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Extinction of the Javan Rhinoceros (*Rhinoceros sondaicus*)

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Abbreviations

AsRSG	Asian Rhino Specialist Group
CEPF	Critical Ecosystem Partnership Fund
CITES	Convention on International Trade in Endangered Species
CTNP	Cat Tien National Park
CTNPCP	Cat Tien National Park Conservation Project (WWF)
DNA	Deoxyribonucleic acid
ETOH	Ethanol
EDTA	Ethylenediaminetetraacetic acid
FPD	Forest Protection Department
GCR	Genome Complexity Reduction
GPS	Global Positioning System
IRF	International Rhino Foundation
IUCN	International Union for the Conservation of Nature
MIKE	Monitoring the Illegal Killing of Elephants
MIST	Management Information System
PCOA	Principle Co-ordinate Analysis
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
RPM	Revolutions per minute
UK	United Kingdom
USA	United States of America
USFWS	United States Fish and Wildlife Service
ZSL	Zoological Society of London

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CONTENTS

EXECUTIVE SUMMARY

The Javan rhinoceros is extinct in Vietnam; the last individual was poached for its horn in late 2009, found dead in 2010. Consequently, the *annamiticus* subspecies is extinct. This leaves only one small population of Javan rhinoceros in Java, Indonesia.

Twenty rhinoceros faecal samples collected by CTNP and WWF between 2003 and 2006 were sent to Queen's University in April 2010 for analysis. Bacterial diversity profiles of these samples concluded that there were at least two individuals present in the population in 2003-2006.

WWF and Cat Tien National Park conducted a comprehensive survey of the Javan rhinoceros population from October 2009 to April 2010, to determine the population status through genetic analysis of rhinoceros dung samples collected. Dung-detection dogs were employed for the survey to increase the detection of rhinoceros dung. The team achieved good coverage, surveying the 6,500ha 'rhino core area' three times and approximately 3,500ha of the wider area, where signs of rhino have not been recorded since 1993, to ensure no individuals were missed.

Twenty-two dung samples were collected by the survey team from the rhino core area between October 2009 and February 2010 and sent to Queen's University, Canada for genetic analysis. No signs of rhinoceros were found outside of the rhino core area at any time during the survey. From 5th February to mid-April, the team did not find any new rhinoceros footprints or dung in Cat Loc.

On 29th April 2010 a Javan rhinoceros was found dead in Cat Loc; samples of skin and teeth were taken from the skeleton and sent to Queen's University to be included in the genetic analyses. The genetic analyses confirmed that all of the dung samples collected in 2009/2010 belong to one individual, the same individual that was found dead in April 2010. Genetic sexing indicates that this individual was female. Bacterial diversity profiles of the faecal samples, which discriminate among different individuals, supported the conclusions from the genetic work that there was 1 individual in 2009-2010, and showed that this individual was one of the two individuals present in 2003-2006.

Given the good survey coverage of the area, the field observations, and the genetic and bacterial diversity work, we can therefore confirm that the Vietnamese population and the *annamiticus* subspecies of Javan rhinoceros is extinct. The Javan rhinoceros is therefore confined to one population on Java, Indonesia.

Poaching was identified as the cause of the extinction of the subspecies; the last individual was shot in the leg, which probably caused its death, and the horn had been removed (Streicher *et al* 2010). Habitat loss due to agricultural conversion and development is also recognised as a driving force behind the loss of this population; the habitat of the species in Vietnam has declined from 75,000ha when it was rediscovered in 1988, to less than 30,000ha today. Furthermore, the population was restricted to only 6500ha of this habitat due to the presence of a heavily used motorbike dirt-track connecting settlements within the park, which restricted access to other parts of Cat Loc, and encroachment of agricultural land within the rhino core area.

The issues of poaching and habitat loss are not unique to Cat Tien National Park but are a nationwide problem in Vietnam, as a result of poor protection and law

enforcement efforts and ineffective protected area management. Consequently, Vietnam is on the verge of an extinction crisis with many other species threatened by hunting and habitat loss. Significant improvements need to be made in law enforcement and protected area management in Vietnam, and the way in which conservation organisations cooperate with protected areas, to ensure that other species do not share the same fate as the Javan rhinoceros.

1. INTRODUCTION

1.1 Javan rhinoceros

The Javan rhinoceros *Rhinoceros sondaicus* is Critically Endangered (van Strien *et al* 2008), until recently surviving in two separate populations, in Indonesia and Vietnam, representing two of the three subspecies (Fernando *et al* 2006, van Strien *et al* 2008). *R. sondaicus inermis* Lesson 1838 formerly occurred in northeastern India, Bangladesh and Myanmar; this subspecies went extinct in the early 1900's. *R. sondaicus sondaicus* Desmarest 1922 formerly inhabited Thailand, Malaysia, Java and Sumatra but only 40-60 individuals remain, in 123,051ha of Ujung Kulon National Park, Indonesia (van Strien *et al* 2008). *R. sondaicus annamiticus* Heude 1892 formerly occurred in Lao, Cambodia, eastern Thailand and Vietnam. *R. sondaicus annamiticus* was presumed extinct by the western world after the Vietnam War until 1988, when reports were received of an individual having been hunted in southern Vietnam (Santiapillai *et al* 1993).

A survey conducted in the same area of southern Vietnam in 1989 confirmed that individuals remained in approximately 75,000ha of habitat at the site known as Cat Loc, just north of the existing Cat Tien National Park (CTNP) (Schaller *et al* 1990) (Figure 1). Cat Loc (30,435ha) was subsequently designated as protected in 1992 and was incorporated into Cat Tien National Park in 1998.

1.2 WWF involvement

WWF have been involved in Cat Tien National Park in strengthening park management and in particular in Javan rhinoceros conservation, since the mid-1990's. The large-scale Cat Tien National Park Conservation Project (CTNPCP) funded by the Netherlands government and implemented by WWF and CTNP ran from 1998 to 2004, with the following aims: i) Effective protection of Cat Tien National Park; ii) Human impacts reduced to sustainable levels; iii) Landscape-level strategy to support the management of CTNP; iv) Effective institutional and administrative support. From 2005 to 2007 WWF continued to provide small-scale support for protection and monitoring of the rhino population and in 2009 funding was raised to support enforcement patrols in Cat Loc and to conduct a comprehensive survey of the population status, which was implemented in 2009/2010.

1.3 Javan rhinoceros surveys and population status in Vietnam

Javan rhinoceros was once common throughout much of lowland Vietnam and was still in high numbers during French colonial times (1859-1956). Hunting of rhinoceros by local people was common in the region before these times and also

popular with colonialists. The widespread availability of military guns during and after the wars in Vietnam with France (1946-1954) and the USA (1955-1975) allowed more efficient hunting, contributing to the dramatic decline of the Javan rhinoceros population. Polet *et al* (1999) present anecdotal reports of a minimum of 39 Javan rhinoceros killings in the CTNP area from before 1957 to 1991. Owing to hunting and habitat loss due to defoliant spraying, it was thought by the western world that the subspecies was extinct until news was received that an individual was poached from the Cat Loc area in 1988 (Polet *et al* 1999).

Although several surveys were conducted following the subspecies re-discovery in Vietnam, no reliable population estimate has ever been obtained. In 1989, researchers estimated that a maximum of 10-15 individuals inhabited the CTNP area (<75,000ha), based on field observations of footprints and interviews with local community members and officials (Schaller *et al* 1990). This was the last survey to document Javan rhinoceros in the Nam Cat Tien sector of CTNP (last sighting 1988) and in Binh Phuoc (Song Be) Province (Figure 1). All rhinoceros surveys that followed reported signs within the Cat Loc sector only. No signs of rhino have been found in the State Forest Enterprise land to the north and south of Cat Loc for at least 20 years (Nguyen Xuan Dang and Osborn 2004; Nguyen Xuan Dang *et al* 2004).

In 1993 (based on a survey conducted in 1991), Santiapillai *et al* estimated a minimum of 8 individuals, or 8-12 individuals, survived in Cat Loc, based on observations of field tracks and anecdotal evidence from ethnic groups residing in Cat Loc. Signs were found in the northeastern part of Cat Loc and the Javan rhinoceros range was estimated at 35,000ha (all of Cat Loc).

By 1999, field surveys and analysis of 111 tracks (plastercasts) conducted by WWF and CTNP, concluded that a minimum of seven and a maximum of eight individuals were present in Cat Loc, surviving in only 6,500ha known as the 'rhino core area' (Polet *et al* 1999). The range of the rhinoceros population had declined by 28,500ha in 6 years although Cat Loc was still an estimated 27,850ha in size. Rapid human population growth and socio-economic expansion resulted in severe encroachment of national park land; much of the best rhinoceros habitat such as the flat alluvial land along the rivers and swamplands was converted to rice paddies, and remaining patches of broadleaved forest were converted to cashew plantations.

The large settlement in the northeast of Cat Loc (village 5) and the new road constructed to this area from outside of the national park virtually disconnects the eastern part of Cat Loc from the west (Figure 2). Rhinos have not been recorded in the northeastern part of Cat Loc (east of the large commune within the protected area) since 1993.

Vital access to water in the dry season has been considerably restricted; rhinoceros used to cross the Dong Nai River into Song Be Province (now known as Binh Phuoc), which marks the northern boundary of Cat Loc. However, much of the forest across the river in Binh Phuoc Province was converted to agricultural land, and a string of human settlements on the Lam Dong Province side of the river near the boundary of Cat Loc restricted access to the river along the edge of much of the rhino core area. Furthermore, the development of dirt-tracks for motorbikes between settlements within and outside of Cat Loc effectively cut off access for the rhinoceros to the eastern part of Cat Loc, limiting the population predominantly to the 6500ha core area (Polet *et al* 1999).

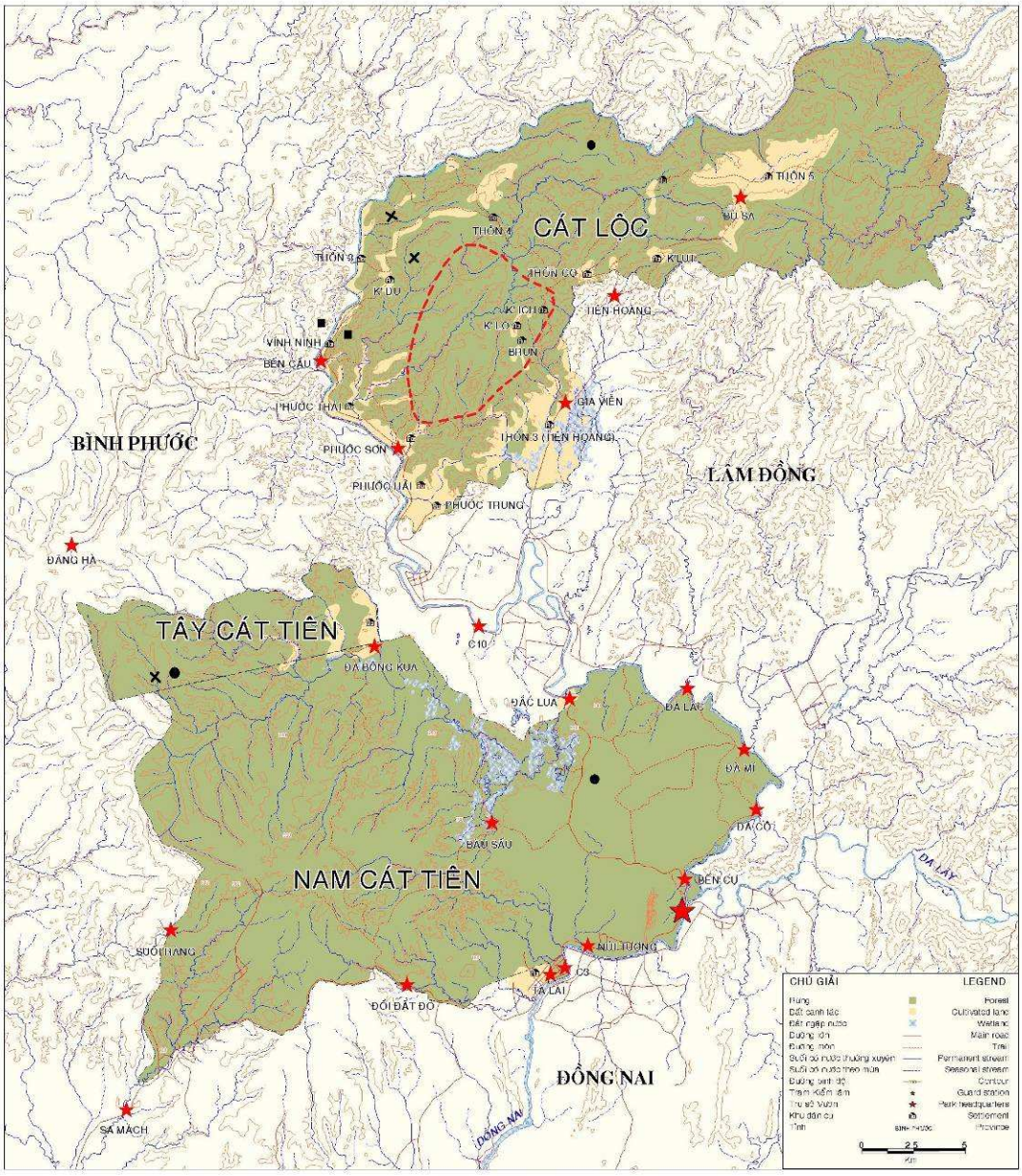

Figure 1. Rhino records in and around CTNP from the 1980's, taken from Schaller et al (1989). Rhinos killed (black cross), rhino sightings (black circles) rhino tracks (black squares). All records after 1993 are from the 'rhino core area' (dotted red line).

