Identification of carnivore feces by local peoples and molecular analyses

Gerald L. Zuercher, Philip S. Gipson, and George C. Stewart

Abstract Two emerging research techniques offer alternatives to historically invasive and expensive carnivore studies: molecular analysis and use of traditional ecological knowledge (TEK) of indigenous and local peoples. We used a molecular analysis of the cytochrome-*b* gene from fecal DNA to validate identification of mammalian carnivore feces collected by indigenous people and local people on the Reserva Natural del Bosque Mbaracayú (RNBM) in eastern Paraguay. At least 16 mammalian carnivore species, several of which are endangered, are known to occur within RNBM. We found a 100% agreement between identification determined through the molecular analysis and identifications provided by indigenous and local peoples. Our results suggest that indigenous and local knowledge may help advance scientific knowledge about natural resources. By validating TEK through molecular techniques, we show that both indigenous and local people can contribute credibly to studies of the natural resources on which they depend.

Key words canids, carnivores, cytochrome-*b*, feces, felids, molecular analyses, scat, traditional ecological knowledge

Mammalian carnivores present unique study challenges to researchers. First, carnivores often are elusive and sparsely distributed, requiring large investments of time and money to adequately sample a given population. Second, carnivores are by nature dangerous to handle. Third, traditional study methods involve capture, restraint, and tissue sampling. Animal handling increases risks to both researchers and carnivores and may influence the behavior of study animals. Two emerging research techniques offer alternatives to more traditional invasive and expensive carnivore studies: the use of traditional ecological knowledge (TEK) of indigenous and local peoples and molecular analyses.

While there is no single definition of TEK, a synthesis of definitions has been proposed (Berkes 1993:3): "TEK is a cumulative body of knowledge and beliefs, handed down through generations by cultural transmission, about the relationship of living things (including humans) with one another and with their environment." Several qualities of TEK have led to resistance of its acceptance by scientists, including that TEK is primarily qualitative, heavily dependent on intuition, largely holistic, and spiritual (Berkes 1993). Despite these differences, the International Union for the Conservation of Nature (1986) predicted that new scientific knowledge could be gained from perceptive studies of traditional environmental knowledge systems. Use of TEK is increasing, as documented in a special section of *Ecological Applications* (October 2000, Volume 10, Number 5).

Involvement of indigenous and local peoples can be particularly important in studies of endangered or elusive species or disappearing habitats where conservation is a goal. Indigenous and local peoples may be directly affected by changes in management of threatened habitats since often they depend on the land for their subsistence. Involving them helps bridge the gap between western scien-

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tists and indigenous and local peoples, which is a common frustration (Byers 1999). When indigenous people work in a collaborative manner with western scientists, they may better understand the goals and outcomes of research and monitoring programs in their communities, thus enhancing the long-term relationship and improving the chances of reaching mutually beneficial outcomes (Byers 1999).

The Aché of the Mbaracayú Reserve in eastern Paraguay were studied (Hawkes et al. 1982, Hill et al. 1984, Hill and Hurtado 1996) and subsequently incorporated into monitoring and research programs (Hill et al. 1997, 2000). The TEK of the Aché is considered central to management of the Mbaracayú Reserve and surrounding buffer zone (Fundación Moisés Bertoni 1997). The Aché also participate in planning for future management. They presently collect most field data for human dimensions research in the reserve (Hill et al. 1997, 2000). The experiences and perceptions that Aché and local people have about the forest and wildlife are extensive, but the level of credibility associated with their beliefs is unknown. One way to assess the accuracy and precision of information provided by indigenous and local peoples is to compare their reports to results from modern analytical methods including molecular scatology.

Molecular scatology (term coined by Reed et al. 1997) helps to eliminate subjectivity in identification of feces. The method is based on identification of feces to the species level by analysis of host DNA (Foran et al. 1997, Paxinos et al. 1997, Hansen and Jacobsen 1999) from sloughed colonic epithelial cells (Albaugh et al. 1992). The conserved regions of the mitochondrial cytochrome-*b* and D-Loop regions are particularly useful to discriminate between closely related mammalian carnivores (i.e., within sympatric Canidae: Paxinos et al. 1997, Pilgrim et al. 1998).

