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iDNA screening: Disease vectors as vertebrate samplers

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Abstract

In the current context of global change and human-induced biodiversity decline, there is an urgent need for developing sampling approaches able to accurately describe the state of biodiversity. Traditional surveys of vertebrate fauna involve time-consuming and skill-demanding field methods. Recently, the use of DNA derived from invertebrate parasites (leeches and blowflies) was suggested as a new tool for vertebrate diversity assessment. Bloodmeal analyses of arthropod disease vectors have long been performed to describe their feeding behaviour, for epidemiological purposes. On the other hand, this existing expertise has not yet been applied to investigate vertebrate fauna per se. Here, we evaluate the usefulness of hematophagous dipterans as vertebrate samplers. Blood-fed sand flies and mosquitoes were collected in Amazonian forest sites and analysed using high-throughput sequencing of short mitochondrial markers. Bloodmeal identifications highlighted contrasting ecological features and feeding behaviour among dipteran species, which allowed unveiling arboreal and terrestrial mammals of various body size, as well as birds, lizards and amphibians. Additionally, lower vertebrate diversity was found in sites undergoing higher levels of human-induced perturbation. These results suggest that, in addition to providing precious information on disease vector host use, dipteran bloodmeal analyses may represent a useful tool in the study of vertebrate communities. Although further effort is required to validate the approach and consider its application to large-scale studies, this first work opens up promising perspectives for biodiversity monitoring and eco-epidemiology.

KEYWORDS
bloodmeal, dipteran, feeding preference, insect, mosquito, sand fly

1 | INTRODUCTION

The current rate of species disappearance is sometimes considered a sixth "mass extinction" crisis (Ceballos et al., 2015). Rapid modifications of biological communities, at both global and local scales, raise concerns as to the concomitant alteration of essential ecosystem functions and services (Cardinale et al., 2012). Developing an indicator system to monitor the state and dynamics of biodiversity through time is a major challenge to reach the 2020 Aichi Targets defined by Parties to the United Nations Convention on Biological Diversity. A set of Essential Biodiversity Variables has been recently defined (Pereira et al., 2013), among which taxonomic diversity of communities is relevant to several Aichi Targets. However, there are still uncertainties regarding the economical and practical feasibility of developing monitoring systems at global and local scales.

Vertebrate community composition is frequently used as an indicator for the state of entire biota, because it is relatively well known, occupies a wide variety of ecological niches, provides key ecosystem functions and contains species that are highly sensitive to human-induced disturbances (Ceballos & Ehrlich, 2002; Ceballos et al., 2015; Collen et al., 2009; de Thoisy et al., 2010). Vertebrate surveys have been relying on time-consuming and expertise-demanding

observational methods such as line transects, track counts or live trapping (Smallwood & Fitzhugh, 1995; de Thoisy, Brosse, & Dubois, 2008; Vieira & Monteiro-Filho, 2003; Voss & Emmons, 1996). Technological progress has allowed the development of alternative approaches, including remote detection through camera trapping (Rovero, Martin, Rosa, Ahumada, & Spitale, 2014) or the sequencing of environmental DNA (Andersen et al., 2012; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012; Valentini et al., 2016). Recently, the use of DNA derived from invertebrate parasites (iDNA) was suggested as a new tool for vertebrate diversity assessment. Preliminary studies conducted on leeches (Schnell et al., 2012) and carrion flies (Calvignac-Spencer, Merkel, et al., 2013; Lee, Gan, Clements, & Wilson, 2016) gave promising results, but the method has not yet been applied to large data sets.

Host identification from bloodmeals of disease-transmitting arthropods has long been performed for estimating their feeding preference (Humair et al., 2007; Tempelis, 1975; Tesh, Chaniotis, Carrera, & Johnson, 1972) because it is an important epidemiological parameter for vector-borne diseases (Hamer et al., 2011; Kent, 2009; Simpson et al., 2012). On the other hand, despite their worldwide distribution, the numerous methods available for their collection and analyses, and the good quality of iDNA they provide (i.e., blood; Calvignac-Spencer, Leendertz, Gilbert, & Schubert, 2013), disease vectors have not been used to investigate the state of vertebrate diversity per se.

