



Prey preference of the common vampire bat (*Desmodus rotundus*, Chiroptera) using molecular analysis

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Morphological identification of prey fragments in vampire bat feces is impossible because of an exclusively blood-based diet. Therefore, studies of their foraging ecology require innovative approaches. We investigated the diet of *Desmodus rotundus* using a PCR–restriction fragment length polymorphism (RFLP) molecular method by amplifying the cytochrome *b* mitochondrial gene (380 bp) from DNA fecal samples collected from captive bats fed with blood from chickens, cattle, pigs, dogs, and humans—the 5 most frequently attacked prey species in rural areas of the Brazilian Amazonia. The prey preference of the vampire bat was investigated in 18 riverine villages, where the availability of domestic animals to bats was quantified. Prey DNA amplified from fecal samples exhibited no visible signals of vampire bat DNA. A PCR–RFLP flowchart and a combination of 2 DNA restriction enzymes allowed the direct identification of prey to species level. The enzymes' restriction profile did not overlap with those of vampire bats or wild mammal and avian species. Chickens were the most attacked prey species (61.4% of the identifications, $n = 27$), but pigs were highly preferred in relation to prey availability. This suggests a preference for mammalian blood in *D. rotundus* diet, with chickens exploited as a secondary food source. No wild vertebrate species was identified in the fecal samples, indicating that vampire bats are selectively feeding on the blood of domesticated animals, probably because they are more predictable and easily accessed resources.

Key words: bat, Brazilian Amazonia, cytochrome *b*, diet preference, foraging behavior, molecular scatology, PCR–RFLP, prey selection

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Detailed analyses of dietary habits of vertebrates are fundamental to answer questions on trophic interactions, how predators and prey interact, and how prey availability affects predator density and distribution (Sheppard and Harwood 2005). Fecal samples have been used extensively in studies of mammal diet based on morphological classification of digested prey remains. However, conventional microscope-based fecal analyses suffer difficulties because prey are often masticated so thoroughly that components cannot be assigned visually to an animal or plant taxon. Thus, this technique rarely provides identification to species level and underestimates prey's soft parts (Whitaker 1988). Prey species that have no exoskeleton, such as caterpillars, may not even be registered in the diet (Kalka and Kalko 2006).

In the last 2 decades, techniques based on investigation of foreign DNA presence in predator's feces have become

a practical tool to identify preyed-upon species (Kohn and Wayne 1997; Symondson 2002). Compared with microscopic examination of postdigestion prey fragments, DNA-based techniques can improve the resolution of taxonomic identification to the species level (Farrell et al. 2000; Adams et al. 2003; Dallas et al. 2003). PCR-based analysis allows the amplification of small DNA fragments resulting from degradation during passage through the digestive tract of the predator. Factors that will affect the performances of DNA analysis from fecal samples include duration of DNA retention in predator gut, age of fecal samples, collection area climate, and storage method (Brinkman et al. 2010; Bubb et al. 2011). Fresh samples, briefly retained in the gut and collected in cold and dry environments, have higher chance of molecular identification of prey species. Additionally, analysis of those genome regions with multiple

copies in cells can maximize chances of DNA amplification (Zaidi et al. 1999).

PCR–restriction fragment length polymorphism (PCR–RFLP) is a molecular method that consists of DNA amplification and subsequent cleavage of the amplified fragments using restriction enzymes (Dowling et al. 1990). Prey assignment is achieved by distinction of restriction patterns between the species analyzed for a combination of different enzymes. The PCR–RFLP technique has been used as a valuable DNA fingerprint method to authenticate animal species in food products (Russell et al. 2000; Bellagamba et al. 2001) to identify the source of vertebrate blood in the intestinal tracts of disease-vectoring arthropods (Kent 2009) and to investigate insects as biological control agents for agricultural pests (Garipey et al. 2007).

For vampire bats, morphological examination of prey is impossible because the diet is composed exclusively of blood, producing semiliquid feces, which prevent material evidence of the prey species. Although feeding behavior and identity of species attacked by vampire bats have been well documented in the literature by direct observation (Greenhall et al. 1969, 1983; Turner 1975), no studies have yet quantified prey consumed and vampire bat food preferences. The common vampire bat *Desmodus rotundus* has a broad distribution from Mexico to Central Chile and southern Uruguay (Gardner 2007). At the regional scale, they have low abundance in primary forest (Bernard 2001; Bobrowiec et al. 2014), but their population increases close to rural villages and farmlands (Delpietro et al. 1992; Bobrowiec 2012) because of the increased availability of prey species such as cattle, horses, mules, goats, pigs, sheep, dogs, chickens, and humans (Greenhall 1988; Delpietro et al. 1992). In consequence, *D. rotundus* is the major vector of the rabies virus for cattle in Latin America, causing great economic damage (Johnson et al. 2014). Attacks on humans are also common in small rural villages in tropical forests of South America (López et al. 1992; Warner et al. 1999; Schneider et al. 2001). Between 2004 and 2005, there were 25 human deaths registered in northern Brazil from rabies transmitted by vampire bats (Rosa et al. 2006).

