

**ASSESSING MAMMAL BIODIVERSITY IN FOREST FRAGMENTS OF THE  
ASIAN TROPICS**

by

Sarah Weiskopf

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Wildlife Ecology

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ASIAN TROPICS**

by

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## ABSTRACT

Deforestation of tropical rainforests is one of the greatest current threats to biodiversity. Asian mammals have been particularly threatened by land use change, yet we lack basic information about many species. This lack of information is a major obstacle to effective conservation, and is driven in part by the high cost and effort required to survey tropical mammals. In the first chapter of this thesis, we used camera traps to investigate the mammalian use of five small forest fragments outside Bukit Barisan Selatan National Park in Sumatra, Indonesia, and compared it to camera trapping which was carried out inside the national park. We found that a significant number of forest species were utilizing the fragments, including endangered species such as the Sunda pangolin (*Manis javanica*) and the Sumatran tiger (*Panthera tigris sumatrae*). The high level of biodiversity found in these fragments suggests that these areas may be of conservation importance, and may increase connectivity across the landscape. In the second chapter of this thesis, we addressed the difficulties of tropical mammal surveys by examining a novel survey technique: genetic sequencing of leech blood meals. We used Sanger sequencing of the 16s rRNA gene to identify recent mammal host species of leeches collected from four forest patches in northeast Bangladesh. We then compared these data to camera trap data obtained from the same locations. We identified a greater number of species in the camera traps than in the leeches; however, leeches may be able to provide a more precise picture of biodiversity in the study area. After comparing the effort, cost, and power associated with each method, both methods have pros and cons. Used together, these methods may provide a more complete monitoring tool for mammal biodiversity in tropical rainforests.

## Chapter 1

### CONSERVATION VALUE OF FOREST FRAGMENTS IN THE INCREASINGLY AGRARIAN LANDSCAPE OF SUMATRA

#### Abstract

Throughout the world, tropical rainforests are being destroyed at an alarming rate, reducing many unprotected habitats to small fragments of remnant forests within the cleared landscape. Understanding the best way to provide habitat for wildlife in the midst of changing landscapes is essential for land use planning, especially in biodiversity hotspots such as Indonesia. However, we still know very little about the conservation significance and mammalian use of forest fragments in Sumatra. In this study, we conducted camera trap surveys within Bukit Barisan Selatan National Park, and within five surrounding forest fragments, and used species composition metrics to compare their use. We found 28 species of mammals in the forest and 21 in the surrounding fragments. The fragments harbored a subset of the species found within the primary forest, as well as several species not observed in the forest. Critically endangered species such as the Sunda pangolin (*Manis javanica*) and Sumatran tiger (*Panthera tigris sumatrae*) were found in the fragments, as well as species of conservation concern such as the marbled cat (*Pardofelis marmorata*) and the Asiatic golden cat (*Pardofelis temminckii*). The biodiversity found within the fragments suggests that these small patches of remnant forest may be of conservation value to certain mammal species, and may facilitate connectivity across the landscape.

## Introduction

Deforestation of tropical forests for agricultural land use is a growing problem throughout the world. Sumatra, Indonesia, which contains about 10% of the world's remaining tropical rainforest, is not exempt from this pattern. Although about a quarter of the island is part of a network of protected areas (Gaveau et al. 2012), deforestation still takes place within the boundaries of protected areas, as well as in the surrounding landscape (Gaveau et al. 2009). This was especially true with the fall of the authoritarian government in 1998, when a chaotic political climate posed major obstacles to conservation. Newly powerful district governments planned roads and issued development permits, ignoring the protected status of the land (Kelman 2013). Protected areas also experienced increased levels of illegal activities such as farming, settlements, poaching, and illegal logging due to lack of capacity by law enforcement (Kelman 2013). The use of slash and burn agriculture has contributed to an estimated loss of 36% of the primary tropical forest between 1990 and 2010 across the island (Margono et al. 2012). With demand for products such as palm oil, coffee, and timber continuing to rise, it is likely that this number will only increase (Margono et al. 2012). This habitat loss has been directly linked to population declines in many mammals, including flagship species such as, the Sumatran tiger (*Panthera tigris sumatrae*), Sumatran rhinoceros (*Dicerorhinus sumatrensis*), and Asian elephant (*Elephas maximus sumatrensis*) (Kinnaird et al. 2003; Orangutan Foundation International 2011).

In the face of substantial habitat loss, there have been conflicting ideas as to how to most effectively conserve species in a human dominated landscape. One approach is land sharing, where farmers maintain forest fragments on the landscape. Supporters of this method argue that low intensity tropical agriculture can support

high levels of biodiversity, and that fragments provide added ecosystem services such as biological pest control and pollination (Gilroy et al. 2014). These fragments are also thought to act as stepping stones for wildlife, allowing animals to travel to forested areas they may not have been able to reach otherwise (Edwards et al. 2010) and as refugia used for resting, and possibly breeding (Rajaratnam et al. 2007; Mohamed et al. 2013). In Indonesia and Malaysia, forest fragments may be beneficial for species like the sun bear (*Helarctos malayanus*; Linkie et al. 2007a, Linkie et al. 2007b), Sumatran tigers (Linkie et al. 2008, Sunarto et al. 2012, Wibisono et al. 2011), leopard cats (*Prionailurus bengalensis*; Rajaratnam et al. 2007) and Asiatic golden cats (*Pardofelis temminckii*; McCarthy 2013), which have all been found to use forest fragments or degraded forest. In addition to benefitting some mammal species, maintaining forest patches near plantations may be important for the life cycles of birds and butterflies that inhabit the plantations, and may act as population sources for these agricultural areas (Koh 2008).

An opposing approach is the use of “land sparing” rather than land sharing. Using this method, farmers do not maintain forest fragments, allowing for maximum productivity on their land. In theory, because of the higher land productivity, greater areas of contiguous forest can be preserved outside of the agricultural landscape. This may be more beneficial for wildlife, as many species need large, undisturbed forest habitat. This approach was supported by Edwards et al. (2010), who found that forest fragments in oil palm plantations had lower overall bird abundance, and lower species richness of priority birds than both primary forest and the oil palm plantations themselves. He also found no evidence that bird abundances in oil palm fields were greater when they were located closer to fragments, although he did find that larger

fragments held more species than smaller ones. To further study the benefits of land sparing versus land sharing, Phalan et al. (2011) looked at bird and tree densities at different levels of agricultural yield in Ghana and India. They concluded that both countries could produce more food at a lower ecological cost if land sparing was used in conjunction with increased forest protection and restoration (Phalan et al. 2011). Hulme et al. (2013) reached a similar conclusion when they examined bird species in Uganda.

Another factor to consider in the land sparing vs. land sharing debate is the effect of forest fragments on human-wildlife conflict. Human-wildlife conflict may be higher in areas containing forest fragments, as they place humans and wildlife in close proximity competing for limited resources. This may be a particular challenge for carnivores, as many have large home ranges and eat domestic livestock to meet their protein needs (Treves and Karanth 2003). Livestock depredation by felids has been found to be greater when herds are managed in more forested areas and closer to riparian corridors that link patches greater than 1000ha (Michalski et al. 2006; Inskip and Zimmermann 2009; Soto-Shoender and Giuliano 2011). Additionally, game farms in South Africa with dense cover (scrub and woodlands) were found to have greater frequency of predation than farms with open cover (grassland and crops), potentially because these areas support greater biodiversity and harbor larger predator populations (Thorn et al. 2012). Carnivores may also cause conflict because of danger to humans. Between 1978 and 1997, 146 people and at least 870 livestock were reportedly killed by Sumatran tigers (Nyhus and Tilson 2004). At least 28 tigers were killed in response to these attacks. The highest levels of tiger conflict were found in intermediate disturbance areas with “diffuse” edges, where tigers and humans had the most overlap

in activity (Nyhus and Tilson 2004). Human-felid conflicts are not restricted to large cats such as tigers. McCarthy (2013) surveyed villagers surrounding Bukit Barisan Selatan National Park in Sumatra regarding their levels of conflict with small felids. She found that many villagers reported livestock depredation by small felids and that Sunda clouded leopards (*Neofelis diardi*), Asiatic golden cats, marbled cats (*Pardofelis marmorata*), and leopard cats were being killed for retribution.

Close proximity to wildlife from the presence of forest fragments may also cause conflict due to agricultural damage. Sumatran farmers have been found to experience frequent conflicts with species such as wild boar (*Sus scrofa*), long-tailed macaques (*Macaca fascicularis*), and Thomas' leaf monkey (*Presbytis thomasi*) (Linkie et al. 2007a; Marchal and Hill 2009). Sumatran orangutans (*Pongo abelii*) can cause extensive crop damage due to their large size. This also makes them extremely visible to farmers, often leading to retribution killing (Campbell-Smith et al. 2012). Okello (2005) found that agricultural expansion has led to increased human-wildlife conflict in Kenya. While the majority of people interviewed believed that wildlife conservation was valuable, most had negative attitudes towards wildlife because of crop damage and danger towards humans, and many farmers suggested that they would support strategies that promoted complete separation of humans and wildlife (Okello 2005).

Clearly, there has been much debate about the utility of forest fragments, as well as the ideal size and location of fragments to maximize benefits for wildlife. This information is essential for land use planning, especially in biodiversity hot spots such as Indonesia. Although Indonesia contains 12% of global mammal diversity and the largest number of threatened mammal species, the majority of which depend on forest

habitats, little is known regarding the effect of widespread landscape changes on Sumatran biodiversity as a whole (Orangutan Foundation International 2011). This information is of the utmost importance to managers faced with prioritizing allocation of limited conservation resources in efforts to maintain diverse ecosystems.

In this study, we conducted camera trap surveys in Bukit Barisan Selatan National Park and five surrounding forest fragments. We compared the presence and relative abundance of species found within the park, and within the fragments to investigate whether forest dwelling species were using the fragments. As the fragments were small, we did not expect to find high levels of biodiversity and suspected that small mammals would make up the majority of the species found. Determining which species are utilizing the forest fragments is an important step in assessing the conservation value of the habitat, and as far as we know, this is the first survey of mammal use of fragments surrounded by coffee plantations in Sumatra. In a broader context, this information is also important to refining our understanding of land sparing vs land sharing conservation practices on the Island of Sumatra.

### **Study Area**

Bukit Barisan Selatan National Park (BBSNP), encompassing 3,245 km<sup>2</sup>, is the third largest protected area in Sumatra (Figure 1). Located in southwest Sumatra between the coordinates 4° 31' to 5° 57' S and 103° 34' to 104° 43' E, the park covers 150 km along the Bukit Barisan Mountain Range, with topography ranging from coastline in the south to mountainous forest in the north. BBSNP experiences seasonal rainfall ranging from 3,000 mm to 4,000 mm, and temperatures range from 22-35°C (O'Brien et al. 2003). It is the major watershed for the southwest region of the island. Declared as a Wildlife Sanctuary in 1935, BBSNP became a National Park in 1982

(World Wildlife Fund n.d.). It is a UNESCO World Heritage Site, part of the Tropical Forest Heritage of Sumatra, and contains some of the largest tracts of lowland tropical rainforest on the island. Because of this, it is considered an important forest area for tiger conservation and is also critical habitat for endangered species such as the Sumatran rhino (*Dicerorhinus sumatrensis*), Sumatran striped rabbit (*Nesolagus netscheri*), and Sumatran elephant (*Elephas maximus sumatrensis*) (Wibisono 2005; McCarthy et al. 2012). The park is surrounded by villages, agricultural fields, and oil palm plantations. With increases in coffee prices, many small scale coffee farmers have moved to the area, and agricultural expansion for coffee production has been the major driver of deforestation in the park in recent years (Suyanto 2007; Gaveau et al. 2009).

We also worked in five randomly selected forest fragments outside the park (Figure 2). Numerous fragments of varying sizes were present but were not walked with the GPS, and thus we did not have the coordinates. Selected fragments were anywhere between 0.59 km and 1.97km distance from the border of the fragment to the border of the primary forest in BBSNP, and were surrounded on all sides by coffee plantations. One of the fragments was partially located within the official park boundary, but was isolated from the primary forest due to significant illegal deforestation. The fragments ranged in size from 0.012km<sup>2</sup> to 0.152km<sup>2</sup>. There are several small, primitive villages nearby (less than 20 houses per village), and scattered houses throughout the area. There are some limited, small scale food crops grown nearby, but the majority of the landscape is composed of coffee plantations.

## Methods

*Camera trapping:* We conducted camera trapping within BBSNP between June and October, 2010, and between January and September 2011 using digital remote cameras (Reconyx HC500, Holmen, Wisconsin, USA). Cameras were motion censored, and operated constantly, using infrared photography at night. Cameras took a series of five photographs each time they were triggered with a one second delay between pictures, and the date and time of the pictures taken were automatically recorded. We placed the cameras within a 1km sampling block that had been the focus of a previous camera trapping study (Figure 2) (McCarthy et al. 2015). We selected a random UTM coordinate within the block, and placed a camera within 100m of that coordinate at the location determined to be most optimal for obtaining vertebrate photographs. These locations were generally along animal trails that showed signs of recent activity. We secured cameras on tree trunks 25 cm above the forest floor. We checked cameras every 30–35 days. In some cases, we moved cameras to a new UTM coordinate within the subunit in order to get better coverage, in other cases the batteries were changed but the camera was not moved.

We also conducted camera trapping in five forest fragments outside of BBSNP between June and September 2013 (Figure 2). Within the forest fragments, cameras (Reconyx HC500, Holmen, Wisconsin, USA, and Bushnell Trophy Cam HD, Overland Park, KS, USA) were set using the same settings described above, except that cameras took a series of three photographs each time they were triggered. Two cameras were placed at separate locations within each fragment. The locations were chosen to be fairly far apart along trails or in clearings. Cameras were deployed for 30-35 days, after which they were checked so that batteries or memory cards could be

replaced, and in some cases, the cameras were moved to different locations within the fragment in order to improve coverage of the entire fragment.