In this study, we first investigate the usefulness of dipteran bloodmeal analyses for vertebrate diversity assessment. In particular, we evaluate the applicability of this approach for between-site comparisons. The study was conducted in the Amazonian territory of French Guiana. This region is more than 80% covered by primary lowland rainforests and as such harbours one of the largest continuous rainforest block that benefits from a relatively favourable conservation status (Hammond, 2005). It thus represents one of the few remaining "natural laboratories" where undisturbed biodiversity can be studied over large spatial scales. However, demographic pressure and illegal gold mining activities constitute rising threats to biodiversity in part of the territory (Hammond, Gond, de Thoisy, Forget, & DeDijn, 2007; de Thoisy, Renoux, & Julliot, 2005; de Thoisy et al., 2010). Blood-fed sand flies (Psychodidae: Phlebotominae) and mosquitoes (Culicidae) were collected in forest sites undergoing contrasting levels of anthropogenic pressure. Dipteran and bloodmeal identifications were performed by PCR amplification and high-throughput sequencing of short mitochondrial markers. The diversity of vertebrates observed through bloodmeals was then compared between sites after rarefying samples down to equal size and dipteran species composition.

2 | MATERIALS AND METHODS

2.1 | Sampling

Sampling was performed in the area of Saint-Georges de l'Oyapock in March 2016. Three study sites were selected to constitute a gradient of anthropogenic pressure, as defined by the Human Foot-Print index (HFP index) which has been shown to be a good predictor of vertebrate diversity (de Thoisy et al., 2010, 2016). In particular, hunting pressure was expected to increase with proximity to Saint-Georges town. To limit environmental variability, the sites were chosen within the same geomorphological landscape harbouring a highly dissected plateau (Guitet et al., 2013) and the same forest type (Guitet, Brunaux, Granville, & Richard-Hansen, 2015). Site "SG1," the most impacted site, was located along the road leading to Brazil, close to the Oyapock river, at 1.2 km from Saint-Georges and exhibited an HFP index of 32. Sites "SG6" and "SG30" were located along the national road 2 (constructed 15 years ago) at 6.8 km and 30.3 km from Saint-Georges and presented an HFP index of 26 and 21, respectively (Figure 1).

Nine Center for Disease Control (CDC) light-traps were set in each site, during four consecutive nights (from 6 p.m.–6 a.m.) for a total of 108 trap-nights. After each night, insects contained in the traps were killed by freezing. Blood-fed mosquito and sand fly females were sorted and kept in individual microcentrifuge tubes with 95% ethanol.

2.2 | DNA amplification and sequencing

We extracted DNA from each engorged female using a without-boiling Chelex protocol (Casquet, Thebaud, & Gillespie, 2012). Two PCR were then performed: the first to amplify the Ins16S_1 marker (Ins16S_1-F: TRRGACGAGAAGACCCTATA; Ins16S_1-R: TCTTAATC CAACATCGAGGTC; Clarke, Soubrier, Weyrich, & Cooper, 2014), which allows species-level identifications of mosquitoes and sand flies (Kocher et al., 2017; Talaga, Leroy, et al., 2017); the second to amplify the 12S-V5 marker (12S-V5-F: TAGAACAGGCTCCTCTAG; 12S-V5-R: TTAGATACCCCACTATGC; Riaz et al., 2011), that was shown to provide accurate identifications of mammals (Kocher et al., 2017). The 12S-V5 marker was designed for metabarcoding of vertebrates. It thus presents several interesting properties for bloodmeal analyses: (i) wide taxonomic coverage (sand flies and mosquitoes may feed on a large variety of vertebrates), (ii) good taxonomic resolution and (iii) very short size (*c.* 100 bp), which allows the detection of highly degraded DNA (Taberlet et al., 2012).

The PCR protocol was the same for both fragments. Amplification was performed in 25 μl mixtures containing 3 μl of DNA template, 12 μl of AmpliTaq Gold PCR Master Mix® (5U/μl; Applied Biosystems, Foster City, CA, USA), 2.5 μl of each primer (5 μM) and nuclease-free water (Promega, Madison, WI, USA). The PCR mixture was denatured at 95°C (10 min) and followed by 35 cycles of 30 s at 95°C, 30 s at 50°C and 30 s at 72°C, completed at 72°C for 10 min. Tags of eight base pairs with at least five differences between them were added at the 5′ end of each primer to enable the sequencing of the multiple PCR products in a single sequencing run (Binladen et al., 2007).