Later surveys estimated progressively fewer animals, with 5-8 estimated in 2004 (Polet and Ling 2004); and less than 5 estimated in 2006 (Fernando *et al* 2006), all from within the rhino core area. DNA analysis performed by Columbia University in 2004 on faecal samples collected by CTNP and WWF in 2001 and 2002, concluded that there were 5-6 individuals present, including both sexes (Vuong Duy Lap *et al* 2004). However, the accuracy of these conclusions is debatable, given that there is no truly accurate method for population estimation from footprint analyses, and primers from Indian rhinoceros (*Rhinoceros unicornis*) had to be used for the DNA analyses, hence creating considerable uncertainty of the results.

The primers for Javan rhinoceros were developed in 2009 by Queen's University, Canada. Consequently WWF (with financial support from WWF, USFWS, CEPF and the Hermsen Foundation) sought to conduct the first comprehensive field survey for Javan rhinoceros in Vietnam, to accurately determine the population status. The survey collected faecal samples for genetic analysis to identify the number and sex of individual rhinoceros. Detection dogs were employed to improve the detection rate of Javan rhinoceros dung. Detection dogs are an efficient method of locating target species dung (Smith *et al* 2003) and have been shown to be four times as effective in detecting dung in comparison to other survey methods (Rolland *et al* 2006).

1.3 Aims and objectives

Although immediate conservation needs for the Javan rhinoceros in Vietnam were clear: to protect the rhinos and their remaining habitat (AsRSG 2000), the accurate population status was urgently required, to: i) identify whether investment in CTNP for Javan rhinoceros conservation was justified (defined by WWF as having individuals of both sexes present in the population); and; ii) to provide the necessary impetus for the Vietnamese Government to endorse more stringent protection measures and conservation actions.

Shortly after the survey was completed, a dead Javan rhinoceros was found in the national park on 29th April 2010.

2. METHODS

2.1 Study site

It is widely accepted that Cat Loc, sub-sector of Cat Tien National Park in Lam Dong Province, Vietnam, held the last remaining population of Javan rhinoceros in mainland Asia. Once tropical lowland semi-deciduous forest, Cat Loc now consists mainly of mixed bamboo and semi-deciduous forest, with some hillsides dominated by dense stands of rattan, as a consequence of heavy defoliant spraying during the Vietnam War. Areas of semi-deciduous forest remain on ridge tops in particular, dominated by Dipterocarpaceae or *Lagerstroemia* spp. The rhino core area is made up of many small steep hills, from 300m to 600m elevation, and lots of streams traverse the area, which drain into the Dong Nai River. Soils are alluvial with heavy clay, helping to create wallows and swampy areas (Polet *et al* 1999).

The 6,500ha known as the 'rhino core area' was the focus of the survey, where tracks and signs of rhinoceros were frequently found up until 2010. Approximately 3,500ha ha of the 'wider area' was also surveyed, where tracks and signs have been observed very infrequently since 1993 (Polet *et al* 1999). The rhino core area and wider area are bisected by a dirt track running south-northwest, on which motorbikes frequently travel between human settlements and cashew plantations inside the national park, creating disturbance and a potential barrier to rhinoceros movements within Cat Loc (Figure 2). The rest of Cat Loc was not surveyed because there have been no records of rhinoceros form these parts for at least 20 years (Santiapillai 1991 survey).

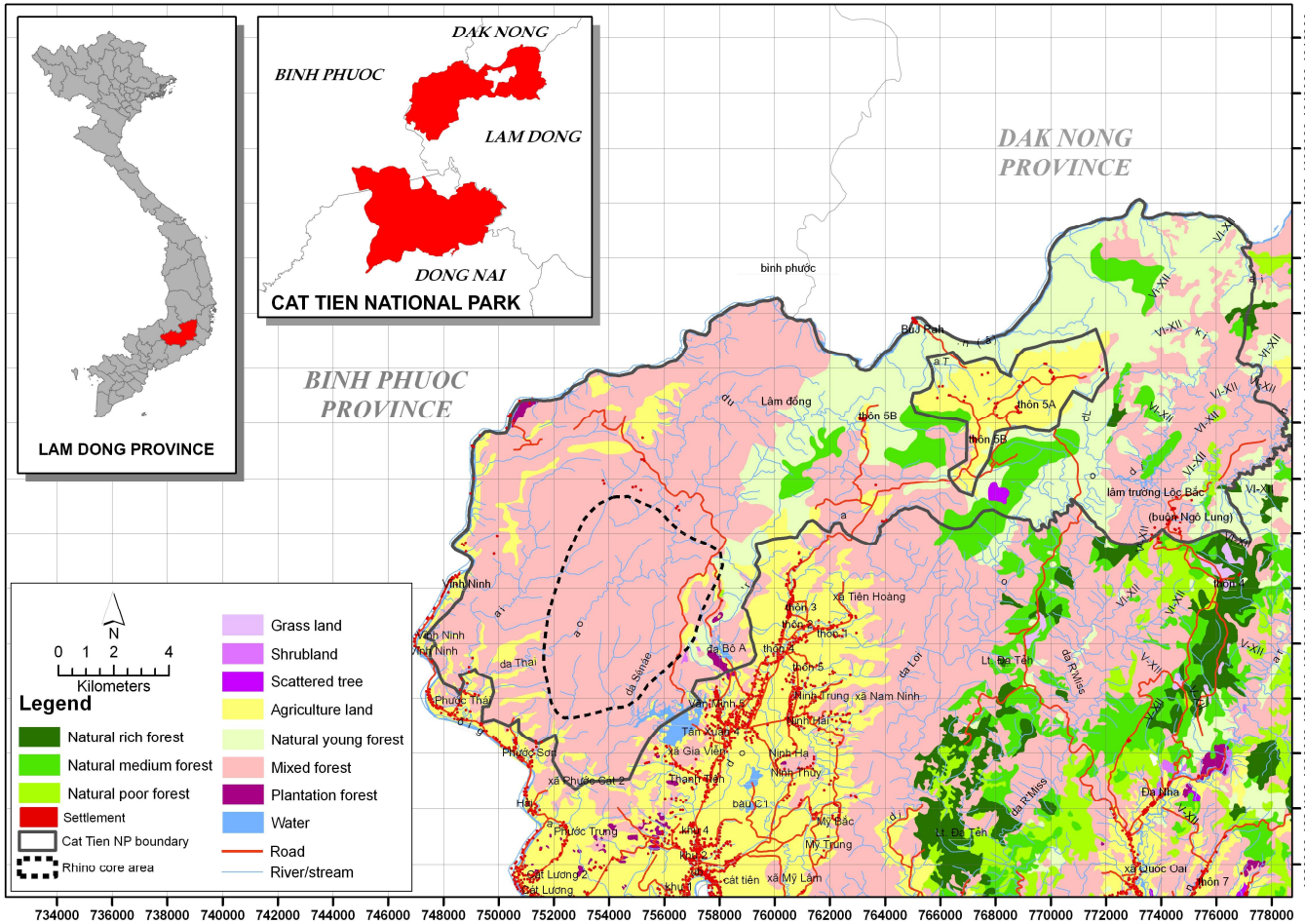

Figure 2. Location of Cat Tien National Park; Nam Cat Tien and Cat Loc sectors, and habitat and land-use of Cat Loc sector. The pink area (mixed forest) within the park boundary in the east roughly delineates the total survey area.

2.2 Detection dogs

Dung detection dogs were contracted from Packleader LLC, USA. Two dogs were selected and trained to recognise and indicate on rhinoceros dung obtained from captive rhinoceros of all species except Javan rhinoceros (of which there are none in captivity), prior to arrival in Vietnam. The dogs and trainer arrived in Vietnam on October 4th2009. Both international ecologists responsible for the survey received 3 weeks of on site training in detector dog handling and the dogs were trained on Javan rhinoceros with dung samples collected from Cat Loc.

2.3 Survey methodology

The survey was conducted from October 27th 2009, to April 8th 2010 during the dry season. During the wet season, dung will break down more quickly and the area also frequently becomes inaccessible after heavy rain. The survey was completed in three phases; phase 1 from 27th October to 13th December, phase 2 from 26th January to 25th February, phase 3 from 3rd March to 8th April 2010.

The survey area (approximately 10,000ha) was divided into 2km x 2km (400ha) grid cells (Figure 2), based on the estimated home range size of female Javan rhinoceros (500ha), with males potentially wandering over larger areas (van Strien *et al* 2008). Each grid cell with suitable habitat was surveyed (all or part of 18 grid cells in the rhino core area and all or part of 17 grid cells in total in the wider area). The team aimed to search the rhino core area in all 3 phases and all the cells of the wider area a minimum of once (Figure 3). Within each cell, 'hotspots' of rhino activity such as swamps, wallows, trails and streams were targeted for searching, to maximise the chances of finding rhino dung. All wallows and swamps which were previously located and mapped by CTNPCP were surveyed a minimum of 3 times if they retained some water (only a few wallows remain wet towards the end of the dry season). If rhinoceros footprints were encountered at any point during the survey, these were followed in both directions for up to 1km to search for faeces.

The survey was conducted by two teams covering adjacent grid cells, each team consisted of one international ecologist/dog handler, one detection dog, one technical staff member of Cat Tien National Park, one Forest Protection Department ranger and one local guide. On average, one grid cell per day was surveyed by each team, covering from 3-8km in terms of human distance walked at a speed of ≤ 1km per hour. 3-5km per day is the optimum daily rate for detector dogs in tropical rainforest conditions (pers. Comm. Steven Weigley). The survey teams were restricted to working for 4 or 5 consecutive days, followed by 2 or 3 days off, to ensure the dogs were well rested (searching ability can be negatively affected by fatigue). It is not possible to accurately estimate the detection distance of scat detection dogs, with so much of this dependent on local conditions, including temperature, time of day, windspeed and direction, topography, habitat type and age of dung. For more information on typical detection dog training methods and scenting ability, see Wasser *et al* (2004).

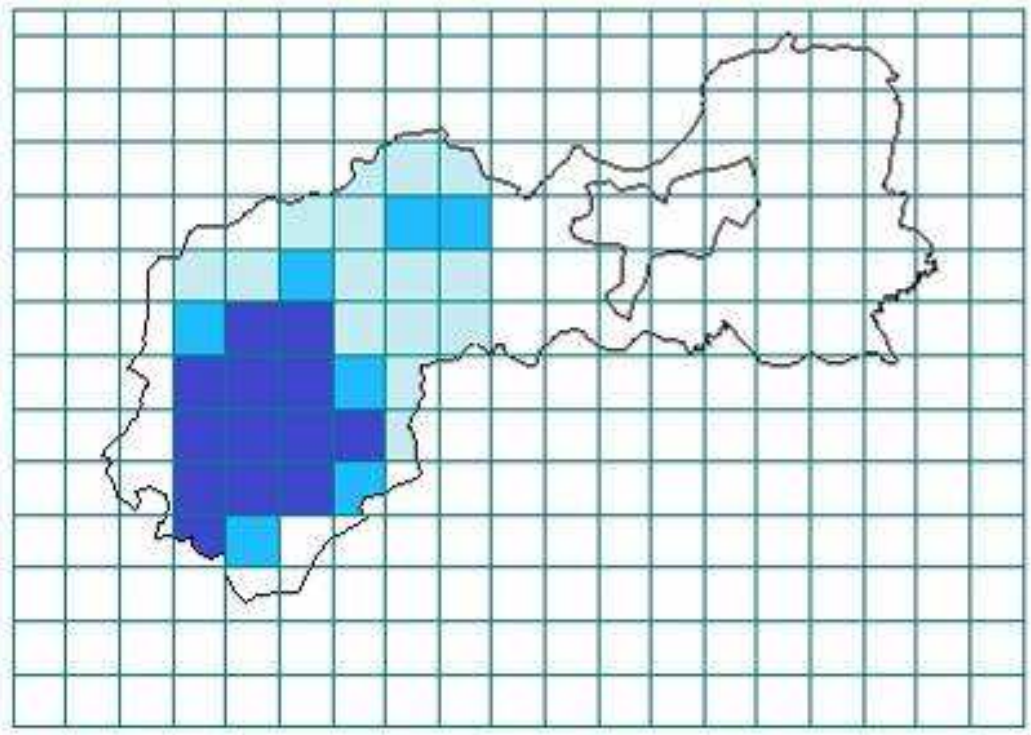

Figure 3. Survey coverage and effort. Cell surveyed 3 times (dark blue), surveyed twice (mid blue), surveyed once (pale blue), not surveyed (white).