There were 2 objectives for our study: 1) develop

Jaguar (*Panthera onca*) feces on a path in the Reserva Natural del Bosque Mbaracayú, Paraguay, August 1999. A pocket tool measuring 11 cm is next to the feces as a reference for size.

a molecular diagnostic technique for identifying canid and felid feces collected within Mbaracayú Reserve, and 2) evaluate the skill of Aché and local rangers in identifying feces by comparing results of their visual field identifications with our molecular identifications of the same feces.

Methods

Study site

Reserva Natural del Bosque Mbaracayú (RNBM; Mbaracayú Forest Nature Reserve) was a 64,400-hectare reserve located within the Department of Canideyú in eastern Paraguay (Figure 1). It was managed by the Fundación Moisés Bertoni, a conservation organization in Paraguay. The reserve was located at approximately 55° west and 24° south, with elevations between 150-450 m above sea level. Rainfall was unpredictable but averaged approximately 1,800 mm per year. Winter in eastern Paraguay was typically dry and lasted from May to September (Sanchez 1973). Marked temperature fluctuations occurred seasonally, with average daily high-low temperatures of 14-25°C in July and 22-34°C in January. The reserve protects the largest remaining fragment of Interior Atlantic Forest in Paraguay. It is a habitat mosaic of 19 natural communities and is home to at least 16 species of mammalian carnivores (Fundación Moisés Bertoni 1997).
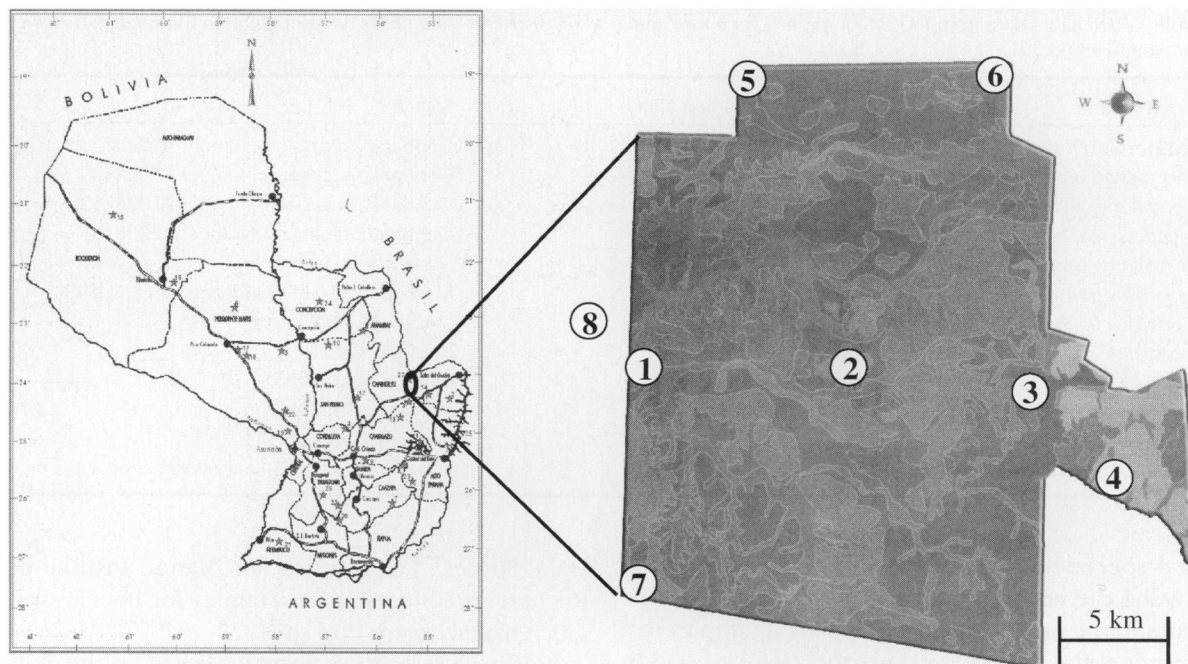
Figure 1. Location of Reserva Natural del Bosque Mbaracayú (RNBM) in eastern Paraguay, where we studied carnivore scat identification in 1999–2001. Map of Paraguay is courtesy of Fundación Moisés Bertoni. Sites 1–7 refer to ranger stations located with RNBM: 1) Jejui'mi–reserve headquarters, 2) Lagunita, 3) Horqueta'mi biological station, 4) Aguara Ñu guard station, 5) Ñandurocai guard station, 6) Carapá guard station, 7) La Morena guard station. Mammalian carnivore scats were collected from sites 1–7 within the reserve. Site 8 was the location of the village where the Aché collaborators live.

