimistic

Long-term management issues of Calauit Island as a protected area remain unresolved, particularly because of overlapping claims by the displaced and now returned original residents in the formerly declared protected area. Direct persecution of wildlife has increased dramatically because of this, and possibly also intrusion by poachers from outside, not only affecting the deer, but a whole range of other wildlife. Even the extinction of the Common Impala *Aepyceros melampus*, one of the eight African ungulates released on the island, was attributed to poaching (F. Sariego, *pers.comm*.). We found trophies of Calamian Deer and African antelopes on display in a restaurant on Busuanga, with at least the latter undoubtedly originating from Calauit.

During our field work in April 2016 large parts of the grasslands of the island were set on fire, also affecting some of the woodlands in the central range. Some fires were started by poachers to encourage new growth of vegetation and to attract deer and other wildlife. In addition to loss of habitat due to human encroachment, other threats to deer are reportedly stray dogs and barbed wire fences in which the animals become entangled (F. Sariego, *pers. comm.*).

Threat factors in the entire range of the species are the same as in Calauit (hunting, habitat conversion, stray dogs). An emerging issue might be the accidental introduction of livestock diseases, since particularly on the island of Busuanga, intensive cattle farming is performing.

Initiatives were underway to establish parts of the range of the species as locally protected areas (Oliver, *in litt*., 2014). However these sites have either not yet been declared, or are not adequately integrated in the regional land use planning process. Based on interviews with forest users and our occupancy surveys, there is no evidence that hunting pressure was reduced of these because of the conservation measures (Kfi, unpubl. data). Very low densities in the entire range of the species (too low, in our experience, to get sufficient data for a reliable population estimate through distance sampling), and the steep decline on Calauit puts the species very possibly in a more precarious position than it was in the past four decades within its range.

Since Calamian Deer were recorded on the main island of Palawan until the late Holocene (Piper *et al.* 2011), the species was suggested as a possible candidate for rewilding within its former range (Louys *et al.* 2014), preferably in areas with reduced poaching pressure. There currently is no viable population of Calamian Deer in captivity. Katala Foundation was granted a Wildlife Farming Permit allowing the capture of up to 25 deer from Calauit to establish a conservation breeding population in a facility on the main island of Palawan, which was newly established specifically for this purpose. This has however experienced several delays, due to pending resolution of legal issues, and due to the current pandemic.

Acknowledgments

The study is part of a conservation and research program on Calamian Deer and Balabac Mousedeer *Tragulus nigricans* under a Memorandum of Agreement with the Palawan Council for Sustainable Development of January 7, 2016. We are grateful for the support of the Palawan Deer Research and Conservation Program, to the North of England Zoological Society (UK), Zoo Landau in der Pfalz (Germany), Zoological Society for the Conservation of Species and Populations e.V. (Germany), Wroclaw Zoo (Poland), Tierpark Berlin (Germany), Los Angeles Zoo and Botanical Gardens (USA), Center for the Conservation of Tropical Ungulates (USA) and an anonymous supporter.

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Molecular phylogenetics and diversification of Swamp deer (*Rucervus duvaucelii*) based on complete mitogenome

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Abstract

Swamp deer (*Rucervus duvaucelii*) is a large cervid confined to isolated patches in the wet grasslands of Himalayan foothills and dry grasslands of central India. There are currently three accepted subspecies of swamp deer: *R. duvaucelii duvaucelii, R. duvaucelii branderi* and *R. duvaucelii ranjitsinhi,* based on their distribution range, habitat preference and morphology. All the subspecies have undergone drastic reduction in their population size and range due to increasing anthropogenic activities and habitat degradation. In this study, we investigated the phylogenetic relationships and diversification among three subspecies of swamp deer using complete mitogenomes. Our study indicates that the swamp deer lineage split into subspecies around 0.14-0.17 Mya ago, during the Late Pleistocene. Low level of genetic differentiation was observed among mitochondrial genomes of the three subspecies. The study highlights the utility of appropriate and selected mitochondrial genes for subspecies level differentiation that will aid in reliable population assessment and evolutionary studies. Moreover, it will also be beneficial for wildlife forensics to track seizures of unknown subspecies, such as meat, skin, antlers and other trophy articles of swamp deer. Considering the small and fragmented populations of swamp deer in the high anthropogenic pressure area, we propose continuous long-term population monitoring and status upgradation of swamp deer in the IUCN database for effective conservation and management attention.

Keywords: mtDNA, Phylogenetic, evolutionary divergence, differentiation

Resumen

El ciervo de los pantanos (*Rucervus duvaucelii*) es un cérvido grande restringido a parches aislados en los humedales de las faldas himalayas y las praderas secas de la India central. Actualmente se reconoce 3 subespecies de ciervo de los pantanos: *R. duvaucelii duvaucelii, R. duvaucelii branderi* y *R. duvaucelii ranjitsinhi*, basados en su distribución, preferencia de hábitat y morfología. Todas las subespecies han sufrido una reducción dramática en su población y rango de distribución debido al aumento de actividades antropogénicas y la degradación del hábitat. En este estudio investigamos las relaciones filogenéticas y la diversificación dentro de 3 subespecies de ciervo de los pantanos usando mitogenomas completos. Nuestro estudio indicó que el linaje del ciervo de los pantanos se dividió en subespecies hace 0.14-0.17 Mya, durante el Pleistoceno tardío. Una baja diferenciación genética fue observada entre los genomas mitocondriales de las 3 subespecies. El estudio destaca la utilidad de seleccionados y apropiados genes mitocondriales para la diferenciación de subespecies que ayudará en asesorar poblaciones y en estudios evolutivos. Además, sería beneficioso en la ciencia forense de vida silvestre para rastrear la procedencia de productos incautados de subespecies desconocidas, como carne, piel, astas y otros artículos de trofeo de ciervos de los pantanos. Considerando las poblaciones pequeñas y fragmentadas de ciervos de los pantanos en las áreas de alta presión antropogénica, proponemos el monitoreo continuo de largo plazo y la actualización del estado de conservación de la especies en la base de datos de la UICN para mejorar la conservación y manejo de la especie.

Palabras clave: mtADN, filogenia divergencia evolutiva, diferenciación

Introduction

The swamp deer (*Rucervus duvaucelii*), commonly known as barasingha, is one of the large cervids endemic to the Indian subcontinent (Schaller, 1967; Qureshi *et al*., 2004). It is the only extant member species of the genus *Rucervus* with Schomburgk's deer (*Rucervus schomburgki*) from Central Thailand believed to have gone extinct by 1938 (Duckworth *et al*., 2015). Swamp deer populations have undergone a steep decline throughout their historical range. During the 19th century, swamp deer were widely distributed throughout the Himalayan foothills from Upper Assam to west of the river Yamuna near Uttarakhand, the Indo-Gangetic plains, Ganges and Godavari (Schaller, 1967; Groves, 1982; Sankaran, 1989). There are currently around 5000 wild individuals inhabiting isolated habitat patches in the Indian subcontinent (Qureshi *et al.,* 2004; Tewari and Rawat, 2013).

Habitat loss and poaching are the most pertinent threats to the swamp deer. Habitat alteration and fragmentation due to anthropogenic conversion of grasslands and wetlands to agricultural land have denuded viable ecosystems where the species has historically thrived (Groves, 1982). Poaching is primarily to supply trophy articles for wildlife trade market and secondarily for meat consumption. It is classified as "Vulnerable" in the Red List of the International Union for Conservation of Nature (IUCN) and included in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In India, this species is protected under Schedule I of the Indian Wildlife (Protection) Act, 1972 (Duckworth *et al*., 2015).

Three subspecies of Swamp deer are recognized based on their current distribution range, distinctive hoof morphology, antlers and habitat usage: the Northern Swamp deer (*R. d. duvaucelii),* the Eastern Swamp deer (*R. d. ranjitsinhi)* and the Central Swamp deer (*R. d. branderi)* (Groves, 1982; Qureshi *et al*., 2004). *R. d. duvaucelii* (Cuvier 1823) or the wetland barasingha is found in the Indo-Gangetic plains and north of the Ganges, Nepal (Shuklaphanta and Bardiya National Park) and previously in Pakistan; *R. d. branderi* (Pocock, 1943) or the hard ground barasingha is limited to Kanha Tiger Reserve (KTR) and has been recently reintroduced in Satpura Tiger Reserve (STR), Madhya Pradesh in Central India; and *R. d. ranjitsinhi* (Groves, 1982) or the eastern barasingha is found in the Brahmaputra plains of Kaziranga National Park (KNP) and Manas Tiger Reserve (MTR), Assam but has now gone extinct from Bangladesh (Qureshi *et al*., 2004).

Swamp deer present an urgent case for effective conservation and management efforts to conserve the dwindling populations as well as to combat the increasing poaching activities. Molecular insight into the phylogenetic and evolutionary relationships among the subspecies is an invaluable tool for efficient conservation. Numerous studies have explored the phylogenetic relationships of swamp deer with other cervids, but were restricted to unidentified subspecies (Pitra *et al.,* 2004; Gilbert *et al*., 2006). A comprehensive database with robust phylogenetic and evolutionary assessment among swamp deer subspecies is still lacking (Gilbert *et al*., 2006; Hassanin *et al*., 2012; Paul *et al*., 2019; Bhaskar *et al*., 2020). A mitochondrial DNA (mtDNA) control region (CR) and microsatellite-based study by Kumar et al. (2017a) revealed weak divergence among the swamp deer's subspecies. The mtDNA has proven to be a useful tool to elucidate molecular phylogenetics and evolution due to its characteristic relatively faster mutational rate, largely uniparental inheritance and lack of recombination (Boore, 1999). Hence, the sequencing of the complete mitogenome offers a useful resource database to compare the evolutionary history of species and subspecies (Kumar *et al*., 2019). Here, we have sequenced the complete mitogenomes of all three subspecies: *R. d. duvaucelii*, *R. d. branderi*, and *R. d. ranjitsinhi,* to explore the phylogenetic relationships along with divergence time estimation. We have also investigated the genetic differentiation between the three subspecies to provide baseline information for genetic monitoring, spatial distribution ranges, and phylogenetic relationships.

Materials and methods

Shed antlers were collected from the three different locations representing, *R. d. duvaucelii* (n=2) from Jhilmil Jheel Conservation Reserve, Uttarakhand, *R. d. ranjitsinhi* (n=2) from KNP, Assam and *R. d. branderi* (n=2) from KTR, Madhya Pradesh (Fig. 1). Genomic DNA was extracted from the antler samples according to the guanidine hydrochloride (Gu-HCl)-based silica-binding protocol (Gupta *et al.*, 2013) in a final elution volume of 100 µl. The extracted DNA was further checked by 0.8% agarose gel and used in a final concentration of 40 ng/µl for the PCR amplification

PCR amplification and sequencing

PCR amplification and sequencing of the entire mtDNA genome was performed using the 24 sets of primers (Hassanin *et al.*, 2009; Gupta *et al.*, 2014; Kumar *et al.*, 2017b). PCR reactions were carried out in 20 µl reactions using 1 × PCR buffer (10mM Tris–HCl, pH 8.3, and 50mMKCl), 1.5mM $MgCl_2$, 0.2mM of each dNTPs, 3 pmol of each primer, 0.5 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems) and 1 µl (~30 ng) of template DNA. The PCR conditions were: initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 40 s and extension at 72 °C for 75 s. The final extension was done at 72 °C for 10 min. PCR amplification was confirmed by electrophoresis and visualized under a UV transilluminator. The amplified PCR products were treated with exonuclease-I and shrimp alkaline phosphatase (Thermo Scientific Inc.) at 37 °C for 20 min to remove any residual primer followed by inactivation of enzymes at 85 °C for 15min. The purified fragments were sequenced directly in an Applied Biosystems Genetic Analyzer 3500 XL from the forward and reverse direction using BigDye v3.1 Kit.