DNA-based techniques have been used to identify prey species in the diet of insectivorous bats (Clare et al. 2009, 2011; Bohmann et al. 2011; Razgour et al. 2011; Zeale et al. 2011; Dodd et al. 2012; Burgar et al. 2014; Sedlock et al. 2014). Carter et al. (2006) reported on techniques for isolation, amplification, and identification of avian DNA from fecal samples of *Diaemus youngi*, the white-winged vampire bat, a specialist avian sanguinivore (Greenhall et al. 1983). However, no molecular methods have been developed yet to identify the range of prey taxa in *D. rotundus* feces. In this study, we used a molecular method based on PCR–RFLP to investigate the diet preference of the vampire bat *D. rotundus*. We analyzed DNA isolated from vampire bat feces to identify and quantify relative frequencies of predation on the 5 vertebrate species (chickens, cows, pigs, dogs, and humans) most commonly attacked in the countryside of Brazilian Amazonia. We optimized a PCR–RFLP protocol using DNA extracted from fecal

samples collected from captive *D. rotundus* fed with blood of these 5 prey species. Following protocol optimization, we investigated the diet of vampire bats in selected riverine villages in the Brazilian Amazonia. We quantified availability of domestic animals to assess *D. rotundus* food preferences. We predicted that close to riverine villages, vampire bats will ingest blood exclusively of domestic animals and in these conditions they will have higher consumption of mammals (pigs and cattle) over chickens. We discuss the potential and limitations of sample storage methods and the use of PCR–RFLP techniques for diet studies of vampire bats.

MATERIALS AND METHODS

Fecal samples collection of bats in captivity.—In order to develop the molecular method for precise prey identification in *D. rotundus*, we collected fecal samples from bats maintained in captivity. A total of 8 adult bats (4 males and 4 females) were kept in individual 0.6- × 0.6- × 1.0-m galvanized iron and wire mesh cages. The cages were placed inside an enclosure located in an urban forest fragment, protected against rain and direct sunlight. We followed the guidelines approved by the American Society of Mammalogists in our procedures (Sikes et al. 2011).

The bats were fed daily with 30 ml of blood collected from 5 prey species: chickens (*Gallus gallus*), cows (*Bos taurus*), pigs (*Sus scrofa*), humans (*Homo sapiens*), and dogs (*Canis lupus familiaris*). Each bat feeding session consisted of blood offering from 1 unique prey species for 5 consecutive nights. To avoid collection of fecal samples containing a mixture of prey species blood, fecal samples were collected beginning 3 days after the feeding session with the blood of 1 prey species was initiated. This protocol was repeated sequentially for all species during the experiment. The blood of cattle and pigs were collected from licensed slaughterhouses, human blood was obtained from a local hematology center (Fundação de Hematologia e Hemoterapia do Amazonas), dog blood was collected from anesthetized animals in a local veterinary clinic, and chickens were provided live within the bat enclosure. The cow and pig blood were stored in anticoagulant-preservative EDTA solution (0.012:1 ml of blood), whereas human and dog blood were stored in citrate phosphate dextrose adenine-1 solution (0.14:1 ml of blood). Preliminary tests showed that the anticoagulants did not affect the feeding behavior of the vampire bats. Fecal samples were collected twice a day (0900 h and 1800 h) and immediately stored at -20°C . We consider as an individual sample all the feces collected up to 18 h after feeding bats.

DNA extraction, amplification, and sequencing.—Total genomic DNA was extracted from standardized blood samples (150 μl) from 6 individuals of each prey species (control samples) and from 190 fecal samples (30–90 mg) of the captive bats (38 samples of each prey species) using a CTAB (hexadecyltrimethylammonium bromide 2%) procedure (Doyle and Doyle 1987). The cytochrome subunit *b* (*Cytb*) mitochondrial DNA gene was amplified by PCR using the vertebrate universal primers L14841 (5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3') and

H15149 (5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3'—Kocher et al. 1989). These universal primers amplify a conserved region of 360 bp of the *Cytb* gene for a variety of vertebrates including *D. rotundus*. Amplification was performed in a final volume of 20 μ l containing 10 \times reaction buffer (10 mM of Tris-HCl, pH 8.4, 500 mM of KCl, Triton X-100 1%), 50 mM of MgCl₂, 2.5 mM of each dNTP, bovine serum albumin (2.5 ng/ μ l), 2 mM of each primer, 5 unit *Taq* DNA polymerase, and 7.5 ng of template DNA. Samples were amplified using the following PCR program: initial denaturation at 94°C for 4 min, followed by 30 cycles of 92°C for 45 s, 56°C for 30 s, and 72°C for 1 h 30 min, and a final elongation step at 72°C for 10 min.

