Complementarity of invertebrate-derived DNA and camera traps as tools in assessing tropical mammals

Introduction

Although mammals are a comparatively well studied taxa, gaps in knowledge regarding species' distributions and taxonomy, exist. This is especially important to species labelled as data deficient and those that are of conservation concern (Burgar et al., 2018). A popular approach to assessing mammalian biodiversity is through camera traps. The use of camera traps in mammalian conservation has surpassed all expectations (Moore et al., 2021); despite this, it is limited in its use in capturing species defined by small population sizes (Schnell et al., 2012), and obscure and mobile species (Weiskopf et al., 2018; Lyet et al., 2021), in areas such as the Tropics, increasing the need for alternatives (Gogarten et al., 2019).

DNA barcoding enables DNA of a specific species to be sampled and sequenced from an environmental source in the absence of the intended organism (eDNA). Sampled eDNA contains unknown DNA that when amplified can be matched with a specific species on the basis of how alike its sequence is in comparison with a reference database of homologous sequences (Cristescu & Hebert, 2018). The use of eDNA has demonstrated its effectiveness to detect the presence of mammalian species in marine ecosystems (Gogarten et al., 2019), and in some cases, terrestrial ecosystems (Lyet et al., 2021), though its use in terrestrial environments is restricted to a handful of studies (Gogarten et al., 2019). This highlights the need for the development of DNA barcoding techniques viable for use in terrestrial ecosystems (Beja-Pereira et al., 2009).

Invertebrates that rely on or feed on vertebrates and their waste products during a stage of their life cycles serve as a potential alternative source of mammalian DNA for use in DNA barcoding techniques to assess mammalian biodiversity. Invertebrate-extracted DNA (iDNA) offers great promise as they are easily obtainable and ubiquitous across the Tropics. While a handful of studies have successfully demonstrated the use of iDNA obtained from hematophagous leeches (*haemadipsa* spp.,) and carrion flies, belonging to the families Calliphoridae (blowflies) and Sarcophagidae (flesh flies) (here after flies, unless specified), in assessing mammalian biodiversity, unclarity persists regarding the use of this new development in describing mammalian biodiversity and how it competes with more commonly used approaches such as camera traps (Gogarten et al., 2019).

This paper aims to draw attention to the potential of iDNA as a tool to assess mammalian biodiversity in the Tropics. Moreover, this paper also has as goal to display the key features of this tool that sets it apart from camera traps and its potential to complement camera traps in assessing mammalian tropical biodiversity and their conservation.

Results & Discussion

One of the first studies to investigate the effectiveness of employing carrion fly iDNA (fly iDNA) as a tool to assess tropical mammalian biodiversity was that of Calvignac-Spencer et al., (2013). To highlight their aim, two different tropical ecosystems – Taï National Park (TNP) in Ivory Coast (moist tropical forest) and Kirindy Forest (KF), Madagascar (dry deciduous forest) – were assessed. TNP and KF are home to a myriad of mammalian species, including 9 primate species and 8 lemur species, respectively, as part of long-standing research programmes. During this study, 75 and 40 flies were randomly sampled in TNP and KF, respectively. iDNA was then extracted from these 115 flies and analysed for the presence of mammalian DNA.

Despite the modest number of flies collected, 40% of the randomly sampled flies in both tropical habitats resulted in amplifiable mammalian DNA, with some containing DNA from multiple mammalian species. In KF, Calvignac-Spencer et al., (2013) found that the collection of 40 flies resulted in the identification of 4 mammalian species making up 13% of the known local mammalian biodiversity in KF. In TNP, 75 flies resulted in the identification of 16 mammalian species, including 6 out of the 9 local primates and the endangered Jentink's duiker (*Cephalophus jentinki*). Furthermore, mammalian DNA was found across tree-and-land-dwelling mammalian species, suggesting that blow and flesh flies have a broad range of host preferences (Calvignac-Spencer et al., 2013).