Collection of mammalian carnivore scats

We collected mammalian predator scats in RNBM beginning in July 1999 and continued through September 2000. We conducted organized scat collections during 2 field seasons (July–August 1999 and July–August 2000), and we opportunistically collected scats found at other times.

We accomplished scat collection by 2 methods. First, we selected 7 areas within RNBM (Jejui'mi, Lagunita, Horqueta'mi, Aguara Ñu, Ñandurocai, Carapa, and La Morena; Figure 1) based on the availability of a guard station. A total of 6 rangers participated in collecting and identifying feces. At each site at least one reserve ranger spent several days walking through the forest or cerrado in search of game trails and other signs. We followed fresh animal trails until we encountered scats. Once we located a scat sample, we placed it inside a sterile WhirlPak® collection bag, using sterilized forceps, and closed the bag tightly. We collected all encountered scats. We wrote a collection code on the outside of the bag with a permanent marker. We determined the location of the sample using a Garmin 12 XL (Garmin International, Inc., Olathe, Kans.) Global Positioning System (GPS) receiver and stored the sample within the device, using the

same code as written on the collection bag. We gave a preliminary species identification for each sample by one of the 6 different rangers during this project. The most experienced ranger made a final examination and species identification for each sample. We stored all samples in the field inside a Coleman® (The Coleman Company, Inc., Wichita, Kans.) cooler and surrounded samples with ice packs. We took samples to the laboratory at the reserve headquarters within 72 hours of collection and stored in a freezer at -20°C.

The second collection effort involved at least 10 indigenous Aché opportunistically collecting scats from mammalian carnivores during their hunting and foraging trips into the reserve from the Arroyo Bandera Reservation (Figure 1). We provided them with WhirlPak® collection bags and instructed them how to place a sample in a bag with minimal contamination, compensating (20,000 Guaranis; ~US$5.00 at that time) for each sample collected. Although it was unclear whether the Aché collected all encountered carnivore scats, several samples appeared old, suggesting that no deliberate attempt was made by the Aché to pass over these samples. The preferred collection method was to use fresh twigs from the forest as modified forceps for each

Table 1. Available data from the cytochrome-*b* gene for canid and felid species potentially occurring within Mbaracayú Reserve, Paraguay 1999–2001.

Species Name	Common Name	Source
Cerdocyon (Dusicyon) thous	Crab-eating fox	GenBank: Accession Number AF028145
Chrysocyon brachyurus	Maned wolf	GenBank: Accession Number AF028139
Pseudalopex gymnocercus	Pampas fox, Azara's fox	GenBank: Accession Number AF028153
Speothos venaticus	Bush dog	GenBank: Accession Number AF028155
Herpailurus yaguarondi	Jaguarundi	Masuda et al. 1996
Leopardus pardalis	Ocelot	Masuda et al. 1996, Janczewski et al. 1995
Leopardus tigrinus	Oncilla, Tigrina	Masuda et al. 1996
Leopardus wiedii	Margay	Masuda et al. 1996
Oncifelis (Lynchailurus) colocolo	Pampas cat	Masuda et al. 1996
Oncifelis geoffroyi	Geoffroy's cat	Masuda et al. 1996
Panthera onca	Jaguar	Janczewski et al. 1995
Puma concolor	Puma	Masuda et al. 1996, Janczewski et al. 1995

new specimen. We identified collected samples by writing the name of the species that deposited the feces in the Aché language and placed samples in a freezer at the reserve headquarters (Jejui'mi station laboratory). We shipped samples from Paraguay in a cooler surrounded by ice packs and stored them at -20°C upon arrival to the United States.