As the fecal samples potentially contained DNA from the excreting bat, enabling a false-positive result for prey species, PCR products from 2 blood and fecal samples of each prey species were purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio) and sequenced using the ABI prism Big Dye III cycle sequencing kit (Life Technologies, Foster City, California), following the manufacturer's instructions. The amplified fragments were sequenced in both forward and reverse directions. The DNA sequences were aligned and edited with Clustal W (Larkin et al. 2007) and compared with homologous *Cytb* sequences in the National Centre of Biotechnology Information (NCBI) database to identify the presence of prey DNA in the *D. rotundus* fecal samples.

Selection of the restriction enzymes for prey identification.—DNA fragments of the 359-bp *Cytb* region available in the NCBI sequence database from *D. rotundus* and each of the prey species were used to check the cleavage sites of the restriction enzymes and to estimate the expected sizes of the fragments using the NEB Cutter 2.0 program (Vincze et al. 2003).

Selection criteria for restriction enzymes were based on the minimum number of enzymes, which in combination produce patterns of restriction fragments capable of differentiating the 5 tested prey species.

In continuous forest, *D. rotundus* usually feeds on a variety of mammals and avian species. To verify that the selected enzymes do allow unequivocal identification of the 5 prey species tested, the restriction profile pattern of the *Cytb* gene homologous region available in the NCBI database for 18 mammals and 19 avian species potentially attacked by vampire bats in the central Amazonia forest were accessed using NEB Cutter 2.0 (Supporting Information S1 and S2). Nucleotide sequences were aligned and edited with 359-bp *Cytb* fragments sequenced from blood of the 5 prey species using Clustal W.

PCR-RFLP analysis.—The RFLP analysis was applied on amplified blood sample products from each prey species and vampire bats, and validated with fecal samples collected from captive vampire bats. The digestion reactions were performed according to recommendations of the manufacturer (Invitrogen, Carlsbad, California) with 5 to 9 μ l of PCR product. Fragment size was estimated by comparison with a 50-bp DNA Ladder (Invitrogen). Cleaved fragment patterns from both blood and fecal samples were compared with fragments produced in the NEB Cutter 2.0 from sequences available in the NCBI database.

Field sampling.—Bats were captured in 18 small *caboclo* villages in Ayapuá Lake (4°26'S, 62°12'W), middle Madeira River (5°27'S, 60°45'W), and Aripuanã River (6°08'S, 60°11'W), situated in the central-western Brazilian Amazonia (Bobrowiec 2012; Fig. 1). Ayapuá Lake is located in the Sustainable Development Reserve Piagaçu-Purus (827,317 ha) in the lower Purus River region. The vegetation of the study area is classified

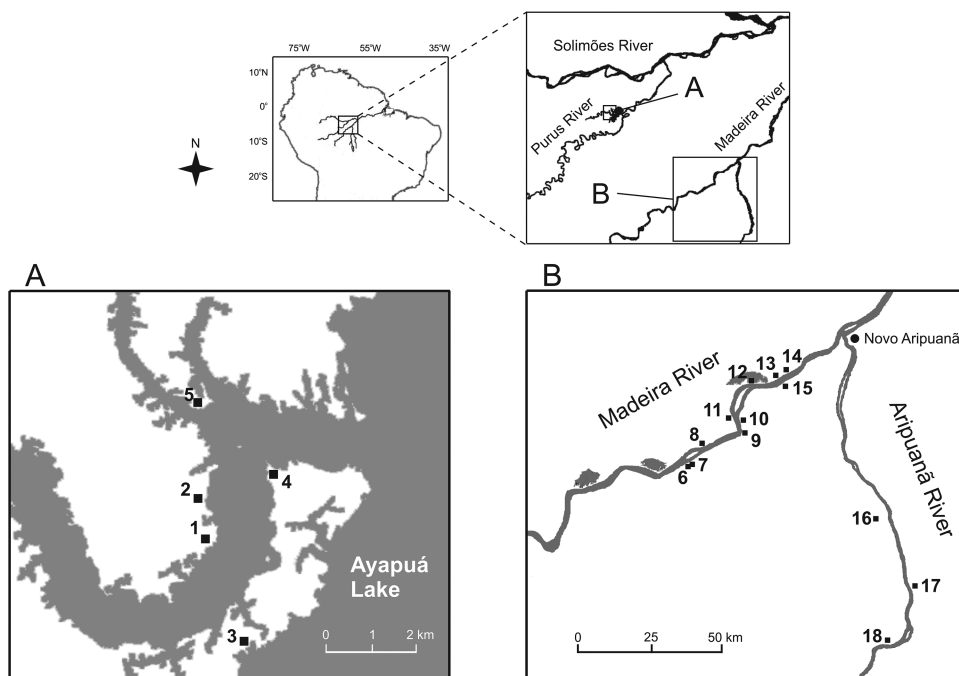


Fig. 1.—Map of the study area showing the A) Ayapuá Lake and B) Madeira River region, Brazilian Amazon. The numbers indicate the riverine villages where vampire bats were captured.

as dense tropical rainforest (Veloso et al. 1991), formed by a mosaic of vegetation types dominated by terra firme forests, with lowland flooded forests at the river margins (Capobianco et al. 2001). Average annual precipitation is 2,600 mm, with the rainy season occurring between January and June (Sombroek 2001). Most of the area has low anthropogenic disturbance (de Deus et al. 2003; Rapp Py-Daniel 2007).