PCR products were pooled and sent for library construction and sequencing to the GeT-PlaGe core facilities of Genotoul (Toulouse, France). Samples were diluted in ultrapure water. A volume of

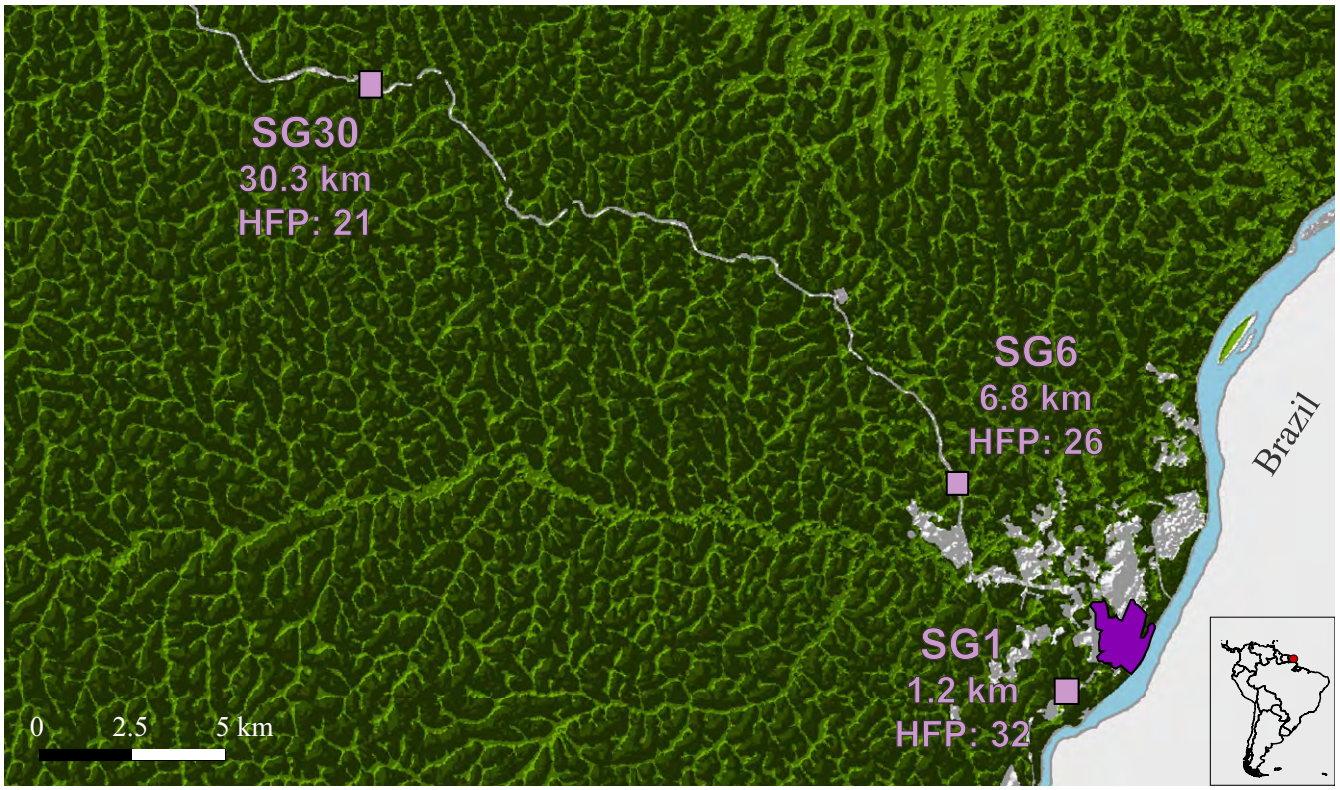

FIGURE 1 Geographical localization of sampling sites in the area of Saint-Georges de l'Oyapock, French Guiana. Human footprint index values (HFP, de Thoisy et al., 2010) and distance from town are indicated. Forested areas are represented in green. Saint-Georges town is displayed in dark purple

130 μl containing 3 μg of DNA was purified using the HighPrep PCR system (Magbio Genomics, Gaithersburg, MD, USA) and used for library construction with the Illumina NEXTflex PCR-Free DNA sequencing kit following the instructions of the supplier (Bioo Scientific corp., Austin, TX, USA). Purified fragments were end-repaired, A-tailed and ligated to sequencing indexed adapters. The quality of the library was controlled using the Fragment Analyzer (Advanced Analytical, Ames, IA, USA) and quantified by qPCR with the Library Quantification Kit-Illumina Genome Analyzer-SYBR Fast Universal (CliniSciences, Nanterre, France). The library was pooled with that of other projects and loaded onto the Illumina MiSeq cartridge according to the manufacturer instructions. The quality of the run was checked internally using PhiX. Quality filtering was performed by the Consensus Assessment of Sequence and Variation (CASAVA) pipeline. The sequencing data were stored on the NG6 platform (Mariette et al., 2012), and all computations were performed on the computer cluster of the Genotoul bioinformatic platform (Toulouse, France).