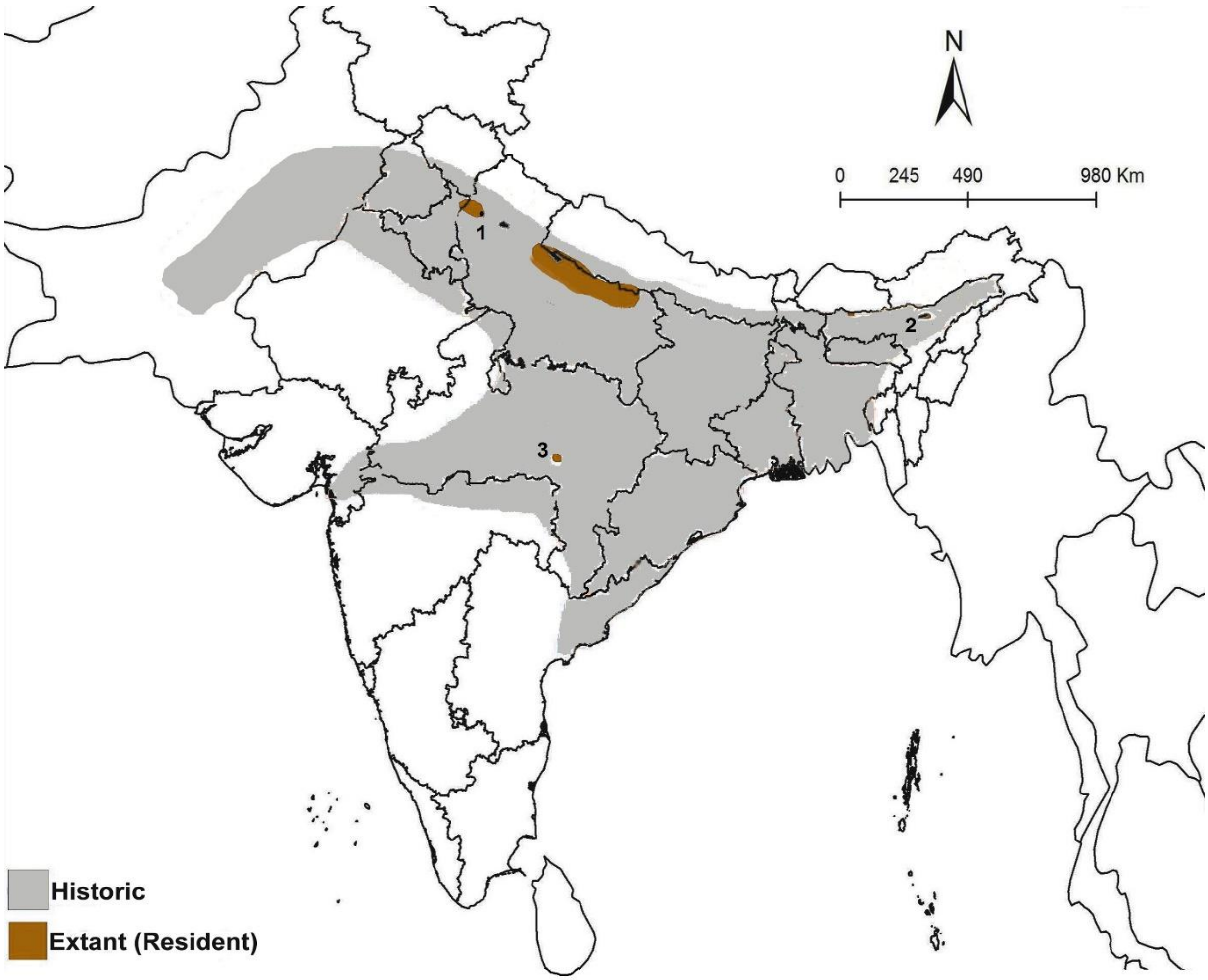

Figure 1. Historic and extant distribution map of *Rucervus duvaucelii* (Duckworth *et al.*, 2015) adapted from Kumar *et al.*, (2017a). 1. Sampling location from Jhilmil Jheel Conservation Reserve, Uttarakhand; 2. Kaziranga National Park, Assam, and 3. Kanha Tiger Reserve, Madhya Pradesh.

Genetic differentiation, phylogenetic relationship and divergence dating

The p-genetic distance among *R. d. duvaucelii*, *R. d. branderi*, and *R. d. ranjitsinhi* was estimated using MEGA X (Kumar *et al.*, 2018). The phylogenetic tree was constructed with 14 additional sequences downloaded from NCBI belonging to: chital (*A. axis*, JN632599); hog deer (*A. porcinus*, MH443786); Eld's deer (*P. eldii*, JN632697); Père David's deer (*E. davidianus*, JN399997); sambar (*R. unicolor*, NC008414); Formosan sambar (*R. u. swinhoei*, EF035448); fallow deer (*D. dama*, JN632629); red deer (*C. elaphus*, AB245427), European roe deer (*C. capreolus*, KJ681491), mule deer (*O. hemionus*, JN632670), Water deer (*H. inermis*, NC011821), and one species of Bovidae, banteng (*B. javanicus*, FJ997262). The concatenated 12 PCGs (excluding the ND6) were used to perform phylogenetic analysis. Alignments of concatenated sequences were conducted in MUSCLE implemented in MEGA X (Kumar *et al.,* 2018) and manually checked. The ND6 gene was excluded as it is encoded on the light strand in contrast to the other 12 PCGs encoded on the heavy strand; both the strands have a highly distinct base composition (Bradshaw *et al.,* 2005). A maximum-likelihood (ML) tree was constructed using MEGA X (Kumar *et al.*, 2018), and the consistency of the phylogenetic relationships was tested by bootstrapping (1000 replicates).

The divergence time among the three subspecies of swamp deer was inferred using a relaxed-clock method in BEAST v.1.8 (Drummond *et al.,* 2012). The TMRCA of Bovids and Cervids was set as a calibration point to 16.6 ± 2 million years (Mya) with a normal prior distribution (Bibi, 2013; Martins *et al*., 2017; Gupta *et al*., 2018). Tree prior categories were set to the Yule-type speciation. An HKY model using gamma + invariant sites with five gamma categories was used. Markov chain Monte Carlo (MCMC) was run for 150 million generations and sampled every 1000 generations. All the runs were evaluated in Tracer v. 1.6. The final phylogenetic tree was visualized in FigTree v.1.4.2. (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Phylogenetic relationship and genetic distance

We obtained 16342 bp long complete mitogenome sequences from all three subspecies of swamp deer and submitted them in NCBI GenBank (MW348977- MW348982). In the ML tree, *Rucervus* formed a sister clade with *Axis* (bootstrap support (BS) 100%), and Cervinae formed a well-supported clade with Capreolinae (Fig. 2), which was congruent with previous studies (Gilbert *et al*., 2006; Hassanin *et al*., 2012). The two subspecies of swamp deer, *R. d. duvaucelii* and *R. d. branderi,* clustered together and nested with *R. d. ranjitsinhi* with high BS (~100%).

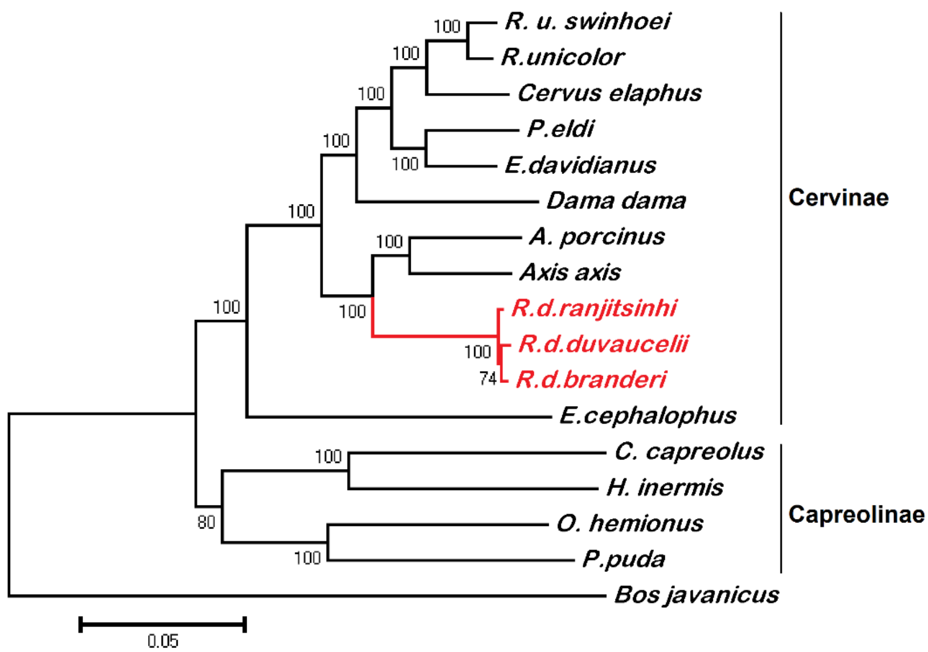

Figure 2. Maximum-likelihood (ML) tree showing the phylogenetic position of three subspecies of swamp deer (*R. duvaucelii*).

The genetic distance was inferred using the complete mitogenome; 13 PCGs, two rRNA genes and the control region (CR) (Fig. 3). Interestingly, the complete mitogenomes showed of all the three subspecies showed high level of genetic similarity among themselves (~99.47%). Hence, we used a gene-wise estimation to differentiate at the subspecies level. The values indicated that out of 13 mitochondrial PCGs, only seven, namely ND1, ND2, COX*II*, ATP6, ND4, ND5, CYTB, along with 16S rRNA and CR, significantly differentiated the subspecies. Low genetic distance was observed within the 16S rRNA gene, whereas it was high for CR. It is noteworthy that the barcoding region: COX*I* and COX*III*, along with 12S, ATP8, ND3, ND4L and ND6 was not able to differentiate between the three Swamp deer subspecies.

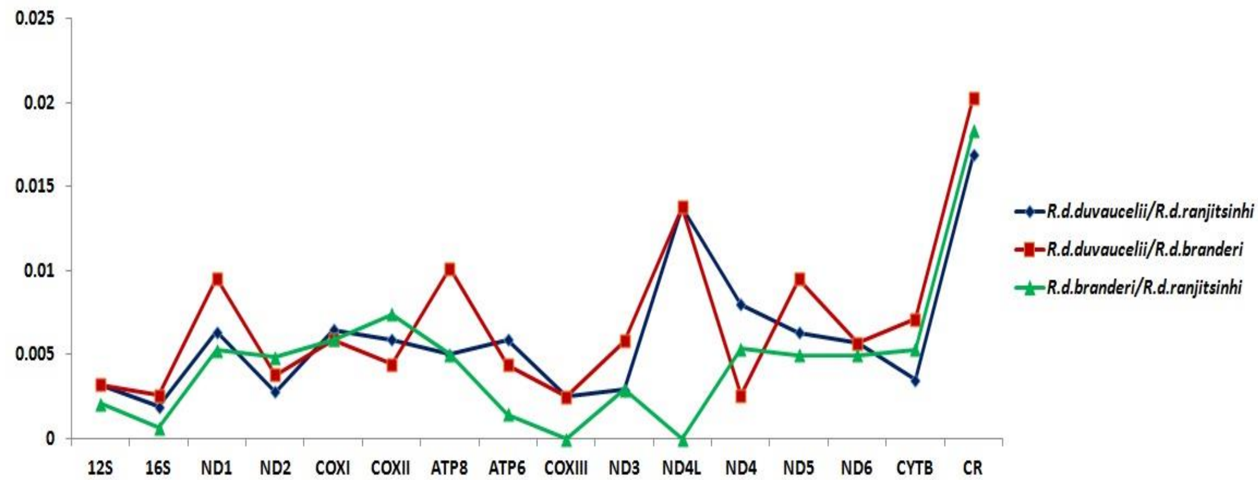

Figure 3. Graphical representation of gene-wise similarity among three subspecies of swamp deer (*R. duvaucelii*).

Divergence dating

The complete mitogenomes from the three subspecies were used to estimate the divergence time with respect to the most recent common ancestor (TMRCA) among cervids and bovids at approximately 15.7 ± 2 million years ago (Mya) with a ($CI_{95\%}$: 11.9-20.0). Our analysis suggested that *Rucervus* split from *Axis* in the Pliocene, around 3.36 Mya ($CI_{95\%}$: 2.3 to 5.0). The diversification among *Rucervus* members began in the late Pleistocene. The *ranjitsinhi* subspecies split from the other two subspecies approximately 0.17 Mya ($CI_{95\%}$:0.103–0.309), while the *duvaucelii* and *branderi* subspecies diverged from each other around 0.14 Mya ($CI_{95\%}$:0.083–0.24) (Fig. 4).

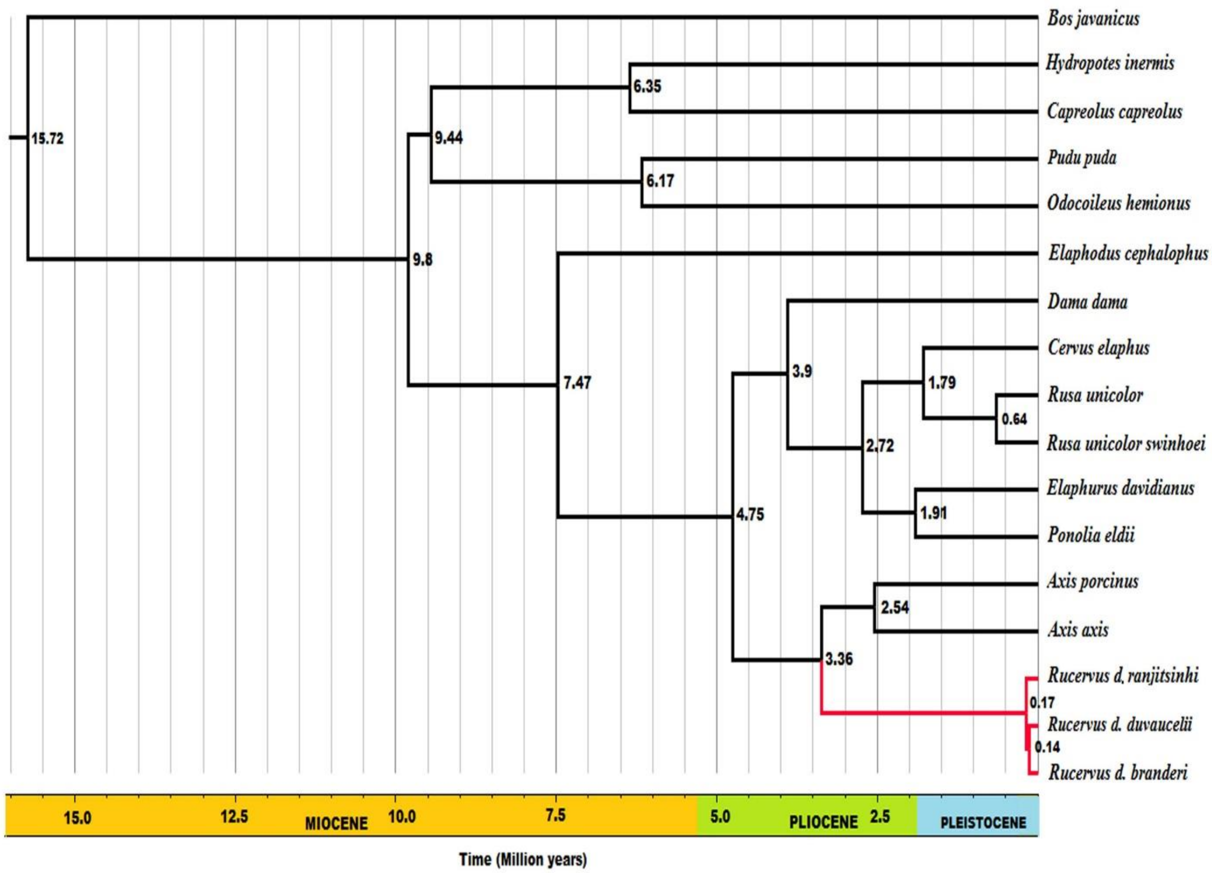

Figure 4. Divergence time estimates using the program BEAST 1.8. The red clade represents the lineage of swamp deer (*Rucervus duvaucelii*). The period's boundary is according to the Geologic Time Scale v. 5.0 (Walker et al., 2018).