Vampire bat fecal samples were collected at villages on the Madeira and Aripuanã Rivers during September 2004 and April–May 2005 and at Ayapuá Lake during June 2005 and February 2007. Each village was sampled for 2–5 nights, totaling 47 nights of bat captures. Two 12- × 3-m and 2 6- × 3-m mist nets were opened at night, positioned close to hen houses, pigsties, corrals, and *caboclo* houses. To maximize the capture of vampire bats with the stomach replete with blood, the nets remained open during the 2nd half of the night from 0000 to 0400 h. Captured vampire bats were placed individually in cotton bags that were inspected for fecal samples up to 6 h after specimen capture. To minimize risk of contamination between individual vampire bats, each cotton bag was used only once. Collected fecal samples were stored individually in 2-ml tubes with silica gel. At the Madeira and Aripuanã River sites, fecal samples were stored at –20°C immediately after collection. At Ayapuá Lake, it was not possible to freeze the fecal samples due to lack of electricity in the region.

Estimation of prey preference.—Bat prey preference was estimated using the resource selection ratio (w_i) with Bonferroni confidence intervals proposed by Manly et al. (2003):

$$w_i = o_i / \pi_i,$$

where o_i is the proportion of attacked prey and π_i is the proportion of available prey species in category i (prey species). If the prey species is used in proportion to their availability, then $w = 1$. If $w_i > 1$, the prey species is used more than expected by chance (preferred). In each village, we counted the number of domestic animals in their enclosures. Prey preference was

calculated for the Ayapuá Lake, Madeira and Aripuanã Rivers, and the 2 localities combined. In the villages of the Madeira and Aripuanã Rivers, the number of dogs was not estimated and this species was not included in the analysis of prey preference. The selection of a particular prey species was determined using log-likelihood chi-squared test (χ_L^2 —Manly et al. 2003). The resource selection function (w_i) was computed using *adehabitat* 1.8.12 package with design I data type (Calenge 2006) in the R computing environment 2.15.3 (R Development Core Team 2013).

RESULTS

DNA amplification and sequencing.—Amplification of a fragment encoding a 359-bp sequence of the *Cytb* gene yielded the expected amplification product for prey blood, fecal samples of all prey species and vampire bat blood. The DNA sequences of these species showed 100% similarity to the *Cytb* gene sequences already published in the NCBI database. DNA from fecal samples subjected to amplification exhibited no visible signals of vampire bat DNA, indicating that bat DNA does not interfere with the amplification of the prey species *Cytb* fragment.

PCR–RFLP analysis from blood and fecal samples of captive bats.—Based on blood sequences of prey species and vampire bats, we established a flowchart that allows direct identification of prey species by a combination of 2 restriction enzymes (Fig. 2). Analysis of the *Hae*III restriction patterns resulted in 4 groups of prey species with fragments close in length (Fig. 2). The *Hae*III fragments of *D. rotundus* did not overlap with those of the prey species. Use of additional restriction enzymes (*Taq*I, *Rsa*I, *Xho*I, and *Bmg*BI) allowed subsequent separation of prey species. The enzymes *Xho*I and *Bmg*BI were specific to humans and cattle, respectively, whereas *Taq*I discriminated the pig from other prey species. *Rsa*I restriction patterns allowed separation of chickens and dogs DNA. A comparative analysis of the fecal sample DNA restriction profiles showed the same restriction pattern obtained from blood DNA for all 5