2.3 | Bioinformatic treatment and taxonomic assignment

The sequencing data were analysed using the OBITOOLS package (Boyer et al., 2016), as described previously (Kocher et al., 2017). Paired-end reads were aligned, merged and then assigned to their corresponding sample based on the tagged primer sequences with two mismatches allowed. Low-quality reads were removed, and reads were then dereplicated. Taxonomic assignment of sequences was performed with `ecotag` (part or the OBITOOLS package). We used reference libraries for Amazonian sand flies (Kocher et al., 2017), mammals (Kocher et al., 2017) and mosquitoes (Data set S3) that we further complemented with sequences from GenBank using `ecoPCR` (Riaz et al., 2011). When a sequence had less than 97% identity with its best match in the reference database, we considered the taxonomic assignment at the generic rank or above. As human presence on the sampling sites was likely scarce and not observed during the sampling period, human sequences identified in bloodmeals were regarded as laboratory contamination and discarded as a conservative measure. In each sample, only the most abundant sequence was considered (i.e., we did not consider potential mixed bloodmeals; see Results and discussion for further explanations).

When dipterans or vertebrates could not be identified at the species level, we defined molecular taxonomic units (MOTUs) within taxa. We used the Poisson Tree Process (Zhang, Kapli, Pavlidis, & Stamatakis, 2013) as implemented in `mPTP` (Kapli et al., 2016). The method seeks to classify the branches of a phylogenetic tree into two processes: within species (corresponding to a coalescence process) and between species (corresponding to a speciation process). Because the method uses a phylogenetic tree, we first performed a

phylogenetic analysis. Sequences were aligned using MUSCLE (Edgar, 2004), and a maximum-likelihood analysis was performed in RAXML v.8 (Stamatakis, 2014), with the GTR+ Gamma substitution model.

2.4 | Bloodmeals diversity analysis

We had to consider two analytical issues when comparing the diversity of dipteran bloodmeals found between sites. First, the number of blood-fed individuals differed among sites, leading to the well-known "sampling" problem of diversity analysis, referring to the fact that the observed number of species is sensitive to sample size (Gotelli & Colwell, 2011). Second, each hematophagous species may exhibit distinct feeding preferences, and their proportions in the samples varied between sites. Hence, the diversity of vertebrates observed through bloodmeals is prone to site-specific bias, precluding the use of classical rarefaction analysis or the direct comparison of diversity estimators.

We thus simulated rarefied bloodmeals pools by randomly drawing, without replacement, a number of individuals from each dipteran species (or MOTU) corresponding to the lowest occurrence of the given species among sites. Using this procedure, the obtained subsamples have identical dipteran composition and can be compared. For each site, we simulated 10 000 rarefied communities and computed mean Hill numbers of diversity (Chao et al., 2014) as well as the range of values containing the middle 95% of their distribution. Sand flies of the same genus appeared to exhibit similar feeding preferences (i.e., *Nyssomyia* feeding mostly on arboreal vertebrates and especially on xenarthrans, *Psychodopygus* feeding mainly on armadillos and *Evandromyia* feeding on terrestrial rodents). Therefore, we grouped sand flies at the generic level to increase the maximum base size of rarefied samples. These analyses can be reproduced using data and scripts available in the Supporting Information.

3 | RESULTS AND DISCUSSION

3.1 | Dipteran sampling and identifications

In total, 209 blood-fed dipterans were collected, including 99 mosquitoes and 110 sand flies. 77, 76 and 56 specimens were collected in SG30, SG6 and SG1, respectively. The insect Ins16S_1 marker (Clarke et al., 2014) was successfully amplified and sequenced for 94.9% of mosquitoes and 84.5% of sand flies, allowing to identify 187 specimens (average sequencing depth = 6465x). Eight mosquitoes and eighteen sand fly species (or MOTUs) were found (Table S1).

3.2 | Bloodmeal analyses

The vertebrate 12S-V5 marker (Riaz et al., 2011) was successfully amplified and sequenced for 94.9% of mosquitoes and 66.4% of sand flies, allowing taxonomic assignment of 167 bloodmeals (average sequencing depth = 2166x). The lower success rate obtained with sand flies is likely explained by the smaller volume of their bloodmeals, which is an important factor for successful iDNA amplification (Calvignac-Spencer, Leendertz, et al., 2013). A total of 22 vertebrate MOTUs were found, of which a majority (nineteen) was identified at the species level (Table S1). These encompassed terrestrial and arboreal mammals of various size, as well as birds, lizards and amphibians (Figure 2). Therefore, on the whole, mosquitoes and sand flies seemed to be efficient vertebrate samplers, allowing the detection of a wide range of species.