Laboratory procedures

Development of diagnostic test. We searched molecular data banks for sequence data from the cytochrome-*b* gene for canid and felid species that occurred within Mbaracayú Reserve (Table 1). We aligned sequences using the DNA analysis software Sequencher™ (Gene Codes Corporation, Ann Arbor, Mich.) and developed 3 distinct primer sets to allow differentiation between canids, small spotted felids, and larger felids (Table 2). Successful amplifications via the canid primers yielded a 338-base-pair (bp) fragment while the small spotted felid primers yielded a 341-bp fragment and the larger felid primers yielded a 276-bp fragment. We further analyzed the sequences from the representative species within each taxonomic group using

DNA Strider™ 1.2 (Christian Marck, Institut de Recherche Fondamentale, France) for species-specific enzyme restriction sites.

We chose restriction enzymes based on the ability to determine species identification from the results of any sample tested against all restriction enzymes for that taxonomic group. For the canids, 3 restriction enzymes (BamH I at position 100, Dde I at position 150, and Btg I at position 179) provided adequate differentiation to identify the 4 possible species occurring at the reserve. We used 4 restriction enzymes (Cla I at position 55, Hpa II at position 140, Alu I at position 258, and Mnl I at position 260) to differentiate the 5 potential small spotted felid species. The large felids (jaguarundis [*Herpailurus yaguarondi*], jaguars [*Panthera onca*] and pumas [*Puma concolor*]) required 3 restriction enzymes (Alu I at position 153, Hph I at position 197, and Rsa I at position 240) to differentiate. We developed a decision-rule flow chart to guide the diagnostic analysis of scats and determine the species identity for each sample (Figure 2). We tested the entire protocol on feces obtained from captive animals held by institutions holding current accreditation by the American Zoo and Aquarium Association (AZA). We used these samples as positive controls and represented at least one species within each group: bush dog (*Speothos venaticus*) (Oklahoma City Zoo, Oklahoma, and Little Rock
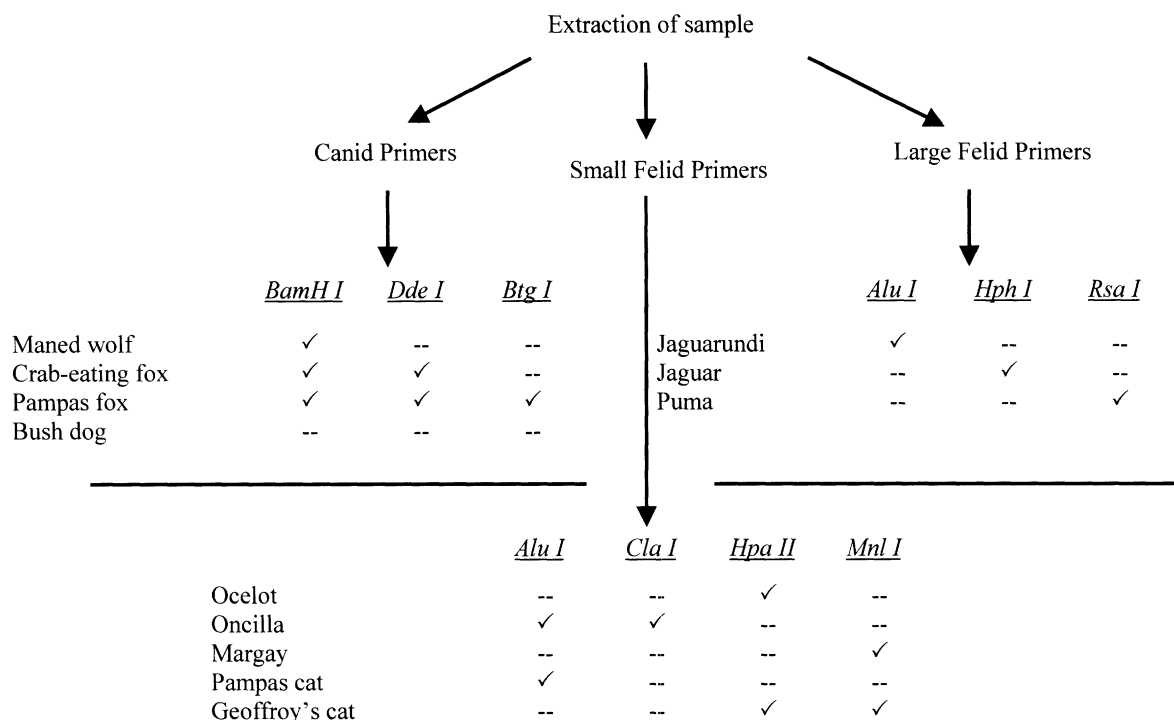
Table 2. Primer sequences used to differentiate between feces from canids, small felids, and large felids occurring within Mbaracayú Reserve, Paraguay during 1999–2001.