Discussion

The three subspecies of swamp deer are experiencing rapid decline in their respective populations due to habitat modification (Fig. 5) and illegal hunting (Qureshi *et al*., 2004). To understand the evolutionary nuances of swamp deer, we analyzed the phylogenetic relationships, divergence, and genetic differentiation among the three extant subspecies. In *Rucervus*, all three subspecies formed distinct clades with subspecies *ranjitsinhi* representing the earliest split lineage. The Eastern subspecies *ranjitsinhi* appears to have diverged from the remaining two subspecies around the Late Pleistocene. Our analysis revealed the sister clade *Axis*, to have separated from *Rucervus* in the Pliocene. The Plio-Pleistocene period was associated with drastic changes in vegetation and rainfall pattern with alteration of forest cover, limiting favorable habitats for swamp deer to isolated pockets of their current distribution range. This chain of events is in concordance with the climatic refugia theory acting on other species (Karanth, 2003). Our finding of the *ranjitsinhi* subspecies to be the earliest diverging clade of swamp deer is also supported by fossil evidence suggesting that *Rucervus schomburgki* (extinct sister species) from Central Thailand is the closest relative, indicating the Eastern subspecies as the oldest lineage of present-day Swamp deer. The Northern and Central subspecies diverged from each other around 0.14 Mya, with sudden alterations of forest cover and other climatic fluctuations (Mani, 1974; Das, 1996; Daniels, 1997).

Figure 5. Herd of Swamp deer in Jhilmil Jheel Conservation Reserve (JJCR), with the prevalence of cattle grazing in the same habitat.

The temporal diversification among the three subspecies was separated by a small-time lapse of 0.03 Mya, suggesting rapid evolutionary diversification. The same was indicated in low genetic differentiation among the mitochondrial genomes of the three subspecies. This warranted mitochondrial genome-wide profiling to assess the reliability of accepted markers to effectively differentiate among the three subspecies. The study of the entire mitogenome and variations in multiple genes provide more interesting insights into lineage delimitation of closely related species/subspecies than short or single-gene analysis (Boore, 1999; Kumar *et al.*, 2019). Gene-wise estimation of genetic distance provides crucial information for the selection of appropriate markers for molecular tracking of swamp deer subspecies. We found seven mitochondrial PCGs with 16S rRNA and the CR to be useful for accurately detecting subspecies level differentiation in swamp deer. It is worth noting that two of the traditionally used Barcoding genes (COX*I* and COX*III*) could not resolve the subspecies level genetic differentiation among swamp deer. It highlights the utility of multiple mitochondrial markers for robust and reliable subspecies level differentiation among the closely related swamp deer subspecies.

Historically, swamp deer have inhabited marshy habitats (Groves, 1982) and the present-day lineages represent subspecies level adaptation and diversification into their current environment amidst climate change and anthropogenic pressure. These large habitat specialist herbivores face the risk of extinction due to their inherent biology as well as the anthropogenic pressure areas their habitats coincide with (Karanth *et al.,* 2010). Most of the remaining wild swamp deer exist in the protected area network of their range countries, which demonstrates the role of *in situ* management practices in effective conservation. To maintain healthy stocks, responsible *in situ* and *ex situ* interventions are warranted. Molecular evidence can be vital in identifying distinct management units to conserve the existing subspecies of swamp deer without unintentional genetic admixture (Kumar *et al.,* 2017a). Moreover, the molecular database will aid in wildlife forensics by tracking seized items of unknown subspecies, such as meat, skin, antlers and other trophy articles. Keeping in view the confined and fragmented distribution range overlapping with high anthropogenic pressure areas, we recommend updated population assessment and status upgradation of swamp deer in the IUCN database for effective conservation attention.

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Camera-trap records of muntjac in the lowlands of Hue Saola Nature Reserve, central Vietnam

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Abstract

Vietnam has a high diversity of muntjac species, but relatively little is known about their ecology and population status. Obtaining this information is important for conservation because all ground-dwelling mammals have declined from historic levels because of intensive snaring across Vietnam. In this study, we employed camera-trapping in a relatively small lowland area of about 850 hectares in the Hue Saola Nature Reserve, Thua Thien Hue Province, central Vietnam to investigate muntjac occurrence and ecology. From 16,486 camera-trapping nights, we confirmed the presence the northern red muntjac (*Muntiacus vaginalis*), with 29 independent detections from 1,428 photos across 12 of 46 stations, but 126 independent detections (82%) from 538 photos of photos were unable to be identified due poor photo quality. We failed to record the Annamite dark muntjac species complex (*Muntiacus rooseveltorum/truongsonensis*), although this result was expected based on known elevational distributions for this muntjac. We also failed to record the large-antlered muntjac (*M. vuquangensis*), a rare and highly-threatened species endemic to the Annamites ecoregion of Vietnam and Laos. Our failure to detect the large-antlered muntjac adds to a growing body of evidence that suggests the species is locally-extirpated in the protected area. Our camera-trapping results provide further insights into ecology of the northern red muntjac in central Vietnam. We also discuss the implications of camera-trap quality and survey design on future muntjac studies.

Keywords: Annamites, camera trap, Hue Saola Nature Reserve, *Muntiacus vaginalis*, northern red muntjac, Vietnam

Resumen

Vietnam tiene una alta diversidad de especies de muntjac, pero se sabe poco sobre su ecología y el estado de sus poblaciones. Es importante obtener esta información para su conservación porque todas las especies de mamíferos terrestres no-voladores han disminuido desde sus niveles históricos como resultado de muchas trampas de alambre puestas por todo Vietnam. En este estudio usamos cámaras trampas en un área relativamente pequeña de 850 ha en la Hue Saola Nature Reserve, Thua Thien Hue Province, Vietnam central, para investigar la ecología y abundancia de muntjacs. Desde 16.486 registros de las cámaras trampas, confirmamos la presencia del muntjac rojo del norte (*Muntiacus vaginalis*), con 29 detecciones de 12 de 46 estaciones, pero no se pudo identificar 126 detecciones independientes (82%), de 538 fotos, como resultado de una pobre calidad fotográfica. No registramos el complejo del muntjac oscuro de los Annamites (*Muntiacus rooseveltorum/truongsonensis*), pero esperamos este resultado porque el área de estudio está fuera del rango altitudinal de la especie. Tampoco registramos el muntjac de astas grandes (*M. vuquangensis*), que es una especie poco común y muy amenazada de la ecorregión Anamites de Vietnam y Laos. También puede indicar que la especie ya ha sido extirpada localmente del área protegido. Nuestros resultados proveen información sobre la ecología del muntjac rojo del norte en Vietnam central. Además, discutimos las implicaciones de la calidad de la información que proveen las cámaras trampas y del diseño de relevamientos para futuros estudios de muntjacs.

Palabras claves: Annamites, cámara trampa, Hue Saola Nature Reserve, *Muntiacus vaginalis*, muntjac rojo del norte, Vietnam

Introduction

Vietnam contains a particularly high diversity of muntjacs (genus *Muntiacus*). At least three muntjac have been recorded in the country: the northern red muntjac (*Muntiacus vaginalis*), the large-antlered muntjac (*Muntiacus vuquangensis*), and the Annamite dark muntjac species complex (*M. rooseveltorum / truongsonensis*) (Schaller & Vrba 1996, Giao et al. 1998, Le et al. 2014). All muntjac species are threatened by intensive snaring to supply the burgeoning wildlife trade in Vietnam (Sterling et al. 2006, Gray et al. 2018). The large-antlered muntjac is endemic to the Annamites ecoregion of Vietnam and Laos, and is listed as Critically Endangered on *The IUCN Red List of Threatened Species* (Timmins et al. 2016a). The northern red muntjac is widespread across mainland South and Southeast Asia, and is categorized as Least Concern, although Indochinese populations have declined in recent years (Timmins et al. 2016b). The Annamite dark muntjac is a little-known species complex that is believed to contain multiple species; it is classified as Data Deficient due to unresolved taxonomy, although it is possible that one or more species in the complex are threatened (Timmins & Duckworth 2016a, Timmins & Duckworth 2016b). Additional studies are needed to resolve the taxonomic status of the dark muntjac complex as a prerequisite to assessing species-level conservation status (Timmins & Duckworth 2016a, Timmins & Duckworth 2016b).

Here, we report muntjac occurrence records from a camera-trapping study conducted in the Hue Saola Nature Reserve, Thua Thien Hue Province, central Vietnam. The original goal of the study was to assess Edward's pheasant occurrence, an endangered lowland galliform species. As there was no record of the pheasants, and the muntjacs were commonly detected, the goal of this paper is then to use such camera-trap data to better understand muntjac ecology. Northern red muntjac and the Annamite dark muntjac have been recently recorded in the Hue Saola Nature Reserve (Schnell et al. 2012, Tilker et al. 2020), and the large-antlered muntjac is expected to occur, or have occurred, there based on limited information from hunter trophies and villager interviews (Trai et al. 2003). The muntjac diversity in the Hue Saola Nature Reserve and its status as one of the reasonably protected areas in Vietnam (Nguyen et al. 2020) make it a good site for further research on muntjac in the Annamites.

Materials and Methods

Study site

Hue Saola Nature Reserve, established in 2013 to protect endangered and endemic species of the Annamites, is located in Thua Thien Hue Province, central Vietnam, and covers approximately 15,500 ha. It borders Quang Nam Saola Nature Reserve and Bach Ma National Park in Vietnam and Xe Sap National Protected Area in Lao PDR. Together, these sites form a large contiguous trans-boundary protected area complex (**Fig. 1**). The area is characterized by a tropical monsoon climate with average annual rainfall of 3,400 – 3,800 mm. The topography contains rugged terrain with deep ravines and numerous streams. The protected area is dominated by closed-canopy multi-tiered broadleaf deciduous rainforest, and although some parts have been subjected to forest degradation from past disturbances, most forests are now classified as at- or near-climax stage, with mature secondary forest patches near the boundary of the reserve. It is one of well protected areas in Vietnam because there has been a group of professional forest guards deployed to patrol and remove snares in the reserve (Hue Department of Forest Protection 2013; WWF-Vietnam 2017; Tilker et al. 2020). As a result, between 2010 and 2015, there have been more than 75,000 snares removed in Hue and Quang Nam Saola nature reserves combined, and it was an extraordinary effort of active protection for any protected areas in Vietnam (Gray et al. 2018).

Data collection

We established 46 camera-trapping stations (Bushnell Trophy Cam®) in the northeastern section of the Hue Saola Nature Reserve between November 2015 and January 2017 (**Fig. 1**). Stations were spaced 194.2 m ± 84.5 m apart, at an average elevation of 282.1 m ± 77.6 m. All stations covered about 850 hectares, or 5.5% total area of the reserve. The high density of camera trapping stations in a small area partly reflected the original goal of the study, and therefore it is different from most camera trapping surveys in Southeast Asia in recent years. Selection of camera-trap locations was based on habitat characteristics with an emphasis on sampling a wide range of microhabitats, including: (i) overall forest structure, (ii) topographic variability, and (iii) understory vegetation and structure that potentially affect animal habitat preference and detection, such as large trees density and nearby water sources. We used one camera per station, with cameras set 20 – 40 cm above the ground. Cameras were active 24 hours/day, triggered by movement, and programmed to take one photo per trigger. All cameras were set to record time and date, and were checked every 60-90 days to replace the batteries and SD memory cards.