2.4 Faecal sample protocol

When rhinoceros faeces were found, three samples were collected from each dung pile, following the MIKE collection protocol (Hedges and Lawson 2006), to investigate the effect of storage type on the genetic analyses.

The samples for DNA analysis were stored in a 50ml tube with: a) ETOH buffer; b) EDTA buffer; c) silica gel sachet. Each of the samples was heated at 72 degrees Celsius for 30 minutes, in accordance with Canadian Food Inspection Agency (CFIA) guidelines. Samples a) and c) were stored in the freezer at zero degrees Celsius and b) samples were stored at room temperature (circa 32 degrees Celsius) before shipping to Queen's University, Canada on April 12th 2010.

The following variables were also recorded with each dung encounter: date, GPS location (northing and easting), habitat type, elevation, bolus intact (y/n), diameter of bolus (if still intact), fungus present in dung (y/n). Each dung pile was marked by tying colour tape to nearby vegetation to ensure they were not sampled more than once during the survey.

In addition, locations of rhinoceros footprints were GPS marked to document the full distribution of rhinoceros within Cat Loc. GPS tracks were downloaded and mapped in MapInfo Professional 8.0.

Two teeth and three tissue samples from the dead Javan rhinoceros were collected by Sarah Brook on behalf of Cat Tien National Park in May 2010; the teeth were stored in an airtight container with silica gel and the tissue samples were stored in a 50ml tube with a) ETOH buffer, b) EDTA buffer (as above) and c) nothing. CITES export

and import permits were obtained and the samples were shipped to Queen's University on 22nd November 2010.

Twenty Javan rhinoceros dung samples collected by CTNP staff and the CTNPCP between 2003 and 2006 were also sent to Queen's University for analysis, to obtain a minimum population estimate for this period (a maximum population estimate for this period cannot be obtained because the survey and collection of dung was not then systematic and comprehensive).

2.5 Genotyping

The extraction of DNA from Javan rhinoceros faecal samples for amplification of microsatellites from target epithelial DNA was optimised. For all samples the extraction procedure detailed in the QIAamp DNA Stool Mini Kit (QIAgen cat# 51504) was followed, with minor modifications in order to process a larger sample volume with an additional pre-extraction step for samples stored in ETOH. Approximately 2.5g were dried in a sterile 15mL conical covered with a Kimwipe (to prevent contamination) for two days inside a fume hood. All samples were homogenized in 2-5mL ASL Buffer (depending on how much sample in the tube, approximately enough to cover the sample) by shaking tubes gently for one minute. The samples were then centrifuged at 12,000rpm in a microcentrifuge Model MB, and the supernatant removed to a clean 15mL conical. One Inhibitex tablet was added teach supernatant, to remove PCR inhibitors. The tablets were suspended into the samples by shaking until the tablet was completely dissolved, followed by one minute of gentle shaking.

Each sample was then centrifuged at 8,000 RPM in an IEC Clinical Centrifuge. Supernatant was then transferred to a clean 15mL tube and 200µL of Proteinase K and an equivalent volume of AL buffer was added to the remaining supernatant and incubated overnight in a 37°C shaker. An equivalent volume of ethanol was added to each sample and mixed by inversion. Samples were then spun through a QIAamp spin column, 700ul at a time. The column was washed with 500µL of Wash buffers 1 and 2 and eluted twice with 200µL of Elution Buffer into a single 1.5mL microcentrifuge tube (for a total volume of 400 µL). Once the DNA had been extracted from the faeces, the samples were concentrated in a LABCONCO Centrivac concentrator with heat, to an approximate volume of 200µL. To confirm that DNA was present in this final eluent, 20µL of each sample was run on a 0.8% agarose gel. 1:100 dilutions of the faecal DNA extraction amplified better in most cases than straight 1:1 extractions. From the primers that were cloned in other rhinos, the amplification conditions for 12 microsatellites that worked in non-faecal Javan rhinoceros tissue and Javan rhinoceros faecal samples (Table 1 & 3) were optimised. For complete details of the primer pair sequence and nature of amplified repeat see Appendix 1. For PCR cycling conditions of microsatellite primers optimized for Javan rhinoceros see Appendix 2. For PCR cocktails used in all PCRs see Appendix 3.

From the original nine primers, one (JR159) contained a PCR artefact from the GCR-PCR and was discarded. The cloned sequences for four of the remaining eight were such that no additional primers could be designed (JR 002A, JR 006, JR 016 & JR 029). For the remaining four – JR003, JR049, JR088 and JR106 –primers were redesigned that performed better than the original primers. Their properties on the control set are detailed in Appendix 1 and Table 3 (the control set comprises 7 samples of Javan rhinoceros bone and tissue, five of which are from museum specimens over 100 years old and two are from recently deceased individuals in

Vietnam and Indonesia). These new loci were not fully optimized to amplify DNA from Javan rhinoceros faecal samples.

Borthakur *et al* (2010) was followed to PCR amplify three replicates of all faecal extracts from the 2009-2010 survey across all optimized microsatellite primers. If there was no consensus (i.e. 2 out of 3 among the replicates), three more replicates were amplified. (If none of the replicates are the same then the microsatellite is not fully optimized for Javan rhinoceros faecal extracts and is no longer assayed).

To err on the side of caution, the findings for sample by locus scores for fully optimized loci where all three replicates or 2 out of 3 of replicates indicated the same genotype are reported (Table 3). In situations where all three replicates indicated different genotypes, they were re-amplified once and if the same results were obtained, that sample x locus datum was scored as 0:0. In this case the target DNA Javan rhinoceros epithelia is compromised and no correct product will be obtained. In some sample x locus cases, two or less replicates would amplify after multiple attempts and for that cell 0:0 was entered. It is likely there were insufficient target DNA Javan rhinoceros epithelia and no product could be obtained. The consensus genotype for the sample x locus cell was entered otherwise.

2.6 Genetic sexing

The multiplex reaction using two primer pairs optimized for the sexing of Indian rhinoceros (Stoop 2009, Borthakur *et al* 2010) and the Zinc Finger single primer pair method (Pepin *et* al 2009) were attempted. Although the multiplex reaction for most of our other rhinoceros species using animals of known sex was optimised, the sex of the Javan rhinoceros samples that worked for this test was not known. In addition, reliable amplification of the larger ZFy product in the faecal samples of Javan rhinoceros was not expected, so this method was abandoned.

The primers of Peppin *et* al (2010) were evaluated for genetic sex determination in Javan rhinoceros on both ABI and Licor Platforms. These primer pairs were chosen because they amplify relatively small pieces of DNA ~ 95-107bp range, and are therefore best suited for the amplification of degraded rhinoceros epithelial DNA found in rhinoceros faeces. The primers were evaluated first on DNA from the Javan rhinoceros control sets (bones and tissue samples) and then on DNA extracts from faeces and the tissue sample from the dead CTNP rhinoceros, which were compared to results from all other extant rhinoceros species with individuals of known sex. All amplifications included a negative control without template DNA. Amplification products were diluted 10-fold in ABI Hi-Di Formamide before capillary electrophoresis on an Applied Biosystems Inc. 3130xl Genetic Analyzer (ABI) or were run directly on a Licor 4200. Alleles were sized against an internal standard (see Figure 9 for Licor Image).

Table 1. Details of the 12 Non-Javan rhinoceros microsatellite loci that amplify Javan rhino microsatellite DNA in the JR control set and the 2009-2010 CTNP faecal samples. Most loci appear to have a single homozygous genotype in the control set except SR 54, WR32A and WR32F, which have 2 or more alleles. It appears some of the loci amplify better in some samples versus others. For some loci DNA from all samples was not available. For example no 146717 (a museum skin) was not available for the control set assay of DB44, IR10, IR11, SR 262, SR 281, WR 32A, WR 32F and WR 35A. This is reflected by 'NA' in corresponding sample x location cells. The samples were amplified at least three times for each locus (except WR32A and WR32F which were only amplified once) at the optimum annealing temp of 58^{o} or with a Touch Up (TU) PCR cycle (Appendix 2). CA 1/10 is the CTNP sample. It appears DNA from 150 year-old bones amplify the best.

	BR06		DB01		DB44		IR10		IR11		IR12		SR54	SR262		SR281		WR32A		WR32F		WR35A	
CA 1/10 (SKIN)	134	134	126	126	194	194	0	0	115	115	0	0	159	159	114	114	0	0	0	0	228	228	202	202
5169 (BONE)	134	134	126	126	194	194	200	200	115	115	187	187	157	157	114	114	220	220	236	232	242	228	202	202
5170 (BONE)	134	134	126	126	194	194	0	0	115	115	187	187	159	159	114	114	0	0	0	0	228	228	202	202
6212 (SKIN _M)	134	134	126	126	194	194	0	0	0	0	187	187		0	0	0	220	220	0	0	0	0	202	202
NS 1/10 (SKIN)	134	134	126	126	194	194	206	200	0	0	0	0	159	159	114	114	220	220	0	0	242	242	202	202
146717 (SKIN _M)	134	134	126	126	NA	NA	NA	NA	NA	NA	187	187	159	159	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NZ 1/10 (SKIN-M)	134	134	126	126	194	194	200	200	115	115	187	187	0	0	114	114	220	220	236	232	228	228	202	202

1/10 = the sample works best at 1/10 dilution of the original extracted concentration.

2.7 Bacterial diversity assay

The samples collected from2003-2006 and the 2009-2010 samples collected by the recent survey were used to evaluate the ability of bacterial diversity profiles of faeces to discriminate among different rhinoceros. This has been done with humans (Ley *et al* 2008). Using degenerate 16s bacterial DNA primers, copies of the DNA from the bacteria present in the faeces were amplified using PCR. Large numbers of these amplified DNA's were sequenced for each extract using 454 sequencing methods. Using UNIFRAC, these sequence data were analysed to produce an unweighted and weighted pairwise distance matrix, which was then analysed using principal co-ordinate analyses. All Javan rhinoceros (N= 104) faecal extracts were analysed using this method.

In some cases only 170 bacterial 16s RNA sequences were produced for each faecal extract, therefore only 170 sequences were selected from each faecal sample for further analyses (rarefaction).

3. RESULTS

3.1 Survey results

A total of 118 survey days were conducted by the two field teams, with a minimum of 429km walked during that time (Figure 4). The first phase covered 18 cells of the core area and 17 cells of the wider area (Figure 5). Phase 2 focused on the rhino core area only, conducting a repeat survey of 14 grid cells (Figure 6). Phase 3 repeat surveyed 16 grid cells of the rhino core area and 10 cells of the wider area (Figure 7).

Eighteen wallows/swamps were recorded, all of which were identified by previous field surveys, with Javan rhinoceros signs found during the survey at all but two of these areas (Figure 4).

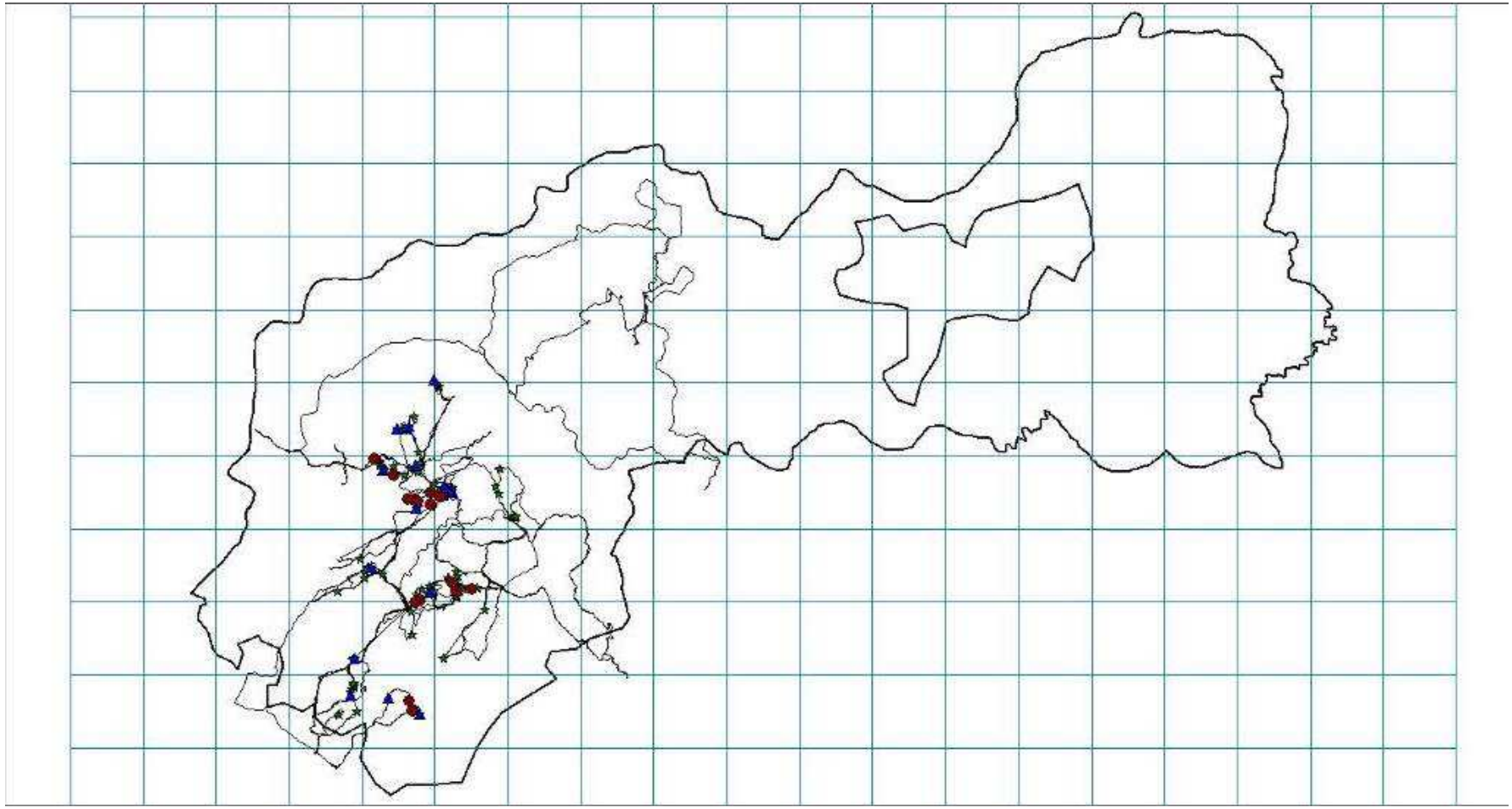

Figure 4. Map of all survey tracks (black lines), location of Javan rhinoceros dung (red circles), footprints (green cross) and wallows (blue triangle).