In response to the study conducted by Calvignac-Spencer et al., (2013), Lee et al., (2016) conducted a study, in 2015, to compare the effectiveness of blowfly-extracted DNA to that of camera traps to assess mammalian biodiversity in the logged tropical forests of Tembat Forest Reserve (TFR), Peninsular Malaysia. The study took place over the course of 30 days during which a total of 1,345 blowflies and camera trap data corresponding to 600 trap nights (20 cameras * a 30-day period) were obtained.

After analysis of the data, 20 mammalian species were identified. Blowfly accounted for a higher species richness and included 11 of the 20 species of the orders Chiroptera, Artiodactyle, Carnivora, Primates and Rodentia, compared to 9 for the camera traps,

belonging to the orders Artiodactyle, Carnivora, Primates Cetardiodactyla, Perissodactyla, and Proboscidae. Actually, blowfly traps accounted for 9 vertebrate orders, however, 4 of them belonged to non-mammal taxa and were thus left out of the study. Furthermore, the only species found in both traps was the genus *Sus* which is likely to be *Sus scrofa* based on photo identification (Lee et al., 2016).

To summarise, studies conducted by Calvignac-Spencer et al., (2013) & Lee et al., (2016) showed that in spite of moderate numbers of flies sampled, a large array of mammal orders and species, including obscure mammals such as the Jentink's duiker could be detected. However, while Calvignac-Spencer et al., (2013) observed no blowfly feeding biases, Lee et al., (2016) found that despite the widespread prevalence of the Long-tailed macaque (*Macaca fascicularis*) within TFR during the time the study took place, the Long-tailed macaque was only observed in camera traps and not in blowfly iDNA.

Despite their widespread prevalence, ease of sampling, and broad host preferences, blowfly derived-iDNA degrades within 24 hours from the time of feeding. In contrast, leech derived-iDNA can be extracted up until 4 months of feeding before it degrades thereby increasing the likelihood that a particular species of interest could be found (Schnell et al., 2012). To investigate the effectiveness of using hematophagous leeches compared to camera traps in assessing mammalian diversity, Schnell et al., (2012) extracted iDNA from 25 leeches to detect for the presence of obscure mammalian species in the Central Annamite rain forests of Vietnam, Asia. The field site used in this study was identical to when camera traps were installed years prior (Schnell et al., 2012).

Schnell et al., found that iDNA from 21 out of the 25 leeches contained matching DNA sequences corresponding with 6 species spread over three orders – Artiodactyla, Carnivora, and Lagomorpha. Of the six species identified, the *Muntiacus truongsonensis* (Truong Son muntjac) and *Nesolagus timminsi* (Annamite striped rabbit) are labelled as 'data deficient' according to the IUCN. Suspicion with regards to the presence of the Annamite striped rabbit in the Central Annamite region has existed since 1996. However, despite more than 2000 camera trap nights having been carried out within this time frame, this was the first ever-record of the species' existence. Of the 16 leeches remaining, six leeches embodied DNA material corresponding to the obscure *Melogale moschata* (small-toothed ferret-badger) and three embodied DNA pertaining to the almost threatened *Capricornis maritimus* or the Indochinese Serow. In fact, despite the *Melogale* having been observed in camera trap footage, the two

Melogale species (*M. moschata and M. personata*) are morphologically indistinguishable from each other (Schnell et al., 2012). Therefore, this study achieved to provide evidence regarding the existence of the *M. moschata*. The remaining leeches embodied DNA pertaining to the *Bos taurus* and *Sus scrofa,* the cow and pig, respectively (Schnell et al., 2012).