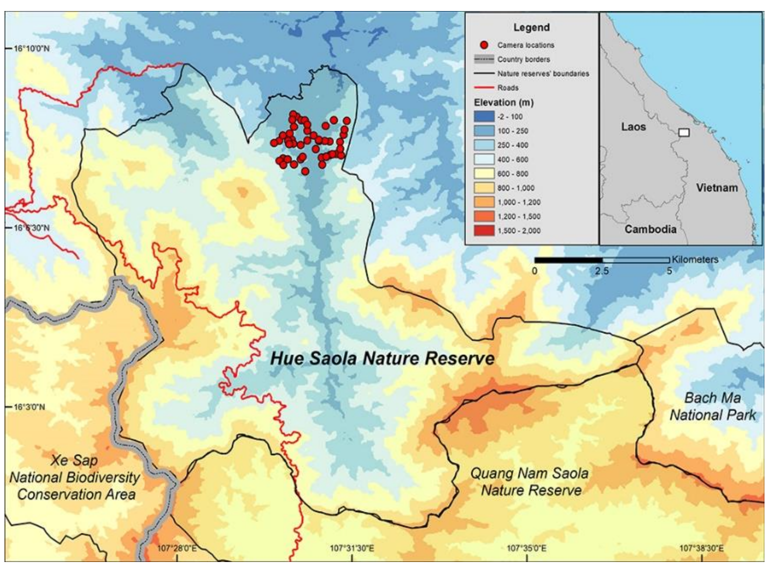

Figure 1. Camera trap locations in Hue Saola Nature Reserve

Camera-trap data were processed using camtrapR version 2.0.2 (Niedballa et al. 2016). Muntjac images were identified by individuals experienced in species muntjac identification. All analyses were performed in R version 3.6.3 (R Core Team 2020). We used a 30-minute threshold determine independent detections (Rovero & Zimmermann 2016). We used the activityDensity function in camtrapR to quantify muntjac activity patterns, and the detectionHistory function to assess time to first detection.

Results and Discussion

From 16,486 camera-trapping days, we recorded 155 independent events of muntjacs at 36 stations. The only muntjac species we documented positively was the northern red muntjac (**Fig. 2A**). Identifications were based on morphological characteristics, including tail length and color, antler configuration in males, and facial striping patterns in females. In total, we recorded 29 independent detections from 1,428 photos of northern red muntjac at 12 stations (naïve occupancy = 26.01%). The average time to first detection was 151.1 days. Northern red muntjac were detected at an average elevation of 265.9 m ± 65.7 m. All other records (126 independent detections from 538 photos – or 82% of all muntjac detections) are likely to also be northern red muntjac based on observable characteristics but because of uncertainties associated with the identification were left as unidentified (*Muntiacus* spp.). No other deer species were recorded.

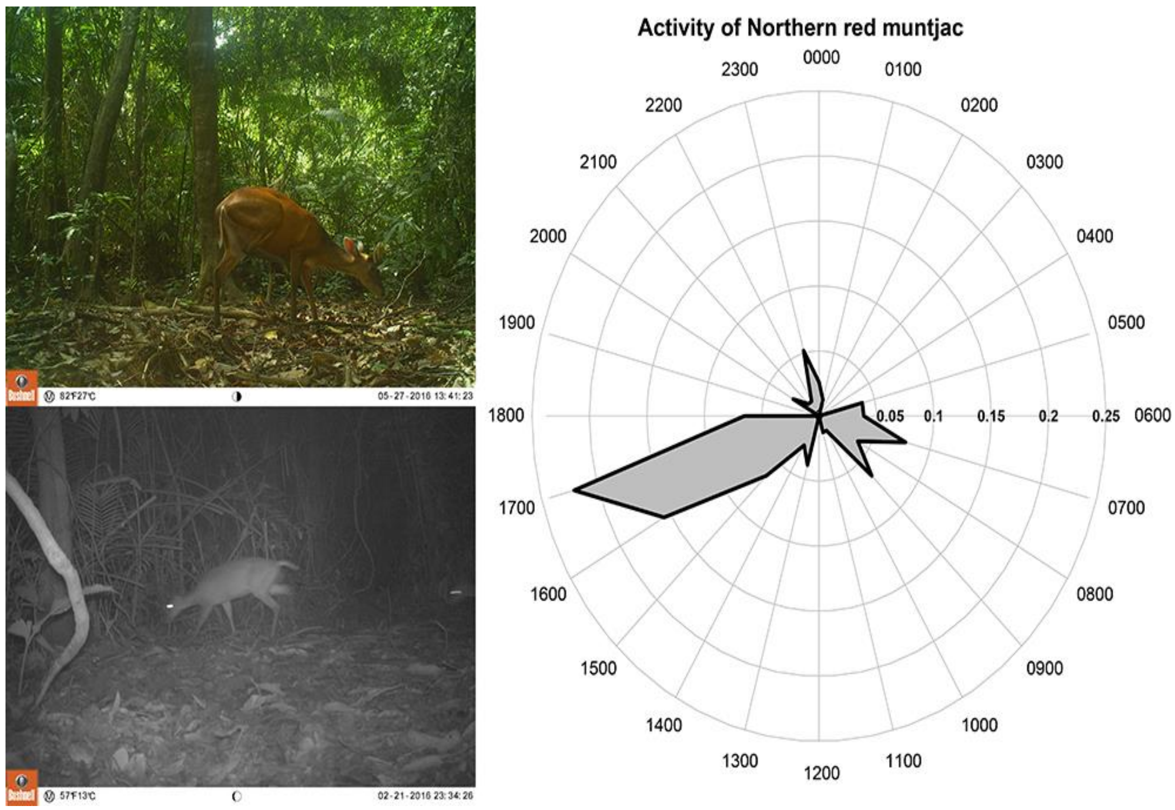

Figure 2 Panel 2 (A-Top) Female northern red muntjac (*Muntiacus vaginalis*) recorded during the camera-trapping survey; (A-Bottom) A pair of unidentified muntjacs recorded (B) daily activity pattern of *M. vaginalis*.

The northern red muntjac was predominantly diurnal (**Fig. 2B**). Approximately 65.5% of independent events for the northern red muntjac occurred between 06:00 and 18:00. Activity patterns peaked in the early morning and late evening. For example, approximately 75.9% of the northern red muntjac events occurred between 05:00 – 08:00 and 15:00 – 18:00. These findings are generally consistent with activity patterns reported for the northern red muntjac in other parts of Southeast Asia (Gray & Phan 2011, Can et al. 2020). Previous studies have proposed that the prevalence of diurnality in many ungulates may come from either food availability or predation risk (Wu et al. 2018, van der Vinne et al. 2019). We believe that this may be the case with the northern red muntjac recorded in our study, but note that further research is needed to assess the drivers of activity patterns. The majority of the independent detections (23 records, ~80%) for the northern red muntjac were one individual only. However, there were three records of two adult northern red muntjacs in December and January, and we recorded two instances of one adult female and one juvenile muntjac in January and March. We also recorded one stance of one adult of unidentifiable sex (but presumably female) and one juvenile in April.

In previous surveys, the northern red muntjac have been commonly recorded in the Hue Saola Nature Reserve (Trai et al. 2003, Hue Department of Forest Protection 2013, Tilker et al. 2020). The Annamite dark muntjac have been recorded in the Hue Saola Nature Reserve by either genetic samples (Schnell et al. 2012) or both genetic and camera trapping surveys (Tilker et al. 2020); and the large-antlered muntjac is expected to occur, or have occurred, there based on limited information from hunter trophies and villager interviews (Trai et al. 2003).

Our study confirms at least one muntjac species reported in previous surveys in Hue Saola Nature Reserve, the northern red muntjac. The fact that we failed to verify the presence the Annamite dark muntjac species complex is not surprising, given that our camera-trapping efforts were concentrated in a lowland area, which appears to be outside the elevational range of where the dark muntjac occurs (Timmins & Duckworth 2016a, Timmins & Duckworth 2016b, Tilker et al. 2020). Therefore, our results provide further evidence that the Annamite dark muntjac complex is a highland species.

We also did not record the large-antlered muntjac, even though the species was reported in the protected area based on wildlife trade surveys, hunting trophies, information from local hunters, and field surveys (Trai et al. 2003). In general, the lack of the large-antlered muntjac records is not surprising. The species has undergone a precipitous range-wide decline in recent years as a result of widespread and intensive snaring across its distribution, with the most severe declines occurring in Vietnam (Timmins et al. 2016a). Our findings are consistent with one other large-scale camera-trap study that did not record this species in the Hue Saola Nature Reserve (Tilker et al. 2020), and support a growing body of evidence that it is approaching local extinction in many areas in Vietnam.

The fact that we were unable to positively identify a large number of our muntjac independent records (81.29% of the total number) to species level highlights the importance of study design and equipment quality in camera-trapping studies that focus on closely-related and morphologically similar species. In our study, the two most common cases that cause difficulty in species-level identification include: (i) the improper de/activation of the flash in different lighting conditions, resulting in unrecognizable over/under-exposed photos, and (ii) the lack of clarity in both color and focus when photos were taken with infrared flash in low light conditions. This issue has been documented in previous studies (Burns et al. 2018), and should be taken into account in planning future surveys. Also, as the majority of muntjac detections were unidentifiable, we could have missed other muntjac species that might still be in the Hue Saola Nature Reserve. Therefore, proper equipment and careful approach in photo identification are of particular important in camera trap surveys for having accurate and usable species records.

Moreover, as our survey focused on a small area, our study design was not optimized to detect rare species that occur at low densities over large geographic areas, as would likely be the case with any large-antlered muntjac individuals that persist in the protected area. A random or systematic camera placement may have increased detection rates, as previous studies have shown that these designs may increase detectability when compared to camera-trapping efforts that concentrated on pre-determined landscape features (Wearn et al. 2013, Tilker et al. 2020).

While we could not confirm the existence of the Annamite dark muntjac complex or large-antlered muntjac, our findings suggest that Hue Saola Nature Reserve may be an important area for remaining populations of red muntjac because of its historical records of other muntjac species and its well management status; and our data provides further, although minor, evidence that large-antlered muntjac is extirpated from these lowland forest blocks. The northern red muntjac has declined precipitously in Vietnam; Timmins et al. (2016b) estimates that Indochinese red muntjac populations may have declined by as much as 30% in the last 10-15 years, primarily as a result of intensive hunting pressure. Even if the species is not globally threatened, its may be important for a variety of ecological reasons, including helping to preserve several ecosystem functions, as has been shown with other ungulates (Ramirez et al. 2018). At least two studies indicate that the northern red muntjac may be important for seed dispersal (Brodie et al. 2009, Chen et al. 2001). We recommend continued snare removal efforts in the Hue Saola Nature Reserve to protect muntjac populations in the protected area. Additional camera-trapping studies are needed to better understand the population status of northern red muntjac in other parts of the reserve, to understand how confirmed populations of northern red muntjac and the Annamite dark muntjac coexist, and to search for possible remaining individuals of large-antlered muntjac.

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Two interesting anecdotes with hog deer in Kaziranga National Park, Assam, India

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The hog deer *Axis porcinus* (Zimmermann, 1780) is an endangered species of deer in the Cervidae. Currently it is found in the Indian subcontinent and Southeast Asia. The Kaziranga National Park (KNP), in the Assam State of India is one of the strongholds for the species (Timmins et al. 2015).

An albino Hog Deer

On 26th April 2015, while conducting a survey of *Gyps* vultures in the central range of KNP, I came across a white deer resting in grasses and understory. It was carefully observed with binoculars (Nikon 10 X 50) and cross checked with the species description in the field guide. After eliminating possibilities of other similar looking deer species, it was identified as an albino hog deer. When it stood up, the deer displayed its typical pig-like gait due to its 'stout rump and the lower front quarters' (Menon 2014). It did not have the bony frontal ridges seen in the Indian muntjac (*Muntiacus muntjack*) and was quite small in size compared to the swamp deer (*Rucervus duvaucelii*) and sambar (*Rusa unicolor*) found in the same areas. It was a female, with the entire body white and the eyes and nostrils pink in colour (Fig. 1).

Figure 1. An albino Hog Deer at Kaziranga National Park Assam, India.

Although a bit restless, the deer could be observed for about fifteen minutes (16:00- 16:15 hrs) before it vanished in tall grasses. Its herd members were around, less timid, and they continued grazing and browsing without being bothered by my presence. There is just a single old record of an albino hog deer from India by Adamson (1916). The author mentioned hunting of an albino hog deer in the then princely State of Cooch Behar (currently Cooch Behar district of West Bengal State). The aerial distance between the Cooch Behar and KNP is about 350 km. Both the locations pertain to the same geographical distribution range described by Gupta et al (2018), for the presence of *Axix porcinus porcinus.* The types of colour aberration in mammals and the list of 56 Indian mammal species with their colour aberrations is well discussed by Mahabal et al (2019), which includes the only record of an albino hog deer.

Figure 2. Hog Deer feeding on algae at Kaziranga National Park, Assam, India

Freshwater algae in the diet of Hog Deer

On 3rd January 2018, I was travelling on national highway NH-37, which passes through the KNP. We stopped at a location near Bura Pahar Range (26.577362 N, 93.081671 E) as sometimes wild animals are visible from the highway itself. As the highway is elevated, it provides a better view of the lowland and maintains a safe distance between the wild animals and road traffic. While watching the grassland bordered by woodland, my attention was seized by a pair of hog deer. The pair was alert yet undeterred. They came out of the woods and approached a stream of water to drink. After that, the female stepped aside, but the male began to feed on the algae in the stream (Figure 2). The algae blooming within the aquatic plants was devoured, while the aquatic angiosperm vegetation was avoided. The deer spent about ten minutes feeding on algae (13:15 to 13:25 Hrs). The algae from this location could not be sampled, but an algae sample was collected from a similar habitat (outside the protected area) and observed under a microscope. Primary observations suggest that there could be an assemblage of filamentous algae of the class Chlorophyceae (Hoek *et al* 1995). Algophagy is well known in invertebrates such as in the green crab (Baeta et al 2006) and a few vertebrates including sheep (Paterson and Coleman 1982), chimpanzee (Sakamaki 1998) and even human beings (Borowitzka 1998). It is possible that a few more herbivorous species have exploited the benefits of this nutrition-rich food source, which could be confirmed through the study of natural history.