Name	Primer sequence	DNA-strand designation
5P Canid	5′-GACCAACATTCGAAAAAC-3′	Heavy
3P Canid	5′-TTTCATGTTTCTATGAATA-3′	Light
5P Felid[a]	5′-ATGACCAACATTCGAAAATCACAC-3′	Heavy
3P Small Felid	5′-ATGTTTCATGTTTCTGA-3′	Light
3P Large Felids	5′-GCAGATAAAGAATATGGAGG-3′	Light

[a] The 5P Felid primer was used for both small and large felids.

Extraction of sample → Canid Primers, Small Felid Primers, Large Felid Primers

Canid Primers

	BamH I	_Dde I_	_Btg I_
Maned wolf	✓	--	--
Crab-eating fox	✓	✓	--
Pampas fox	✓	✓	✓
Bush dog	--	--	--

Large Felid Primers

	Alu I	_Hph I_	_Rsa I_
Jaguarundi	✓	--	--
Jaguar	--	✓	--
Puma	--	--	✓

Small Felid Primers

	Alu I	_Cla I_	_Hpa II_	_Mnl I_
Ocelot	--	--	✓	--
Oncilla	✓	✓	--	--
Margay	--	--	--	✓
Pampas cat	✓	--	--	--
Geoffroy's cat	--	--	✓	✓

Figure 2. Flow chart for decision rule used to determine the species identification of feces from Mbaracayú Reserve, Paraguay during 1999–2001. Species are designated by their assigned codes (see Introduction, Table 2). A "√" indicates the PCR product was cut by the specified restriction enzyme (written in italics for each species group). A "--" indicates the restriction enzyme did not cut the PCR product.

Zoo, Arkansas) and maned wolf (*Chrysocyon brachyurus*) (Sedgwick County Zoo, Wichita, Kansas, and Little Rock Zoo) for canids, jaguar (Sedgwick County Zoo and Little Rock Zoo) for large felids, and ocelot (*Leopardus pardalis*) (Little Rock Zoo) for small spotted felids.

Molecular analysis of scat samples. We extracted DNA from 30–60 mg of frozen scats using the IsoQuick extraction kit (ORCA Research Inc., Bothell, Wash.). We treated samples with 200 μl each of lysis buffer and sample buffer in a 1.5-ml microcentrifuge tube, vortexed for 15 seconds, stored at room temperature for 2 hours and vortexed again for 15 seconds. We transferred approximately 200 μl of lysate to a new microcentrifuge tube and added 700 μl of extraction matrix. We vortexed the sample for 15 seconds. We added extraction buffer (400 μl), and vortexed the sample again for 15 seconds. After 30 minutes of storage at room temperature, we placed the sample in a centrifuge for 5 minutes at 12,000 rpm. We transferred the aqueous phase (~250–350 μl) to a new microcentrifuge tube and added sodium acetate to equal one-tenth (0.1) the final volume. We added an equal volume of 100% isopropanol and shook the sample

by hand to mix and precipitate DNA. We next placed the sample in the centrifuge for 10 minutes at 12,000 rpm. We discarded the supernatant and added 1 ml of 70% ethanol. We inverted the sample 5 times and placed in the centrifuge for 5 minutes at 12,000 rpm. Again, we discarded the supernatant and dried the sample using a CentriVap® Centrifugal Concentrator (Labconco Corporation, Kansas City, Miss.) and EC-353 Gel Dryer Pump (Thermo EC, Holbrook, N.Y.). We resuspended the remaining pellet with 100 μl of RNase-free water (provided with the kit). We confirmed DNA extraction by gel electrophoresis, using 10 μl of extract and a 2% agarose gel and visualized it with ultraviolet light.