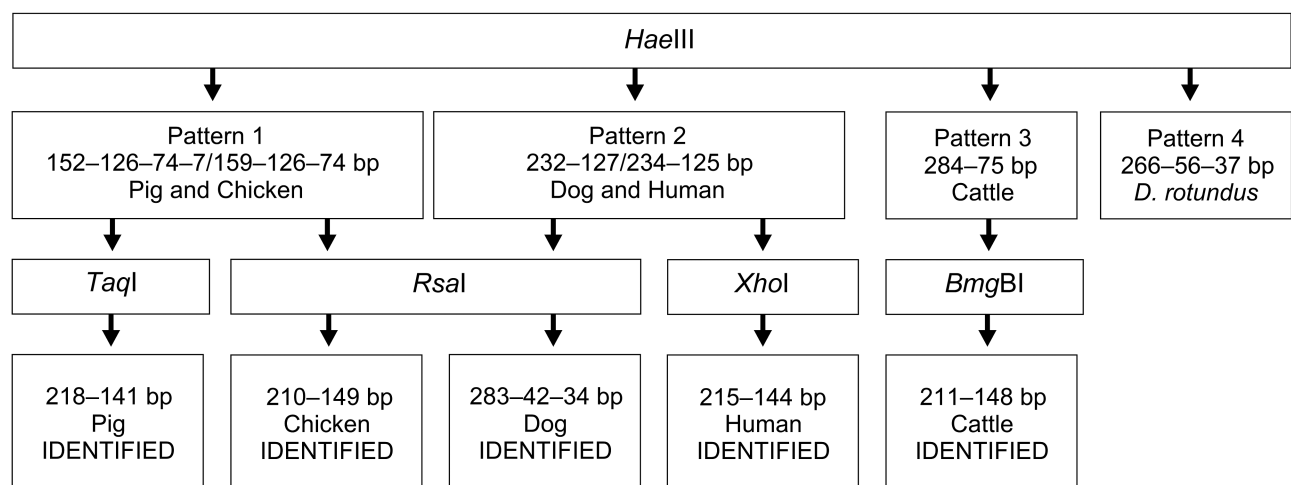


Fig. 2.—Proposed flowchart of restriction patterns found for unequivocal identification of 5 prey species mostly attacked by the vampire bat (*D. rotundus*) in riverine villages in the Brazilian Amazonia.

prey species, validating prey species identifications made using fecal samples (Supporting Information S3).

Discrimination between the 5 prey species and wild species.—When the 2 restriction enzymes were used in combination as per the flow chart, the 380-bp sequences of the *Cytb* gene published in the NCBI database from wild mammals ($n = 18$ species) and avian species ($n = 19$ species) considered potential prey of vampire bats in the central Amazonia did not show restriction profiles similar to any of those found for the 5 target prey species (Supporting Information S1 and S2). Some mammal and avian species were cleaved by 1 or 2 selected enzymes, but the length of fragments was very different when compared with the studied prey species (Supporting Information S1 and S2).

Diet of vampire bats in the riverine villages of the Brazilian Amazonia.—During 47 nights, we captured 163 vampire bats (157 *D. rotundus* and 6 *D. youngi*) in 18 villages and collected 88 fecal samples (Table 1). We identified the prey species from 48 fecal samples (54.5% of the total). Of the remaining 40 samples, 30 failed in DNA amplification and 10 failed in RFLP analysis. DNA sequencing of the 10 fecal samples that failed in RFLP analysis revealed only the presence of *D. rotundus* DNA. Freezer-stored fecal samples (those collected on the Madeira and Aripuanã Rivers) had 20% higher sample amplification and 25.6% greater prey identification than those stored in silica gel (Ayapuá Lake samples).

The analysis of the restriction enzyme profiles showed consumption of chicken, pig, cattle, and dog blood by *D. rotundus* in the sampled localities. Chickens represented two-thirds of the prey used by *D. rotundus* ($n = 27$ samples; 61.4%), followed by pigs ($n = 14$; 31.8%), cattle ($n = 2$; 4.5%), and dogs ($n = 1$; 2.3%; Table 2). There was no difference in the consumption of chickens ($\chi^2 = 0.21$, $d.f. = 1$, $P = 0.65$) and pigs ($\chi^2 = 1.40$, $d.f. = 1$, $P = 0.24$) between Ayapuá Lake and Madeira and Aripuanã Rivers. During the field work, in 1 village (Porto Seguro), local residents reported attacks by vampire bat on people.

Overall, all 18 villages had chickens and dogs, whereas cattle were recorded in only 4 villages and pigs in 5 villages (Table 2). The resource selection ratio (w_i) indicated that pigs were significantly more selected than the other prey species (all localities: $\chi_L^2 = 56.28$, $d.f. = 2$, $P < 0.0001$; Ayapuá Lake: $\chi_L^2 = 42.66$, $d.f. = 2$, $P < 0.0001$; Madeira and Aripuanã Rivers: $\chi_L^2 = 24.77$, $d.f. = 2$, $P < 0.0001$; Fig. 3). The selection ratio for pigs was 6 to 7.3 times higher than for chickens, the 2nd-most preferred prey. Selection ratios for chickens were significantly

higher than for cattle and dogs. However, the mean values and the lower confidence limits of chicken-selection ratios were very close to 1, suggesting that *D. rotundus* ingest the blood of chickens in proportion to their availability (Fig. 3). PCR-RFLP analysis of the 4 fecal samples collected from 3 sampled individuals of *D. youngi* identified chicken ($n = 3$) and pig ($n = 1$) blood ingestion.

DISCUSSION

Our study provides an optional method for species identification of mammals and birds attacked by vampire bats. We chose the combined PCR-RFLP method, which consisted of the amplification of a short variable region of the mitochondrial *Cytb* gene using universal primers that amplify a wide variety of vertebrate species (Kocher et al. 1989), followed by cleavage of the amplified DNA fragment using selected restriction enzymes, which produced different and nonoverlapping restriction patterns for *D. rotundus* and other wild vertebrate species in the rain forest, thus allowing their discrimination. The short fragment of the mitochondrial *Cytb* gene is a multiple copy DNA ideal for amplifying degraded DNA during digestion. This DNA technique is advantageous because 1) fecal samples have sufficient amount of DNA for amplifications; 2) the universal *Cytb* region primers amplify DNA for several prey species, so producing large amounts of DNA for cleavage by enzymes; 3) *Cytb* sequences are available for an increasing number of vertebrate species in public databases, so allowing identification of potential restriction enzymes without having to sequence products of prey species; 4) this technique can be replicated in any location and can include other prey species; and 5) PCR-RFLP can be used whenever DNA sequencing is cost-prohibitive or infeasible.