The largely dominant mosquito MOTU (*Culex* MOTU 1) appeared to exhibit an opportunistic behaviour, because it had fed on ten mammal species belonging to four different orders, as well as on two bird species (Table 1). *Culex* MOTU 4 was found to feed on two lizards and a frog. All other mosquito species had fed on birds, but were represented by few specimens. For some sand flies, our observations were consistent with existing knowledge: the canopy-dwelling *Nyssomyia umbratilis* feeding mainly on arboreal mammals including a large proportion of xenarthrans (Christensen, Arias, de Vasquez, & de Freitas, 1982; Le Pont & Pajot, 1980) and the ground-dwelling *Bichromomyia flaviscutellata* feeding on small rodents (Lainson & Shaw, 1968; Tesh, Chaniotis, Aronson, & Johnson 1971). In particular, these known vectors of *Leishmania guyanensis* and *L. amazonensis*, respectively, were found to have fed on the main known respective *Leishmania* reservoirs in the region (*Choloepus didactylus* and *Proechimys cuvieri*; Rotureau, 2006). For other species, this study provides the first bloodmeal data. Five *Psychodopygus* species (*Ps. amazonensis*, *Ps. ayrozai*, *Ps. hirsuta*, *Ps. s. maripaensis* and *Ps.* MOTU 1) fed almost exclusively on armadillos. The nine-banded armadillo (*Da. novemcinctus*) is the known reservoir of *L. naiffi* (Lainson & Shaw, 1988), and *Ps. ayrozay* and *Ps. s. maripaensis* are suspected vectors of the same parasite (De Freitas, Biancardi, & Castellon, 1985; Fouque et al., 2007). The association between sand flies and armadillos has been known for a long time, as it can be attested by the Amerindian (*Tupi*) name for sand fly, "tatuquira" (literally armadillo-fly; Von Ihering, 1968). We here report the first direct evidence of this vector–host interaction. The strong observed feeding preference of *Psychodopygus* sand flies for armadillos may explain the relatively low number of human cases caused by *L. naiffi*, as previously suspected, *Ps. ayrozai* being considered as a nonanthropophilic species (Lainson & Shaw, 2010). Finally, sand flies of the genus *Evandromyia*, for which no diet data were available, were observed to feed mainly on terrestrial rodents. Of anecdotal but surprising mention is the detection of the rodent *Isothrix sinnamariensis* (the brush-tailed rat, weighing ca. 200 g) in a sand fly bloodmeal. This rare echimyid was previously know from only three localities in French Guiana, one locality in Guyana and one in Suriname (Lim & Catzeflis, 2014).

Several studies have reported the detection of multiple bloodmeal sources in single hematophagous dipterans (González et al., 2015; Sales et al., 2015), or the possibility to amplify vertebrate host DNA from specimens that are not visibly blood-fed (Moreno et al., 2017). In our case, several vertebrate species were identified in 68% of the specimens, with up to 6 and for a mean of 1.95 species. However, only considering the majority sequence from visibly blood-fed specimens appeared to us as a necessary conservative measure. PCR-based methods are extremely sensitive to laboratory contaminations, and the use of high-throughput sequencing leads to