We amplified a fragment of the cytochrome-*b* gene (~400 base pairs) via the polymerase chain reaction (PCR) using the following conditions for a 100-μl sample: 4 μl DNA, 63.5 μl sterile water, 10 μl of 10-mg/ml bovine serum albumin (BSA), 10 μl of 10X PCR reaction buffer, 4 μl of 25-mM $MgCl_2$, 3 μl each for heavy and light primers (Table 2), 2 μl of 10-mM dNTP's (2.5 mM for each NTP), and 0.5 μl Taq DNA polymerase. We treated each sample was treated separately with all 3 primer sets. Reactions

took place in a programmable thermocycler (GeneAmp PCR System 9600, Perkin Elmer Cetus, Norwalk, Conn.) with a 3-minute denaturation step at 94°C, 35 amplification cycles (94°C for 60 seconds, 42°C for 60 seconds, and 72°C for 45 seconds), a 3-minute final extension at 72°C, and an indefinite concluding step at 10°C. We verified PCR products through electrophoresis on a 1% agarose gel and examined them with ultraviolet light.

We then subjected PCR products to digestion by each restriction enzyme for the appropriate taxonomic group with the following 1-hour 20-µl reaction: 15 µl PCR product, 2 µl 10X restriction enzyme buffer, 1 µl restriction enzyme, and 2 µl sterile water. We visualized restriction enzyme results after electrophoresis on a 2% small fragment agarose gel with ultraviolet light. We compared final species identifications as determined through the molecular diagnostic analysis to identifications provided during field collection by the RNBM rangers and the Aché of the Arroyo Bandera Reservation.

Results

We attempted molecular analysis on 172 scats collected by the rangers and 66 scats collected by the Aché. We achieved identification via molecular analysis on 124 (72.1%) of ranger-identified samples and 62 (93.9%) of Aché-identified samples.

Canids constituted the largest group of feces collected and identified by the rangers that also were identified by molecular analysis. We identified only one canid from the samples provided by the Aché. The majority of feces successfully identified by molecular analysis from the samples provided by the Aché were felid in origin; these were represented almost equally by large and small, spotted species.

We identified 11 total samples from bush dogs (Table 3), 17 from maned wolves, and 34 from crab-eating foxes (*Cerdocyon thous*) as determined by results of each sample to each of the 3 designated restriction enzymes for canids (Figure 3). We identified no scats from pampas foxes (*Pseudalopex gymnocercus*). We identified 36 samples as jaguar, 26 as puma, and 5 as jaguarundi based on the comparison of each sample to each of the 3 restriction enzymes designated for large felids (Figure 3). Within the small spotted felids, we identified 11 as ocelot, 29 as oncilla (*Leopardus tigrinus*), 10 as margay (*L. wiedii*), and 7 as Geoffroy's cat

Table 3. Canid and felid scats from Mbaracayú Reserve, Paraguay identified by rangers and Aché during 1999–2001 and also analyzed by molecular techniques. Successful and unsuccessful refers to whether identification was achieved with the molecular analysis and does not refer to whether rangers and Aché accurately identified specimens. Numbers in parentheses are the percent each category represents in terms of successful/unsuccessful molecular analyses for both ranger and Aché samples.

Source Outcome	Total samples	Canids	Small spotted cats	Large cats
Rangers				
Successful	124	61 (49.19)	26 (20.97)	37 (29.84)
Unsuccessful	48	42 (87.5)[a]	4 (8.33)[b]	2 (4.17)[c]
Aché				
Successful	62	1 (1.61)	31 (50.00)	30 (48.39)
Unsuccessful	4	0 (—)	3 (75.00)[d]	1 (25.00)[e]

[a] Forty-one of these samples were identified as crab-eating fox (*Cerdocyon thous*) in the field with the other being identified as maned wolf (*Chrysocyon brachyurus*).

[b] Three of these samples were identified as oncilla (*Leopardus tigrinus*) in the field and 1 as margay (*L. wiedii*: Lewi).

[c] These samples were identified in the field as 1 jaguar (*Panthera onca*) and 1 puma (*Puma concolor*).

[d] In the field, one of these samples was identified as ocelot (*L. pardalis*) and 2 as "little spotted cat" ("Kajamini" in the Aché language could be oncilla [*L. tigrinus*], margay, [*L. wiedii*] or Geoffroy's cat, [*Oncifelis geoffroyi*]).

[e] This sample was identified as a jaguar in the field.