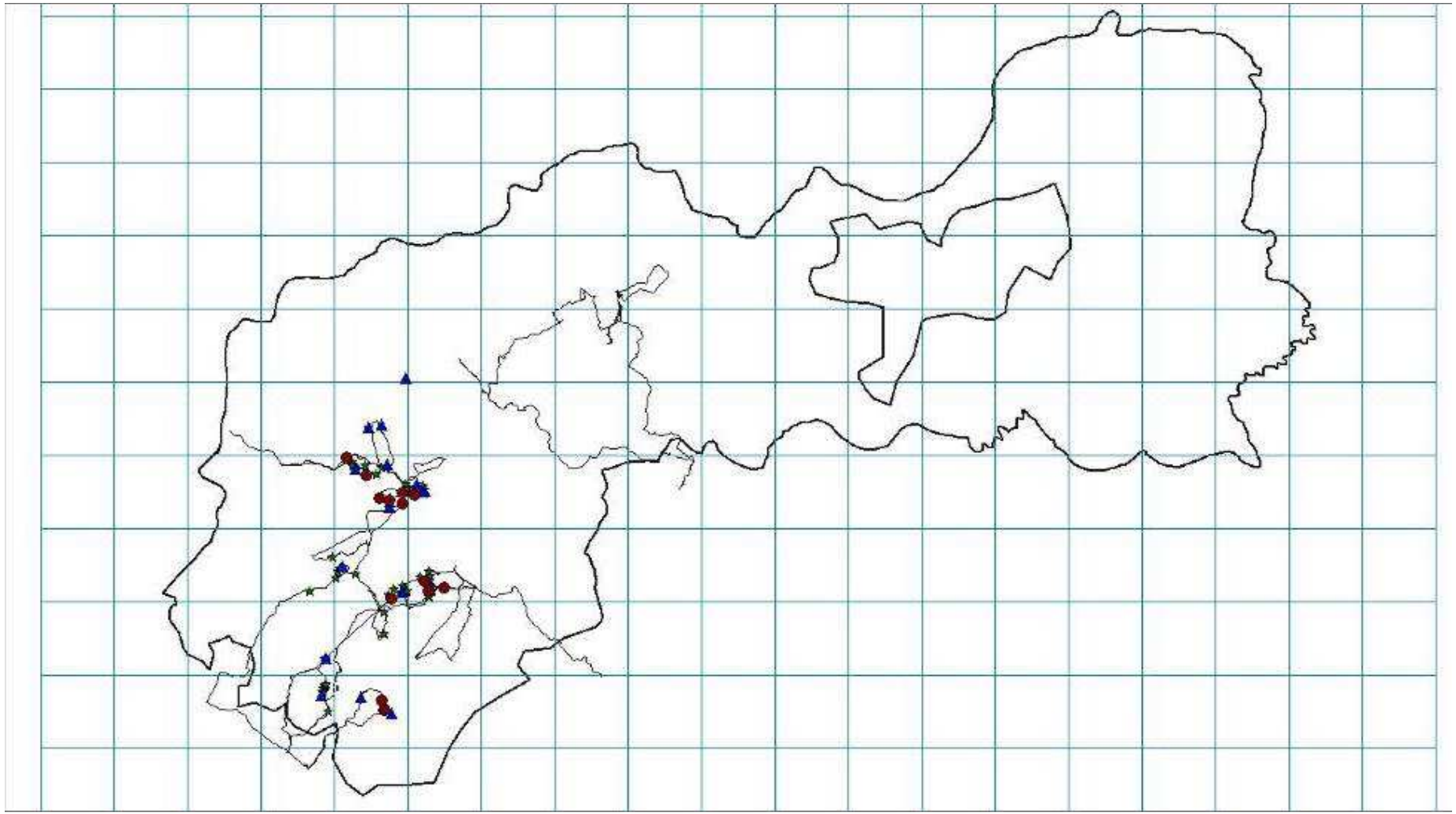

Figure 5. Map of phase 1 of the survey (29/10/09 – 13/12/09); tracks (black lines), Javan rhinoceros dung (red circles), footprints (green star) and wallows/swamps (blue triangle).

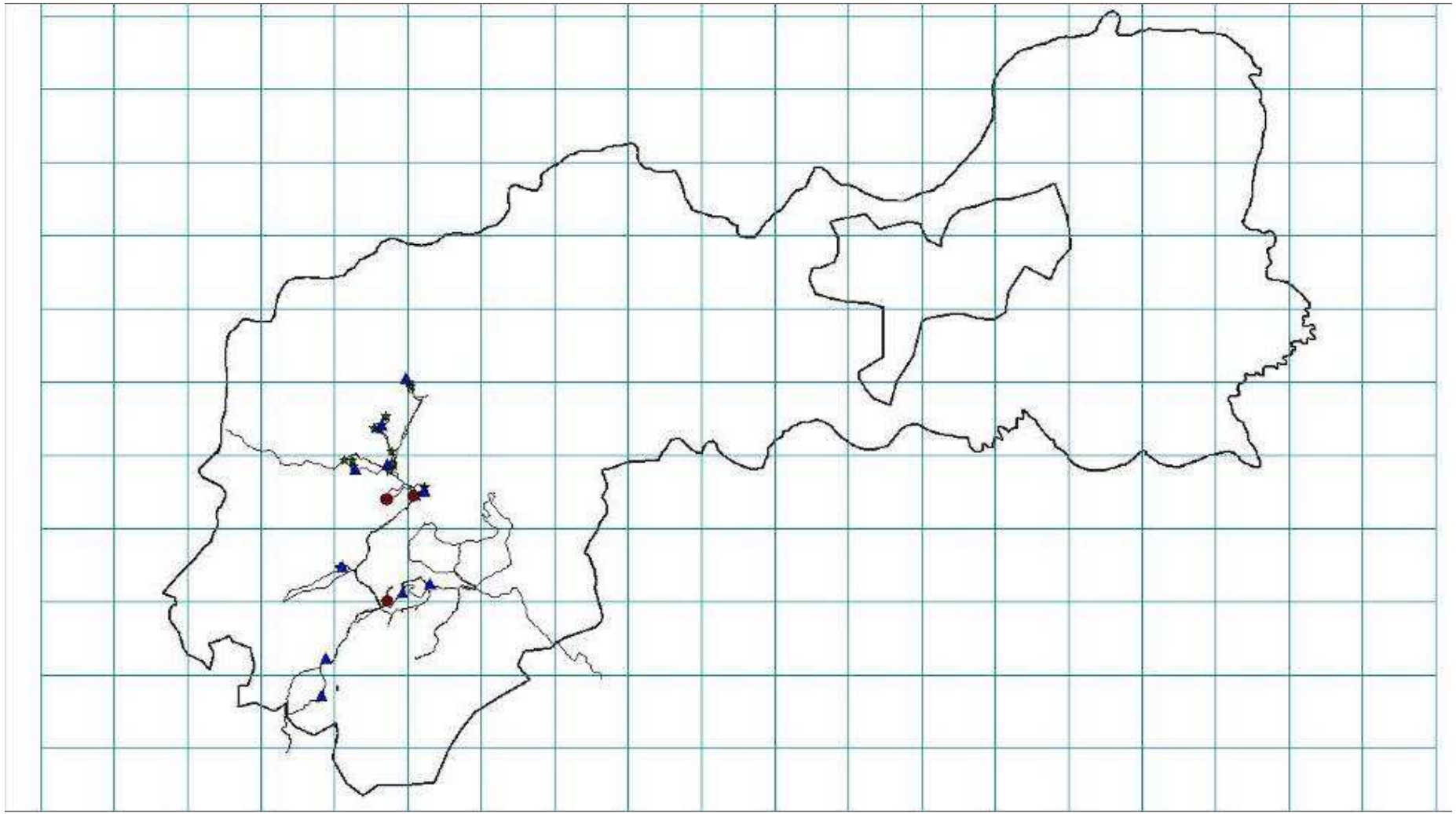

Figure 6. Map of phase 2 of the survey (26/01/10 – 25/02/10); tracks (black lines), Javan rhinoceros dung (red circles), footprints (green star) and wallows/swamps still wet (blue triangle).

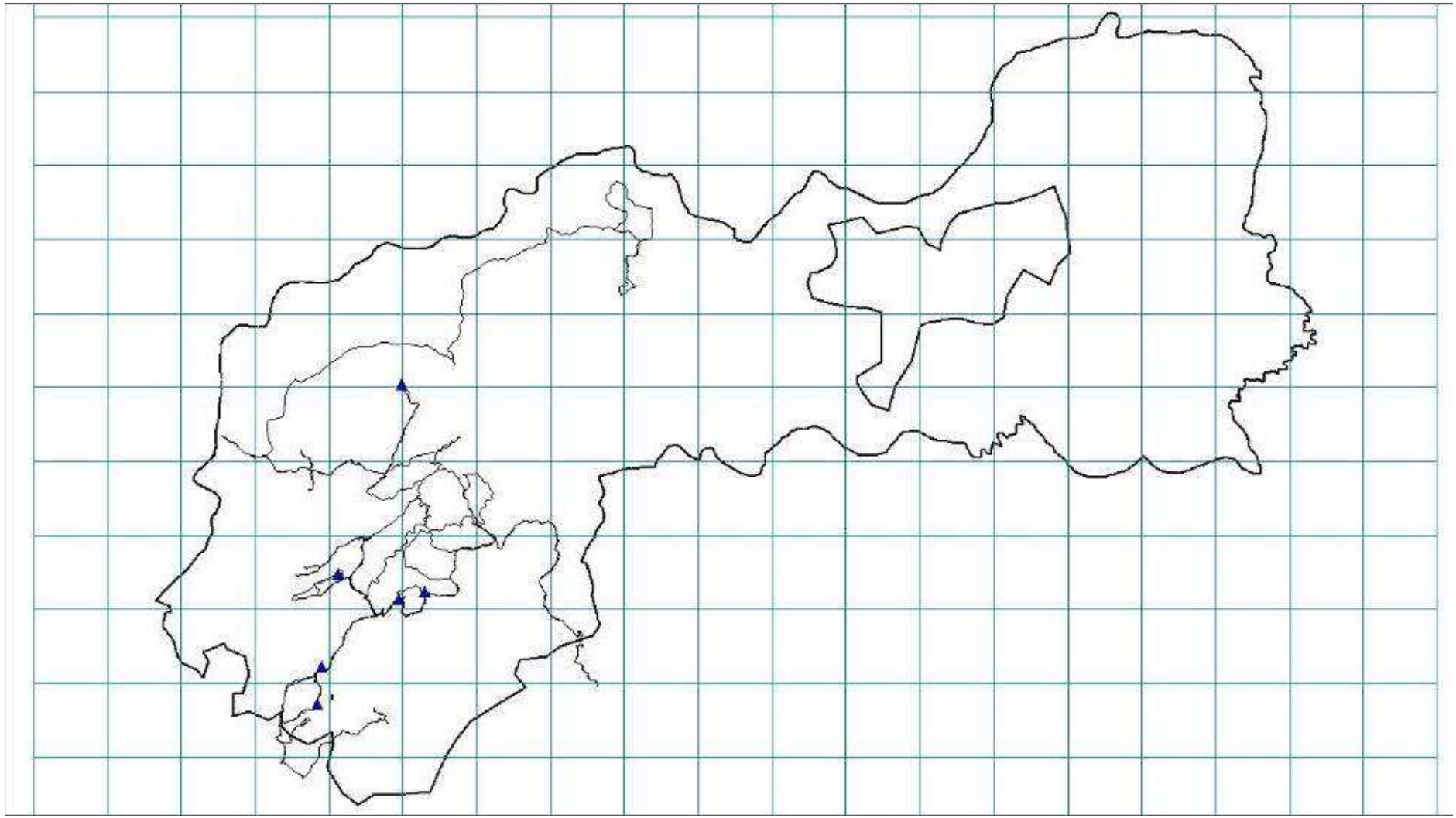

Figure 7. Map of phase 3 of the survey (03/03/10 – 08/04/10); tracks (black lines), wallows/swamps still wet (blue triangle).