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Mother and fawn interactions in captive red brocket deer (*Mazama americana*) during 72 hours after birth

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Abstract

The behavior of a mother-fawn dyad of red brocket deer kept in captivity was continuously recorded during the first 72 h post-birth. Maternal care started immediately after delivery. It took the fawn 6 min to stand up and 8.5 min to take its first steps. The first suckling was observed at 17.67 min postpartum, and a total of 94 suckling bouts averaging 111.32 ±73.85 sec each occurred during the 72 h period. The total licking bouts was 129, totaling 6.3 h. The total mother-fawn investment time during the three days was 17.63 h. This report is the first description of maternal care after the birth of a red brocket deer in captivity.

Keywords: animal behavior, captive wild animal, maternal care.

Resumen

El comportamiento de un par madre-cría de la corzuela roja en cautiverio fue registrado continuamente durante las primeras 72 h después del nacimiento. Los cuidados maternales posteriormente al parto fueron inmediatos. La cría se levantó a los 6 min y dio sus primeros pasos a los 8,5 min. La primera mamada fue observada a los 17,67 min post parto, y un total de 94 registros de 111.32 ± 73.85 seg en promedio ocurrió durante las 72 h de observación. El número total de lamidas fue de 129 veces, con duración total de 6,3 h en las 72 h de observación. Este reporte es la primera descripción de los cuidados maternales posterior al nacimiento en cautiverio.

Palabras claves: comportamiento animal, animal silvestre en cautiverio, cuidado maternal

Introduction

Parental care in ungulates is defined by the energy allocated by the mother determining, consequently the number, size and growth of offspring (Evans 1990). Andersen et al. (2000) defined maternal care as the amount of resources invested in an offspring multiplied by the number of offspring, with prenatal care used to produce and postnatal care used to raise, the offspring. Therefore, coordinated expression of appropriate behaviors of maternal care is important to the establishments of early recognition and young survival during the first week of life (Hernández et al., 2012; Nowak et al., 2000). After birth, the mothers focus interest on the newborn and in the amniotic fluids, as an important behavior for the establishment of maternal responsiveness and chemosensory information that facilitates the bonding (Poindron et al., 2010).

The genus *Mazama* includes small deer with simple antlers that inhabit Neotropical forests with dense vegetation (Eisenberg; Redford, 1999). Currently, the genus *Mazama* represents five of the eight deer species occurring in Brazil, with *Mazama americana* as the largest species and the most widely distributed throughout the Brazilian territory (Rossi, 2000).

Commonly known as the Red brocket deer, *Mazama americana* is characterized by its reddish color with whitish areas in the ventral region (Eisenberg; Redford, 1999) (Fig. 1). Hunting and other human activities directly affect the species, making it difficult to observe brocket deer individuals in open areas, limiting behavioral study of the species in nature (Duarte et al., 2012).

In this context, captivity allows being near the animals, thus facilitating the observation and better understanding of behaviors, such as territory marking, feeding, and reproduction (Azevedo, 2008). *Mazama americana* has been described as a "hider" species, where the mother forages alone and the fawn hides, lies down and waits for the mother to return for nursing (Lickliter, 1987). Other studies of reproductive behavior of this species in captivity described the birth of a single fawn after 220 days of gestation (± 6d) (Krepschi et al., 2013). However, there is no report of the birthing process and mother-fawn relationship in captivity during the first hours after birth. Due to the importance of maternal care behavior to guarantee the survival of the fawn, it is important to study the initiations and duration of suckling, licking and approximations among mother and newborn as important maternal care in the first hours of life (Therrien et al., 2008).

The objective of this study was to describe the behavior of mother-fawn red brocket deer kept in captivity from birth to 72 h post-birth, aiming to evaluate the time invested in postnatal interaction that are essentials for the survival of the fawn.

Figure 1. The dyad, mother and fawn (*Mazama americana*) at Deer Research and Conservation Center (NUPECCE).

Material and methods

The Red brocket deer female analyzed was 19 years old, multiparous and had 9 previous parturitions. Known to originate from Tocantins, Brazil, the female in captivity at the Deer Research and Conservation Center (NUPECCE) in a cement stall 3 meters wide by 4 meters deep, with walls 2 meters high and the floor covered with straw. Water was provided ad libitum and 0.5 kg of concentrated feed for horses with 1 kg/animal day of ramíe (*Boehmeria nivea*) and blackberry leaves (*Morus rubra*) during the pre-partum phase and ad libitum during the post-partum phase.

The mating occurred after detection of estrus by mucus discharge and receptivity to the male, with pregnancy confirmed by the absence of heat at 21 days after mating. Both female and male were Paraná cytotype (Abril et al. 2010).

An Intelbras Multi HD 3000 video camera was installed fixed to the ceiling in the female's stall for behavioral recording. The birthing occurred after 211 days of pregnancy, two weeks before the gestation time reported for the species of 225 days after mating (Krepshi et al., 2013), delivering a female fawn. The mother remained lying down from the beginning of the appearance of the newborn head until the appearance of the newborn hindlegs, when the mother stood up to complete the delivery. Fawning occurred quickly with a duration of 2 min and 13 sec and the delivery of the placenta occurred at 102 min after birth. Starting at the end of the birth, all behaviors of the female and offspring were classified into seven categories (Table 1), and recorded in frequency and duration for a 72 h period after delivery.All recordings were analyzed using VLC software version

3.0.8. The first behaviors of the newborn, and the duration, and frequency of each behavioral category of both mother and offspring were recorded during the first 72 h postpartum.

Table 1. Behavioral categories of a mother-fawn dyad of red brocket deer kept in captivity during the first 72 hours after delivery.

Behavioral categories	Descriptions
Approximation	The mother comes closer to the fawn or vice-versa. The act of sniffing each other can happen and additionally, the mother can occasionally give a single lick to the fawn.
Licking	The mother moves the tongue across the surface of the fawn's body more than twice, with pauses between one set of licks and another of up to 15 seconds.
Walking with the fawn	Mother walks around the facility, looking at the fawn, apparently stimulating the fawn to follow her.
Suckling	Fawn tries to or succeeds in getting a mother's teat into its mouth and sucks.
Rest	Act of lying down upright with legs folded under the body, usually closer to each other.
Stand up	The fawn, after trying, stands up, remaining stable on the forelegs and hindlegs
Walking	The fawn, already stable in a standing position, takes steps towards the mother.

Results and discussion

Maternal care started immediately after the fawn was delivered and continued, with few intervals, for 182 min until the mother assumed a resting posture, which was considered to be the first time that the mother lay down. That time may be sufficient for the doe to become familiar with that fawn´s unique signature and discriminate her fawn (Keller et al., 2003).

The first behavior expressed by the mother was licking the fawn as described by Nowak et al. (2000), facilitating the removal of the fetal membranes from the neonate head and neck, and stimulating the teat-seeking activity of the fawn. The fawn's behavior was also crucial in determining survival,

providing the fawn nutrients and thermoregulation as observed by Dwyer et al. (2015) in small ruminants.
The newborn stood up 6 min after hitting the floor and started walking 8 min and 30 sec after birth, with four previous attempts for each behavior (Table 2). During this process, the fawn got closer to its mother each time that she walked away from her. These results were congruent with other mother-young relationships described in domestic ungulates, with an interval of 10 to 30 min after birth for the time it takes for a newborn to stand on its feet (Nowak et al., 2007). On the other hand, Arman (1974) described the latencies for both standing up and walking as far longer (50 min), in *Cervus elaphus* (Table 2). The fawn walked, following its' mother on 23 occasions in the 72 h of observation, and each walk lasted 178 ± 129 sec, on average.

Table 2. First behaviors of the postnatal interaction observed in the mother-fawn dyad of *Mazama americana* in the first 72 hours after birth.

Behavioral categories	**First time after birth**
Fawn	
Suckling	17 min 40 sec
Stand up	6 min
Walk	8 min 30 sec
Mother	
Licking the fawn	1 second
Resting	3 h 4 min 16 sec
Get close to the fawn	Immediately (less than 1 second)

The first suckling was recorded 17 min and 40 sec after birth (Table 2), and it were repeated at intervals of two minutes for up to two hours, totaling 92 sucking bouts in the first 72 h. Each suckling bout lasted 94.34 ± 65.57 sec, on average. The mother usually licked the perianal region of the fawn when it was suckling, stimulating it to defecate and urinate, and she usually ingested the feces of the offspring, exactly as described by Gosling (1969) for Coke's Hartebeest. Licking this region of the fawn's body (during sucking or not) occurred 132 times, totaling 9 h and 33 min in the 72 h of observation, resulting in a mean duration of 117 ± 136.643 sec per licking bout. The licking time decreased over time, with a mean of 191 ± 197 sec on the first day, declining to 78.66 ± 69.60 on the second day and 76.47 ± 53.04 on the third day, respectively.
The total time of the mother-fawn interaction during the 72 h after delivery was 17 h and 38 min, suggesting an important investment by the mother to provide the necessary care for fawn development in the first hours of life

(Fouda et al., 1990). This great investment in maternal care may be influenced by the multiparous condition as described by Andersen et al. (2000) in wild-living and captive *Capreolus capreolus*.

In the first 10 h after birth, the durations of the fawn's suckling and the doe´s licking behaviors were the longest. Later, as described in other ungulates (Hernández et al. 2012) these behaviors decreased (Fig. 2). The frequencies of both behaviors were also high initially, but they peaked later, between 41–50 h after delivery (Fig. 3).

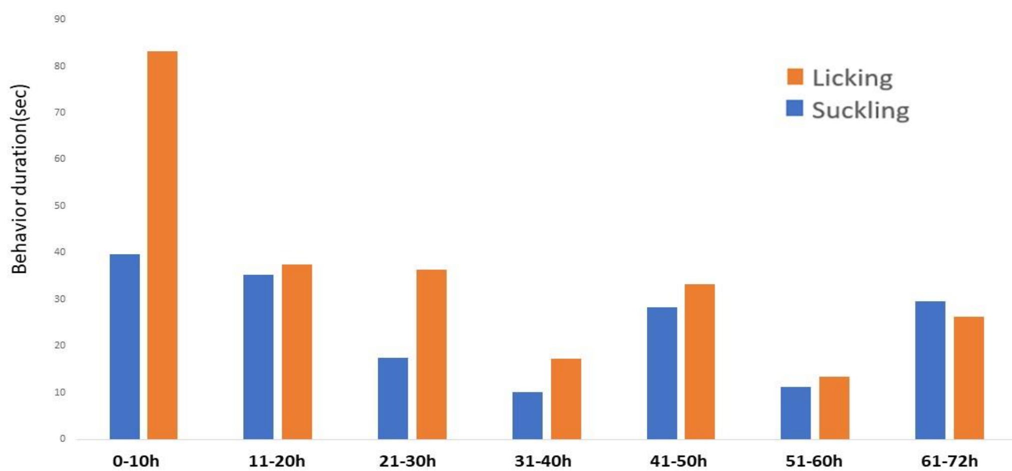

Figure 2. Distribution of the duration of doe licking (orange) and fawn suckling (blue) in the mother-fawn interaction of captive Red brocket deer *Mazama americana* in intervals of 10 hours during 72 hours of continuous observation after birth.

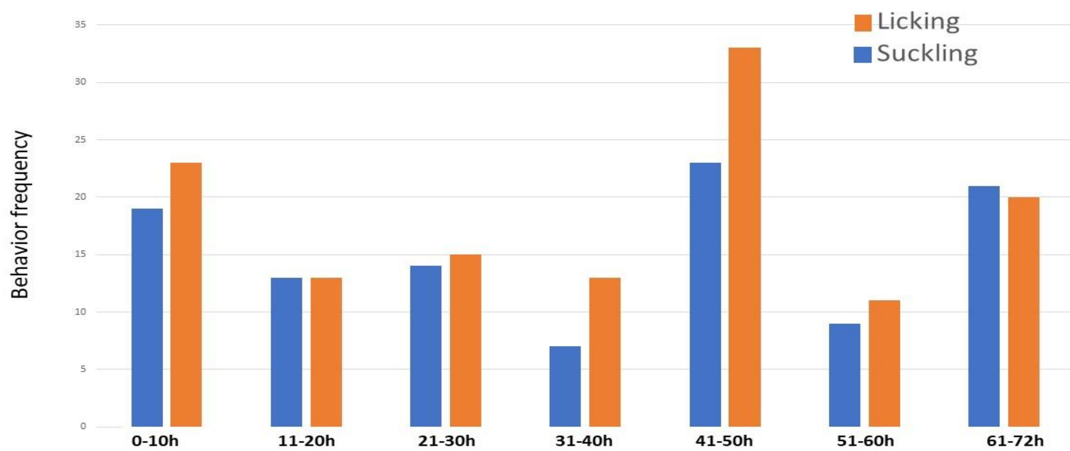

Figure 3. Distribution of the frequencies of doe licking (orange) and fawn suckling (blue) in the mother-fawn interaction of captive Red brocket deer *Mazama americana* in intervals of 10 hours during 72 hours of continuous observation after birth.