The number of trap nights for each camera was calculated as the number of days between when it was first deployed until it was retrieved. Each photograph of a mammal was identified to species where possible. If we were unable to determine the exact species but were able to identify it as a separate taxonomic unit, we included it in the analysis as a separate species (ex. *Sciuridae* sp., *Tragulus* sp.). Poor quality photographs in which we could not identify the animal to an order were excluded. Photographs of the same species taken within one hour of the first picture were considered a single photographic event if we were unable to identify it as a separate individual. A sensitivity analysis of 15, 30, 60, and 120 minute intervals suggested one hour was a good compromise to limit either over or under representing individual capture events. If multiple individuals were seen in a photograph, we counted it as multiple independent photos, e.g., if there were two wild boars in a photograph, we counted it as two independent photos. Photo rates were calculated as the number of photographs of the species per 100 trap nights.

*Data analysis:* All analyses were conducted in R version 3.2.0 (R Core Team 2016). We constructed species accumulation curves using function *specaccum* in the *vegan* library using Kindt's exact method (Oksanen et al. 2015). This method does not rely on the underlying distribution of individuals, but gives very similar results to the classic randomization method (Ugland et al. 2003, Kindt et al. 2006) We then used function *poolaccum* in the *vegan* library to estimate the overall species richness (including observed and unobserved species) in both the forests and the fragments. We computed the estimates using three methods: Chao's method, jackknife, and bootstrap.

Chao's method is beneficial when many individuals are only captured a few times, because jackknife and bootstrapping tend to underestimate species richness if there are a high number of rare species, or too few samples. However, Chao's method is less precise than the other two methods, and may not work if the average capture probability is large (Smith and Belle 1984; Chao 1987).

To compare the species composition between the forest and the fragments, we also calculated a Sorenson species dissimilarity index using function *vegdist* in the *vegan* library, which is based only on the presence-absence of species.

## Results

We had a total of 904 trap nights and 386 independent photos in the fragments, and 1,381 trap nights and 247 independent photos in the forest. Twenty-eight mammal species were observed in the forest, and 21 mammal species were observed in the fragments (Table 1, Figure 3), but species accumulation curves did not reach their asymptote in either the forest or the fragments (Figures 4 and 5). Estimates for total species richness were higher for the forest across most metrics (Table 2), and the forest and the fragments were moderately dissimilar in terms of mammalian species composition (Sorenson index = 0.429).

The number of species observed in each fragment ranged from 7-11 (Table 1). The size of the fragments and distance from the fragment edge to the intact forest edge did not have an apparent impact on the number of species observed in each fragment. The largest fragment did have the largest number of observed species (11), but the smallest fragment contained 10 observed species. The fragment located closest to the forest had 8 observed species, while the farthest fragment had 11 species. All fragments contained at least one felid species, with a leopard cat and a golden cat

observed in the smallest fragment, and a Sumatran tiger and a leopard cat observed in the second smallest fragment. A marbled cat was seen only in the largest fragment. Sunda pangolins (*Manis javanica*) were also exclusively observed in the smallest two fragments.

### **Discussion**

Consistent with previous studies, we observed fewer species in the fragments than in the primary forest (Fischer and Lindenmayer 2007; Sampaio et al. 2010). Because different species have different habitat size requirements, smaller patches of habitat tend to support fewer species than larger ones, and the composition of smaller patches is often a subset of the species in larger habitat patches (Fahrig 2003). However, the lower species richness of the fragments does not necessarily indicate that forest fragments have low conservation value. While large mammals may be unable to survive in small fragments, mid-sized generalist species have been found to be more abundant in small neotropical forest fragments than in the primary forest, and small remnant patches (<100ha) in Amazonian Brazil have been found to support some forest bird and mammal species (Turner and Corlett 1996; Sampaio et al. 2010). Similarly, in our research, banded linsangs (*Prionodon linsang*), yellow-throated martens (*Martes flavigula*), leopard cats, and hog badgers (*Arctonyx collaris*) had higher photo rates in the fragments than in the forest. In addition, we observed some species, i.e., long-tailed macaques, mouse-deer (*Tragulus* sp.), tree shrews (*Tupaiaidae* sp.), and pangolins, only in the fragments, although this could also be due to sampling only a small area of the park, as several of these species have been observed in other areas of the forest.

Some of the species that we did not observe in the forest fragments included endangered species such as the dhole (*Cuon alpinus*), Malayan tapir (*Tapirus indicus*), and Sumatran elephant, and vulnerable species such as the sambar deer (*Rusa unicolor*). It is likely that the forest fragments were not large enough to provide sufficient resources for these large, and wide ranging species. Tapirs have an estimated home range of 13km<sup>2</sup>, although they have been reported to move through rubber and oil palm plantations, and have been observed in degraded forest (Magintan et al. 2012; Linkie et al. 2013). Degraded forests have also been used by Sumatran elephants, but elephant families require 60-170km<sup>2</sup>, and the fragments in our study were far smaller (Kinnaird et al. 2003; Sitompul et al. 2013). Similarly, dholes are generally shy animals that avoid disturbed areas. However, they were observed using rainforest fragments in a tea plantation in India (Kumara et al. 2004). This is likely because the area was an attractive grazing location for their main prey species, the sambar deer, and also because streamside vegetation facilitated their movement through the plantation (Kumara et al. 2004). However, it is possible that some of these species do utilize the fragments but were not observed during the camera trapping period, as elephant signs were found in the fragments. Additionally, while camera trapping in the forest took place over multiple seasons, camera trapping in the fragments only took place in June-September, thus may have missed seasonal variation in fragment use.

Although not found exclusively in the fragments, four of the five felid species observed in the primary forest (golden cat, marbled cat, Sumatran tiger, and leopard cat) were observed in the fragments. In particular, leopard cats had a much higher photo rate in the fragments. In previous studies, leopard cats have been found in

greater relative abundance closer to forest edges (Azlan and Sharma 2006; McCarthy et al. 2015). These results are also consistent with the conclusions of Rajaratnam (2007), who found that leopard cats may depend on forest fragments for survival. They are a felid species that is commonly found in close proximity to open areas and human settlements, and may use the forest fragments as a refuge. While tigers may not depend on fragments to the same degree as leopard cats, their observation in the fragments indicates that they may use them for movement, supporting the Sunarto et al. (2012) conclusion that fragments may act as stepping stones between more suitable tiger habitat.

In addition to felids, the fragments also contained several species that were not observed in the forest, including small rodents such as tree shrews, long-tailed macaques, and the Sunda pangolin, which is considered critically endangered by the IUCN (Challender et al. 2014). Small mammal abundance may be higher in the fragments because they are feeding on coffee or crops. The presence of long-tailed macaques in the forest fragments is unsurprising, as they inhabit a wide variety of habitats and have been observed in secondary forest, disturbed areas, and agricultural areas (Fooden 1995). While we only observed pangolins in the fragments, they have been seen in different areas of the park. Although we are lacking information on the ecology and population status of Sunda pangolins, they are thought to be in serious decline throughout their range. The biggest threat to pangolins is illegal hunting for their meat and scales, mostly to supply the traditional medicine trade in China (Challender 2011). Much of this supply is obtained from Indonesia and Malaysia (Challender 2011). It is therefore noteworthy that we captured multiple photographs

of pangolins in the two smallest forest fragments in areas completely surrounded by coffee plantations and humans.

Our fragments were close to the primary forest. This may increase the number of species that can be supported, because individuals in our study area can utilize both the fragments and the primary forest, assuming that they are able to cross non-forested areas. If individuals can use a network of interconnected fragments, it may increase ecosystem resilience by providing functional diversity and connectivity (del Castillo 2015). Maintaining interconnectedness in the landscape through a network of forest fragments surrounded by coffee plantations may be beneficial for mammal species in Sumatra, as it appears that at least some are able to move between them across the human dominated matrix. Additionally, although all of our fragments were very small, the size of our fragments in this study did not appear to impact the number of species present. This could be due to the fact that the fragments were very close together, possibly allowing for movement between them, or because they were close to the primary forest.

Increased movement of wildlife through human dominated areas may increase human-wildlife conflict. We found problem species, such as tigers, Asiatic golden cats, marbled cats, leopard cats, wild boar, and long-tailed macaques, inhabiting the forest fragments. Human-wildlife conflict should be taken into consideration in creating management strategies, as any initiative that is undertaken needs to take into account the needs of local communities to be successful (Bhagwat et al. 2008). However, increased human-wildlife conflict does not rule out the benefits of forest fragments, especially if appropriate mitigation strategies can be initiated. McCarthy et al. (2013) found that despite reports of human-wildlife conflict around BBSNP,

attitudes towards felid species were not extremely negative. After basic education and mitigation strategies, conflict levels and negative attitudes towards clouded leopards, golden cats, and leopard cats decreased. Another study focusing on a hyper-fragmented landscape in Brazil where human-felid conflict was frequent found that over half of respondents were open to non-lethal control methods (Michalski et al. 2006). Moreover, human-wildlife conflict may still take place in a land sparing conservation strategy along the borders of contiguous forest and agricultural areas. Mitigation strategies should be context dependent, as conflict species likely vary by location, and locals in different areas are likely to respond better to different approaches.

Despite the concerns of increased human-wildlife conflict, forest fragments may have high conservation value. Our five small forest fragments outside BBS National Park harbored a surprisingly high level of biodiversity, including species of conservation concern. The fact that pangolins, golden cats, marbled cats, and Sumatran tigers were utilizing this habitat is significant, especially because habitat loss is such an important reason for decline in many species. Increasing habitat connectivity around BBSNP may help sustain populations of species with large home ranges and may alleviate problems associated with small populations and lack of gene flow by connecting BBSNP to nearby primary forest. Although human-wildlife conflict may be greater in areas that contain fragments, working with local populations on farming and livestock practices to reduce predation and agricultural damage, as well as education about the importance of biodiversity, may help mitigate some of the problem (McCarthy 2013, Michalski et al. 2006). Although studies of other forest fragments have found that smaller patches support less biodiversity and that greater

distances between fragments or primary forest may decrease the number of species, these responses can be species specific and dependent on the landscape matrix (Laurance et al. 2002). Factors such as greater human density or increased disturbance in the matrix may decrease connectivity. It appears that a large number of species are utilizing relatively small forest fragments surrounded by coffee plantations around BBSNP. Fragments such as these are present throughout Sumatra, and therefore should be considered as part of the conservation landscape. Future studies should examine whether forest fragments in areas farther from core forests and of varying sizes in Sumatra still support high levels of biodiversity, and whether species are in fact utilizing the areas as stepping stones between core habitats. If so, managers should develop methods to preserve forest fragments in coffee plantations on the landscape in Sumatra.

## TABLES

Table 1 Species photographed in Bukit Barisan Selatan National Park (cameras deployed 2010-2011) and five surrounding forest fragments (cameras deployed 2013).

| Common Name       | Scientific Name                 | Primary Forest | Fragment (Size in km/Distance to Primary Forest in km) |                        |                       |                        |                        |
|-------------------|---------------------------------|----------------|--|------------------------|-----------------------|------------------------|------------------------|
|                   |                                 |                | 1<br>(0.13/<br>0.594)                                  | 2<br>(0.012<br>/1.315) | 3<br>(0.15/<br>1.967) | 4<br>(0.047/<br>1.145) | 5<br>(0.018/<br>1.826) |
| Banded linsang    | <i>Prionodon linsang</i>        | X              | X  |                        |                       |                        |                        |
| Banded palm civet | <i>Hemigalus derbyanus</i>      | X              |  |                        |                       |                        |                        |
| Binturong         | <i>Arctictis binturong</i>      | X              |  |                        |                       |                        |                        |
| Clouded Leopard   | <i>Neofelis diardi</i>          | X              |  |                        |                       |                        |                        |
| Dhole             | <i>Cuon alpinus</i>             | X              |  |                        |                       |                        |                        |
| Domestic dog      | <i>Canis lupus familiaris</i>   | X              |  |                        | X                     |                        |                        |
| Golden cat        | <i>Pardofelis temminckii</i>    | X              | X  | X                      |                       |                        |                        |
| Hog badger        | <i>Arctonyx collaris</i>        | X              |  |                        | X                     |                        |                        |
| Leopard cat       | <i>Prionailurus bengalensis</i> | X              |  | X                      |                       | X                      | X                      |

|                     |                             |   |   |   |  |   |   |
|---------------------|-----------------------------|---|---|---|--|---|---|
| Long-tailed macaque | <i>Macaca fascicularis</i>  |   |   | X |  | X |   |
| Malayan tapir       | <i>Tapirus indicus</i>      | X |   |   |  |   |   |
| Marbled cat         | <i>Pardofelis marmorata</i> | X |   |   |  | X |   |
| Masked palm civet   | <i>Paguma larvata</i>       | X | X | X |  |   | X |
| Mouse-deer          | <i>Tragulus sp.</i>         |   |   |   |  | X | X |
| Muntjac             | <i>Muntiacus muntjac</i>    | X | X |   |  | X | X |
| Sunda pangolin      | <i>Manis javanica</i>       |   |   | X |  |   | X |
| Pig-tailed macaque  | <i>Macaca nemestrina</i>    | X |   |   |  |   |   |
| Porcupine           | <i>Hystrix sp.</i>          | X |   | X |  |   |   |
| Rodentia sp. A      |                             |   |   |   |  |   | X |
| Rodentia sp. B      |                             |   |   |   |  | X |   |
| Rodentia sp. C      |                             | X |   |   |  |   |   |
| Rodentia sp. D      |                             | X | X |   |  |   |   |
| Rodentia sp. E      |                             | X |   |   |  |   |   |