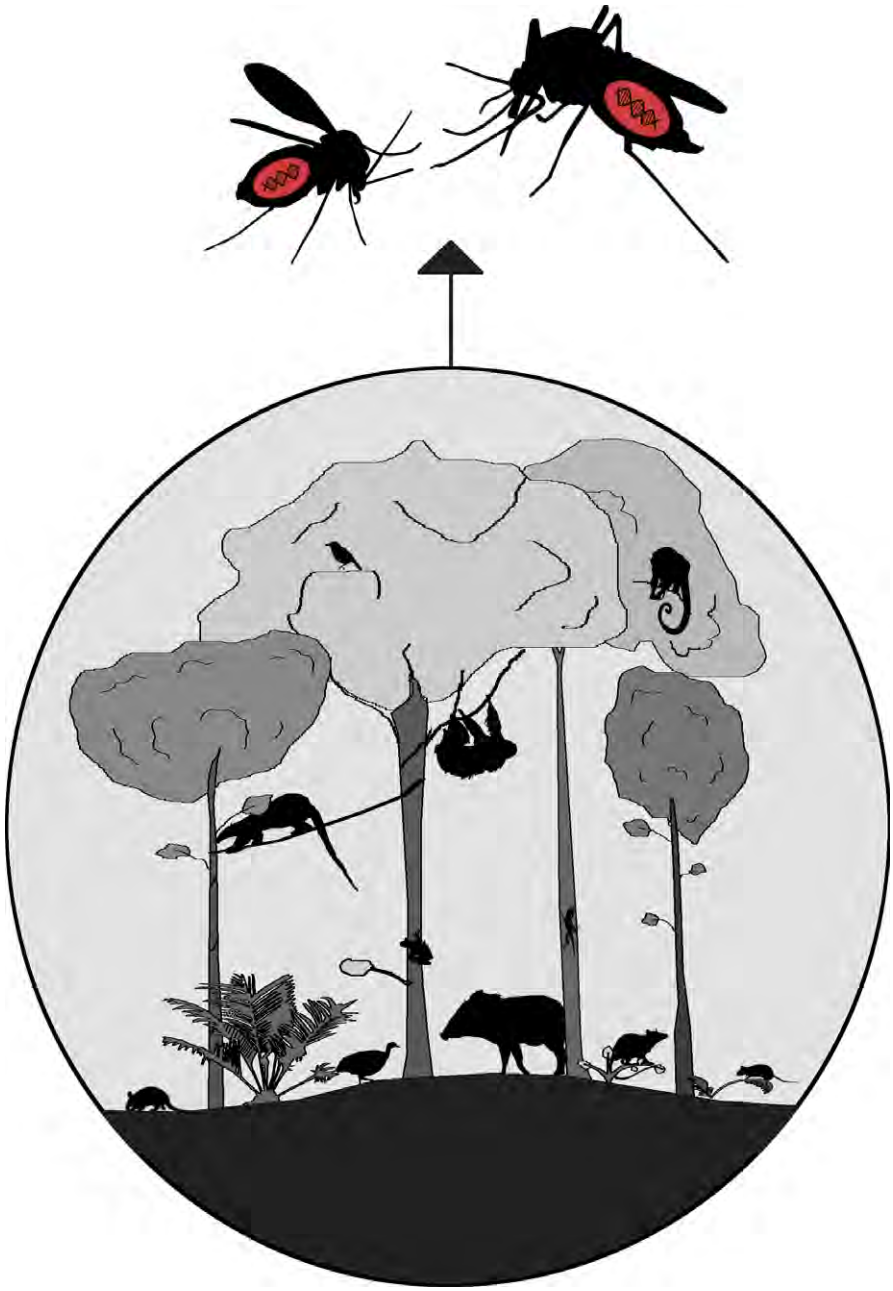

FIGURE 2 Schematization of the methodology employed in this study. Dipteran bloodmeal analyses allowed identifying arboreal and terrestrial mammals of various body size as well as birds, lizards and amphibians. This would typically require the implementation of multiple observational methods, wide taxonomic expertise and considerable fieldwork

frequent intersample "leakage" due to tag-switching events (Esling, Lejzerowicz, & Pawlowski, 2015; Schnell, Bohmann, & Gilbert, 2015). Of important note, although considering the possibility for mixed bloodmeal sources would have nearly double the size of our data set, it would have led to the detection of only one additional vertebrate species (*Potos flavus*, without considering obvious laboratory contaminants that may not be present in the sampling region). In our opinion, this is strong evidence that most of vertebrate identifications we could have considered to originate from mixed bloodmeals were rather laboratory or sequencing artefacts. We argue that results obtained from not visibly blood-fed specimens, or the detection of multiple bloodmeal sources should be taken with caution when using high-throughput sequencing of multiplexed PCR products. Furthermore, proper negative controls (e.g., strictly phytophagous dipteran males collected in the same traps) should be used when reporting the detection of frequent field and laboratory contaminants such as human DNA.

3.3 | Diversity comparisons

The percentage of bloodmeal pairwise identity was higher within trap-nights than within sites, for both mosquitoes (87.6% vs. 32.4%; $X^2 = 347.68$; $p < 10^{-15}$) and sand flies (53.5% vs. 31.6%; $X^2 = 7.91$; $p < .005$), indicating that insects collected in the same trap and during the same night were likely to have fed on the same individual or group of conspecifics. Therefore, bloodmeal data were converted into occurrences by trap-night for each dipteran species (or genus for sand flies, see Material and Methods) before diversity analysis.

TABLE 1 Identifications of bloodmeals in sand flies and mosquitoes collected the area of Saint-Georges de l'Oyapock in French Guiana. Only the 152 specimens for which both dipteran species and bloodmeals were successfully identified are included