The animals walked together at the same time and in the same direction, usually when the mother was looking for food or going to a place where she preferred to lie down. The mean duration of this behavior was 120.20 ± 86.30 sec and occurred 20 times during the 72 h of observation, with a peak (12 times) between 24 and 48 h. Walking behavior in nature is related to maternal protection; in addition to allowing the fawn to hide, she can call it and go to a safer place (Ozoga; Verme, 1986).

Resting behavior occurred five times during the 72 h period of observation and lasted on average 4481 ± 3289 sec per bout. The walking and resting behavior of the mother-fawn interaction has been described by Ozoga and Verme (1986) in open environments; the fawn stayed next to the mother, who is more experienced at detecting predators

In conclusion, maternal care started immediate after delivery, attending to the basic needs of the fawn, such as standing, walking, suckling, defecating, urinating, and sleeping. The duration and frequency of each of these behaviors during the first 72 h postpartum indicated the great investment in maternal care of the female deer in captivity. Further studies of the mother-fawn interaction in Red brocket deer species are recommended in order to compare the significance of different factors that may be involved in maternal care among females. This is the first description of the mother-fawn interaction and the behaviors associated with the birth of *M. americana* in captivity.

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Fecal progestins during post-partum period of one captive Amazonian brown brocket deer (*Mazama nemorivaga*)

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Abstract

The Neotropical region has one of the richest diversities regarding deer species, with 17 currently identified. The genus *Mazama* is endemic to this region and the most diverse, with 10 species, although, certainly, there are more to be described. One of the least known *Mazama* species is *M. nemorivaga*, which has been recently recognized as a distinct taxon from *M. gouazoubira*. Understanding the basic reproductive physiology of this species is still a challenge due to the scarce number of specimens in captivity. This study aimed to characterize behavioral estrus and fecal progesterone metabolite (FPM) concentration during the post-partum period in one captive female of *M. nemorivaga*. FPM concentrations were measured by non-invasive hormonal dosing. Thus, fecal samples were collected once a day from parturition until detection of the second post-partum estrus for FPM analysis by enzyme immunoassay (EIA). Post-partum behavioral estrus was detected on the 24th and 43rd days, coinciding with a decrease in FPM levels. FPM concentration reached baseline values on the 8-9th post-partum days with subsequent increasing, which possible represents a pattern of ovarian activity (ovulation and corpus luteum functionality) in the puerperium, as reported in other species of the genus *Mazama*.

Keywords: Neotropical deer; reproductive biology; enzyme immunoassay; puerperium.

Resumen

La región Neotropical posee actualmente17 especies de cérvidos identificadas. El género *Mazama*, endémico de esta región, es el más amplio con 10 especies, aunque ciertamente hay más por describir. Una de las especies menos conocida del género es *M. nemorivaga*, recientemente reconocida como taxón distinto de *M. gouazoubira*. Comprender su fisiología reproductiva básica es un gran desafío, debido al escaso número de individuos en cautiverio. Así, este estudio tuvo como objetivo caracterizar el estro comportamental y la concentración de metabolitos fecales de progesterona (MFP) durante el período posparto en una hembra de *M. nemorivaga*. Las concentraciones de MFP fueron medidas mediante dosaje hormonal no invasivo. Para ello, fueron colectadas heces una vez al día desde el parto hasta la detección del segundo estro posparto, y la concentración de MFP fue analizada mediante inmunoensayo enzimático (EIA). La hembra manifestó el primer y segundo estro posparto a los 24 y 43 días, respectivamente, coincidiendo con una disminución de las concentraciones de MFP. Los MFP alcanzaron valores basales a los 8-9 días posparto con posterior incremento, representando un patrón de actividad ovárica (ovulación y funcionalidad del cuerpo lúteo) en el puerperio, como ya relatado en otras especies del género *Mazama*.

Palabras clave: Ciervos Neotropicales, biología reproductiva, enzimas inmunoensayo, puerperio

Introduction

The family Cervidae includes 55 species distributed on all continents, except Antarctica (IUCN 2020). Among the family Cervidae, the genus *Mazama* includes 10 species, which are widely distributed in the Neotropical region. Nevertheless, there are still gaps regarding its taxonomy and the potential existence of a greater number of species (Duarte et al. 2008). Indeed, there is also a gap in knowledge regarding reproduction, genetics and ecology for some of these species, as in the case of Amazonian brown brocket deer (*Mazama nemorivaga*) (Rossi et al. 2010). This was considered, in its classification, as a synonym for *M. gouazoubira* (Ávila-Pires 1959, Cabrera 1960) until a few years ago, which has been corrected by both morphological (Rossi 2000) and genetic data (Duarte et al. 2008, Fiorillo et al. 2013). It is classified as Least Concern by the IUCN (Rossi & Duarte 2016), but more studies are needed to define its population status, since its actual geographical distribution is not yet clearly known (Rossi & Duarte 2016). Amazonian brown brocket deer was thought to have a restricted geographic distribution in the Amazon (Rossi et al. 2010), but recent evidence showed the presence of some populations in the Atlantic Forest region (Oliveira et al. 2020). Furthermore, there is a potential existence of cryptic species within the taxon (Duarte et al. 2008, Fiorillo et al. 2013, Morales-Donoso 2017), which may be under severe threat.

The conservation of captive and free-ranging wildlife populations requires a different set of strategies, including the development of reproductive biotechniques, which are essential for the management and conservation of the biodiversity of populations (Comizzoli et al. 2000, Duarte 2005). However, this is not possible without a basic understanding of the reproductive physiology of the species. With respect to the family Cervidae, temperate zones species have been more fully addressed than Neotropical species. Deer species from higher latitudes have sexual activity limited to a certain time of year (Lincoln 1992, Asher et al. 1996). These reproductive pattern differences are associated with environmental issues such as photoperiod, rainfall and temperature, which vary from region to region. In contrast, Neotropical deer are presumably non-seasonal breeders, showing reproductive activity all year round, and some species have already shown the presence of post-partum estrus (Pereira et al. 2006, Krepschi et al. 2013, Rola et al. 2013, Polegato et al. 2018, Pereira et al. 2020). The presence of a post-partum estrus, if fertile, could result in a rapid replacement of the population size, so that the species can maintain sustainable limits of predatory hunting (Hurtado-Gonzales & Bodmer 2004, Rossi et al. 2010, Guimaraes et al. 2016).

Assessing the reproductive hormonal status of wildlife species with non-invasive protocols is a fundamental strategy for the conservation programs of endangered species (Micheletti et al. 2014), mainly in the case of deer where studies are hampered by their reactive behavior, great susceptibility to stress, and rejection of handling. Thus, non-invasive techniques are adequate and advantageous, as they allow the elimination of stress caused by physical or chemical containment of the animal and enable daily and long-term monitoring of endocrine activity (Monfort et al. 1990, Christofoletti et al. 2010, Duarte 2010).

The main steroids related to the reproductive cycle of mammalian females are progesterone and estradiol (Schwarzenberger et al. 1996, Micheletti et al. 2014). In the specific case of female deer, both hormones are secreted as unconjugated metabolites in feces (Kapke et al. 1999, Yamauchi et al. 1999). Thus, non-invasive monitoring of fecal progesterone metabolites (FPM) has been established as an important methodology for the characterization of the reproductive physiology of female deer in recent decades (Pereira & Polegato 2010). Regarding Neotropical deer, this method has been already used to characterize ovarian activity in the puerperium in *Blastocerus dichotomus* (Polegato et al. 2018), *M. americana* (Krepschi et al. 2013), and *M. gouazoubira* (Pereira et al. 2006).

This research aimed to characterize the behavioral estrus and fecal progestin concentration during the post-partum period in one captive female from *M. nemorivaga*.

Material and Methods

Animal maintenance

A female of the species *M. nemorivaga*, belonging to the Deer Research and Conservation Center (NUPECCE), located in Jaboticabal, São Paulo - Brazil, was used, being the only center with the species in captivity. For this reason, obtaining a larger sample is not possible. During the experiment, the female was maintained in an individual stall (4 m x 3 m). The female was fed with a diet consisting of 0.5 kg of pelleted ration (Equi Tech MA – Presence®) and approximately 1 kg/deer/day of fresh perennial soybean (*Neonotonia wightii*), ramie (*Boehmeria nivea*), or mulberry branches (*Morus alba*), and water *ad libitum*. The pelleted ration was provided once a day in the morning.

Pregnancy

The female was placed in the presence of a male of the same species once a day (8 am), until the estrus was identified by the receptivity to the male and subsequent mating (Pereira et al. 2006). Mating was allowed every 4 hours until the end of estrus. To confirm pregnancy, estrus was monitored for thirty days after the last mating, once a day (8 am). The female did not accept the male during this period and was considered pregnant.

Estrus detection in the puerperium

After parturition, estrus detection was performed once a day (8 am) until the second estrus was identified. Mating was not permitted on any of the occasions, being interrupted by a handler. A second pregnancy was not the objective of the study. Suckling by the fawn was allowed during the entire estrus monitoring period.

Feces sampling and storage

Fecal sampling was done once a day, always at the same time, from the first post-partum day, until the female displayed the second post-partum estrus. For the collection of fresh feces, the female was placed in an empty stall of a male of the same species to stimulate defecation. Feces were collected immediately, packed in plastic bags, identified and stored in a freezer at -20ºC.

Fecal samples processing

Fecal samples were placed in an oven at 56°C for approximately 72 hours (Yamauchi et al. 1997, Hamasaki et al. 2001) and then pulverized with a rubber hammer. FPM were extracted as described by Graham et al. (2001). Briefly, 5 ml of 80% methanol was added to 0.5 g of pulverized feces, this mixture was vortexed for 30 seconds at high speed and, subsequently, for 12 hours on a mechanical shaker (Mod. AP22® - Phoenix Ltda. - Araraquara - Brazil). Then, the solution was centrifuged at 400 x g for 20 minutes and the supernatant was collected and stored in a freezer at -20ºC.

Enzyme immunoassays (EIA)

The FPM concentration was determined using CL425 (California University; Davis, CA, USA) for progestogens at NUPECCE's endocrinology lab. This antibody shows reactivities for the following progestins: 4-pregnen-3,20-dione (progesterone) 100.0%; 4-pregnen-3α-ol-20-one 188.0%; 4-pregnen-3β-ol-20-one 172.0%; 4-pregnen-11α-ol-3,20-dione 147.0%; 5α-pregnan-3β-ol-20-one 94.0%; 5α-pregnan-3α-ol-20-one 64,0%; 5α-pregnan-3,20-dione 55.0%; 5β-pregnan-3β-ol-20-one 12.5%; 5β-pregnan-3,20-dione 8.0%; 4-pregnen-11β-ol-3,20-dione 2.7%; 5β-pregnan-3α-ol-20-one 2.5%; 5β-pregnan-3α, 20α-diol (pregnanediol) < 0.1%; other metabolites < 0.1%) (Polegato 2004). Validation of FPM concentration was conducted according to Brown et al. (2004) by observation of parallel arrangement between the standard curve and the curve formed by the pool of fecal extracts prepared by serial dilution ($y = -0.0267x + 2.5176$, $R^2 = 0.9691$). The coefficients of variation between assays for two separate internal controls were 5.4% (n = 3, 62% of binding) and 5.7% (n = 3, 24% of binding). Intra-assay variations were < 10%. All results were expressed based on the fecal dry weight (ng/g).

Analysis of results

Data were described and hormone concentration and behavioral estrus were presented.

Results and discussion

The female gave birth to a male fawn 205 days after mating (Fig. 1). According to NUPECCE records, this female presented pregnancies before and after the study of 210 and 209 days, respectively. Thus, a gestational period was established of 208 ± 1.53 days (mean ± SEM) for this animal. Our data are in agreement with a previous report for the same species by Oliveira et al. (2016), who observed a 210-day gestation, after transcervical artificial insemination. Our data are also similar to those reported for *M. gouazoubira*, 208 to 215 days (Pereira et al. 2006), and slightly lower than observed for *M. americana*, 217 to 226 days (Krepschi et al. 2013).

Figure 1. Female and male fawn of Amazonian brown brocket deer (*Mazama nemorivaga*).

The behavioral results indicate that the animal showed post-partum estrus on the 24th and 43rd days after parturition. Similar results were observed in other species of the genus, such as *M. americana*, where the reestablishment of the behavioral estrus occurred about 26.9 ± 3.4 days after the parturition (Krepschi et al. 2013). In *M. gouazoubira* the display of post-partum estrus was reported on the 2nd (two females) and 46th (one female) post-partum day (Pereira et al. 2006). Suckling was allowed during the puerperium period to prevent early weaning from being a factor for the return to ovarian activity, as observed in other deer species, such as *Cervus elaphus hispanicus* (García et al. 2002) and *Rusa timorensis* (Sudsukh et al. 2016).

The FPM pattern verifies the corpus luteum functionality during the post-partum period, indicating estrogenic activity and potential follicular activity (Fig. 2). The occurrences of behavioral estrus coincided with a decline of FPM concentration and showed an adequate relationship with the behavioral observations, which allowed physiological validation of the results. The increase of FPM concentration after the estrus display indicates the possible occurrence of ovulation and subsequent development of a corpus luteum, which could reflect a period of fertility (Pereira et al. 2006). This association between variations in the endocrine profile and behavioral estrus has already been observed in other species of deer from other regions, such as *Cervus eldi thamin* (Monfort et al. 1990, Hosack et al. 1997), *Elaphurus davidianus* (Monfort et al. 1991), *Alces alces* (Schwartz et al. 1995), and *Odocoileus virginianus* (Knox et al. 1992).