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|                               |  |   |   |   |   |   |   |   |
|-------------------------------|--|---|---|---|---|---|---|---|
| Rodentia<br>sp. F             |  |   |   |   |   |   | X |   |
| Sambar<br>deer                | <i>Rusa<br/>unicolor</i>                   | X |   |   |   |   |   |   |
| Squirrel sp.                  | Sciuridae sp.                              | X | X | X | X | X | X | X |
| Sumatran<br>elephant          | <i>Elephas<br/>maximus<br/>sumatrensis</i> | X |   |   |   |   |   |   |
| Sumatran<br>serow             | <i>Capricornis<br/>sumatraensis</i>        | X |   |   |   |   |   |   |
| Sumatran<br>striped<br>rabbit | <i>Nesolagus<br/>netscheri</i>             | X |   |   |   |   |   |   |
| Sumatran<br>tiger             | <i>Panthera<br/>tigris<br/>sumatrae</i>    | X |   |   |   |   |   | X |
| Sun bear                      | <i>Helarctos<br/>malayanus</i>             | X |   |   |   |   |   |   |
| Sunda stink<br>badger         | <i>Mydaus<br/>javanensis</i>               | X |   |   |   |   |   |   |
| Tree shrew<br>sp.             | Tupaiaidae sp.                             |   |   | X | X | X | X | X |
| Wild boar                     | <i>Sus scrofa</i>                          | X | X | X | X | X | X | X |
| Yellow-<br>throated<br>marten | <i>Martes<br/>flavigula</i>                | X | X | X | X |   |   |   |

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Table 2 Estimated mammal species richness in the forested study area of Bukit Barisan Selatan National Park and five forest fragments in the surrounding landscape. Species is the observed species richness. The Chao, Jackknife, and Bootstrap are species richness estimators that account for species that were unobserved by the camera traps.

| Estimate Type | Forest   |                | Fragment |                |
|---------------|----------|----------------|----------|----------------|
|               | Estimate | Standard Error | Estimate | Standard Error |
| Species       | 28       | -              | 21       | -              |
| Chao          | 35.13    | 5.90           | 36.98    | 16.5           |
| Jackknife     | 37.99    | 3.16           | 28.99    | 2.83           |
| Bootstrap     | 32.67    | 1.78           | 24.41    | 1.51           |

## FIGURES

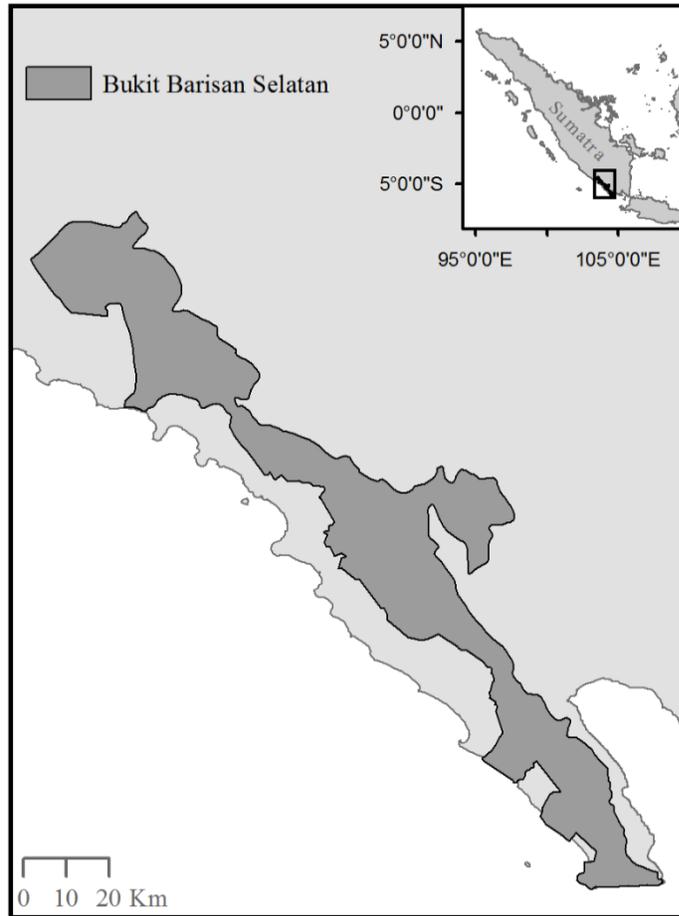


Figure 1 A map of Bukit Barisan Selatan National Park, Sumatra.

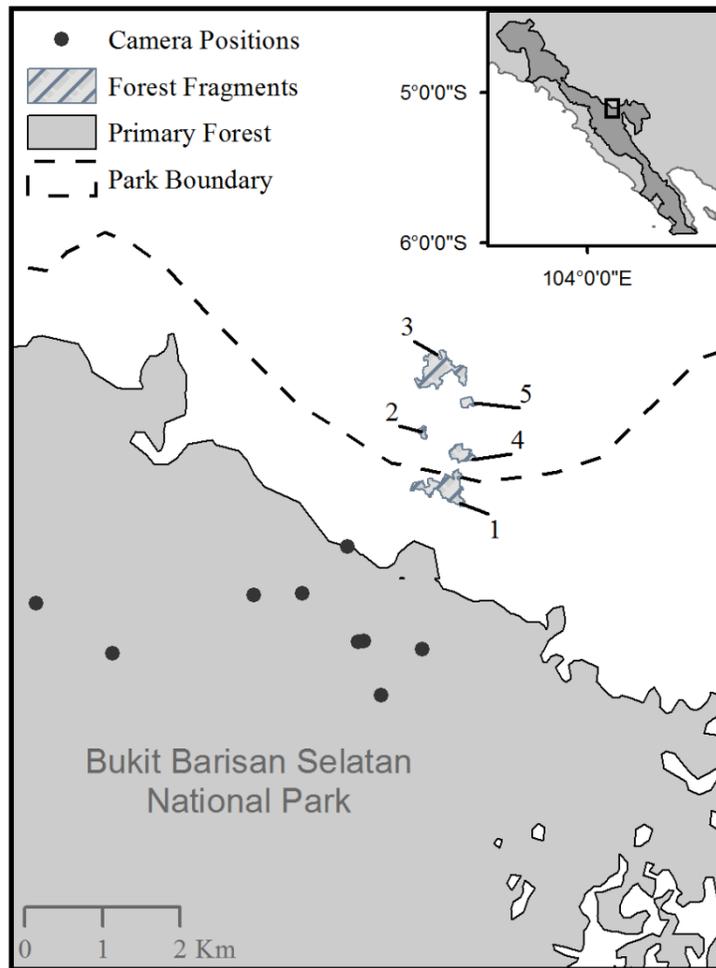


Figure 2 A map showing the location of the camera traps inside the primary forest, as well as the five forest fragments outside of Bukit Barisan Selatan National Park. More fragments were present in this area, but we did not have GPS locations to add them to the map. The exact locations of the cameras within the forest fragments were lost, however there were 4 camera locations in fragment 1, six camera locations in fragment 2, six camera locations in fragment 3, five camera locations in fragment 4, and 4 camera locations in fragment 5.

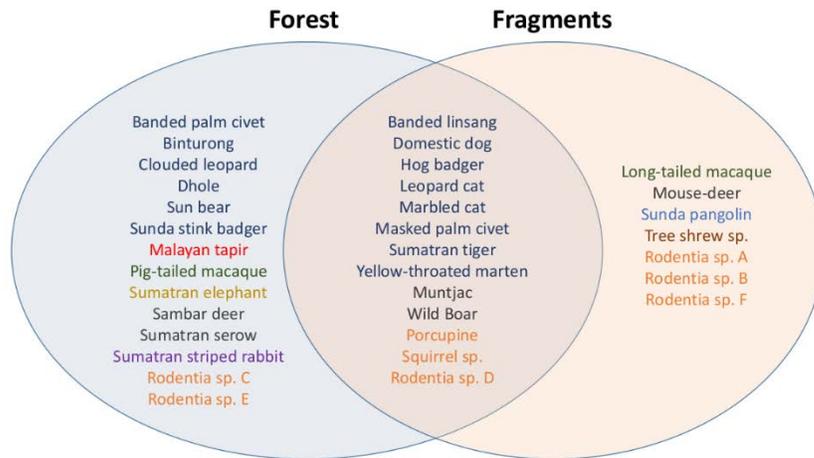


Figure 3 A comparison of the mammalian species composition of the primary forest of Bukit Barisan Selatan National Park (cameras deployed 2010-2011) and the five surrounding forest fragments (cameras deployed 2013). Species are color coded by order.

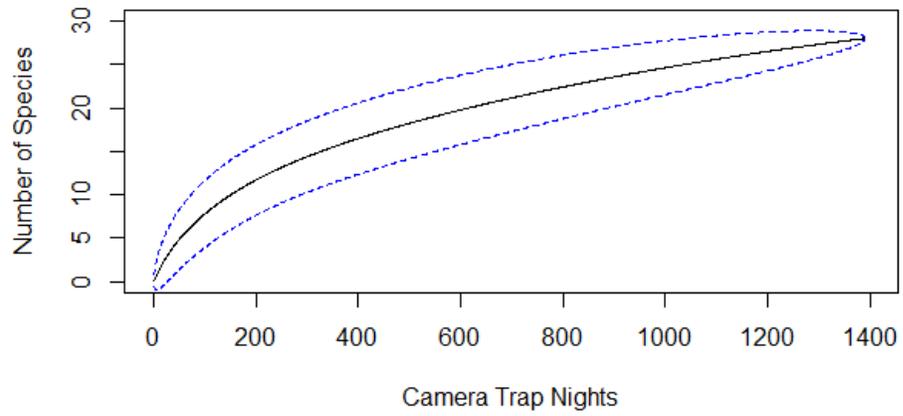


Figure 4 Species accumulation curve for the primary forest study site in Bukit Barisan Selatan National Park, Sumatra. The curve was created using camera trap data collected between 2010 and 2011.

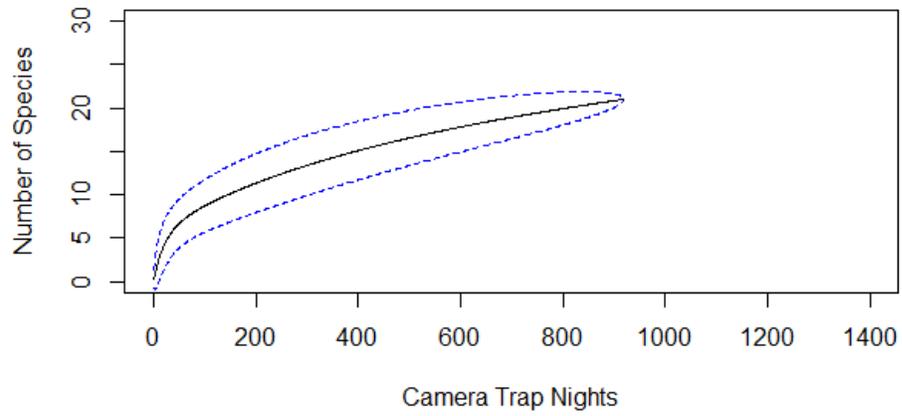


Figure 5 Species accumulation curve for five forest fragments outside of Bukit Barisan Selatan National Park, Sumatra. The curve was created using camera trap data collected in 2013.

## Chapter 2

### USING TERRESTRIAL HAEMATOPHAGOUS LEECHES TO ENHANCE TROPICAL BIODIVERSITY MONITORING PROGRAMS

#### Abstract

Measuring mammal biodiversity in tropical rainforests is challenging, and methods which reduce effort while maximizing success are critical to long-term monitoring programs. Commonly used methods to assess mammal biodiversity are biased toward certain species groups (e.g. camera trapping may be better at detecting larger mammals), and may require substantial sampling effort to be effective. Genetic methods are a new and important sampling tool on the horizon, but obtaining sufficient DNA samples can be a challenge. In this study, we evaluated the efficacy of using parasitic leeches (*Haemadipsa* spp.) to sample biodiversity as compared to camera-trapping. We collected 200 leeches from four forest patches in northeast Bangladesh, and identified recent vertebrate hosts using Sanger sequencing of the 16S rRNA gene extracted from the leeches' blood meals. We then compared this data to species data from camera-trapping conducted in the same forest patches. Overall, 40.8% of sequenced leeches contained amplifiable non-human mammal DNA. Four days of collecting leeches led to the identification of 12 species, compared to the 26 species identified in 1334 camera trap nights. After assessing the cost, effort, and power of each technique, there are pros and cons to both methods. Camera-trapping and leech collection appear to be complementary approaches that, when used together, may provide a more complete monitoring tool for vertebrate biodiversity in tropical rainforests.

## **Introduction**

Deforestation is a critical issue worldwide. Between 2000 and 2010, 400,000 square kilometers of primary forest was lost (Secretariat of the Convention on Biological Diversity 2010). With forests supporting over half of terrestrial animal and plant species, this loss is severely damaging to global biodiversity (Secretariat of the Convention on Biological Diversity 2010). The loss of tropical rainforests in particular is considered one of the greatest current threats to biodiversity (Kinnaird et al. 2003). Asian species have been especially threatened by this loss, and in 2008, Asia and the Pacific reported the highest number of threatened species. Of the threatened Asian species, mammals have had the sharpest increase in extinction risk, likely due to the combination of habitat loss and hunting (Squires 2013).

In order to create effective conservation strategies to combat these declines, we need baseline data such as density, occupancy, and abundance; as well as effective monitoring programs to assess the effectiveness of conservation interventions, and to guide management decisions (Burton 2012, Wong et al. 2013). While we know more about mammals than many other taxa, we still have many knowledge gaps about species' distributions and taxonomy (Francis et al. 2010). This information is of the utmost importance to managers faced with prioritizing the allocation of limited conservation resources in efforts to maintain diverse ecosystems. However, monitoring mammal biodiversity is challenging, particularly when target species are rare, cryptic, and highly mobile, as are many species in the tropics (Linkie et al. 2007, Wibisono et al. 2011).