Table 2. Javan rhinoceros dung sample collection details.

Date	Sample no.	Easting	Northing	Habitat	Fungus present in dung	Bolus intact	Amplified
27/10/2009	D-1	753396	1287043	Ridge, rattan	Yes	Degraded	Yes
27/10/2009	D-2	753289	1287292	Ridge, bamboo	Yes	Degraded	Yes
27/10/2009	D-3	753369	1287042	Ridge, rattan	Yes	Degraded	Yes
27/10/2009	D-4	753365	1287043	Ridge, rattan	Yes	Degraded	No
27/10/2009	D-5	753367	1287041	Ridge, rattan	Yes	Degraded	No
12/11/2009	D001	753559	1290070	Ridge, rattan, bamboo	Yes	None	Yes
13/11/2009	D003	753857	1292664	Slope, bamboo	No	None	No
18/11/2009	D002	752333	1293920	Ridge, rattan, bamboo	No	None	Yes
19/11/2009	D004	752873	1293464	Ridge, bamboo	No	None	Yes
20/11/2009	D006	754188	1292918	Ridge, bamboo	No	Degraded	Yes
20/11/2009	D008	754186	1292919	Ridge, bamboo	No	Degraded	Yes
20/11/2009	D010	753876	1292990	Ridge, bamboo	No	None	Yes
20/11/2009	D012	753866	1292988	Ridge, bamboo	No	None	Yes
20/11/2009	D014	753501	1292770	Slope, bamboo	No	None	Yes
20/11/2009	D016	753237	1292831	Slope, bamboo, rattan	No	None	Yes
13/12/2009	D018	755001	1290368	Ridge, rattan, bamboo	No	Degraded	Yes
13/12/2009	D020	754464	1290546	Ridge, rattan, bamboo	No	None	Yes
13/12/2009	D022	754587	1290296	Fern swamp	Yes	Intact	No
28/01/2010	D005	753454	1290017	Ridge, bamboo	Yes	Degraded	Yes
28/01/2010	D024	754150	1292890	Ridge, bamboo	No	Intact	No
04/02/2010	D007	753425	1292791	Slope, bamboo	No	Intact	Yes

NB. Samples were also taken from a single additional Javan rhinoceros dung bolus collected by CTNP prior to the beginning of the survey in 2009, not included in this table.

Footprints were largely concentrated around the wallows and swampy areas but this could be an artefact of the season, with footprints not holding in anything but wet muddy ground during the dry season.

In total, between 27th October 2009 and 4th February 2010, twenty-two Javan rhinoceros dung piles were located and sampled and sent for DNA analysis (Figure 4, Table 2). Notably, no new dung piles were found after 4th February, for the last 9 weeks of the survey and no fresh footprints (footprints that had not been recorded by the survey before) were found after mid February (Figure 7).

3.2 Genotyping

The material collected in 2003-2006 was difficult to work with so long after its collection; the success of the amplifications was low after serial dilutions and multiple re-extractions of these faecal samples. Consequently, genotyping the 2003-2006 faecal samples was stopped, to concentrate on genotyping the 2009-2010 Javan rhinoceros faecal samples.

Only one Javan rhinoceros genotype is present in the 2009-2010 Javan rhinoceros faecal samples (Table 3). This genotype matches the genotype of the skin samples collected from the deceased individual found in CTNP.

Different Javan rhinoceros faecal samples have different quality Javan rhinoceros epithelial DNA and so have different amplification success across microsatellite loci. Five samples did not yield any data despite repeated extraction and amplification so they were not analysed further. Of the five samples that did not amplify, they are from four different locations, which are some distance apart. Two were from five dung samples collected at the same time and place at the beginning of the survey in October 2009 (D-4, D-5) and were relatively degraded. The third sample was very degraded (D003); there were no boli and predominantly fibrous material remaining. The fourth sample was found in a swamp (D022), partially submerged in water, which probably removed the epithelial cells. The fifth sample was partially degraded, collected in late January 2010 (D024). All of the samples that did not amplify were found close to several other samples, which did amplify (Figure 8).

Considering the data for 17 samples, a given faecal sample had a 57% of amplifying across all 12 loci. When only samples that amplified at 50% or more loci where considered (dropped six samples) this probability increase to 69%. Not all loci were as reliable as others as on average each locus amplified 53% of samples. When four loci were removed - IR12, WR32A, WR32F and WR35A - this increased to 67%.

Table 3. The genotypes of seventeen 2009-2010 Javan rhinoceros faecal samples that amplified microsatellites after repeated attempts with all 12 loci listed in Table 1. These data suggest a single rhino in CTNP in 2009-2010, a conclusion supported by the bacterial diversity survey of the Javan rhinoceros faeces. The rules for each sample x locus cell are described in the text. Five of the 22 Javan rhinoceros faecal samples collected in 2009-2010 did not work in our microsatellite genotyping assays. The last column reflects the average success rate for a sample over the 12 loci and the last row reflects the average success rate for a locus across 17 samples (see text for details).

Faecal Sample	BR06		DB01		DB44		IR10		IR11		IR12		SR54		SR262		SR281		WR32A		WR32F		WR35A		Across Samples
001[Full]	134	134	126	126	194	194	200	200	132	132	187	187	157	157	114	114	220	220	0	0	0	0	202	202	0.85
002[Full]	134	134	126	126	194	194	0	0	132	132	187	187	157	157	114	114	0	0	0	0	242	228	202	202	0.77
004[Full]	134	134	126	126	194	194	0	0	132	132	187	187	0	0	114	114	228	220	236	232	242	228	202	202	0.85
005 [1/10]	0	0	0	0	194	194	200	200	0	0	0	0	0	0	114	114	0	0	0	0	0	0	0	0	0.31
006[1/10]	134	134	0	0	194	194	0	0	132	132	187	187	157	157	114	114	220	220	236	232	0	0	0	0	0.69
007[1/100]	134	134	0	0	0	0	200	200	132	132	187	187	157	157	0	0	0	0	0	0	0	0	202	202	0.54
008[1/10]	0	0	0	0	194	194	0	0	0	0	0	0	157	157	114	114	0	0	0	0	0	0	0	0	0.31
009[1/100]	134	134	126	126	0	0	200	200	132	132	0	0	157	157	0	0	220	220	0	0	0	0	202	202	0.62
010[1/100]	134	134	126	126	194	194	200	200	132	132	0	0	157	157	0	0	0	0	236	232	242	228	0	0	0.69
012[1/100]	134	134	126	126	194	194	200	200	132	132	0	0	157	157	114	114	220	220	0	0	0	0	202	202	0.77
014[1/100]	134	134	126	126	0	0	200	200	132	132	0	0	157	157	0	0	220	220	0	0	0	0	0	0	0.54
016[1/10]	134	134	126	126	0	0	0	0	132	132	0	0	157	157	0	0	220	220	0	0	0	0	0	0	0.46
018[1/100]	134	134	126	126	194	194	200	200	132	132	0	0	157	157	114	114	220	220	0	0	0	0	0	0	0.69
020[Conc.]	134	134	126	126	0	0	0	0	132	132	0	0	157	157	114	114	220	220	0	0	0	0	202	202	0.62
D1 [1/10]	0	0	0	0	194	194	200	200	0	0	0	0	0	0	114	114	0	0	0	0	0	0	0	0	0.31
D2[1/100]	0	0	0	0	0	0	200	200	132	132	0	0	157	157	0	0	0	0	0	0	0	0	0	0	0.31
D3[1/100]	0	0	0	0	194	194	200	200	0	0	0	0	157	157	114	114	0	0	0	0	0	0	0	0	0.38
Across Loci		0.71		0.59		0.65		0.65		0.76		0.29		0.82		0.65		0.53		0.18		0.18		0.41	

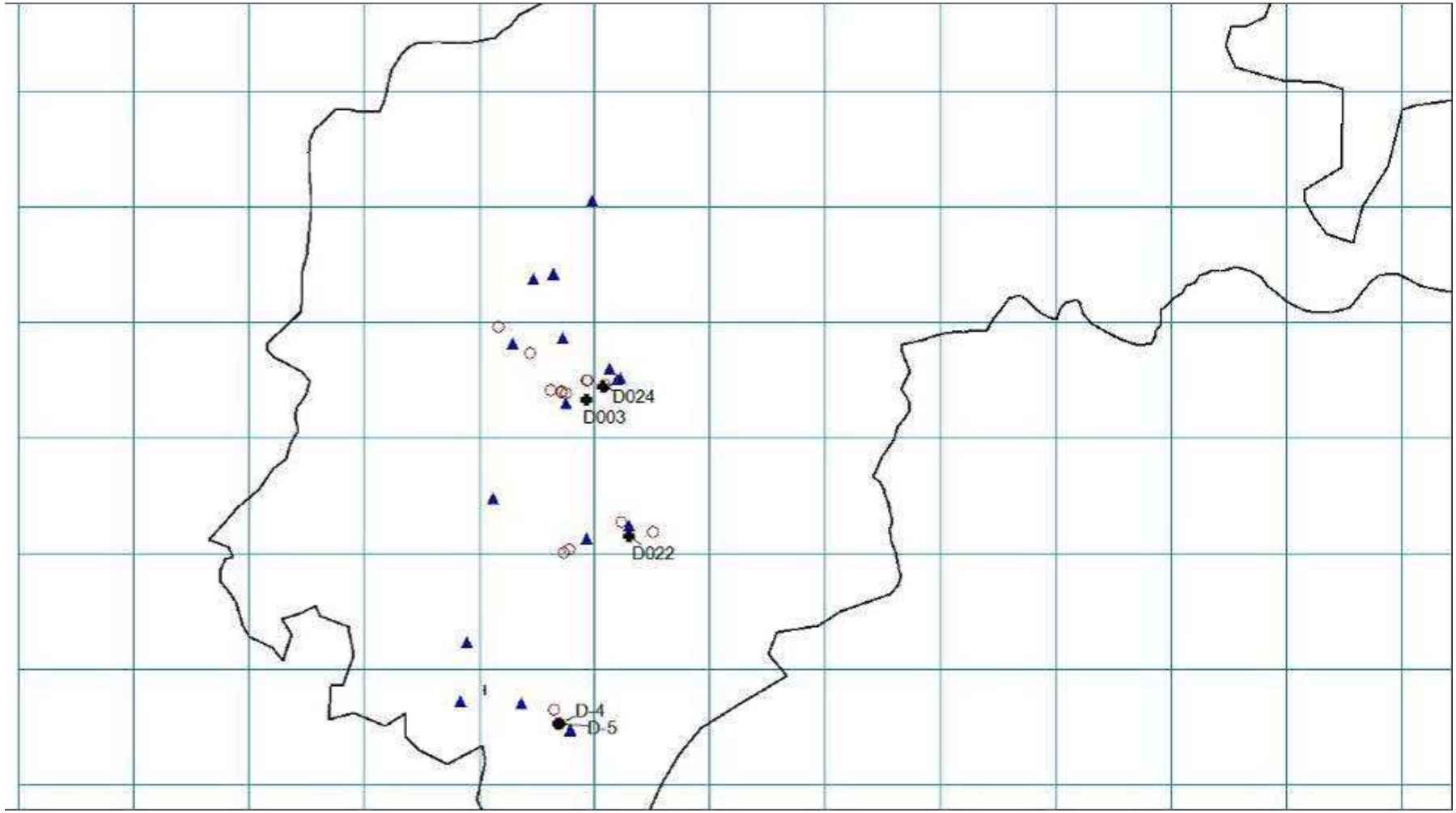

Figure 8. Location of dung samples that did (open circle) and did not (black cross) amplify DNA for analysis, and wallows (blue triangles).

3.3 Genetic sexing

According to the genetic sexing methods of Peppin *et al* (2010), the female sex is indicated by one or both of the lower two bands 95/99 while the presence of the male includes these two and an additional band of 107bp.In all of the six control set samples for Javan rhinoceros, two products of 95/99 were generated. This was the same pattern seen in the females of the black (*Diceros bicornis*), Indian, Sumatran *(Dicerorhinus sumatrensis)* and white rhinoceros. No upper band of 107 as seen in the males of the other species was detected in the Javan rhinoceros samples. This suggests that all of the sampled Javan rhinoceros were female (including the tissue samples from the most recently deceased Vietnamese animal). The faecal extracts that did amplify all showed the female band only. As they are likely all from the same individual, the genotype indicates it was female.