Table 2.—Availability of prey species used by *Desmodus rotundus* in riverine villages in the study area. The number of prey species identified in the fecal samples of *D. rotundus* by PCR-RFLP is presented in parenthesis. nd, not determined.

| Prey species | Madeira-Aripuanã Rivers | Ayapuá Lake | Total |
|--------------|-------------------------|-------------|----------|
| Chicken | 24 (15) | 127 (12) | 164 (27) |
| Cattle | 10 (2) | 97 (0) | 107 (2) |
| Pig | 3 (6) | 8 (8) | 21 (14) |
| Dog | 30 (0) | nd (1) | 30 (1) |

Table 1.—Number of bats captured, fecal samples collected, DNA amplifications, and quantity of prey identified using PCR-RFLP molecular analysis.

| Localities | Nights | Bats captured | | Fecal samples | | PCR amplified | | Prey identified | |
|-----------------------------|--------|--------------------------|-----------------------|--------------------------|-----------------------|--------------------------|-----------------------|--------------------------|-----------------------|
| | | <i>Desmodus rotundus</i> | <i>Diaemus youngi</i> | <i>Desmodus rotundus</i> | <i>Diaemus youngi</i> | <i>Desmodus rotundus</i> | <i>Diaemus youngi</i> | <i>Desmodus rotundus</i> | <i>Diaemus youngi</i> |
| Madeira and Aripuanã Rivers | 17 | 71 | 2 | 33 | 2 | 26 | 2 | 23 | 1 |
| Ayapuá Lake | 30 | 86 | 4 | 55 | 4 | 32 | 4 | 21 | 3 |
| Total | 47 | 157 | 6 | 88 | 6 | 58 | 6 | 44 | 4 |

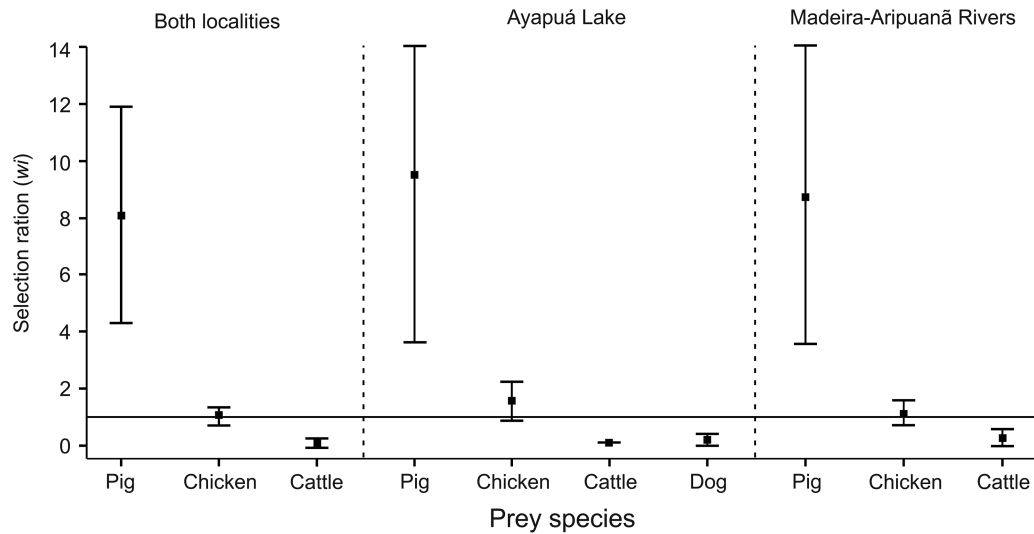


Fig. 3.—Resource selection ratio (w_i) of preferred prey species used by *Desmodus rotundus* in riverine villages in the Brazilian Amazonia. Values above the horizontal line ($w_i = 1$) indicate vampire bat preference for a prey species. Vertical lines represent Bonferroni CIs.

Less than one-half (45.8%) of the fecal samples from the Ayapuá Lake could be identified, compared with 71.4% of the material collected from the Madeira and Aripuanã Rivers. The rapid freezer storage of the fecal samples from the Madeira and Aripuanã Rivers probably contributed to slow the DNA degradation. The samples collected from captive bats were immediately frozen and all DNA sequences obtained from these feces could be identified. DNA degradation by endonucleases can be avoided under conditions of low temperature, rapid drying, and high salt concentration (Frantzen et al. 1998; Nsubuga et al. 2004). The amplification of mitochondrial DNA from fecal samples of wolves (*Canis lupus lupus*) collected during winter in Italian Alps was 11.8% higher than samples collected in summer (Lucchini et al. 2002). Although the silica dryer technique allowed the DNA preservation in 40.7% ($n = 24$) of the fecal samples, we highly recommend the immediate freezing of the fecal samples after collection for better maintenance of the DNA integrity and quality.