Commonly used methods to assess mammal biodiversity, e.g., camera traps, track plates, and genetic analyses of hair or feces, tend to be better at detecting certain species groups, and, when used individually, may fail to provide an accurate

assessment of biodiversity (Gompper et al. 2006). For example, cameras can be effective for detecting certain carnivores and large herbivores, but may fail to detect smaller mammals due to trigger sensitivity or placement issues, and images may not be clear enough for species identification (Tobler et al. 2008). Track plates tend to work best for small and medium sized species, and trail-based scat surveys have been found to miss species known to occur in the area (Gompper et al. 2006). Additionally, many of these methods can be difficult to implement; for instance, deploying a sufficient number of cameras is time consuming and expensive, and finding scat samples can be challenging and require a large survey effort. Moreover, rare and elusive species are difficult to capture, and thus substantial sampling efforts may be required to obtain sufficient data (Tobler et al. 2008).

The difficulty in gathering ecological information on tropical mammals has led the IUCN to list a large proportion of these species as “data deficient,” which is a significant obstacle to effective conservation (Schipper Jan et al 2008, Schnell et al. 2012). In order to rectify these data deficiencies, ecologists need an expanded set of complementary tools to address current limitations. One recent addition to the biodiversity monitoring toolkit is the use of DNA extracted from blood meals of haematophagous insects (Rovie-Ryan et al. 2013, Votýpka et al. 2015, Calvignac-Spencer et al. 2013) and from haematophagous leeches (Schnell et al. 2012). Leeches of the genus *Haemadipsa* are haematophagous micropredators commonly found in Indo-Pacific rainforests that are easy to collect due to their prevalence and willingness to attack humans (Borda and Siddall 2010). They also have a wide vertebrate prey base, and do not appear to be species specific in their host selection (Sawyer 1986).

Schnell et al. (2012) found that 84% of collected leeches (N=25) contained mammalian DNA of sufficient quantity and quality to be extracted and amplified by PCR. Extracted blood from each leech contained DNA sequences from only one species, suggesting that DNA concentration decreases over time such that only the most recent blood meal is detectable. As leeches have a wide prey base, are prevalent in tropical rainforests throughout Southeast Asia, and are easy to collect; this method has the potential to provide previously inaccessible information regarding biodiversity in tropical rainforests.

However, although leeches are a promising tool for sampling biodiversity, there are still questions about their efficacy. Any method chosen to sample biodiversity will have different levels of accuracy and varying cost-benefit ratios (Gaidet-Drapier et al. 2006). Understanding these costs and benefits is essential to create effective study designs, as different methods may lend themselves better to particular research objectives. In many cases, vertebrate monitoring programs are cost-limited, thus an understanding of the tradeoffs between cost and performance is vital for project success (Lyra-Jorge et al. 2008). In this study, we evaluated the efficacy of using terrestrial haematophagous leeches as a means to estimate mammalian biodiversity in Bangladesh. Our objectives were: 1) determine whether sequencing leech blood meals to estimate vertebrate biodiversity is effective, 2) determine whether leech size impacts the amplification success rate of mammalian DNA, 3) compare the performance of leech blood meal sequencing and camera trapping in terms of the detection of mammalian biodiversity, and 4) assess and compare the costs and benefits of both methods.

## Study Area

Bordering the Indo-Burma biodiversity hotspot, Bangladesh is a small country with a great diversity of flora and fauna, but also a rapidly diminishing tropical rainforest (Chowdhury and Koike 2010, Myers et al. 2000, Mukul 2007). With a population growth rate of 1.7% per year, deforestation is likely to continue in the country, which will have a significant impact on biodiversity (Chowdhury and Koike 2010). This study was conducted in northeast Bangladesh, a once highly forested area containing tropical evergreen and mixed evergreen forests. Most of the area has been deforested for roads, highways, plantations, and agriculture; and the remaining forest is now contained in 10 fragmented forest patches (Islam et al. 2013, Quazi and Ticktin 2016). These forest patches are located between 24°4' and 24°21' N and 91°15' and 91°7' E, and range from 10-100km<sup>2</sup>. They are predominantly bordered by industrial plantations or rural settlements (Bangladesh Forest Department 2012), although some are connected to forests in Bhutan, Myanmar, and India. Most of the patches are managed by the forest department as “reserve forest,” a classification defining the forest as protected, but with certain extraction activities permitted. The area also contains Satchari and Lawachara national parks, and Rema-Kalenga wildlife sanctuary, where no extraction activities are allowed. The topography of the region is hilly, with elevations between 50 and 300m above sea level. The patches consist of hill forest, shrubs, and mixed bamboo forest, with many streams and swampy areas (Bangladesh Forest Department 2012). Annual temperature ranges from 7-23°C and rainfall in the region is 3,334mm per year, with most occurring between May and September.

Four forest patches, Aora Hill Reserve Forest (AHRF, ~100km<sup>2</sup>), Patharia Hill Reserve Forest (PHRF, ~60km<sup>2</sup>), Rajkandi Reserve Forest (RRF, ~62km<sup>2</sup>), and

Tarap Hill Reserve Forest (THRF, ~82km<sup>2</sup>) were selected for this study (Figure 6, Figure 7). AHRF and RRF are extensions of larger forest tracts in India that expand into Bhutan and Myanmar. Similarly, PHRF is connected with a larger forest in India, although geopolitics (i.e. fence building at the border) and development have reduced the connectivity. THRF is the most isolated of the patches, and contains the Rema-Kalenga wildlife sanctuary.

## Methods

*Leech collection*-Leeches were collected from the four forest patches in October, 2015. We collected 50 leeches in each of the patches over four collection days. At each patch, we picked five locations from which we had previous camera trap data. We attempted to collect 10 leeches from each of these sites, however if we were unable to find 10 at a particular camera site, we collected additional leeches at another site (generally close to the intended camera site or along trails) to ensure we had 50 leeches from each patch. We collected leeches by hand, using nitrile exam gloves to limit DNA contamination, and placed them into individual 1mL test tubes. We then filled the test tube with RNAlater® to preserve the DNA for extended periods of time without refrigeration. At each collection site, we recorded a GPS coordinate, and auxiliary information such as the weather and time of collection.

*Leech processing and sequencing*- All leeches were identified to species based on external morphological characteristics. Eight leeches were kept as voucher specimens and one leech was missing, leaving us with 191 leeches from which to sequence blood meals. We also measured leech length and width at the widest point. Many of the leeches were curled when they were removed from the RNAlater®. We straightened the leeches using our fingers, and then measured the leeches using a

micrometer on a compound microscope. This is not an accurate measure of leech size at collection, as they tend to shrink in the RNALater®; however, knowledge of relative sizes of successful leeches may provide a guideline for collection of leeches in the field.

Blood meal sequencing was conducted at the American Museum of Natural History's (AMNH) Sackler Institute of Comparative Genomics Center for Conservation Genetics, as they have the means to analyze degraded DNA from non-typical sources. They also have an ongoing DNA barcoding project which aims to create cost effective methods to identify species using short DNA segments (DNA Barcoding Initiative for Conservation 2014). We used a Qiagen DNeasy 96 Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) to extract the DNA following manufacturer's instructions with slight modifications to improve extraction quality. To prepare the leeches for sequencing, we removed a 2.5mm segment of the digestive tract from between the rear sucker and the midpoint of the leech. We then chopped these segments into quarters and performed the extraction with longer incubation (overnight) and longer elution wait periods (20 minutes instead of 1 minute) than specified in the manufacturer's instructions.

After extraction, we performed polymerase chain reaction (PCR) using 16s primers known to amplify vertebrate DNA. The 16s rRNA DNA regions are particularly useful for identifying low-quantity or degraded DNA samples, as there are many copies in each cell (a benefit over nuclear DNA). Additionally, this gene typically has a lot of variation between species, probably due to the faster rate of mitochondrial DNA evolution, which makes species identification easier (Kocher et al. 1989, Yang et al. 2014). We added 21.3µL water, 0.7 µL of 10X forward primer,

0.7  $\mu\text{L}$  of 10X reverse primer, 0.3  $\mu\text{L}$  of BSA, and 2  $\mu\text{L}$  of template to illustrate PuReTaq Ready-To-Go PCR Beads.

We then performed Ampure PCR purification, using a 2:1 ratio of Ampure to template to remove everything under 125 base pairs (Bekman Coulter). We used 20 $\mu\text{L}$  of template and 40 $\mu\text{L}$  of Ampure for each well. Following Ampure purification, we checked several rows of the plate for successful amplification on a 2% agarose gel stained with SYBR® Safe.

After ensuring that at least some of the wells had successful amplification, we performed cycle sequencing, adding 1 $\mu\text{L}$  of 1X forward primer, 1 $\mu\text{L}$  of extension buffer, 1 $\mu\text{L}$  of big dye, and 3 $\mu\text{L}$  of template DNA to each well. After completion of cycle sequencing, we performed ethanol precipitation. We sequenced genes on an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA) and compared sequences with the NCBI Nucleotide BLAST Database.

For our analysis, we only included sequences that had an E-value (expectation value) of less than  $e^{-30}$ . The E-value is a corrected version of a p-value for multiple testing. Because there are so many sequences in BLAST, sequences with E-values above  $e^{-30}$  are unreliable. Several blood meals had high match scores and low E-values for species that are not known to occur in Bangladesh. The true species in these blood meals are likely missing sequences in BLAST. We therefore reported these only to family.

*Camera trapping:* Camera trapping was conducted between May 1, 2014 and January 29, 2015 using digital remote cameras (Bushnell Trophy Cam HD, Overland Park, KS, USA) as part of a research project on felid conservation (Hasan Rahman, unpublished data). Cameras were set to operate continuously, using infrared

photography at night, and were set to take 2 pictures when triggered with a 15 second delay before another photograph could be triggered. Date and time of the photographs were automatically recorded. Camera trap locations were chosen so that there was about 1.1km between cameras, and cameras were placed within 200m of the chosen trap site in areas where felids were most likely to travel, such as along trails. Twenty seven cameras were deployed and moved periodically so that there were 44 camera sites in total. Cameras were placed at approximately 25 cm above the ground, inside a theft proof box made of steel, and attached to a tree using a metal. They were checked every 15-20 days to change batteries and memory cards.

To maximize the potential of capturing felids, scent lures were used at trap stations. Calvin Klein Obsession for Men (CK Obsession) was used at 36 trap stations, while chicken body parts were used at 8 locations. At stations using CK Obsession, cotton balls were sprayed with 4-6 sprays of cologne and subsequently placed inside plastic bottles on the ground or attached to a tree. At sites where chicken body parts were used, portions of the chicken were placed in a plastic bag with holes that allowed the scent to be released. The bag was placed on a tree at least 3m above the ground to decrease the chance of scavenging. At 26 locations, visual attractants in the form of chicken feathers were also used. Chicken feathers attached to a wire were attached to a tree branch 25-30cm above the ground. These were not used at sites where they might increase visibility of the cameras to humans, and increase the chance of theft. All attractants were placed 2-3m in front of the cameras.

The number of trap nights for each camera was calculated as the number of days between when it was first deployed until it was retrieved. Each photograph of an animal was identified to species where possible. If the quality of the photograph was

so low that we were unable to identify the animal, we excluded the picture from analysis. Photographs of the same species taken within one hour of the first picture were considered one photographic event.

*Analyses-* To compare the efficacy of using leech blood meal analysis or camera trapping as a biodiversity sampling technique, I first used a Bayesian paired t-test to determine differences in raw species richness (number of species identified) within sites and between the two methods. This test was implemented using the *bayes.t.test* function in the program *BayesianFirstAid* (Bååth 2014). Next, I constructed species accumulation curves in R version 3.2.0 (R Core Team 2016) using Kindt's exact method in the function *specaccum* of the *vegan* library (Oksanen et al. 2015). This method does not rely on the underlying distribution of individuals, but gives very similar results to the classic randomization method (Ugland et al. 2003, Kindt et al. 2006). I developed separate curves based on leech data and camera trap data, both across and within the four study sites.

To enable comparison of effort between methods I first used an iterative model to randomly sample camera sites to match the number of leech collection sites per forest patch. I then created a species accumulation curve using this subset of camera trap data. Within each of 5,000 iterations of this model, I extracted the number of trap nights needed to reach 12 species, the number found within our leech dataset. Finally, I created a dataset using a random sample of cameras sites, again matching the number of leech collection sites, and truncated it to the median number of trap nights needed to obtain 12 species as determined by the iterative model. I used this random, truncated, subset of the data to create a final species accumulation curve representing

an example of camera trapping at the same number of locations and reaching the same number of identified species as our leech collection efforts.

To evaluate the effect of leech size and leech species on DNA amplification success, I applied four candidate logistic models in a Bayesian framework (Table 3). Because length and width were highly correlated ( $r=.87$ ) I did not place them together in a model. I ran the models in program *rstan* (Stan Development Team 2016) using the package *rethinking* (McElreath 2015) and used WAIC to select the best model.

Finally, I compared both the monetary and time costs of each method. Monetary costs for the leech method included the salary, travel, and field supplies for two technicians, RNALater, test tubes and caps, shipping materials to Bangladesh, and DNA extraction and sequencing supplies. Time costs included the amount of time spent collecting leeches in the field as well as the amount of time spent conducting the genetic sequencing. Monetary costs for camera trapping included the cost of field supplies, cameras, and salaries and travel for field technicians. Time costs included the amount of time spent in the field conducting the camera trapping as well as the time spent identifying the species in each photograph. After determining that we needed an ~ 13-fold increase in camera trap nights to progress from 12 identified species to 26 identified species, I subsequently estimated the cost of collecting and analyzing 13-fold more leeches ( $n = 2,600$ ) using both Sanger sequencing and Next-Generation sequencing (NGS).