Similar to the study conducted by Schnell et al., (2012) was that of Weiskopf et al., (2018). Having identical aims in mind, the latter study was conducted in four dense, but fragmented, tropical forest fragments of Northeast Bangladesh. In total, fifty leeches were sampled per forest fragment over the course of four days, one forest fragment a day. After analysis of the fragments, Weiskopf et al., (2018) found that over the course of 1,334 camera trap nights, 863 individual mammals were recorded corresponding to 26 identified mammal species, in comparison to the 12 mammal species identified by analysis of 200 leech blood meals. Of these 12 mammalian species, however, nine could be identified and three could not. Furthermore, the effectiveness of each method differed with camera traps, on average, accounting for 9.1 more species, but the confidence interval (CI) was very wide (95% CI: 2.0-16.3). However, it is noteworthy to point out that camera traps were deployed for a longer period of time in comparison to when leech sampling took place.

In both the leech blood meals and camera traps, *Bos taurus* (cows) and *Sus scrofa* (pigs) were the most frequently sighted species. While camera traps recorded higher rodent diversity, they were only partially successful in this as several were unable to be identified using camera trap footage. Two felid species, the Asiatic golden cat *Pardofelis temminckii* and leopard cat *Prionailurus bengalensis,* were captured on camera but were not found to be present in leech iDNA. On the other hand, many more leeches contained mammalian DNA belonging to the Rhesus macaques (*Macaca mulatta*) compared to in the camera trap data. In addition, the rodent, *Rattus tanezumi,* could only be identified in the leech blood meals but not on camera. Finally, only leech blood meals detected the presence of three unknown species which did not correlate with list of species known to exist in Bangladesh. These 'false positives' are a result of insufficient database coverage, a problem known to persist throughout the tropics due to absent sequences in the GenBank (Weiskopf et al., 2018). Moreover, a higher species diversity was observed when using camera traps compared to leech blood samples, however, leech blood samples identified species that were not observed on camera traps (Weiskopf et al., 2018).

To summarise, results obtained by Schnell et al., (2012) & Weiskopf et al., (2018) point out that that the use of leeches also prove to be effective in detecting obscure and mobile mammals. However, interestingly, the Asiatic golden cat and the Leopard cat were not detected in leech blood meals while some smaller mammals were missing on camera traps, highlighting the importance of both these techniques in obtaining a good coverage of mammalian biodiversity. However, three species could not be identified in leech blood meals, a challenge opposing the use of this technique.

Conclusion

While all the studies included in this summary highlight the potential of the use of iDNA in mammalian biodiversity assessments, they form the majority of the handful of studies that have investigated the effectiveness of this approach in Tropical regions (Carvalho et al., 2021). Furthermore, due to its infancy, shortcomings regarding the methodologies used, and insufficient understanding of dispersal, distribution and feeding biases with regards to hematophagous leeches and carrion flies in tropical regions, persist throughout these studies (Carvalho et al., 2021). Of importance, is the unreliability and inaccuracy associated with the methodological approached used, especially pertaining to the impact of climate on DNA persistence in tropical environments, contamination in the process of sampling and laboratory analysis, thereby contributing to the quality of mammalian DNA found in iDNA. This, in combination with the limited number of Tropical species represented in GenBank, leads to an increase in false positives (species is missing but DNA is detected) or false negatives (species is not detected when it is known to exist) associated with this technique (Carvalho et al., 2021; Calvignac-Spencer et al., 2013; Lee et al., 2016; Schnell et al., 2012; Weiskopf et al., 2018).

In order to understand the full capacity of this approach to mammalian biodiversity conservation, it is imperative that more such studies need to be conducted in the Tropics, together with studies aiming to investigate the dispersal and feeding biases of hematophagous leeches and carrion flies. Furthermore, an increased number of studies would also help in adding more species to GenBank, and invaluable tool in this approach's utility and success. However, the main reason for the limited number of studies up to this date is the amount of funds available to carry out mammalian research in tropical areas (Carvalho et al., 2021). This is a consequence of two reasons: many tropical areas lie in low-income countries; secondly, there is a lack of urgency when it comes to the preservation of mammalian biodiversity within the political circle, something that has to be addressed. Moreover, with increased funding and

more studies, this tool could fill the void left by camera traps, and serve to complement rather than compete with camera traps, improving mammalian biodiversity in the Tropics in pivotal times like these.

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