Freezing is considered to be the best method for long-term storage of fecal DNA (Buś and Allen 2014). However, this method cannot be usually applied for samples collected during extended fieldwork expeditions, especially in tropical forests. Ethanol (70–95%) and buffer solutions may be alternative storage methods when it is infeasible to deposit the fecal samples immediately in the freezer. However these methods may affect the DNA amplification success in different ways so we do not recommend them to preserve vampire bat fecal samples.

Our data show that chickens were the prey species most commonly attacked by *D. rotundus*. However, when the abundance of available prey was estimated, our results indicated preference for pigs. The differences in nutritional quality of pig blood accessed by the hematocrit parameters of the blood and anticoagulant action of bat saliva may help to explain this preference. Hematocrit values of the pig blood is statistically higher than that found for chickens, cattle, and dogs (analysis of variance: $F = 41.54$, $d.f. = 3, 56$, $P < 0.0001$; hematocrit values: pig, 390 ± 32 ml/l, $n = 14$; dog, 317 ± 43 ml/l, $n = 9$;

chicken, 297 ± 23 ml/l, $n = 20$; cattle, 282 ± 22 ml/l, $n = 17$; data from Borges et al. 1999; Jorge et al. 2002; Silva et al. 2003, 2007; Corassa et al. 2006; Soares et al. 2007; Almeida et al. 2009; Ferreira et al. 2009). *Desmodus* saliva liquefies mammal blood more readily than it does avian (Hawkey 1988), providing easier and faster blood ingestion. As it is highly susceptible to starvation due to an inability to maintain adequate glucose levels, *D. rotundus* needs food every night (Freitas et al. 2003), and the large size of pigs can attract more bats in the same night compared with a single chicken.

Desmodus populations are generally higher in areas with cattle and pig livestock (Greenhall 1988; Delpietro et al. 1992). When chickens and cattle are maintained in proximity, *D. rotundus* does not attack chickens every night, exploiting them as a secondary food source when cattle are absent (Uieda 1992). It is likely that in our study area the number of pigs was insufficient to sustain the vampire bat population, which led them to adopt chickens as a complementary food source.

That dogs and cattle were poorly represented in our sampling ($n = 3$) may have been due to these animals being left to roam free in most villages, making it easier for them to avoid *D. rotundus*. Delpietro (1989) observed a reduced incidence of *Desmodus* predation when cattle and horses are dispersed within their pasture or when they were free to move during the night.

We found only DNA from farmed animals in the feces of *D. rotundus* captured in the villages, suggesting that bats analyzed had not fed on rainforest mammals and avian species. Herrera et al. (1998) found that *D. rotundus* will occasionally take blood from rainforest mammals, such as peccaries, tapirs, and deer. However, our captures occurred solely within village boundaries, mainly around the prey shelters. This could have led to the capture of bats carrying only the blood of domestic animals. Voigt and Kelm (2006) showed that vampire bats from a colony within forest but close to pastures preferred to feed on cattle rather than wild animals. Domestic animals are easier to detect for vampire bats. They represent a more

predictable and constant food source than rainforest mammals as they are kept confined at night, and are generally larger than wild prey species. However, the total absence of any vestiges of DNA wild animals in *D. rotundus* fecal samples is somewhat intriguing considering that villages sampled are surrounded by continuous forests with low levels of hunting pressure. As the domesticated species arrived in the Amazon only in the last few centuries, this suggests a rapid behavioral adaptation to the anthropogenic environment, which has allowed *D. rotundus* populations to successfully exploit them. Further investigations using fecal samples collected from *D. rotundus* individuals captured deep into primary forest are necessary to clarify how human settlements in the Amazon have affected *D. rotundus* feeding behavior.

Our study confirms the consumption of pig blood by *D. youngi* under natural conditions in the Brazilian Amazonia. *D. youngi* is known to feed preferentially on avian blood, but few reports indicate the ingestion of mammal blood by this vampire bat species in the wild and/or captivity (Greenhall and Schutt 1996; Sazima and Uieda 1980; Uieda 1994). We do not know why mammals are only rarely attacked by *D. youngi*, but this may occur when chickens and wild birds are not available. We captured *D. youngi* twice. Upon analysis, both fecal samples showed that pig and chicken blood had been ingested, indicating feeding flexibility in *D. youngi*. Like *D. rotundus*, *D. youngi* saliva also has a protease that greatly slows mammalian blood coagulation (Tellgren-Roth et al. 2009). However, it is known that *D. youngi* has difficulty digesting high amounts of dry matter from mammal's blood (Coen 2002). This physiological constraint means *D. youngi* cannot survive by consuming exclusively mammal blood for consecutive nights (Coen 2002).