## Results

Two-hundred leeches were collected in-situ, one leech was lost during transit, and eight leeches were maintained as voucher specimens, resulting in 191 leeches for genetic analysis. We collected four species of leeches; 137 *Haemadipsa ornata*, 33 of

an unidentified species, 15 *H. montevidicus*, 13 *H. c.f. sylvestris*, and 1 leech that was too damaged to be identified. Overall, 40.8% (N=78) of our leeches contained amplifiable non-human mammal DNA, 10.5% (N=20) contained human DNA, and 2.1% (N=4) contained chicken (*Gallus gallus*) DNA; the remainder (46.6%, N=89) did not contain amplifiable vertebrate DNA. *Haemadipsa c.f. sylvestris* had the greatest percentage of amplifiable non-human mammal DNA with 54.5% (N=6) of leeches successfully amplifying. Amplification success was lower for the remaining three species of leech, with 46.7% of *H. ornata* (N=63), 7.7% of *H. montevidicus* (N=1), and 25.8% of the unidentified species (N=8) containing amplifiable non-human mammal DNA. The percent of successful leeches varied by collection site, with 43.8% (N=21) success from AHRF, 33.3% (N=16) from PHRF, 37.5% (N=18) from RRF, and 48.9% (N=23) from THRF.

Due to camera theft (11 units) and permanent malfunction (3 units), only 30 camera trap stations were effective. We captured a total of 863 independent mammal photographs in 1,334 camera trap nights. Twenty-six mammal species were identified from the photographs compared to 12 mammal species identified in the leech blood meals. The Bayesian paired t-test of species richness at each site estimated that camera traps found an average of 9.1 more species, but the credible interval was wide (95% credible interval: 2.0 to 16.3).

Across sites, in both the leech blood meals and the cameras, cows (*Bos taurus*) and wild boar (*Sus scrofa*) were in the top three most frequently captured species (Figure 8). We captured a greater amount of rodent diversity on the cameras, but several were unable to be identified to species. The leeches also failed to detect any

felid species. When looking at the species composition at each site, the results between the cameras and the leeches differed (Figure 9).

The species accumulation curve made using all 191 sequenced leeches did not reach its asymptote, and neither did the curve constructed from all camera trapping sites combined (Figures 10 and 11). Using the iteratively produced species accumulation curves, based on sub-setting our camera trap data, a median of 99 (95% CI: 78-133) trap nights was required to reach 12 species, which is equivalent to 6.2 days of actual trapping using 16 cameras.

The best binomial model from our candidate set for predicting amplification success of non-human mammal DNA was model 4, which included a random effect for species. The length parameter in this model overlapped 0 (Table 4, Figure 12). However, the model that included length but did not account for species did have a significant, non-zero parameter for leech length.

Total monetary costs for collecting and analyzing the 200 leeches using Sanger sequencing was \$4,019.64 (Table 5). I estimated the costs of collecting and analyzing 2,600 leeches using Sanger sequencing to be \$38,483.90 and the estimated costs of collecting and analyzing 2600 leeches using NGS to be \$5,487.56. Total monetary costs for collecting and analyzing all 1,334 nights of camera trapping was \$24,000 (Table 5). Time estimates for collecting and analyzing 200 leeches and 99 camera trap nights were comparable, except for differences in the time required to obtain permits (3 months for the leeches vs 1 day for camera trapping).

## **Discussion**

We collected leeches in Bangladesh after the peak rainy season. While leeches were still prevalent and easy to collect, leeches may be a more efficient sampling

method if field work is conducted during the rainy season. Despite this we were able to find 12 mammal species in only four days of leech collection. Our leech collection points were also fairly close together and occurred in a single day in each patch. Increasing the sample size, spatial range, and/or collection time of leeches is likely to improve results, as our species accumulation curve was not yet near to its asymptote and was very similar to the species accumulation curve for our subset of 99 camera trap nights (Figure 13).

In addition to the mammal species reported above, 10.5% (n=20) of leeches contained human DNA sequences. We suspect that a large portion of these leeches actually fed on humans, because precautions were taken both in the lab and in the field to prevent contamination, and humans were the species captured most frequently on the camera traps, however, we are unable to quantify potential contamination in the laboratory or the field. Leeches may be a better method of sampling in areas of lower human density where leeches are less likely to have fed on humans. Similarly, a large percent (34.6%) of our mammal records were of cattle, again an artifact of the high level of human presence in the area. Anecdotally, there were also a number of bird species captured on the cameras, but red junglefowl (*Gallus gallus*) was the only avian species found in the leeches. This is likely because red junglefowl are ground-dwelling birds, making them more accessible to the leeches than birds that spend less time on the ground.

Like camera traps, leeches tended to be biased towards ground-dwelling species, as we did not identify any arboreal species in the blood meals. We also did not find any felid species in the leech blood meals, but we did catch Asiatic golden cats (*Pardofelis temminckii*) and leopard cats (*Prionailurus bengalensis*) on the

cameras. Conversely, we had a much greater frequency of Rhesus macaques (*Macaca mulatta*) in the leeches than we did on the cameras. We were also able to identify a rodent, *Rattus tanezumi*, which we were not able to identify to species on the camera traps. Leeches may therefore be valuable for obtaining more precise information about the species composition of an area. Leeches may also be useful for identifying rare species. Using DNA analysis of leech blood meals, Schnell et al. (2012) confirmed the presence of the Annamite striped rabbit (*Nesolagus timminsi*), which although long suspected to inhabit the region had never been caught in over 2,000 nights of camera trapping.

Further knowledge about life history characteristics of leeches may improve our understanding of the biases associated with the technique. Information such as host preferences and leech activity patterns may affect what species are predated. Based on our top model, leech species may have an impact on the amplification success rate of non-human mammalian DNA. The fact that this model did not have a significant length parameter, but the model that included only length did, implies that length does not likely have an effect on amplification success after accounting for leech species, but it may still be a viable proxy in the field where leech species identification may be difficult or impractical. Even so, it is difficult to determine if there is a significant species effect because we had so few individuals of several species. Leech species vary by location, but at least in our study site, if biologists are unable to identify leeches in the field, collecting larger leeches may act as a proxy and may improve amplification success rate.

Overall, our non-human mammal amplification success rate was lower than that of Schnell et al. (2012, 40.8% compared to 84%). This may be due to slightly

different amplification techniques. The AmpliTaq Gold, used by Schnell et al. (2012), has been found to be more specific and enhance PCR yields (Kebelmann-Betzing et al. 1998, Moretti et al. 1998), and may have improved our amplification success rate if we had used it. Our amplification success was also lower than studies of several other haematophagous insect species (Townzen et al. 2008, Garipey et al. 2012, Janssen et al. 2015, Gorchakov et al. 2016). This may be due to the length of time between feedings. Leeches spend a long time digesting a blood meal and can go several months without feeding, thus extracted DNA may be lower quality compared to DNA from the blood meal of an insect that has most likely fed recently. It is also possible that time of collection could affect the success rate of DNA amplification, as leeches may be more likely to have fed at different points in the rainy season.

We used Sanger sequencing in this study so that we could examine the blood meals of individual leeches. In the future, NGS is likely to be the most common way to sequence leech blood meals. Pooling of leeches may improve the efficiency of the sequencing step and decrease the costs of higher sample sizes. It may also improve identification success if leeches contain multiple blood meals, as the presence of multiple species' DNA can create background noise and give low quality sequences (Logue et al. 2016). Another aspect to consider when using genetics to identify species is that the findings are only as good as the databases with which sequences are compared. If the true host species is not represented in the database or sequences in the database are inaccurate, the blood meal could be misidentified (Kent 2009). In our study, two of our blood meal sequences were most closely matched to species that do not occur anywhere near Bangladesh, i.e. African civet (*Civettictis civetta*), common kusimanse (*Crossarchus obscurus*). These false positives are a result of poor database

coverage, something that will likely prove a particular challenge for sampling tropical species, until missing sequences are added to GenBank. For several blood meals, the best match in BLAST was *Muntiacus muntjac*. It is possible that these leeches fed on *M. vaginalis*, which was upgraded from a subspecies of *M. muntjac* to its own species in 2003 and does not have a sequence in GenBank (Groves 2003). We also had a BLAST match with gray wolf (*Canis lupus*). This is likely domestic dog (*Canis lupus familiaris*), a subspecies of *Canis lupus*, as gray wolves are unlikely to occur in Bangladesh.

Research on hematophagous insects suggests it may be possible to use leech blood meals to learn other useful information about the host species, e.g., animal age, using gene expression (Kent 2009), population estimates, using individual identification (Darbro et al. 2007, Ligon et al. 2009, Burkett-Cadena et al. 2010, Martínez-de la Puente et al. 2015), or disease prevalence, using host immunoglobulins or viral or microbial DNA in the blood meals (Jasinskas et al. 2000, Wickramasekara et al. 2008, Baskova and Zavalova 2001).

Ultimately, both camera traps and leeches have benefits and drawbacks. Using leeches to sample biodiversity was a cheaper, but slightly less efficient way to sample 12 non-human mammal species in the same number of collection sites. Most of the difference in time costs was spent obtaining an export permit for the leeches. Permit regulations are highly variable by country, and can greatly affect the feasibility of using leeches to sample biodiversity. Anecdotally, we attempted to perform the same study in Sumatra, Indonesia, but were unable to obtain export permits for biological samples. Conversely, it was possible to conduct camera-trapping in that study site. In

Bangladesh, a change in leadership in the Forest Department concurrent with our export permit application slowed the process down considerably.

Collecting 200 leeches proved insufficient to sample biodiversity in our study area. However, we estimate that increasing the sample size of leeches to 2,600 would provide equivalent results to our 1,334 camera trap nights. This would require only a slight increase in field effort as leeches were readily available in large numbers throughout each forest patch. Using NGS on this increased sample size may also allow for cost and time savings, dependent on the desired spatial resolution, i.e., how much you partition your data into pools for analysis. The NGS estimate was based on the costs of pooling the leeches by site (thus 4 samples total), sequencing using an Illumina HiSeq 2500, and sending the samples to an outside vendor for sequencing. Therefore, this method may be even cheaper if researchers plan to perform sample preparation on their own. Ultimately the cost and effort of using leeches as a monitoring tool for biodiversity will be context specific, but in our example could be both less expensive and less labor intensive. One important caveat is that leech blood meal analysis requires access to a laboratory in which to conduct the sequencing and highly trained personnel with knowledge of degraded DNA analysis and species identification.

Monitoring programs are desperately needed in the tropics, where biodiversity and threats to biodiversity are high and data is limited (Burton 2012). In order to be effective and sustainable in the long term, monitoring programs must be supported financially, politically, and logistically (Lindenmayer 1999). Using leech blood meals to monitor biodiversity is potentially cheaper and more efficient than camera trapping for large sample sizes. While the method does not require extensive technology in the

field, it does require sophisticated laboratory analysis. Leeches may also be able to provide a more precise description of the species composition of an area than camera trapping, although further studies are needed to determine whether they can detect comparable levels of species richness. In this study, we presented some of the cost to performance tradeoffs of camera trapping and leech blood meal sequencing, allowing managers to make more informed decisions about which technique to utilize. Perhaps using the two methods together would improve the efficiency and capacity of monitoring programs, allowing researchers to create more effective conservation plans.

## TABLES

Table 3 Candidate models to evaluate the effect of leech size and leech species on mammalian DNA amplification success from extracted blood meals. Models were implemented in program rstan using the package rethinking, with vague normal priors (with a mean of 0 and a standard deviation of 10) for each coefficient.

|         | Response              | Link function | Intercept      | Covariate              |
|---------|-----------------------|---------------|----------------|------------------------|
| Model 1 | Amplification success | Logit         | Pooled         | Leech length           |
| Model 2 | Amplification success | Logit         | Pooled         | Leech width            |
| Model 3 | Amplification success | Logit         | Fixed Species  | Leech length           |
| Model 4 | Amplification success | Logit         | Random Species | Leech length           |
| Model 5 | Amplification success | Logit         | Pooled         | Leech length*<br>width |
| Model 6 | Amplification success | Logit         | Pooled         | Leech<br>length/width  |
| Model 7 | Amplification success | Logit         | Pooled         | None                   |

Table 4 Model comparison for candidate models, described above, evaluating the effect on leech size and species on mammalian DNA amplification success from extracted blood meals.

| Model   | WAIC  | Estimated Effective Number of Parameters | Delta WAIC | Akaike weight | Standard Error of Delta WAIC |
|---------|-------|--|------------|---------------|------------------------------|
| Model 4 | 252.5 | 5.1                                      | 0          | 0.42          | NA                           |
| Model 3 | 253.1 | 5.8                                      | 0.6        | 0.31          | 2.85                         |
| Model 1 | 255.1 | 2.1                                      | 2.6        | 0.12          | 5.07                         |
| Model 2 | 256.1 | 2  | 3.6        | 0.07          | 5.73                         |
| Model 5 | 256.4 | 2.1                                      | 3.9        | 0.06          | 5.25                         |
| Model 7 | 259.3 | 1.0                                      | 6.8        | 0.01          | 7.04                         |
| Model 6 | 260.3 | 2.1                                      | 7.8        | 0.01          | 6.6                          |

Table 5 Cost breakdown of camera trapping (based on the costs of a camera trap survey conducted May 2014-January 2015) vs the collection and blood meal sequencing of 200 leeches (based on work conducted October 2015) vs the estimated costs of Sanger sequencing and NGS (using 4 pooled samples) of 2600 leeches in four forest patches in northeast Bangladesh.