Group	Species	Nb	Bloodmeals
Phlebotominae	*Bichromomyia flaviscutellata*	4	*Echimys chrysurus* (1); *Proechimys cuvieri* (2); *Pr.* MOTU 1 (1)
	Evandromyia brachyphalla	2	*Pr. cuvieri* (1); *Pr. guyannensis* (1)
	Ev. infraspinosa	1	*Dasyprocta leporina* (1)
	Ev. sericea	1	*Cuniculus paca* (1)
	Ev. walkeri	1	*Isothrix sinnamariensis* (1)
	Nyssomyia MOTU 1	3	*Choloepus didactylus* (2); *Tamandua tetradactyla* (1)
	Ny. umbratilis	12	*Alouatta macconelli* (1); *Ch. didactylus* (7); *Coendou melanurus* (2); *Pecari tajacu* (1); *Ta. tetradactyla* (1)
	Pintomyia damascenoi	1	*Ta. tetradactyla* (1)
	Psychodopygus amazonensis	3	*Dasypus kappleri* (1); *Du. novemcinctus* (2)
	Ps. ayrozai	17	*Du. kappleri* (2); *Du. novemcinctus* (14); *Pe. tajacu* (1)
	Ps. claustrei	1	*Dr. leporina* (1)
	Ps. hirsuta	5	*Du. novemcinctus* (5)
	Ps. s. maripaensis	8	*Du. novemcinctus* (7); *Pe. tajacu* (1)
	Ps. MOTU 1	5	*Du. novemcinctus* (5)
	Sciopemyia sordellii	1	*Pr. cuvieri* (1)
Culicidae	*Aedes serratus*	1	*Thamnophilus* MOTU 1 (1)
	Culex mollis	1	*Tinamus major* (1)
	Culex MOTU 1	78	*Ch. didactylus* (3); *Co. melanurus* (1); *Cu. paca* (6); *D. leporina* (2); *Du. novemcinctus* (1); *M. nudicaudatus* (3); *Pe. tajacu* (1); *Pr. cuvieri* (37); *Pr. guyannensis* (1); *Pr.* MOTU 1 (4); *Th. nigrocinereus* (16); *Th.* MOTU 1 (3)
	Culex MOTU 2	1	*Th. nigrocinereus* (1)
	Culex MOTU 3	2	*Th. nigrocinereus* (2)
	Culex MOTU 4	3	*Kentropyx calcarata* (1); *Osteocephalus MOTU* (1); *Polychrus marmoratus* (1)
	Wyeomyia ypsipola	1	*Ti. major* (1)

Decreasing biodiversity is a typical signature of human-induced disturbance (Brashares et al., 2004; Turner, 1996) and was expected along our sampling sites. Taking raw bloodmeal data, we observed a marked decrease in vertebrate species richness with increasing HFP and proximity to town (seventeen, eleven and seven species in SG30, SG6 and SG1, respectively, Figure 3).

However, as different numbers of specimens were collected in each site, with varying proportions of dipteran species characterized by specific feeding habits, direct comparisons of observed bloodmeal richness are inevitably biased. Rarefaction is a commonly employed procedure to account for sampling size in diversity analyses (Gotelli & Colwell, 2011). Here, we rarefied our sample to estimate Hill numbers of diversity (Chao et al., 2014) based on bloodmeals originating from identical pools of dipterans.

Rarefied samples included 13 randomly drawn specimens: four *Culex* MOTU 1, one *Evandromyia*, three *Nyssomyia* and five *Psychodopygus*. Resulting estimated Hill numbers of order zero, one and two (i.e., species richness, exponential of the Shannon–Wiener index and Simpson index) only slightly differed between SG30 and SG7, but still markedly decreased in SG1 (Figure 3). Of note, some species that are typically rare or absent in highly disturbed habitat were found only in site SG30 or SG6 (e.g., *Alouatta maconnelli*, *Metachirus nudicaudatus*, *Pecari tajacu*, *Tamandua tetradactyla*; *Tinamus major*; de Thoisy et al., 2008, 2010). This suggests that dipteran bloodmeals may provide a useful tool for vertebrate diversity comparisons, even with moderate sampling effort. However, the main limitation of this study is the lack of results simultaneously obtained with classical methods, and further work is needed to validate such an approach for vertebrate diversity assessment. Of note, other studies are currently being conducted in the same area in the framework of the "Observatoire Hommes-Milieux Oyapock" (http://ohm-oyapock.in2p3.fr/). Those involve classical camera trapping, measures of seed dispersal, bioacoustic monitoring and dung beetles sampling. This should bring complementary information on vertebrate diversity along the same anthropogenic pressure gradient and allow interesting comparisons with our results in a near future.

Dipteran species collected in this study exhibited a variety of ecological features and complementarity feeding behaviour. Therefore, the analyses of the whole dipteran community, rather than specific species, allowed the detection of a broad spectrum of vertebrate hosts. We argue that future studies should develop integrated sampling protocols that are able to maximize the diversity of iDNA-carrying arthropods collected on study sites. However, such a community-based approach also comes with drawbacks, because between-site comparisons are prone to biases related to arthropod community compositions. In this study, we adopted a conservative approach in this regard, but the development of specific analytical tools is another main challenge to be addressed in future iDNA studies.