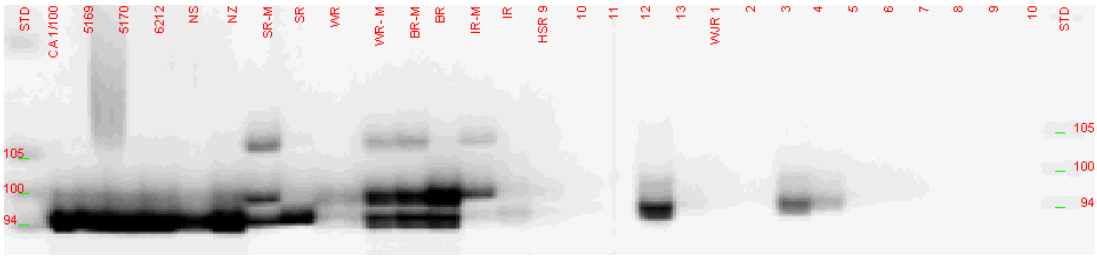

Figure 9. The genetic sexing of Javan rhinoceros samples using the methods of Peppin et al (2010). This analysis indicates that the recently deceased CTNP Javan rhinoceros (CA 1/100) was female. The 94, 100 and 105 base pairs size standards are indicated. The first six samples are 6 of the 7 Javan rhinoceros control set, followed by 2 Sumatran, 2 White, 2 Black and 2 Indian rhinoceros. Then follows 5 Sumatran rhino (HSR9 to 13) and 10 Javan rhinoceros faecal samples (WJR1 to 10). The sex of the rhinoceros if known is indicated (–M). The males of the Black (BR-M), Indian (IR-M), Sumatran (SR-M) and White rhinoceros (WR-M) are clear. The sexing of Javan rhinoceros faecal genotypes was completed using fresh 1:10 extracts from ten 2009-2010 Javan faeces. Two of the Javan extracts amplified with this single pass. To complete a target dataset the PCRs were completed using both undiluted and 1:100 dilutions of DNA extracts.

3.3 Bacterial diversity assay

The findings suggest that a single Javan rhinoceros is present in the 2009-2010 samples and that the 2003-2006 samples are from two animals. Most of the unique 2003-2006 samples form a (blue) cloud except for three samples in the un-weighted analysis (Figure 10) and four samples in the weighted analysis (Figure 11), that are immersed in a the set of unique 2009-2010 samples (green samples). These patterns suggest that between three and four of the samples collected between 2003-2006 are from the animal that was alive in 2009-2010.

This result must be viewed with caution as it is based on a relatively small number of sequences. However other work has shown that the increased number of sequences is not expected to change our main findings (Ley *et al* 2008). Indeed the similarity of the un-weighted (Figure 10) and weighted (Figure 11) analyses suggest the sampling of only 170 sequences may not have led to biased findings as the weighted distance matrix does not appear to have been affected by the high relative proportion of a single sequence.

Storage of triplicate samples of Javan rhinoceros faecal samples for DNA analysis and bacterial diversity assay was optimised in 95% ethanol.

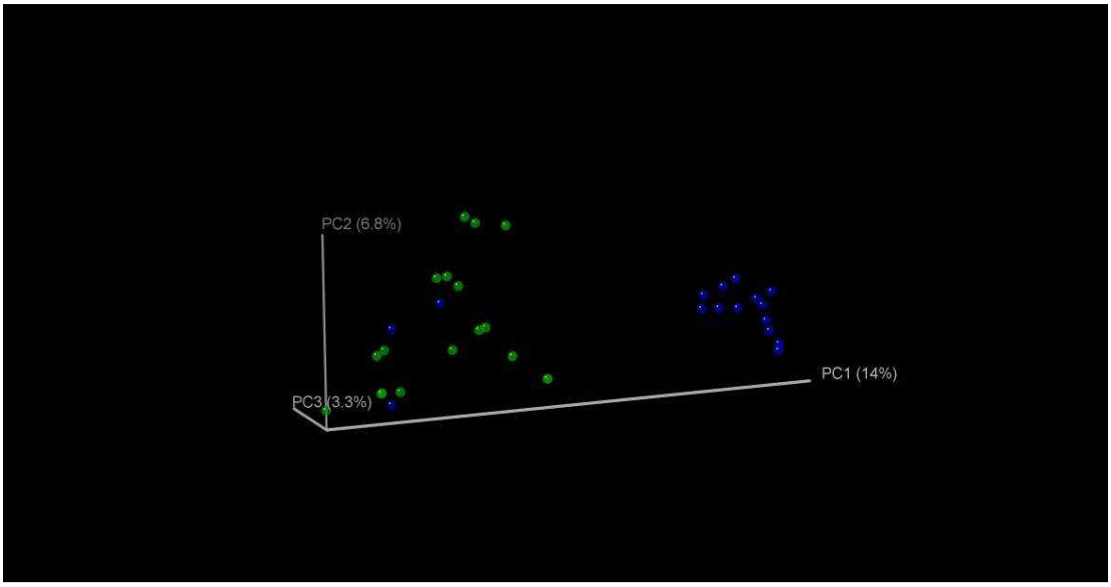

Figure 10. Principal co-ordinate analysis of bacterial diversity of Javan rhinoceros faeces collected in 2003-2006 (blue dots) and 2009-2010 (green dots). The PcoA was conducted on an unweighted matrix. Three of the 2004 faecal samples are closely associated with the 2009 faecal samples suggesting that there was more than one animal alive in 2004 and a single animal alive in 2009.

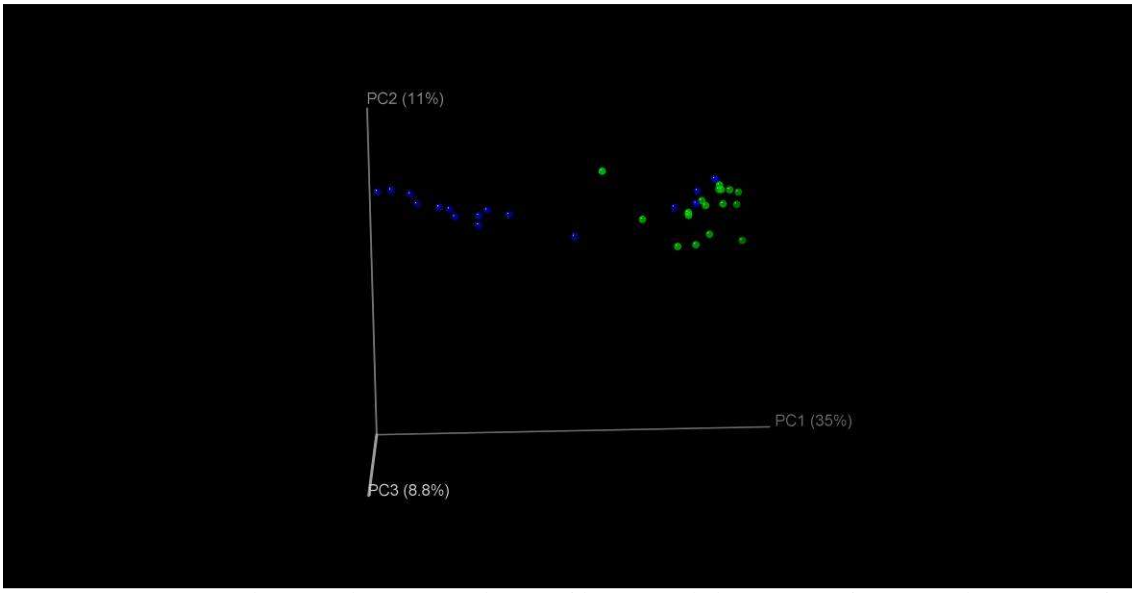

Figure 11. Principal Co-ordinate analysis of bacterial diversity of Javan rhinoceros faeces collected in 2003-2006 (blue dots) and 2009-2010 (green dots). The PcoA was conducted on distance matrix weighted by the relative proportion of each sequence. Four of the 2004 faecal samples are closely associated with the 2009 faecal samples suggesting that there was more than one animal alive in 2003-2006 and a single animal alive in 2009-2010. More variation in this distance matrix is explained by the ordination than in the case of the un-weighted matrix.

4. DISCUSSION

This survey achieved good coverage of the known and possible range of Javan rhinoceros in Cat Loc. Repeat surveys of the rhino core area were conducted, ensuring that the survey effort was sufficient to collect dung samples from a significant area of Cat Loc. The wider area was also surveyed to ensure that no individual was missed by the survey team. No signs of rhinoceros were found outside of the rhino core area at any point during the survey.

The genotyping work, bacterial diversity assays, and the field survey records combined, confirm the extinction of the Javan rhinoceros in Vietnam. Genetic data was extracted from seventeen of the twenty-two faecal samples (77%) collected by the survey teams; the remainder were likely too old and decomposed from which to extract DNA. The genetic work identified that all of these seventeen faecal samples had the same genotype and that this matched the genotype of the skin samples which were taken from the Javan rhinoceros found dead in CTNP in April 2010. As the survey effort and coverage were sufficient, all amplified samples were from the same individual, and the genotype matched that of the rhinoceros found dead in April 2010, we confirm that there are no rhinoceros remaining in CTNP.

The bacterial diversity profiles provide support for this conclusion, with principal co-ordinate analysis identifying only a single Javan rhinoceros in 2009-2010. Interestingly, from this work it appears that this and at least one other Javan rhinoceros was alive when the other samples were collected in CTNP, from 2003-2006. This suggests that another individual has been lost from CTNP between that time and prior to the beginning of the WWF survey in 2009, the remains of which have never been found.

The field survey data provides yet further evidence that all of the samples belonged to only one individual, the individual that was found dead in CTNP in April 2010. The teams repeatedly surveyed Javan rhinoceros 'hotspots' of activity such as wallows, swamps and streams, throughout a 6-month period. All twenty-two dung samples were collected in the first four months and no new dung samples or footprints were found after 4th February 2010, even though prior to this these were recorded on a regular basis. Although a pathological examination could not determine precisely when the animal died (Streicher *et al* 2010), due to the absence of most of the skin and soft tissue which had already decomposed, it is therefore suspected from the field data that the last rhinoceros died in late January/early February 2010.

To ensure that the rhinoceros had not simply moved out of the core area, the teams surveyed the wider area to search for tracks and signs targeting potential hotspots, but no signs of rhinoceros were found. By that time of year, most of the swamps and streams in the wider area were almost or completely dry; since Javan rhinoceros require wallows and swampy areas, it is very unlikely that the rhinoceros would be able to inhabit the wider area in the dry season. In the past, rhinoceros used to be recorded in this area, but there have been no records of this since access to the Dong Nai River has been restricted and the forest over the other side of the river was converted to agricultural plantations. Furthermore, anecdotal reports from local people indicate that the rhinoceros population of Cat Loc rarely leave the core area, with very few confirmed records outside of the core area since 1999.The Javan rhinoceros has not been recorded outside of Cat Loc for more than 20 years; little opportunity remains for the rhinoceros to inhabit other areas, with much of the surrounding habitat to the north, south and west of the protected area converted to agricultural land and urban areas.

Consequently, we are confident in reporting that the death of the individual Javan rhinoceros in 2010 represents the extinction of Javan rhinoceros in Vietnam and of the *annamiticus*

subspecies. The only remaining population of Javan rhinoceros survives in Java, Indonesia. The protection and expansion of this population is the utmost priority for conservation of this critically endangered species.

The extinction of the Javan rhinoceros in Vietnam is a major conservation failure. When the subspecies was rediscovered in 1988, the population was estimated at up to 10-15 individuals (although this was probably an over-estimate), and adequate habitat remained. Javan rhinoceros home range size is estimated to be no more than 500ha for females and larger for males (van Strien *et al* 2008). Cat Loc is expected to be sub-optimal habitat for the species so this home range size is likely to be an underestimate, however, 75,000ha of habitat in 1988 could have supported a sizeable population. Had the rhinoceros and its habitat been preserved, it may have been possible to affect a population recovery in the style of the Indian (*Rhinoceros unicornis*) and African rhinoceros species through an intensive management programme. The southern white rhinoceros (*Ceratotherium simum simum)* was on the brink of extinction by the late 19th century, reduced to one small population of approximately 20 individuals. However, more than 100 years later, due to effective protection and translocations of individuals to establish new populations, the southern white rhinoceros now numbers close to 20,000 individuals (IUCN 2010).

In 2009 and 2010, with only one individual remaining in CTNP, which was subsequently poached, it was far too late to implement intensive population management to try to save the subspecies. The results of this work have shown that there were at least two individuals alive in 2003-2006 however, and undoubtedly there were more between 1988 and 2003. Regardless of the population size, the recommendations of conservation groups from 1988 onwards, to protect the population and conduct intensive habitat management were still valid, but never carried out sufficiently in Cat Tien National Park. Ultimately, conservation efforts were inadequate to prevent the extinction of the Javan rhinoceros in Vietnam; below we discuss the two driving forces behind the loss of the subspecies.

4.1 Poaching

The loss of the last individual Javan rhinoceros from Vietnam, probably as a result of being shot (Streicher *et al* 2010), highlights the lack of effective protection within Cat Tien National Park. CTNP is a well-funded national park with a relatively large number of staff; theoretically a good standard of protection should be possible from a human resource point of view. WWF and other organisations have supported building CTNP staff capacity in law enforcement, and the establishment of a good monitoring system for Javan rhinoceros and other key species.