| Item   | Cost for 10 months of camera trapping (USD) | Cost for 200 leeches (USD) | Estimated costs of Sanger sequencing 2600 leeches (USD) | Estimated costs of NGS 2600 leeches (USD) |
|--|---|----------------------------|---|---|
| Field assistant salary                         | 2700  | 197.37                     | 986.85  | 986.85                                    |
| Field guide salary                             | 1000  | 39.47                      | 197.35  | 197.35                                    |
| Travel   | 4250  | 105.26                     | 105.26  | 105.26                                    |
| Lodging  | 1000  | 78.94                      | 394.7   | 394.7                                     |
| Food   | 4500  | 73.68                      | 368.4   | 368.4                                     |
| Miscellaneous field costs                      | 1170  | 400                        | 600   | 600                                       |
| 30 Camera traps                                | 6000  | NA                         | NA  | NA  |
| Taxes on supplies                              | 3000  | 305                        | 305   | 305                                       |
| Theft proof box                                | 680   | NA                         | NA  | NA  |
| Batteries                                      | 500   | NA                         | NA  | NA  |
| RNA Later                                      | NA  | 270                        | 2316  | 2316                                      |
| Test tubes                                     | NA  | 55.5                       | 492   | 214                                       |
| Sanger sequencing                              | NA  | 2500                       | 32500   | NA  |
| Qiagen extraction kit                          | NA  | NA                         | NA  | 161                                       |
| Illumina HiSeq 2500 library prep for 4 samples | NA  | NA                         | NA  | 1200                                      |
| Illumina HiSeq 2500 analysis                   | NA  | NA                         | NA  | 3000                                      |
| Quality control                                | NA  | NA                         | NA  | 50  |
| <b>Total</b>                                   | <b>24800</b>                                | <b>4025.22</b>             | <b>38265.56</b>   | <b>5487.56</b>                            |

## FIGURES

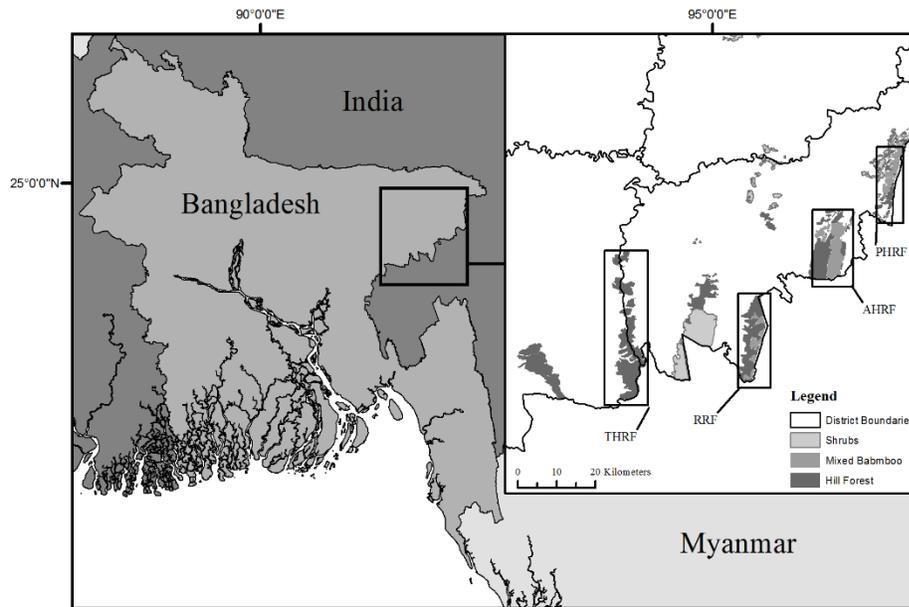


Figure 6 Map depicting four forest patches in northeast Bangladesh that were the focus of camera trap surveys from May 2014-January 2015 and from which leeches were collected in October 2015.

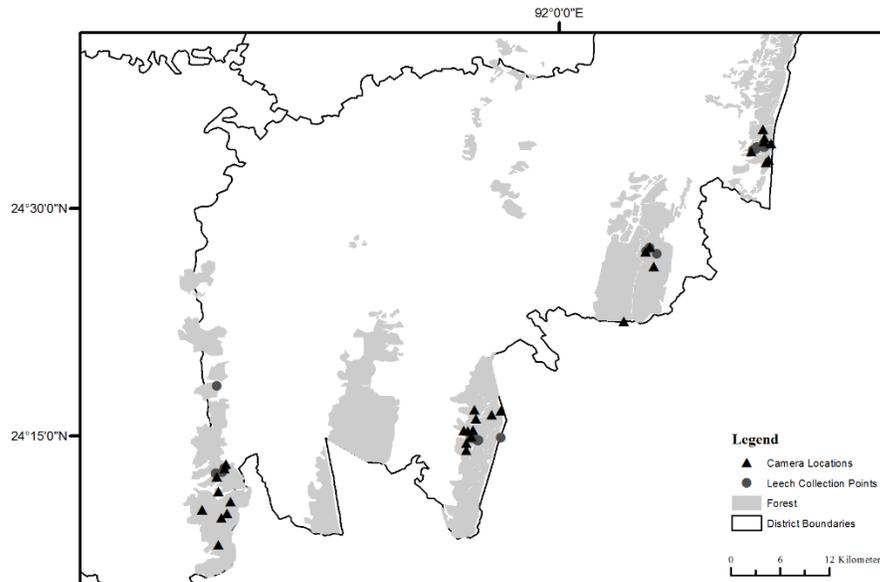


Figure 7 Map depicting camera trap locations (cameras deployed May2014-January 2015) and leech collection points (October 2015) in four forest patches in northeast Bangladesh.

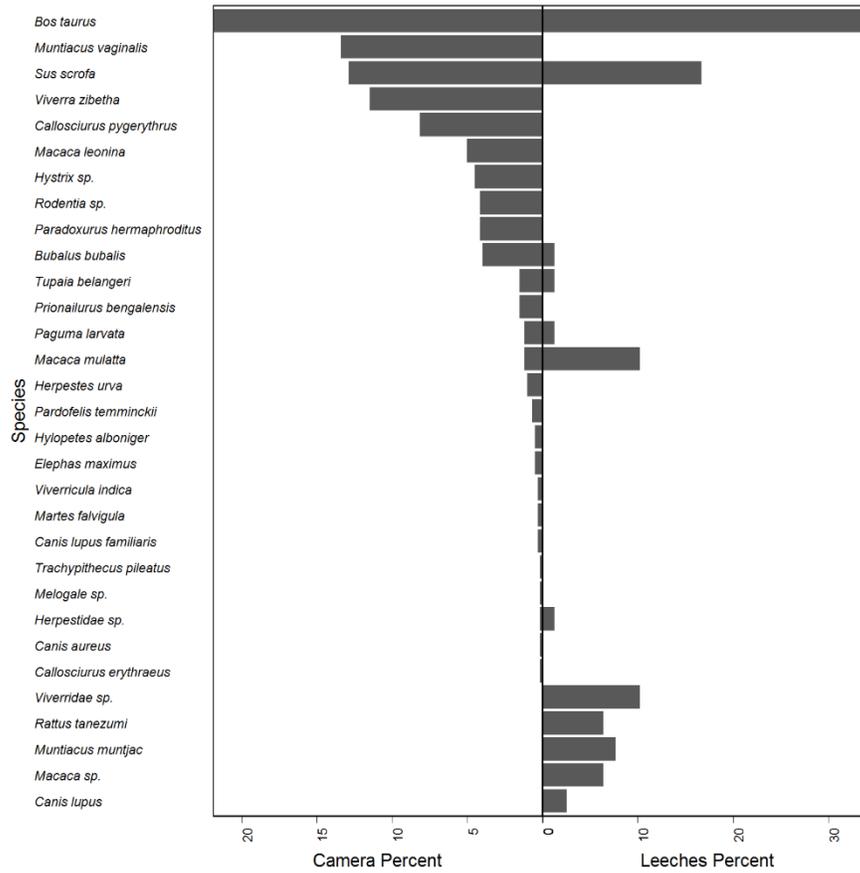


Figure 8 Non-human mammalian species composition of camera trap photographs from 1334 nights of camera trapping (left) and non-human mammalian species composition of blood meals (right) identified from 78 leeches that successfully amplified non-human mammalian DNA. Camera trapping was conducted in northeast Bangladesh in 2014-2015. Leeches were collected in Bangladesh in 2015.

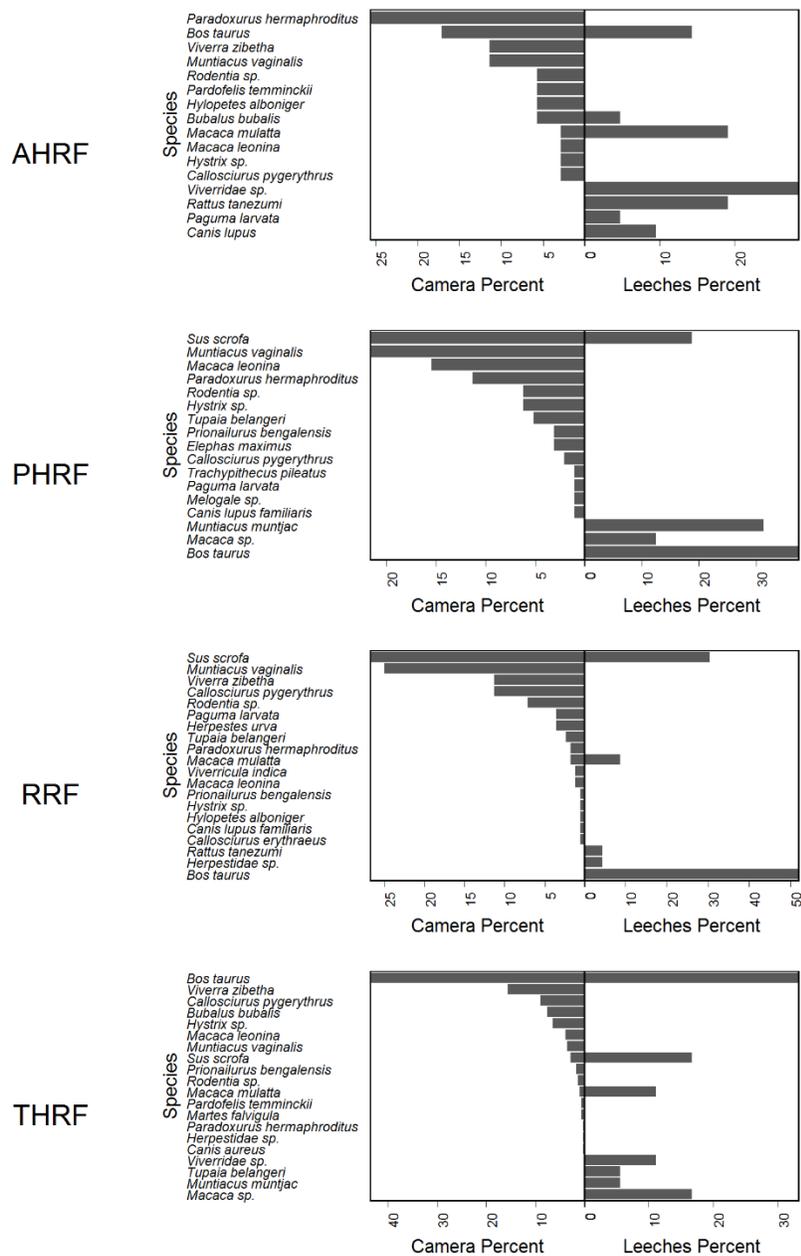


Figure 9 Non-human mammalian species composition found using camera trapping (left) and leech blood meals (right) at four sites in northeast Bangladesh (AHRF= Atora Hill Reserve Forest, PHRF= Patharia Hill Reserve Forest, RRF= Rajkandi Reserve Forest, THRF= Tarap Hill Reserve Forest). Camera trapping was conducted 2014-2015, and leeches were collected in 2015.

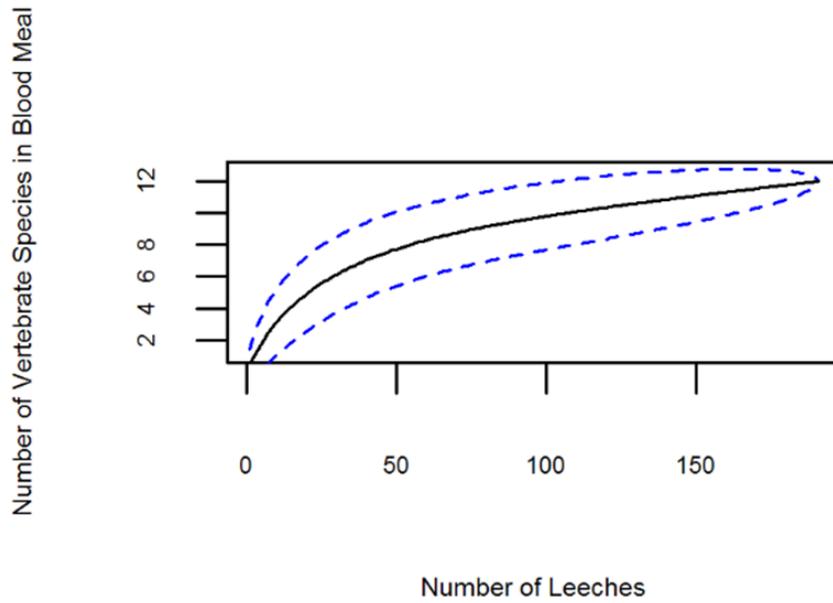


Figure 10 Species accumulation curve constructed in R version 3.2.0 (R Core Team 2016) using function specaccum in the vegan library using Kindt's exact method (Oksanen et al. 2015). All 191 sequenced leeches (collected in Bangladesh in 2015) were used to construct the curve.