4 | CONCLUSIONS

Although of preliminary nature, this work demonstrates that dipteran bloodmeal analyses can be used as a single source of information to screen for the presence of a wide range of vertebrates. Our study also suggests that the approach may be suitable for highlighting variation in vertebrate community composition between sampling sites.

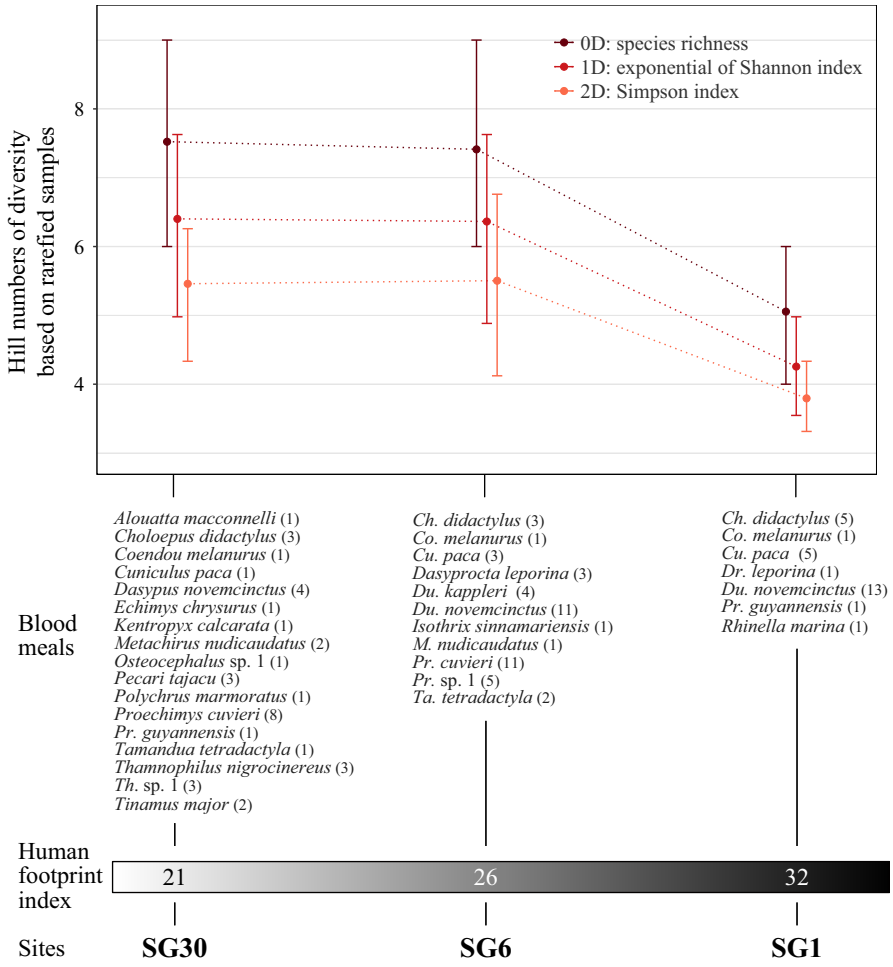

FIGURE 3 Vertebrates identified in dipteran bloodmeals (numbers in brackets indicate occurrences per trap-night) and estimations of mean Hill numbers of diversity (^{q}D) on rarefied bloodmeal pools among sites, along an anthropogenic pressure gradient defined by the human footprint index (de Thoisy et al., 2010). Error bars indicate the middle 95% of rarefied Hill number distributions

Knowing the large expertise existing for the study of disease vectors, these results set the stage for promising perspectives. Nevertheless, further studies involving comparisons of dipteran bloodmeal analysis and classical vertebrate surveys are required to better evaluate the efficiency of the method.

Arthropods themselves are already frequently used as biodiversity indicators (Dedieu, Vigouroux, Cerdan, & Céréghino, 2015; Talaga, Dézerald, et al., 2017), and bloodmeal identifications could easily be complemented with insect community analyses to extend the scope of such studies. Furthermore, our results provide precious contributions to the knowledge of disease vector feeding habits. In recent years, questions have been raised about the impact of biodiversity changes on the transmission risk of zoonotic infectious diseases (Ostfeld & Keesing, 2012). In combination with DNA-based pathogen detection, the present approach could open up new avenues for both biodiversity assessment and eco-epidemiology.

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DATA ACCESSIBILITY

All data and scripts are available in the Supporting Information.

AUTHOR CONTRIBUTIONS

A.K., A.-L. B., B.T. and J.M. designed research; A.K., J.M., F.C. and B.T. performed field work; A.K. and S.V. performed laboratory work; A.K. analysed the data; A.K. wrote the article and all authors contributed to its improvement.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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