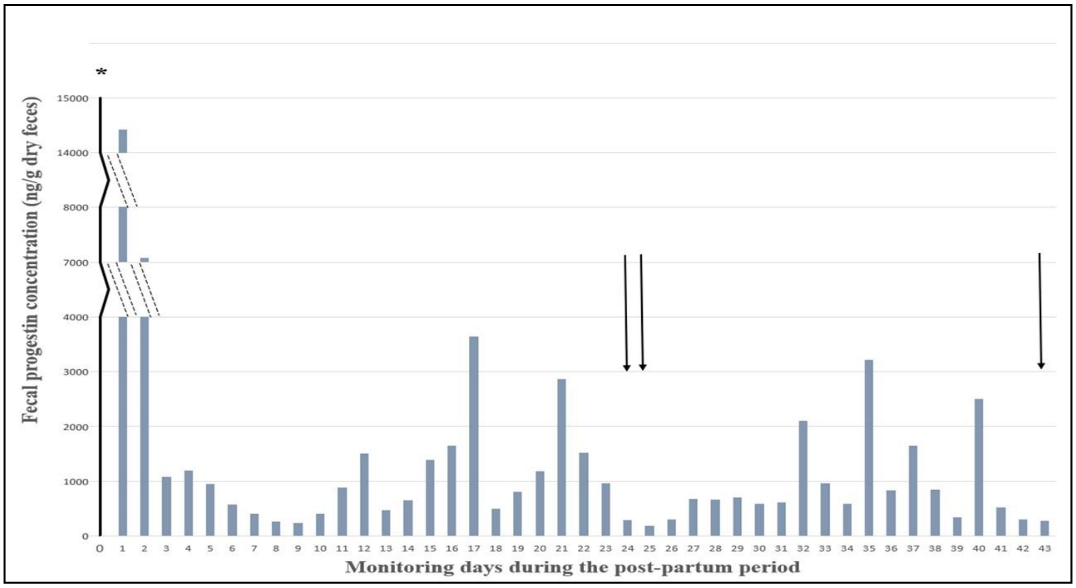

Figure 2. Concentration of fecal progestin's during post-partum period in one captive female of *Mazama nemorivaga*. The asterisk indicates the parturition day, and the arrows indicate the days of behavioral estrous.

Moreover, Polegato et al. (2018) described a shorter estrous cycle during the post-partum period in marsh deer (*B. dichotomus*), which was characterized by lower concentrations of progestins in the puerperium preceding normal cycles. The authors did not observe a similar pattern in the present study. Although it was not an objective of the present study, it was possible to estimate the duration of a female's single estrous cycle from the behavioral observations and the endocrine profile, which was estimated at 19 days. The duration of the observed estrous cycle for *M. nemorivaga* is similar to that observed in other species of the same genus, such as *M. americana* (21.3 ± 1.1 days) and *M. gouazoubira* (24.7 ± 1.2 days). However, as it is a single description, it is expected that later studies with a larger sample size will give a better indication of the exact interval of the estrous cycle for the species.

Thus, it was possible to conclude that the female studied presented behavioral estrus and a pattern of ovarian activity that possible represents ovulation and corpus luteum functionality in the puerperium, similar to other species of the genus *Mazama*. This suggests that these animals would be able to gestate soon after parturition, with a potential production of 1.5 fawns per year.

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First record of axis deer (*Axis axis* – Erxleben, 1777) in the State of Paraná, southern Brazil

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Abstract

The invasion of exotic species is considered one of the major causes of the current biodiversity crisis. Exotic ungulates are the world's most intentionally introduced vertebrates. The axis deer is native to Asia and was successfully introduced in different regions of the world. In South America this deer was initially recorded in Argentina and Uruguay; in Brazil the species has been reported from two states, Rio Grande do Sul and Santa Catarina State. Here we describe the first record of *Axis axis* at the border of Iguaçu National Park, Paraná State, southern Brazil. This new record indicates a rapid and concerning increase of the species' distribution in Brazil. Further assessments of axis deer in Paraná State are necessary to evaluate the current situation and generate data to support the elaboration of a strategy to manage and control the species in the region, as it represents a threat to the local biodiversity.

Keywords: Camera trap, Iguaçu National Park, Conservation, Atlantic Forest

Resumen

La invasión de especies exóticas se considera una de las principales causas de la crisis actual de biodiversidad. Los ungulados exóticos son los vertebrados que más han sido introducidos intencionalmente en el mundo. El ciervo axis es originario de Asia y se introdujo con éxito en diferentes regiones del mundo. En América del Sur este venado se registró inicialmente en Argentina y Uruguay; en Brasil, la especie se registró en dos estados, Rio Grande del Sur y Santa Catarina. Aquí describimos el primer registro del *Axis axis* en el límite del Parque Nacional Iguaçu, Estado de Paraná, en el sur de Brasil. Este nuevo registro indica un aumento rápido y preocupante de la distribución de la especie en Brasil. Son necesarias más evaluaciones del venado axis en el estado de Paraná para evaluar la situación actual y generar datos que sustenten la elaboración de una estrategia de manejo y control de la especie en la región, ya que representa una amenaza para la biodiversidad local.

Palabras clave: Trampa cámara; Parque Nacional Iguaçu; Conservación; Bosque Atlántico

Introduction

Invasive species are considered one of the main causes of animal extinction worldwide (Clavero & Garcia-Berthou 2005), and their distribution is usually related to human actions (Di Caspri 1989). Among the mammalian invasive species, the order Artiodactyla has the greatest invasive success (Clout & Russell 2008). In general, deer and wild boar (*Sus scrofa*, L 1758) are the world's most intentionally introduced vertebrates (Clout & Russell 2008; Novillo & Ojeda 2007), as ornamental species, food resources and for hunting (their antlers are trophies) (Belden 1994; Nentwing 2007).

The axis deer (*Axis axis)* is native to Asia (India, Nepal and Sri Lanka) and was successfully introduced in different regions of the world, such as North America, South America, Europe, Hawaii, and Australia (Long 2003; Romero et al. 2008). This deer is of medium size, with head and body length ranging from 1.00 to 1.75 m, shoulder height 0.60 to 1.00 m and body mass between 27 to 110 kg (Schaller 1967; Nowak 1991). Males are larger than females (females ≤ 70 kg and males ≤ 110 kg) (Schaller 1967; Nowak 1991). Adults have a reddish-brown coat with white spots (Schaller 1967), and males have a pair of three-pointed antlers during the mating season, which are replaced annually (Schaller 1967; Nowak 1991). The axis deer has crepuscular habits, inhabiting a variety of field habitats but rarely dense forests (Nowak 1991). This deer is considered a social species, living in groups of 5 to 10 individuals, although Nowak (1991) reported groups with more than 200 individuals.

In South America this deer was reported from Argentina and Uruguay; in Brazil the species has been reported from two states in the south of the country, Rio Grande do Sul (pampas) (Sponchiado et al. 2011) and Santa Catarina (Preuss et al. 2020). In Iguaçu National Park there are two species of native deer: red brocket deer (*Mazama americana*) and Brazilian dwarf brocket (*Mazama nana*) (Vogliotti, 2008). The Brazilian dwarf brocket is listed as Vulnerable by IUCN (Duarte et al. 2015). In this manuscript we describe the first record of the invasive alien species *Axis axis* from the border of Iguaçu National Park, Paraná State, southern Brazil.

Material and Methods

The species was reported from a stud farm monitored by the Project Jaguars of Iguaçu, during jaguar monitoring in the area. The farm (Haras Cataratas) has an area of 31 ha and is located on the boundaries of Iguaçu National Park; a mosaic of grassland and forest patches connects the farm to the park (Figure 1). The Iguaçu National Park (INP), is a public federal protected area of Brazil, and it is located in the Western region of Paraná State, comprising an area of 185,262.5 ha in the Atlantic Forest. Two predominant phytophysionomies are found in the Park: Interior Atlantic forest and Araucaria moist forest (Ribeiro et al. 2009). The Iguaçu National Park is one of the country's most important remnants of Interior Atlantic Forest, and it is immersed in a landscape composed of monoculture plantations (mostly corn and soybean), reforestation with exotic species, urban areas and small forest remnants (Olegário et al. 2014).

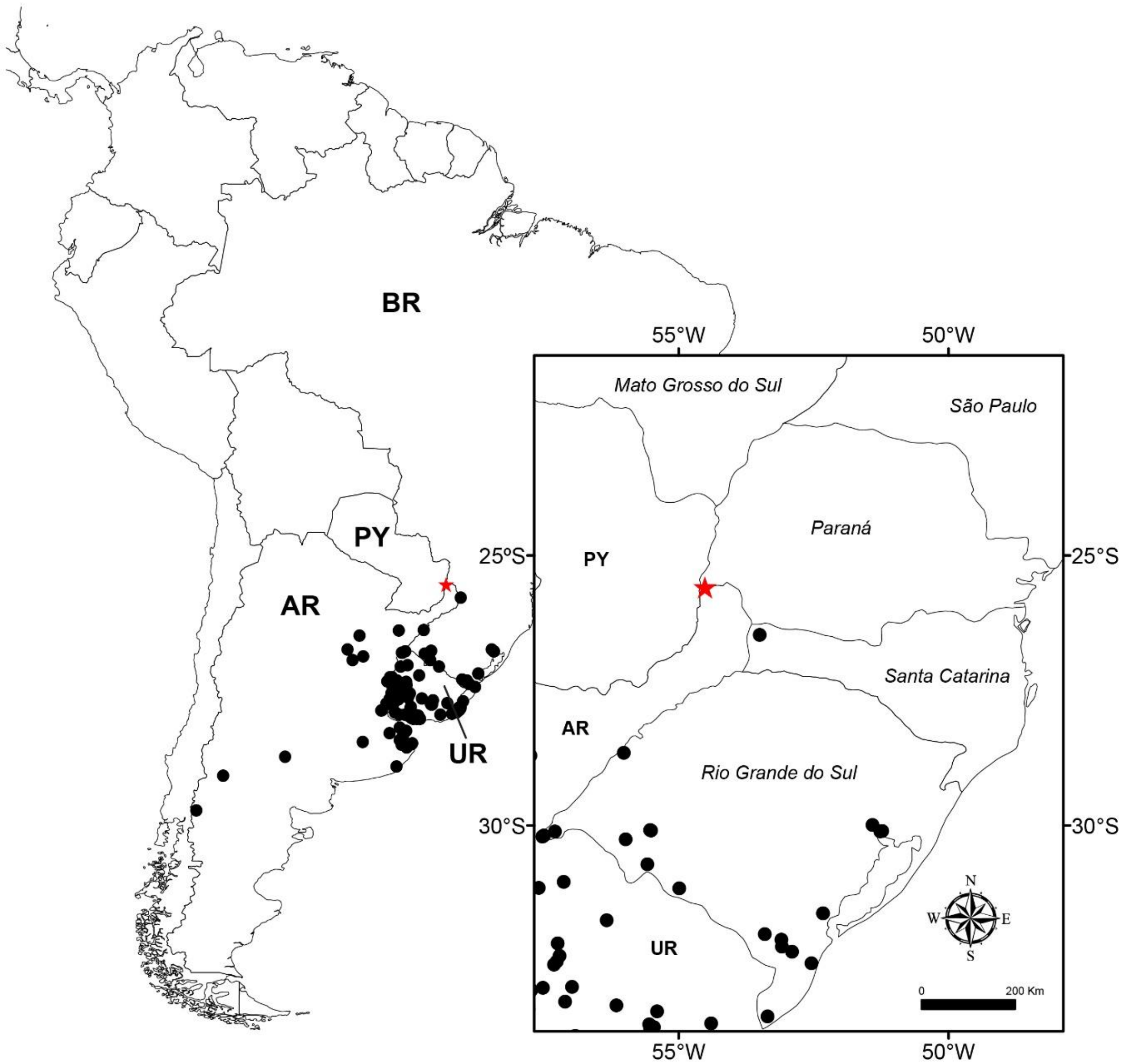

Figure 1. Localities with records of *Axis axis* in southern South America (da Rosa et al., 2020). Red star shows the locality where *Axis axis* was recorded in Iguaçu National Park (25°35'S, 54°30' W).

We documented one individual of axis deer during a big cat species monitoring project (jaguars and pumas) after an episode of livestock predation by a puma on the property. One camera trap (Bushenell ThophyCam HD Camo) was installed in the property, which remained active between September 2019 and August 2020. The camera trap is activated by movement or heat and operates continuously (24/day), taking one 15s video with a six second interval between videos. Videos have date and time recorded. The camera trap was checked at 20-30 days interval for downloading videos and changing batteries.

Results and Discussion

The total sampling effort was 330 camera-days, and we obtained only one record of a single individual of axis deer. The record was obtained on August 14$^{th°}$, 2020 at 2:11 AM, from a juvenile male (25°36'8.75"S, 54°30'20.56"W) (Figure 2). The specimen was identified as *Axis axis* by its external characteristics such as the lines with white spots and big tail that differentiate this animal from other native deer species, such as *Mazama americana* (Erxleben 1777). The identification was confirmed by a specialist, the PhD J. M. B. Duarte (Núcleo de Pesquisa e Conservação de Cervídeos (NUPECCE) Universidade Estadual Paulista (UNESP), Jaboticabal city, Brazil).