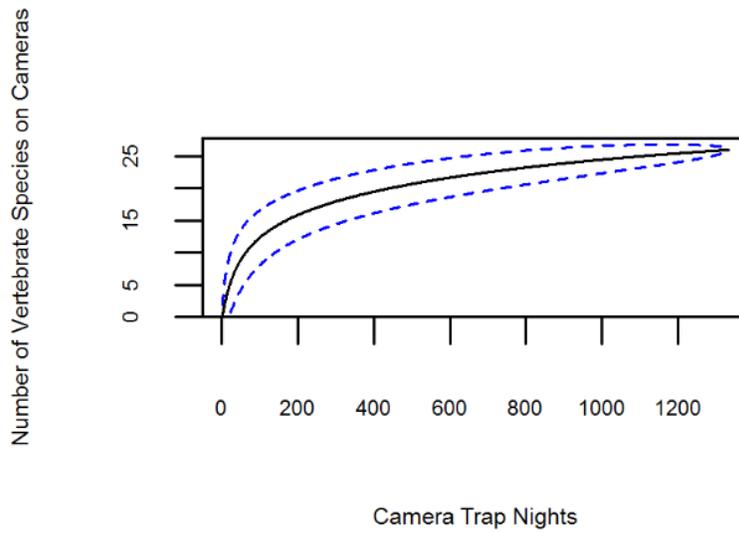


Figure 11 Species accumulation curve constructed in R version 3.2.0 (R Core Team 2016) using function `specaccum` in the `vegan` library using Kindt's exact method (Oksanen et al. 2015). The curve was created from 1,334 camera trap nights collected from four study sites in northeast Bangladesh.

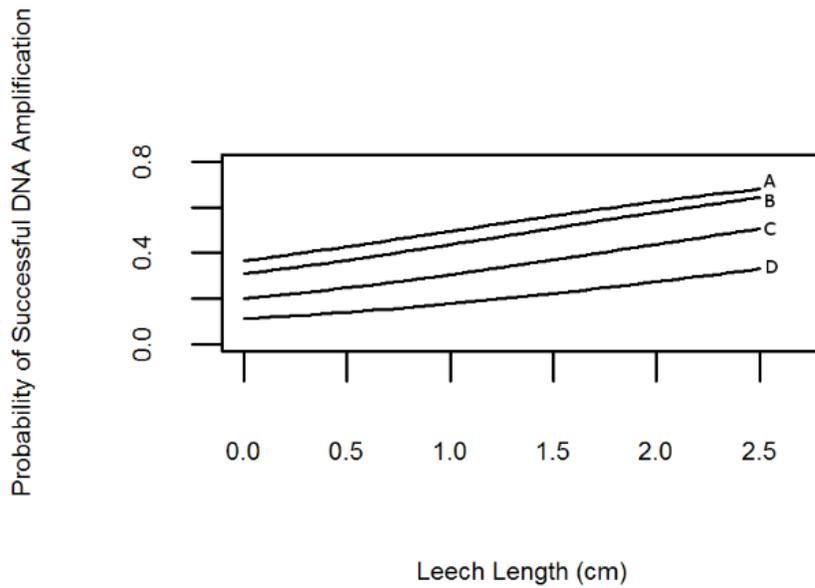


Figure 12 Predicted probability of successful non-human mammalian DNA amplification as a function of leech size. Predictions were based the output of Model 4 described above. The four different lines represent the mean probability of successful amplification at different leech lengths for the four different leech species; “A” is *Haemadipsa c.f. silvestris*; “B” is *H. ornata*; “C” is the unknown species; and “D” is *H. montevidicus*.

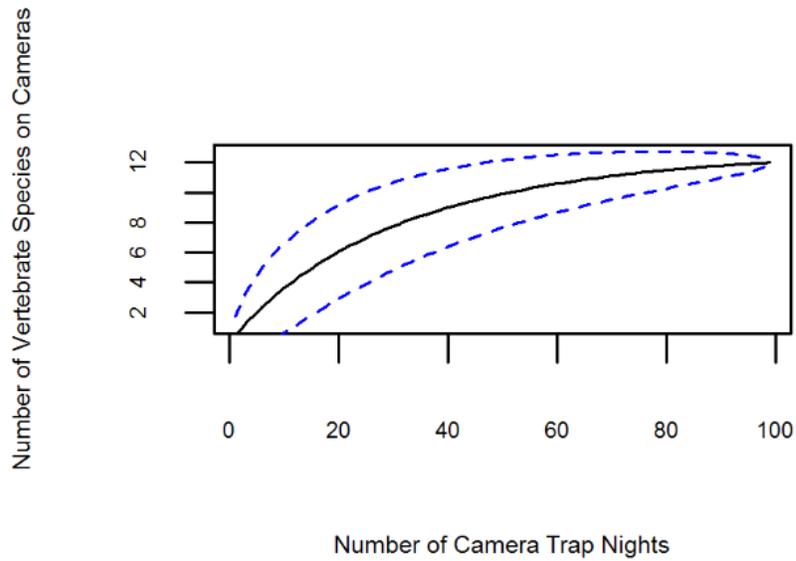


Figure 13 Species accumulation curve constructed in R version 3.2.0 (R Core Team 2016) using function `specaccum` in the `vegan` library using Kindt's exact method (Oksanen et al. 2015). The curve was created from 99 randomly selected camera trap nights from 16 randomly selected cameras collected from four study sites in northeast Bangladesh.

## REFERENCES

- Al-Khleif, A., M. Roth, C. Menge, J. Heuser, G. Baljer, and W. Herbst. 2011. Tenacity of mammalian viruses in the gut of leeches fed with porcine blood. *J. Med. Microbiol.* 60:787–92.
- Azlan, J. M., and D. S. K. Sharma. 2006. The diversity and activity patterns of wild felids in a secondary forest in Peninsular Malaysia. *Oryx* 40:36.
- Bååth, R. 2014. Bayesian First Aid: A Package that Implements Bayesian Alternatives to the Classical \*.test Function. in *User! 2014 - the International R User Conference*.
- Bangladesh Forest Department. 2012. GIS map of forest cover in Bangladesh. Dhaka.
- Baskova, I. P., and L. L. Zavalova. 2001. Proteinase inhibitors from the medicinal leech *Hirudo medicinalis*. *Biochemistry* 66:703–14.
- Borda, E., and M. E. Siddall. 2010. Insights into the evolutionary history of Indo-Pacific bloodfeeding terrestrial leeches (Hirudinida : Arhynchobdellida: Haemadipidae). *Invertebr. Syst.* 24:456–472.
- Burkett-Cadena, N. D., R. a. Ligon, M. Liu, H. K. Hassan, G. E. Hill, M. D. Eubanks, and T. R. Unnasch. 2010. Vector-host interactions in avian nests: Do mosquitoes prefer nestlings over adults? *Am. J. Trop. Med. Hyg.* 83:395–399.
- Burton, A. C. 2012. Critical evaluation of a long-term, locally-based wildlife monitoring program in West Africa. *Biodivers. Conserv.* 21:3079–3094.
- Calvignac-Spencer, S., K. Merkel, N. Kutzner, H. Kuhl, C. Boesch, P. M. Kappeler, S. Metzger, G. Schubert;, and F. H. Leendertz. 2013. Carrion fly-derived DNA as a tool for comprehensive and cost effective assessment of mammalian biodiversity. *Mol. Ecol.* 22:915–924.
- Campbell-Smith, G., R. Sembiring, and M. Linkie. 2012. Evaluating the effectiveness of human-orangutan conflict mitigation strategies in Sumatra. *J. Appl. Ecol.* 49:367–375.
- Challender, D., K. Nguyen Van, T. Shepherd, C. Krishnasamy, A. Wang, B. Lee, E. Panjang, L. Fletcher, S. Heng, J. Seah Han Ming, A. Olsson, A. Nguyen The Truong, Q. Nguyen Van, and Y. Chung. 2014. *Manis javanica*.
- Challender, D. W. S. 2011. Asian Pangolins: increasing affluence driving hunting pressure. *TRAFFIC Bull.* 23:92–93.

- Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43:783–791.
- Chapman, C. A., R. Ghai, A. Jacob, S. M. Koojo, R. Reyna-hurtado, J. M. Rothman, D. Twinomugisha, M. D. Wasserman, and T. L. Goldberg. 2013. Going, going gone: a 15 year history of the decline of primates in forest fragments near Kibale National Park, Uganda. Pp. 89–100 in *Primates in Fragments: Complexity and Resilience*. Springer New York.
- Chowdhury, M. S. H., and M. Koike. 2010. An overview on the protected area system for forest conservation in Bangladesh. *J. For. Res.* 21:111–118.
- Darbro, J. M., A. A. Dhondt, F. M. Vermeulen, and L. C. Harrington. 2007. *Mycoplasma gallisepticum* infection in house finches (*Carpodacus mexicanus*) affects mosquito blood feeding patterns. *Am. J. Trop. Med. Hyg.* 77:488–494.
- del Castillo, R. F. 2015. A conceptual framework to describe the ecology of fragmented landscapes and implications for conservation and management. *Ecol. Appl.* 25:1447–1455.
- Edwards, D. P., J. a. Hodgson, K. C. Hamer, S. L. Mitchell, A. H. Ahmad, S. J. Cornell, and D. S. Wilcove. 2010. Wildlife-friendly oil palm plantations fail to protect biodiversity effectively. *Conserv. Lett.* 3:236–242.
- Fahrig, L. 2003. Fragmentation on of Habitat Effects Biodiversity. *Annu. Rev. Ecol. Syst.* 34:487–515.
- Fischer, J., and D. B. Lindenmayer. 2007. Landscape modification and habitat fragmentation: a synthesis. *Glob. Ecol. Biogeogr.* 16:265–280.
- Fooden, J. 1995. Systematic review of Southeast Asia long-tail macaques, *Macaca fascicularis* Raffles (1821). *Fieldiana Zool.* 64:1–44.
- Francis, C. M., A. V. Borisenko, N. V. Ivanova, J. L. Eger, B. K. Lim, A. Guillén-Servent, S. V. Kruskop, I. Mackie, and P. D. N. Hebert. 2010. The Role of DNA Barcodes in Understanding and Conservation of Mammal Diversity in Southeast Asia. *PLoS One* 5:e12575.
- Gaidet-Drapier, N., H. Fritz, M. Bourgarel, P. C. Renaud, P. Poilecot, P. Chardonnet, C. Coid, D. Poulet, and S. Le Bel. 2006. Cost and efficiency of large mammal census techniques: Comparison of methods for a participatory approach in a communal area, Zimbabwe. *Biodivers. Conserv.* 15:735–754.

- Gaveau, D. L. a, L. M. Curran, G. D. Paoli, K. M. Carlson, P. Wells, a. Besse-Rimba, D. Ratnasari, and N. Leader-Williams. 2012. Examining protected area effectiveness in Sumatra: Importance of regulations governing unprotected lands. *Conserv. Lett.* 5:142–148.
- Gaveau, D. L. a, M. Linkie, Suyadi, P. Levang, and N. Leader-Williams. 2009. Three decades of deforestation in southwest Sumatra: Effects of coffee prices, law enforcement and rural poverty. *Biol. Conserv.* 142:597–605. Elsevier Ltd.
- Gilroy, J. J., F. a. Edwards, C. a. Medina Uribe, T. Haugaasen, and D. P. Edwards. 2014. Surrounding habitats mediate the trade-off between land-sharing and land-sparing agriculture in the tropics. *J. Appl. Ecol.* 51:1337–1346.
- Gompper, M. E., R. W. Kays, J. C. Ray, S. D. Lapoint, D. A. Bogan, and J. R. Cryan. 2006. A Comparison of Noninvasive Techniques to Survey Carnivore Communities in Northeastern North America Peer Reviewed A Comparison of Noninvasive Techniques to Survey Carnivore Communities in Northeastern North America. *BioOne* 34:1142–1151.
- Groves, C. 2003. Taxonomy of ungulates of the Indian Subcontinent. *J. Bombay Nat. Hist. Soc.* 100:341–361.
- Hulme, M. F., J. a Vickery, R. E. Green, B. Phalan, D. E. Chamberlain, D. E. Pomeroy, D. Nalwanga, D. Mushabe, R. Katebaka, S. Bolwig, and P. W. Atkinson. 2013. Conserving the birds of Uganda’s banana-coffee arc: land sparing and land sharing compared. *PLoS One* 8:e54597.
- Inskip, C., and A. Zimmermann. 2009. Human-felid conflict: a review of patterns and priorities worldwide. *Oryx* 43:18.
- Islam, M. A., M. Uddin, M. A. Aziz, S. Bin Muzaffar, S. Chakma, S. U. Chowdhury, G. W. Chowdhury, M. A. Rashid, S. Mohsanin, I. Jahan, S. Saif, M. B. Hossain, D. Chakma, M. Kamruzzaman, and R. Akter. 2013. Status of bears in Bangladesh : going , going , gone ? *Ursus* 24:83–90.
- Jasinskas, a, D. C. Jaworski, and a G. Barbour. 2000. *Amblyomma americanum*: specific uptake of immunoglobulins into tick hemolymph during feeding. *Exp. Parasitol.* 96:213–221.
- Kelman, C. 2013. Governance Lessons from Two Sumatran Integrated Conservation and Development Projects. *Conserv. Soc.* 11:247.