The CTNPCP provided significant support for the improvement of law enforcement and patrolling, including a comprehensive training course in 2003 for all park rangers in implementing more effective patrolling techniques, and a refresher course in 2004. The law enforcement consultant involved in this work concluded that although trainees retained the knowledge gained from the training course, some significant recommendations were not implemented on the ground (such as achieving good patrolling coverage of Cat Loc). A lack of law enforcement management capacity and supervisory presence were thought likely to hinder the effectiveness of patrolling in CTNP (Havemann 2004).

WWF supported enforcement and monitoring patrols during the CTNPCP in Cat Loc by park rangers, and also in 2005 and 2006 following the conclusion of the CTNPCP, however the level of patrolling declined during this time. WWF again attempted to increase protection for the rhinoceros population from July 2009 and provided support for monthly patrols by 3 CTNP guard stations within the rhino core area in Cat Loc. Patrolling methodology was

agreed with CTNP, funding was provided and the implementation of the patrols was monitored by the transfer of GPS tracks for each patrol to WWF.

Unfortunately, the patrolling in CTNP was not implemented to the standard required. GPS tracks were not provided to WWF on a monthly basis, often being sent several months later, making it difficult to monitor the implementation of the project. When data were received it was clear that the teams were not achieving sufficient coverage within Cat Loc and were not patrolling for the minimum time stipulated, or even at all in some months (Appendix 6). This was a consequence of a lack of management and supervision of the rangers from CTNP headquarters. Rangers were not held accountable for not maintaining patrols, or for failing to provide protection for the Javan rhinoceros population.

The insufficient enforcement effort during the period 2009-early 2010 of course cannot be attributed to the population decline over several years and the final extinction of the rhino in Vietnam, however, it clearly illustrates the challenges faced in achieving the appropriate levels of protection in CTNP over a period of many years. This lack of basic protection for the rhinoceros and other wildlife in Cat Loc, where hunting pressure is very high, ultimately resulted in the extinction of the Javan rhinoceros in Vietnam. These issues are not unique to CTNP but are easily demonstrated here owing to the plight of the Javan rhinoceros population. This case demonstrates that there needs to be minimum standards (e.g. patrolling coverage, patrolling length and frequency etc.) for protection in parks and reserves where high-value species such as rhinos, tigers, elephants, or turtles are concerned. The high levels of threat to wildlife in Vietnam are clearly not being adequately dealt with by the authorities charged with the protection of wildlife.

4.2 Habitat Loss

Since the discovery of the Javan rhinoceros in Vietnam in 1988, the range of the species has steadily declined from circa 75,000ha (including Nam Cat Tien) in 1988, to approximately 6,500ha of Cat Loc in 2010. A significant area of Cat Loc was excised from CTNP following its conversion to agricultural land and development of urban areas. Although there are more than 6,500ha of habitat remaining in Cat Loc, the Javan rhinoceros population appeared to be restricted to this small area due to human disturbance from the use of dirt-roads, and development of settlements and agricultural land around the edge of the rhino core area.

The short-term target of the national action plan for Vietnamese rhinoceros of 'Extension of the secure rhinoceros habitat to at least 15,000ha in 5 years time and a proportional increase in number of rhinoceros between 2000/2010' was never achieved (IUCN AsRSG 2000). The expansion of agricultural areas around and into the national park was an ongoing problem not effectively dealt with. Although the resettlement of two villages inhabiting the rhino core area to outside of the park was successful in reducing some of the pressures and disturbance, they continue to harvest cashews from this area and two villages remained and continue to rely on Cat Loc to some level for natural resources extraction. In addition nearly 200,000 people live in the buffer zone of the park, a significant number of which also undertake some level of natural resource extraction from the park (Polet *et al* 2003).

Although CTNP planned to buy back some of the cashew plantations within the rhino core area, government funds are yet to be allocated to allow them to do this. Encroachment and expansion of agricultural plantations into the rhino core area and around the edge of the national park is commonplace on a small scale in many locations (Appendix 5). Existing plantations are typically expanded by a few metres per year, to avoid aversive action by the authorities.

Consequently, considering the insufficient political will to support the protection of the Javan rhinoceros and its habitat, the long-term prospects for the Javan rhinoceros population were unlikely to have been good even if the population was still extant. The proposal several years ago to connect Cat Loc to Nam Cat Tien (which is believed to be better quality habitat for Javan rhinoceros) via a corridor would have provided ample habitat for the rhinoceros population. However, this was not achieved and the lack of available habitat in Cat Loc due to infrastructure development, disturbance and an expanding human population and agricultural land, would have eventually limited population growth. It should be noted that in India, Kaziranga National Park covers 42,900ha (which is considerably smaller than all of CTNP) and with strong protection and effective park management, it supports the largest population of Indian rhinoceros, of more than 2,000 individuals.

4.3 A common problem

The issue of inadequate law enforcement within protected areas, whether related to prevention of poaching or habitat loss and encroachment is by no means unique to Cat Tien National Park, or to Vietnam. Reports from organisations working within protected areas throughout the country highlight that this national issue of uncontrolled illegal poaching to supply the commercial wildlife trade is the major threat to biodiversity in Vietnam, and improved protection and law enforcement is the most important solution to this crisis (BirdLife *in Indochina* 2008, Le Trong Trai *et al* 2008, Nadler *et al* 2003).

The fate of the Javan rhinoceros highlights a much larger problem; many other species are on the verge of extinction in Vietnam, a large proportion of which are endemic to Vietnam or the region, and will almost certainly be lost without better protection and management of protected areas. Hog deer (*Axis porcinus*) is almost certainly extinct in Vietnam due to widespread habitat loss and high hunting pressure (Timmins *et al* 2008). Saola is critically endangered, with a highly dispersed and fragmented population, estimated to be no higher than the low hundreds in total. The saola's decline is due largely to hunting and to a lesser extent habitat loss (Timmins *et al* 2008; Ming Hoang *et al* 2004). Tonkin snub-nosed monkey, endemic to Vietnam, has a significantly reduced range, restricted to only a few areas in northern Vietnam. There are thought to be 250 individuals remaining, due to massive deforestation and intensive hunting pressure (Xuan Canh 2008), similar to the plight of many other primates in Vietnam. Other species, which in the past shared swamp forest with the Javan rhinoceros, are already extinct or almost extinct in Vietnam due to the widespread loss of this habitat (Wege *et al* 2000). The white-shouldered ibis is extinct as a breeding species in Vietnam (individuals or small flocks might occasionally visit from neighbouring Cambodia) as a result of habitat loss and hunting (BirdLife International 2011). Siamese crocodile was hunted to extinction in Vietnam but was reintroduced by WWF under the CTNPCP; the species survives in Bau Sau lake; the only place where they are adequately protected due to the presence of a ranger station and significant numbers of tourists which deters hunters.

More widespread species are also declining in Vietnam. Asian elephant has been reduced to very small and isolated populations in the central and southern parts of Vietnam and are being persecuted where they remain (Choudhoury *et al* 2008). The tiger population is estimated at fewer than 30 individuals in Vietnam, a direct result of hunting for wildlife trade (Chundawat *et al* 2010). Gaur is reported to be in serious decline in Vietnam (Duckworth *et al* 2008) and banteng are in a similar situation, having been lost from many sites in which the species formerly occurred (Timmins *et al* 2008).

The widespread decline of Vietnam's wildlife populations is fuelled by an increasing demand for wildlife in the traditional medicine trade in Vietnam, China and other parts of Asia, and

the domestic wild meat trade. With Vietnam's rapidly growing urban middle and upper classes, consumption of wildlife, which is seen as a symbol of status and wealth (Wyler and Sheikh, 2008; TRAFFIC, 2007), is becoming more available for a greater proportion of society. Vietnam is also known as a major trader of wildlife in the region and internationally. As Vietnam's wildlife is depleted, traders are travelling further afield to source highly valued animals such as rhinoceros, tigers, turtles and pangolins to satisfy demand. Rhinoceros are perhaps the most highly prized species, with their horns fetching up to $100,000 per kg. Throughout their African and Asian ranges, all species of rhinoceros are facing increasing pressure from poachers, targeting the animals for their horns. Several seizures and other incidents indicate most of these horns, particularly those from southern Africa, are being smuggled to buyers in Vietnam.

Given the tremendous pressures on rhinoceros and other highly valued species for the commercial wildlife trade, protection of these species requires intensive site-based protection and law enforcement, which is focused on these species to prevent their extirpation. The levels of patrolling undertaken by CTNP were clearly not adequate to protect the rhinoceros population and even under the CTNPCP, protection levels could have been improved. WWF standards now suggest that a minimum of 16 days per month should be spent on patrol, achieving good coverage of the area is very important and a number of patrolling tactics should be used to combat the threats posed by hunters with guns, dogs and snare traps, all of which were encountered in Cat Loc.

4.4 The role of WWF and other conservation organisations in CTNP

Given the sub-standard uptake of enforcement techniques within CTNP following training from a law enforcement expert, and the priority to provide high levels of protection for the rhinoceros population, it was perhaps a little premature for WWF to discontinue its presence in CTNP following the completion of the CTNPCP. Although small amounts of funding were provided from 2005-2006 to continue providing support for rhinoceros enforcement and monitoring patrols, without supervision from WWF this was being implemented to a lower standard than under the CTNPCP. By the time WWF had an on-site presence for the rhinoceros survey in 2009/2010, there was little interest from CTNP staff in adhering to technical advice on patrolling and enforcement from WWF. Had WWF maintained their advisory presence on site throughout the 2000's, the implementation of patrolling in Cat Loc may not have declined.

Furthermore, the failure to establish a headquarters for rhinoceros conservation within Cat Loc itself probably led to the neglect of Cat Loc as a management unit, in comparison to Nam Cat Tien where the CTNP headquarters are based. The rangers in Cat Loc operate with little supervision from the headquarters and as such do not have the incentive to patrol; they are not monitored sufficiently or held accountable for poor performance. WWF and CTNP could have cooperated to establish a management unit within Cat Loc itself, with a member of WWF staff permanently based there to provide technical advice and supervision of protection and monitoring activities.

Having a separate management unit in Cat Loc may have increased political support from Lam Dong Province in conserving the rhinoceros population, who were wary of supporting conservation actions such as village resettlement. Cat Loc is located in Lam Dong Province and is therefore this province's responsibility, but CTNP falls under the jurisdiction of Dong Nai Province. A lack of coordination and differing agendas between provinces hindered the implementation of conservation actions for the Javan rhinoceros.

5. CONCLUSIONS

Vietnam is facing an extinction crisis due to the largely uncontrolled illegal wildlife trade and rampant, ubiquitous poaching of wildlife. Current protected area management practices and conservation interventions have proved inadequate for dealing with this threat. The extinction of the Javan rhinoceros from Vietnam is a direct result of this inadequate protection and protected area management from all parties involved in its conservation.

This extinction was the result of a number of failings that are indicative of the conservation challenge in Vietnam. There was insufficient political support to secure adequate habitat, prevent encroachment, and protect the remaining rhinoceros from hunting. Although Cat Tien National Park is a relatively well funded protected area, as is typical for National Parks in Vietnam a much greater proportion of government funding is spent on activities such as infrastructure development, than on addressing threats to the protected area. Moreover, there is little or no accountability of rangers, their managers, and protected area managers, which means that protection efforts are mostly ineffective within the current protected area management system in Vietnam.

Significant investments in CTNP by international organisations have attempted to address these issues, with the Javan rhinoceros as the flagship species for many projects. However, as this report has shown, these measures ultimately failed. The failure to conserve this population should act as a warning that the mode of operation for such organisations investing in protected areas has typically not been effective in Vietnam, especially when management responsibility and accountability for the conservation intervention is unclear.

With a rapidly growing demand for wildlife and wildlife products and the increasing sophistication of poaching and trading, site-based protection of species and their habitats needs to be prioritised and dramatically improved. Wherever the Government of Vietnam requests the support of international or other non-government organizations, more consideration should be given to the role of each partner and to respective accountabilities if wild species conservation in Vietnam is going to be effective. And irrespective of if there is international support or not, conservation investments in protected areas need to focus on major improvements in protection and enforcement of laws against poaching, trading and selling of wildlife. So far, the scale of the response has not matched the scale of the threats to species. Without these changes there is a very high probability that other species will soon share the same fate as the Vietnamese Javan rhinoceros.

Specifically, WWF makes the following recommendations for improved protection and law enforcement in all protected areas in Vietnam, and especially in protected areas where other critically endangered species populations remain:

1. Increase the number of trained forest rangers patrolling in all protected areas;
2. Increase or reallocate budgets to ensure adequate patrolling operations;
3. Adopt and implement the internationally-used *MIST* law enforcement patrolling; monitoring and management system across the whole protected area network;
4. Establish a national system of protected area management accountability that manages the performance of protected area Directors and staff.

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Appendix 1. Locus name, amplified repeat motif, forward and reverse primers, annealing temp [Tm].