In conclusion, our study showed that PCR-RFLP is a reliable molecular method for identification of vampire bat prey at the species level. With this technique, we found that vampire bats can take advantage of feeding on the blood of a variety of domesticated animals in the Brazilian Amazon, probably because they are more easily accessed than wild species in the region. Under such conditions, *D. rotundus* do prefer feeding on pig's blood when they are available. This preference may be related to the night-time corralling of the pigs, the higher nutritional value of the pig blood compared with other farm animal species, as well as the high anticoagulant efficiency of the vampire bat saliva on the mammal blood to maintain its fluidity, and hence digestibility.

SUPPORTING INFORMATION

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Restriction profiles of an amplified 359-bp fragment of *Cytb* generated from different mammal species potentially attacked by vampire bats in Brazilian Amazonia using 5 restriction enzymes. The *Cytb* sequences of each mammal species was obtained from the National Centre of Biotechnology Information (NCBI) database. The restriction profiles in bold indicate similarity with the profiles of the 5 target prey species.

Supporting Information S2.—Restriction profiles of an amplified 359-bp fragment of *Cytb* generated from different avian species potentially attacked by vampire bats in Brazilian Amazonia using 5 restriction enzymes. The *Cytb* sequence of each avian species was obtained from the National Centre of Biotechnology Information (NCBI) database. The restriction profiles in bold indicate similarity with the profiles of the 5 target prey species.

Supporting Information S3.—Restriction profiles of an amplified 359-bp fragment of *Cytb* generated from 5 prey species after digestion with *Hae*III (A), *Rsa*I (B), *Bmg*BI (C), *Taq*I (D), and *Xho*I (E). Lines 1–2: chicken, lines 3–4: cattle, lines 5–6: pig, lines 7–8: human, lines 9–10: dog, lines 11–12: vampire bat (*Desmodus rotundus*). M represents 50-bp marker.

RESUMO

A identificação morfológica de fragmentos de presas nas fezes de morcegos vampiros é impossível por causa da sua dieta exclusivamente baseada em sangue. Portanto estudos sobre sua ecologia de forrageio exigem abordagens inovadoras. Nós investigamos a dieta do morcego vampiro comum *Desmodus rotundus* usando o método molecular PCR-RFLP, amplificando o gene Citocromo *b* (380 pb) do DNA de amostras fecais coletadas de morcegos em cativeiro, alimentados com sangue de galinha, gado, porco, cachorro e humano - as cinco espécies de presas mais frequentemente atacadas em zonas rurais da Amazônia brasileira. A preferência por espécie de presa pelo morcego vampiro foi investigada em 18 comunidades ribeirinhas, onde a disponibilidade de animais domésticos usados pelos morcegos foi quantificada. O DNA das presas amplificado a partir de amostras de fezes não apresentou sinais visíveis do DNA do morcego vampiro. A sequência de uso e a combinação de duas enzimas de restrição permitiram a identificação direta das presas ao nível de espécie. O perfil de restrição gerado pelas enzimas não coincidiu com os perfis do morcego vampiro e de mamífero e aves selvagens. Foram coletadas 88 amostras de fezes e identificadas as espécies de presas em 54,5% (48 amostras). Amostras fecais armazenadas congeladas tiveram 25,6% mais identificação das espécies de presas do que as amostras armazenadas em sílica. Galinhas foram as presas mais atacadas (61,4% das identificações, $n = 27$), mas os porcos foram altamente preferidos em relação à disponibilidade das presas ($w_i = 8,14 \pm 1,79$ SE). Isto sugere uma preferência por sangue de mamíferos por *D. rotundus*, com galinhas exploradas como fonte de alimento secundário. Nenhuma espécie de vertebrados selvagem foi identificada nas amostras fecais, o que indica que os morcegos vampiros se alimentam seletivamente do sangue de animais domésticos, provavelmente porque são recursos mais previsíveis e de fácil acesso.

ACKNOWLEDGMENTS

This research was part of the PhD thesis of P. E. D. Bobrowiec. We acknowledge financial support from Instituto Nacional de Pesquisas da Amazônia (Laboratório de Genética e Biologia Reprodutiva de Plantas—Labgen, Programa de Pós-graduação em Genética—GCBEV, Conservação e Biologia Evolutiva, and Conservation and Sustainable use of Brazilian Biological Diversity Project—PROBIO). P. E. D. Bobrowiec was supported by a fellowship from Fundação de Amparo à Pesquisa do Estado do Amazonas and Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brazil. R. Gribel is a productivity research fellow from CNPq/Brazil. We thank the local people from the Madeira and Aripuanã Rivers and Ayapuá Lake for their collaboration during this research, and the Fundação de Hematologia e Hematoterapia do Amazonas (HEMOAM), Fazenda Experimental da Universidade Federal do Amazonas, Instituto Piagaçu (IPI), and Fundação Nacional de Saúde (FUNASA) of Novo Aripuanã for logistical support. We also thank W. Melo de Sousa, P. Piagaçu, and A. Pavan for field assistance. We are grateful to Labgen staff for technical assistance during lab work. A. Barnett helped with the English revision.

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Submitted 17 June 2014. Accepted 27 July 2014.

Associate Editor was Jorge Ortega.