- Kent, R. J. 2009. Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. *Mol. Ecol. Resour.* 9:4–18.
- Kindt, R., P. Van Damme, and A. J. Simons. 2006. Patterns of species richness at varying scales in western Kenya: Planning for agroecosystem diversification. *Biodivers. Conserv.* 15:3235–3249.
- Kinnaird, M. F., E. W. Sanderson, T. G. O. Brien, H. T. Wibisono, and G. Woolmer. 2003. Deforestation Trends in a Tropical Landscape and Implications for Endangered Large Mammals. *Conserv. Biol.* 17:245–257.
- Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U. S. A.* 86:6196–6200.
- Koh, L. P. 2008. Can oil palm plantations be made more hospitable for forest butterflies and birds? *J. Appl. Ecol.* 45:1002–1009.
- Kumara, H. N., M. A. Kumar, A. K. Sharma, H. S. Sushma, M. M. Singh, and M. M. Singh. 2004. Diversity and management of wild mammals in tea gardens in the rainforest regions of the Western Ghats, India: A case study from a tea estate in the Anaimalai Hills. *Curr. Sci.* 87:1282–1287.
- Laurance, W. F., T. E. Lovejoy, H. L. Vasconcelos, E. M. Bruna, R. K. Didham, P. C. Stouffer, C. Gascon, R. O. Bierregaard, S. G. Laurance, and E. Sampaio. 2002. Ecosystem decay of Amazonian forest fragments: A 22-year investigation. *Conserv. Biol.* 16:605–618.
- Laurance, W. F., H. E. M. Nascimento, S. G. Laurance, A. Andrade, J. E. L. S. Ribeiro, J. P. Giraldo, T. E. Lovejoy, R. Condit, J. Chave, K. E. Harms, and S. D'Angelo. 2006. Rapid decay of tree-community composition in Amazonian forest fragments. *Proc. Natl. Acad. Sci. U. S. A.* 103:19010–19014.
- Ligon, R. A., N. D. Burkett-Cadena, M. Liu, G. E. Hill, H. K. Hassan, and T. R. Unnasch. 2009. Short report: Assessing mosquito feeding patterns on nestling and brooding adult birds using microsatellite markers. *Am. J. Trop. Med. Hyg.* 81:534–537.
- Lindenmayer, D. B. 1999. Future directions for biodiversity conservation in managed forests: indicator species, impact studies and monitoring programs. *For. Ecol. Manage.* 115:277–287.

- Linkie, M., Y. Dinata, a. Nofrianto, and N. Leader-Williams. 2007a. Patterns and perceptions of wildlife crop raiding in and around Kerinci Seblat National Park, Sumatra. *Anim. Conserv.* 10:127–135.
- Linkie, M., Y. Dinata, A. Nugroho, and I. A. Haidir. 2007b. Estimating occupancy of a data deficient mammalian species living in tropical rainforests: Sun bears in the Kerinci Seblat region, Sumatra. *Biol. Conserv.* 137:20–27.
- Linkie, M., G. Guillera-Arroita, J. Smith, A. Ario, G. Bertagnolio, F. Cheong, G. R. Clements, Y. Dinata, S. Duangchantrasiri, G. Fredriksson, M. T. Gumal, L. S. Horng, K. Kawanishi, F. R. Khakim, M. F. Kinnaird, D. Kiswayadi, A. H. Lubis, A. J. Lynam, Maryati, M. Maung, D. Ngoprasert, W. Novarino, T. G. O'Brien, K. Parakkasi, H. Peters, D. Priatna, D. M. Rayan, N. Seuaturien, N. M. Shwe, R. Steinmetz, A. M. Sugesti, Sunarto, M. E. Sunquist, M. Umponjan, H. T. Wibisono, C. C. T. Wong, and Zufahmi. 2013. Cryptic mammals caught on camera: Assessing the utility of range wide camera trap data for conserving the endangered Asian tapir. *Biol. Conserv.* 162:107–115. Elsevier Ltd.
- Linkie, M., I. A. Haidir, A. Nugroho, and Y. Dinata. 2008. Conserving tigers *Panthera tigris* in selectively logged Sumatran forests. *Biol. Conserv.* 141:2410–2415.
- Lyra-Jorge, M. C., G. Ciocheti, V. R. Pivello, and S. T. Meirelles. 2008. Comparing methods for sampling large- and medium-sized mammals: Camera traps and track plots. *Eur. J. Wildl. Res.* 54:739–744.
- Magintan, D., C. Traeholt, and K. V Karuppanannan. 2012. Displacement of the Malayan Tapir (*Tapirus indicus*) in Peninsular Malaysia from 2006 to 2010. *Newsl. IUCN/SSC Tapir Spec. Gr.* 21:13–17.
- Marchal, V., and C. Hill. 2009. Primate crop-raiding: A study of local perceptions in four villages in North Sumatra, Indonesia. *Primate Conserv.* 24:107–116.
- Margono, B., S. Turubanova, I. Zhuravleva, P. Potapov, A. Tyukavina, A. Baccini, S. Goetz, and M. Hansen. 2012. Mapping and monitoring deforestation and forest degradation in Sumatra (Indonesia) using Landsat time series data from 1990–2012. *Environ. Resaerch Lett.* 7:1–16.
- Martínez-de la Puente, J., M. Méndez, S. Ruiz, J. a. Godoy, R. C. Soriguer, and J. Figuerola. 2015. Individual identification of endangered species using mosquito blood meals: a proof-of-concept study in Iberian lynx. *Parasitol. Res.* 114:1607–1610.

- McCarthy, J. L. 2013. Conservation and Ecology of Four Sympatric Felid Species in Bukit Barisan Selatan National Park, Sumatra, Indonesia. University of Massachusetts, Amherst.
- McCarthy, J. L., T. K. Fuller, K. P. McCarthy, H. T. Wibisono, and M. C. Livolsi. 2012. Using camera trap photos and direct sightings to identify possible refugia for the Vulnerable Sumatran striped rabbit *Nesolagus netscheri*. *Oryx* 46:438–441.
- McCarthy, J. L., H. T. Wibisono, K. P. McCarthy, T. K. Fuller, and N. Andayani. 2015. Assessing the distribution and habitat use of four felid species in Bukit Barisan Selatan National Park, Sumatra, Indonesia. *Glob. Ecol. Conserv.* 3:210–221. Elsevier B.V.
- McElreath, R. 2015. rethinking: Statistical Rethinking book package.
- Michalski, F., R. L. P. Boulhosa, A. Faria, and C. A. Peres. 2006. Human-wildlife conflicts in a fragmented Amazonian forest landscape: Determinants of large felid depredation on livestock. *Anim. Conserv.* 9:179–188.
- Mohamed, S. Rahel, H. Bernard, L. N. Ambu, P. Lagan, S. Mannan, H. Hofer, A. Wilting, A. Mohamed, and R. Sollmann. 2013. Density and habitat use of the leopard cat (*Prionailurus bengalensis*) in three commercial forest reserves in Sabah, Malaysian Borneo. *J. Mammal.* 94:82–89.
- Mukul, S. A. 2007. Biodiversity Conservation Strategies in Bangladesh : the State of Protected Areas. *Tigerpaper* 34:28–32.
- Myers, N., G. a B. Fonseca, R. a Mittermeier, G. a B. Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–8.
- Nyhus, P. J., and R. Tilson. 2004. Characterizing human-tiger conflict in Sumatra, Indonesia: implications for conservation. *Oryx* 38:68–74.
- O'Brien, T. G., M. F. Kinnaird, and H. T. Wibisono. 2003. Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. *Anim. Conserv.* 6:131–139.
- Okello, M. M. 2005. Land Use Changes and Human–Wildlife Conflicts in the Amboseli Area, Kenya. *Hum. Dimens. Wildl.* 10:19–28.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, H. H. Stevens, and H. Wagner. 2015. vegan: Community Ecology Package. R package version 2.3-0.

- Orangutan Foundation International. 2011. Indonesian Forest Facts.
- Phalan, B., M. Onial, A. Balmford, and R. E. Green. 2011. Reconciling Food Production and Biodiversity Conservation: Land Sharing and Land Sparing Compared. *Science* 333:1289–1291.
- Quazi, S. A., and T. Ticktin. 2016. Understanding drivers of forest diversity and structure in managed landscapes: Secondary forests, plantations, and agroforests in Bangladesh. *For. Ecol. Manage.* 366:118–134. Elsevier B.V.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rajaratnam, R., M. Sunquist, L. Rajaratnam, and L. Ambu. 2007. Diet and habitat selection of the leopard cat (*Prionailurus bengalensis borneoensis*) in an agricultural landscape in Sabah, Malaysian Borneo. *J. Trop. Ecol.* 23:209.
- Rovie-Ryan, J. J., Z. Z. Zainuddin, W. Marni, A. H. Ahmad, L. N. Ambu, J. Payne, and M. T. Abdullah. 2013. Blood meal analysis of tabanid fly after it biting the rare Sumatran rhinoceros. *Asian Pac. J. Trop. Biomed.* 3:95–9. Asian Pacific Tropical Biomedical Magazine.
- Sampaio, R., A. P. Lima, W. E. Magnusson, and C. a. Peres. 2010. Long-term persistence of midsized to large-bodied mammals in Amazonian landscapes under varying contexts of forest cover. *Biodivers. Conserv.* 19:2421–2439.
- Sawyer, R. T. 1986. *Leech Biology and Behaviour*. Oxford University Press, Oxford.
- Schipper Jan et al. 2008. The status of the World's land and marine mammals : diversity, threat and knowledge. *Science* 322:225–230.
- Schnell, I. B., P. F. Thomsen, N. Wilkinson, M. Rasmussen, L. R. D. Jensen, E. Willerslev, M. F. Bertelsen, and M. T. P. Gilbert. 2012. Screening mammal biodiversity using DNA from leeches. *Curr. Biol.* 22:R262–3. Elsevier.
- Secretariat of the Convention on Biological Diversity. 2010. *Global Biodiversity Outlook 3*.
- Sitompul, A., C. Griffin, N. Rayl, and T. Fuller. 2013. Spatial and Temporal Habitat Use of an Asian Elephant in Sumatra. *Animals* 3:670–679.
- Smith, E. P., and G. Van Belle. 1984. Nonparametric estimation of species richness. *Biometrics* 40:119–129.

- Soto-Shoender, J. R., and W. M. Giuliano. 2011. Predation on livestock by large carnivores in the tropical lowlands of Guatemala. *Oryx* 45:561–568.
- Squires, D. 2013. Biodiversity Conservation in Asia. *Asia Pacific Policy Stud.* 1:n/a–n/a.
- Stan Development Team. 2016. Rstan: the R interface to Stan, Version 2.9.0.
- Sunarto, S., M. J. Kelly, K. Parakkasi, S. Klenzendorf, E. Septayuda, and H. Kurniawan. 2012. Tigers need cover: Multi-scale occupancy study of the big cat in Sumatran forest and plantation landscapes. *PLoS One* 7.
- Suyanto, S. 2007. Underlying cause of fire: Different form of land tenure conflicts in Sumatra. *Mitig. Adapt. Strateg. Glob. Chang.* 12:67–74.
- Thorn, M., M. Green, F. Dalerum, P. W. Bateman, and D. M. Scott. 2012. What drives human-carnivore conflict in the North West Province of South Africa? *Biol. Conserv.* 150:23–32. Elsevier Ltd.
- Tobler, M. W., S. E. Carrillo-Percastegui, R. Leite Pitman, R. Mares, and G. Powell. 2008. An evaluation of camera traps for inventorying large-and medium-sized terrestrial rainforest mammals. *Anim. Conserv.* 11:169–178.
- Treves, A., and K. U. Karanth. 2003. Human-Carnivore Conflict and Perspectives on Carnivore Management Worldwide. *Conserv. Biol.* 17:1491–1499.
- Turner, I. M., and R. T. Corlett. 1996. The conservation value of small, isolated fragments of lowland tropical rain forest. *Trends Ecol. Evol.* 11:330–333.
- Ugland, K. I., J. S. Gray, and K. E. Ellingsen. 2003. The species-accumulation curve and estimation of species richness. *J. Anim. Ecol.* 72:888–897.
- Votýpka, J., J. Rádová, T. Skalický, M. Jirků, D. Jirsová, A. D. Mihalca, G. D'Amico, K. J. Petrželková, D. Modrý, and J. Lukeš. 2015. A tsetse and tabanid fly survey of African great apes habitats reveals the presence of a novel trypanosome lineage but the absence of *Trypanosoma brucei*. *Int. J. Parasitol.*, doi: 10.1016/j.ijpara.2015.06.005.
- Wibisono, H. T. 2005. Population ecology of Sumatran tigers (*Panthera tigris sumatrae*) and their prey in Bukit Barisan Selatan National Park, Sumatra, Indonesia. University of Massachusetts Amherst.

- Wibisono, H. T., M. Linkie, G. Guillera-Arroita, J. a. Smith, Sunarto, W. Pusparini, Asriadi, P. Baroto, N. Brickle, Y. Dinata, E. Gemita, D. Gunaryadi, I. a. Haidir, Herwansyah, I. Karina, D. Kiswayadi, D. Kristiantono, H. Kurniawan, J. J. Lahoz-Monfort, N. Leader-Williams, T. Maddox, D. J. Martyr, Maryati, A. Nugroho, K. Parakkasi, D. Priatna, E. Ramadiyanta, W. S. Ramono, G. V. Reddy, E. J. J. Rood, D. Y. Saputra, A. Sarimudi, A. Salampessy, E. Septayuda, T. Suhartono, A. Sumantri, Susilo, I. Tanjung, Tarmizi, K. Yulianto, M. Yunus, and Zulfahmi. 2011. Population status of a cryptic top predator: An island-wide assessment of tigers in sumatran rainforests. *PLoS One* 6.
- Wickramasekara, S., J. Bunikis, V. Wysocki, and A. G. Barbour. 2008. Identification of residual blood proteins in ticks by mass spectrometry proteomics. *Emerg. Infect. Dis.* 14:1273–1275.
- Wong, W.-M., N. Leader-Williams, and M. Linkie. 2013. Quantifying changes in sun bear distribution and their forest habitat in Sumatra. *Anim. Conserv.* 16:216–223.
- World Wildlife Fund. n.d. Bukit Barisan Selatan National Park.
- Yang, L., Z. Tan, D. Wang, L. Xue, M.-X. Guan, T. Huang, and R. Li. 2014. Species identification through mitochondrial rRNA genetic analysis. *Sci. Rep.* 4:4089.
2014. DNA Barcoding Initiative for Conservation.