Whether the primer has a influorescent tag at the 3' end of the forward (F) primer – tagged – or has to have an influorescent tail added into the PCR - tailed – which adds 19bp to the product is indicated. Db44 and SR 262 preform best when combined as a single multiplex reaction. The PCR cycles and cocktails are indicated in Appendix 2 and 3 respectively. (1 = Brown & Houlden 1999. 2 = Cunningham *et al* 1999. 3 = Scott, Van Coeverden de Groot & Boag unpublished. 4 = Scott *et al*. 2004. 5 = Florescu *et al*. 2003).

Locus	Repeat Motif	Direction	Primer	Tm	
BR06 [1]	(CA)15	F	TCATTTCTTTGTTCCCCATAGCAC		
		R	AGCAATATCCCACGATATGTGAAGG	58	tagged
DB01 [2]	(CA)14	F	AGATAATAATAGGACCCTGCTCCC		
		R	GAGGGTTTATTGTGAATGAGGC	58	tagged
DB44 [2]	(CA)4g(CA)16	F	GGTGGAATGTCAAGTAGCGG		
		R	CTTGTTGCCCCATCCCTG	58	muliplex
DB52 [2]	(CA)21	F	CATGTGAAATGGACCGTCAGG		
		R	ATTTCTGGGAAGGGGCAGG	58	tailed
IR10 [3]	(CA)22	F	CAGTGAGGAAGATTGGTTGC		
		R	CCTGACTCACACATCACCAG	58	tagged
IR11 [3]	(GT)12	F	CATCCATCACCTCACATAGTTAC		
		R	GCATGGCGACTACGATTAAC	58	tailed
IR12 [3]	(CA)18	F	GAATGCTGATCATTTAGTGAC		
		R	GGGTCCAGTTGAGATATCAC	58	tagged
JR003 [3]	(AC)37	F	TCTGGTTCTATAGTGGCAGCAC		
		R	GTATGGACCAGATGCTGCAA	TU	tailed
JR049 [3]	(AG)8a(AG)aa (AG)2	F	CAATATCCGATTCCAATTGATG		
		R	GGAAGGTGATGGTATCTTCGAG	TU	tailed
JR088 [3]	(AG)21	F	GGAGATGGGAGAATGACGAA		
		R	TGCAAAAGACAGCCACAAAC	58	tagged
JR106 [3]	(TC)2tt(TC)t(TC)8	F	CGTTCGATCAAGTGGAAGGT		
		R	CCAACATGGACTCAGCAGTTAG	TU	tagged
SR54 [4]	(CA)26	F	CAATATCCAGGCTTCCAGG		
		R	CTGTTTACTGTTATCGATGCTC	58	tagged
SR63 [4]	(AC)19	F	CTTGAGCAGAGTAGAATTTGG		
		R	CTCTGTATCCACCTCATTCC	58	tagged
SR 262 [4]	(GT)28	F	CTGCCTTAACAACTGAACTGC		
		R	TGGAGGTTATCTCATGCCAC	58	tagged
SR268 [4]	(CA)25	F	GTTTATACTATGCCCTGCAC		
		R	GGATGCTACCGAATAGATTG	58	tagged

Locus	Repeat Motif	Direction	Primer	Tm	
SR281 [4]	(GT)23	F	AGGTGATTAGGGAATTGCTGG		
		R	TTCTTCTGTCCTGGCATTGC	58	tagged
WR35A [5]	(CA)20	F	AGCCTGCTTTGCTGCCTTGC		
		R	AGGTGCACACATCCCACTCG	58	tagged
WR32A [5]	(CA)15	F	CCTGGTGGTTGAGCACTG		
		R	GCTGAGGGAATGACAGAAGG	58	tagged
WR32F [5]	(CA)17	F	CTGGAAATGGAAACCCCGAC		
		R	GCACACTCCATCGGACTGTC	58	tagged

Appendix 2.The two PCR cycles for the amplification of microsatellite DNA from Javan faecals using a Biomtera T-Gradient machine.

Annealing Temp 58°C	Touch Up PCR
Lid – 110°C 94°C – 5 minutes 94°C – 20 seconds 58°C – 30 seconds 72°C – 30 seconds 72°C – 10 minutes 35cycles	Lid- 110 94°C - 5 minutes 94°C - 20 seconds 50°C up to 58°C - 30 seconds 72°C - 40 seconds Repeat 16 times increasing by 0.5°C each cycle 94°C – 20 seconds 58°C – 30 seconds 72°C – 30 seconds 72°C – 7 minutes 24 Additional cycles at 58°C

Appendix 3.Components of a typical PCR used in this study:

1.2uL DNA
7.9uL dH2O
1uL 10x PCR Buffer (final concentration of 1x)
0.015uL dNTP's(final concentration of 0.15mM)
0.015uL of each Forward and Reverse Primers(final concentration of 0.15uM)
0.25U of Taq
0.2uL M13 Tag

Appendix 4.Photographs of the Javan rhinoceros survey

Swamp forest

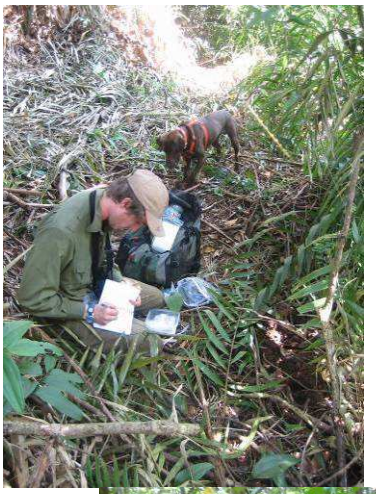
Wallow

Wallow

Semi-deciduous forest

Bamboo stream

Ridge top forest, hillside of rattan and wallow.



Sampling dung

Detection dog Chevy with rhinoceros dung

Javan rhinoceros footprints

Javan rhinoceros skeleton Javan rhinoceros skull with horn removed

Appendix 5. Photographs of threats to Cat Loc and the Javan rhinoceros population

Many snares were removed by the survey team, including this large animal snare removed by Bach Thanh Hai

Traps (in baskets) on sale in nearby town.

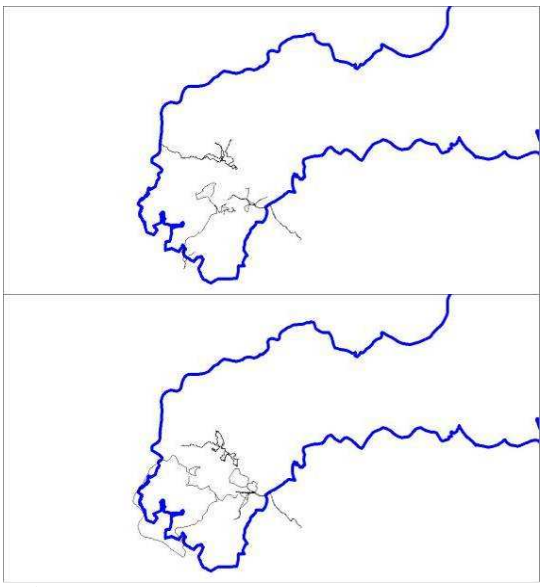
Hunting camp destroyed by survey team.

Illegal conversion of forest to agricultural land within the core zone of Cat Loc.

Illegal forest conversion.

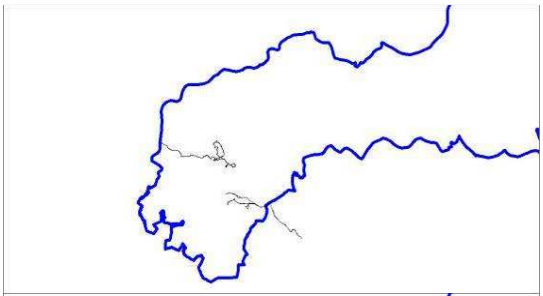
Forest loss to agricultural land and infrastructure zdevelopment (new road in background)

Appendix 6.Maps of patrolling coverage of Cat Loc by the Forest Protection Department, CTNP.

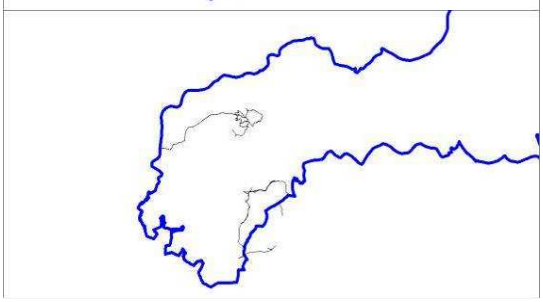


July 2009

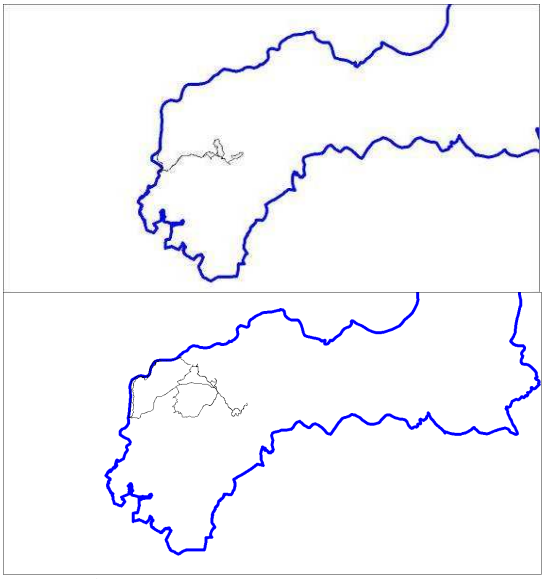
August 2009



September 2009

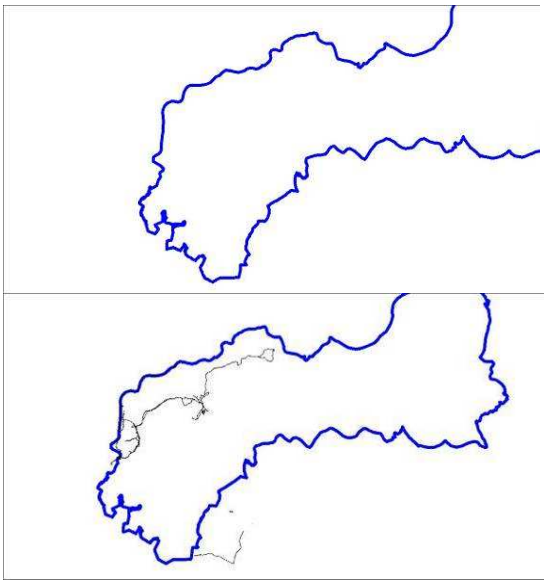


October 2009



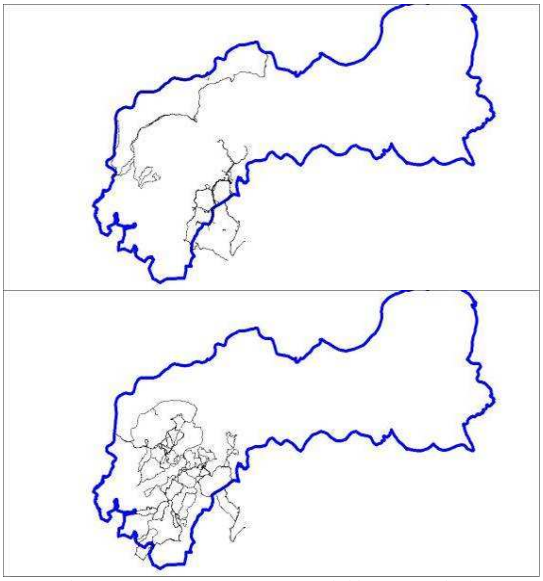
November 2009

December 2009 (motorbike tracks)



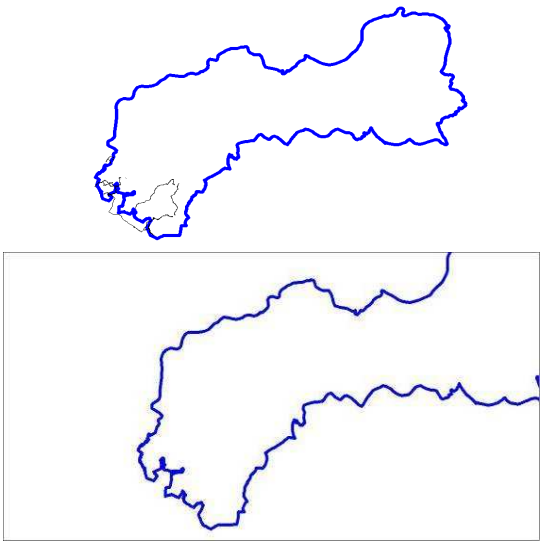
January 2010

February 2010 (motorbike tracks, not in core area)



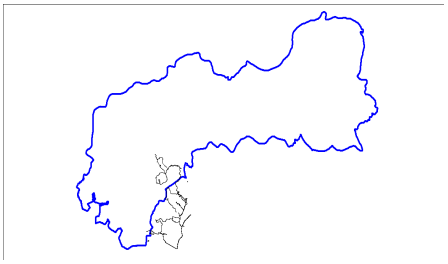
March 2010 (some motorbike tracks)

April 2010 (with WWF supervision)



May 2010

June 2010



July 2010