Figure 2. The new record of exotic species *Axis axis* (Erxleben, 1777), in the border of the Iguaçu National Park, Paraná State, southern Brazil.

Our new record may represent a range expansion of this species, considered a successful invader in several countries (Clout & Russell 2008; Sponchiado et al. 2011). This record was made around 1,3 km from the Argentinean border, in Brazilian territory, in the municipality of Foz do Iguaçu (Paraná State). The last record of axis deer in Brazil was from 30 August, 2019, a male, in the city of São José do Cedro, Santa Catarina State.

In fact, since 2015 local people on the Brazilian border with Uruguay have reported sightings of this exotic species (Preuss et al. 2020). However, the recent record in Foz do Iguaçu is approximately 138 kilometres from the record obtained in Santa Catarina State, indicating a possibly rapid and concerning increase of the axis deer' geographic distribution in Brazil. We hypothesize that the species reached Paraná State migrating from Santa Catarina State, which is the closest site with the most recent records of axis deer.

The first axis deer introduction in southern South America occurred in 1906, in the province of La Pampa, Argentina (Lever 1985), and since then the species has been expanding its distribution across the country and crossing borders (Novillo & Ojeda 2008; Sponchiado et al. 2011, Rosa et al. 2020) (Figure 1). In Uruguay this species was introduced in 1930, and is also expanding its distribution and invading the south-western region of Brazil (Nowak 1991, Sponchiado et al. 2011, Rosa et al. 2020). To date, there are no records of the species in Paraguay (Figure 1). Etges (2016) indicated that axis deer can adjust its habitat requirements to adapt to new areas where it is recently introduced, and this behaviour favours invasion and establishment of populations on different continents. Studies demonstrated that the occurrence of axis deer can have negative impacts on the ecosystems, for a variety of animals and plants (Davis et al. 2016; Sponchiado et al. 2011). For example, the axis deer can compete with native deer species, resulting in competitive exclusion since they have similar ecological requirements (Etges 2016, Sponchiado et al. 2011). This competition is a reality for the endangered Pampas deer (*Ozotoceros bezoarticus* L1758) in the Brazilian pampas biome (Nowak 1991; Sponchiado et al. 2011). Dolman & Waber (2008) recorded aggression between exotic and native deer. Another negative point is related to the direct effects of herbivory pressure of axis deer on invaded areas, modifying habitats and causing extinction of some plant species, as well as indirect effects, such as the dissemination of diseases that can result in declines of populations of rare and threatened species (Davis et al. 2016; Faas &Weckerly 2010; Flueck 2010; Mohanty et al. 2016). It is important to note that the axis deer reached an important core area of both red brocket deer and Brazilian dwarf brocket deer. Therefore, the risk of competition for resources, and especially disease transmission, could lead to a serious conservation problem for these native local species, especially to Brazilian dwarf brocket deer, a vulnerable species with a small geographic distribution (Oliveira et al. 2019).

Therefore, this first record of axis deer in the State, which may represent an expansion of its range, signals an alert, and indicates the urgent need of a strategy to manage and control the species in the region, as it represents a potential threat to the local biodiversity.

Acknowledgements

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Reintroduction of the Persian Fallow Deer in Iran

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In the mid-twentieth century the Persian fallow deer =*Dama*) *mesopotamica* was documented as extinct throughout its range. However, in 1955 a small remnant population of this species was discovered in the groves around the rivers of Dez and Karkheh in Khuzestan province in southwestern Iran. Six individuals were captured from this population between 1963 and 1964 and were transferred to a breeding center in northern Iran (Dasht-e Naz, Sari). Today, Persian fallow deer are bred in nine captivity and semi-captivity centers in Iran.

During February 2020, 17 (12♂, 5♀) Persian Fallow Deer were captured from the breeding sites of Ilam province (Manesht and Qalarang) and transferred to Khuzestan province for reintroduction. Due to the large number of males at the Ilam breeding sites, we had to consider the sexual composition of the transfer in such a way as to transfer a larger number of males to Khuzestan for release. Of the 17 animals, six males and three females were reintroduced in Dez National Park, and the rest were transferred to a small site in Karkheh to be released in the following years. Dez National Park is located in southwestern Iran, near the city of Dezful in Khuzestan province (Fig.1). With an area of 17,895 hectares, it is one of the most prominent habitats for Persian fallow deer, with dense vegetation as well as access to water, which are important to the survival of the Persian fallow deer. A breeding facility for Persian fallow deer, named Mianroud, existed previously in this park not far from the present release site, but in 2016 its fence was destroyed by floods and the deer dispersed. There have been reports of deer sighted in the region after the flooding event.

Figure. 1. Map of Iran showing release site of Persian fallow deer in Dez & Karkheh national parks located in Khuzestan province.

The release protocol was as follows:

Initially, a small enclosure to habituate the deer was built in this area. After capture, the deer were transferred from the Ilam site to this enclosure (Fig.2) and after about 10 days, they ventured out to the natural habitat following the opening of the gate. The group was released in two stages: in the first stage 5 males and one female were released, and in the second stage the remaining 1 male and 2 females were also released to the wild.

Figure. 2. The Persian fallow deer marked by ear tag and transferred in special crate to a small enclosure in Dez national park before release.

All deer were ear tagged before their release and, after release, local people as well as the rangers of Dez National Park have constantly monitored them and reported whenever they saw a deer. Persian fallow deer were observed a total of 11 times after release, from February to August 2020. The nearest observation point was at 300 meters and the farthest observation point was at 3.56 km from the release point. A total of 24 deer were observed, of which 4 males and 2 females were identified through ear tags; all of the observations pertained to these same individuals. The highest number in one observation was 5 deer including 4 males and one female and the lowest was 1 fallow deer.

In 2016, floods destroyed the Mianroud breeding site in Dez National Park and there is not much information about the fate of a number of deer that left the site after the destruction of the fence. But there are reports of fallow deer being seen around the site in later years. The area where deer were released in 2020 is close to this site and it is possible that, if there are still deer that survived the 2016 flood, they will join this new reintroduced gr

Study finds new population of rare deer — but in Brazil's Arc of Deforestation

Aimee Gabay

A Pampas deer in Campos Amazônica National Park. Image by Ana Rafaela D'Amico.

Scientists have discovered new populations of Pampas deer in the savanna region along the southern edge of the Brazilian Amazon, hundreds of miles away from the species' historical range.

The findings illustrate the need for more detailed studies to assess the deer's conservation status and that of other unrecorded species.

While finding new populations is good news, it's tempered by the fact that the largest of those groups is in an area known as Brazil's Arc of Deforestation, where the land is fast being taken over for agriculture.

https://news.mongabay.com/2020/02/study-finds-new-population-of-rare-deer-but-in-brazils-arc-of-deforestation/

Antler poaching decimates wild reindeer herds in Russia

Herders gather semi-domesticated reindeer in a corral near Kyusyur, Yakutia. Their wild cousins on the Taymyr peninsula, to the west, undertake a yearly migration of up to 1,800 miles.

Photograph by Max Avdeev

By Alec Luhn
PUBLISHED August 12, 2020

The Tamyr Peninsula in northern Siberia used to be the home of the world's largest herd of wild reindeer (*Rangifer tarandus*), of over a million animals, but since the early 2000s, the numbers have plunged to around 400,000. The causes have been illegal poaching of hides and meat during the autumn migration south to the wintering grounds and harvesting of antlers in velvet during the spring migration. The reindeer migrate almost 3000 km in the autumn, spending the winter in forested areas in the Evenkiya district and Yakutia region. In the spring, after the calves are born, the deer migrate north across the Khatanga river system back into Taymyr,

In the spring, hunters in boats targeted swimming reindeer as they crossed the Ketyr or Katanga Rivers on the Tamyr Peninsula as they migrated toward their summer grounds. They sawed off the antlers of the helpless deer, swimming with just their heads above water. It is estimated that 70% of deer subjected to these amputations die of sepsis or blood loss. In 2016, 22 tons of harvested velvet antlers were recovered, estimated to be from around 4000 reindeer. In 2019 a 5 year ban on harvesting velvet antlers from wild reindeer was instituted, which has reduced the number of reindeer killed. Antler harvesting is still legal for domestic reindeer, and the legal trade offers cover for illegal harvesting of wild antlers, considered to be superior for Chinese medicine.

Legal hunting of reindeer for meat and furs is permitted for indigenous peoples and hunting permits are also issued for recreational hunters. In 2019, 41,500 permits were issued, a number considered to be above the sustainable limit. Legal hunting and poaching are still dangers to these populations. Reindeer and caribou are now listed as Vulnerable by the IUCN.

https://www.nationalgeographic.com/animals/2020/08/poachers-target-largest-reindeer-herd-antler-velvet/

Bukhara (Bactrian) deer (*Cervus elephas bactrianus*) reintroduced to Kazakhstan

Photo by Sarefo, CC license

Last year 5 Bukhara or Bactrian deer (*Cervus elephas bactrianus*) were reintroduced to the Ili-Bakash Nature Reserve near Lake Balkhash in central Kazakhstan, where they have been absent for 100 years. Formerly occupying central Asian riparian areas, Bukhara deer were reduced to around 400 animals by the end of the last century. As a result of re-introduction programs, they now number around 1400 animals in the wild in several countries. WWF Russia and the Kazakhstan Ministry plan to re-introduce several hundred deer into the area over the next 5-6 years. Reintroductions of Bukhara deer are also taking place in Tajikistan and Uzbekistan. The ultimate aim of this project is to re-introduce a tiger subspecies into these areas. As the Caspian tiger native to the zone went extinct in the 1970s, it is planned re- reintroduce the almost identical Amur tiger, also known as the Siberian tiger.

https://www.goodnewsnetwork.org/once-numbering-less-than-400-majestic-bukhara-deer-return-to-the-wilds-of-kazakhstan/

Eld's deer (*Rucervus eldii*) observed in Kratie Province, Cambodia, for first time after five years

Mom Kunthear

A camera trap study found evidence of the presence of Eld's deer (*Rucervus eldii*) in Sambor Wildlife Sanctuary in the Mekong flooded forest landscape in Kratie province, Cambodia, for the first time in 5 years. Eld's deer is classified as Endangered by the IUCN and its continued presence in the area was in doubt. Four deer were seen in photographs in the study in August and September of 2020, out of 1710 photographs taken in various parts of the sanctuary. The Sambor Wildlife Sanctuary covers 50,093ha and is regularly patrolled by rangers from the Ministry of the Environment.

Cambodian Eld's deer belong to the subspecies *Rucervus eldii siamensis,* and they have declined more than 90% since 2008. The IUCN red list from 2015 considers that there are <700 deer left in Cambodia, scattered in isolated subpopulations with few being found in protected areas. Thus, this study represents good news; it was reported by the World Wildlife Fund.

https://www.phnompenhpost.com/national/endangered-elds-deer-spotted-first-time-kratie-after-five-years?mc_cid=c2bb903d79&mc_eid=01b0c0d611

The large-antlered muntjac (*Muntiacus vuquangensis*), the most threatened muntjac

Joel Berger and Minh Nguyen
3 August 2020

The large-antlered muntjac has been classified as Critically Endangered by the IUCN. Its range is restricted to small parts of the Annamites mountainous region in Vietnam, Lao and possibly Cambodia. It was only discovered by the West by scientists Rob Timmins and Bob Evans in 1993 and has become vanishingly rare. It is one of 13 species of muntjacs, which are native to various parts of Southeast Asia. The northern and southern red muntjacs are abundant and listed as Least Concern, while a number of species have been recently discovered and are listed as Data Deficient. They are all threatened by extensive snaring by native peoples for the wildlife trade. Other human activities such as hydropower projects, timber harvesting, mining and new roads also fragment populations and facilitate access to remote areas where muntjacs are found.

Muntjacs are small, solitary forest deer with simple antlers, similar to their ecological Neotropical equivalents, the brocket deer. They are not easy to study and thus relatively little is known of their population status, ecology or behavior.

The large-antlered muntjac has disappeared from most of its previously known range, which has often been determined more by the presence of trophies in the homes of villagers than actual population data. There is some hope for its protection *in situ* on the Dalat Plateau in Vietnam and in Lao's Nakai–Nam Theun National Protected Area. The DSG has also recommended the creation of an *ex situ* breeding site.

https://news.mongabay.com/2020/08/the-large-antlered-muntjac-southeast-asias-mystery-deer-commentary/?mc_cid=6a7d123472&mc_eid=01b0c0d611

Silver backed chevrotain (*Tragulus versicolor*) seen after 30 years

The silver backed chevrotain (*Tragulus versicolor*), the rarest of all chevrotains, had not been detected since 1990 in its range in Viet Nam and some feared it had gone extinct. However, in a camera trap study near the city of Nha Trang, in forests near the southern coast of Viet Nam, it was detected and identified. It is difficult to distinguish the silver backed chevrotain from a closely related species, the lesser chevrotain. Since the first discovery, more than 200 photos have been taken that show the chevrotain alone and in pairs. The study was conducted by Andrew Tilker and others at the Global Wildlife Conservation, and they now want to determine the size of the population and establish a conservation plan.

https://www.newscientist.com/article/2222920-we-thought-this-tiny-deer-like-animal-was-extinct-for-almost-30-years/

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