(*Oncifelis geoffroyi*). We based these identifications on comparisons of each sample to each of the 4 restriction enzymes designated for small cats (Figure 3). We identified no scats from pampas cats (*Oncifelis colocolo*).

A comparison of the identifications of scat samples determined by the molecular diagnostic test with identifications given by reserve rangers and local indigenous Aché revealed 100% agreement. The 11 samples identified as bush dog by molecular analysis corresponded to those identified as bush dog by the rangers and Aché. All 124 ranger-identified samples successfully identified by molecular analysis exactly matched the species identification given the sample by the ranger in the field. Of the 62 Aché-identified samples that were successfully identified by molecular analysis, 42 exactly matched the species identification given at the time of collection. We identified the remaining 20 samples as margay, oncilla, or Geoffroy's cat, all of which matched the Aché word "kajamini" that applied to these 3 distinct species of small spotted cat.
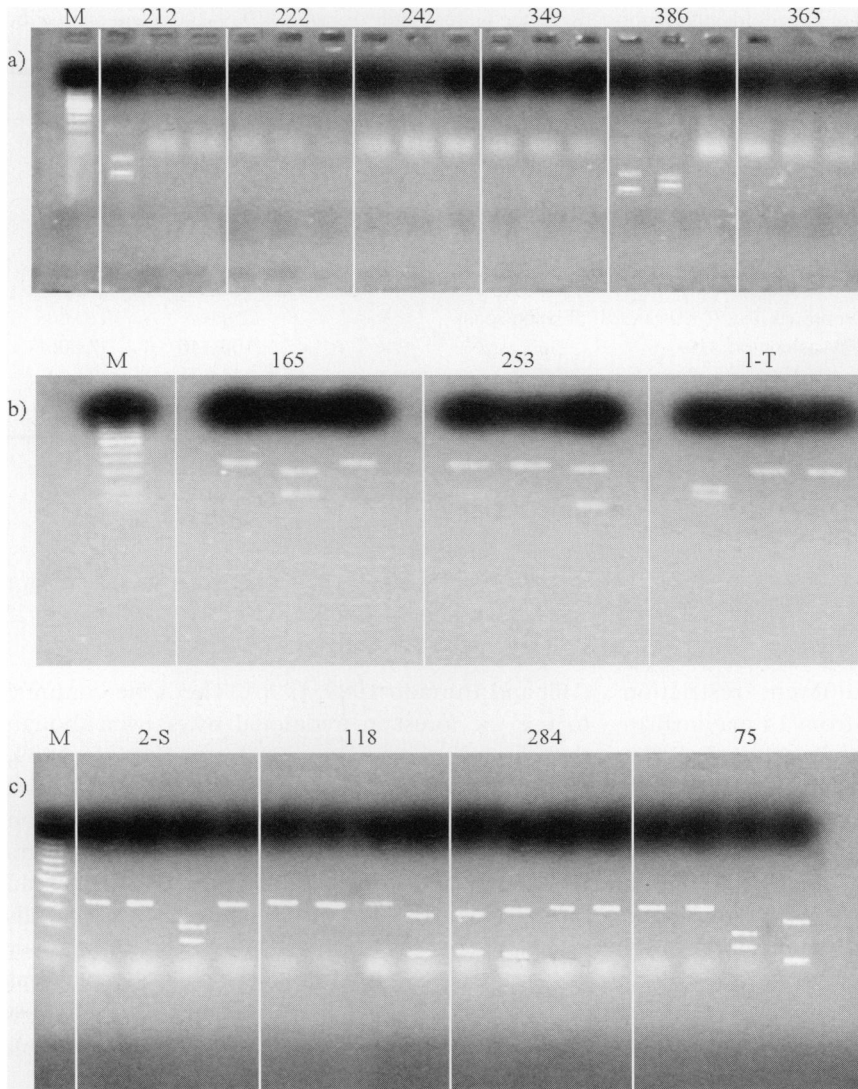
Figure 3. Gel (2% small-fragment size agarose) electrophoresis of carnivore samples after treatment with restriction enzymes. Canids are presented on a), large felids on b), and small felids on c). The "M" above each gel refers to the super-marker. On a), 212 is a crab-eating fox; 222, 242, 349, and 365 are bush dogs; 386 is a maned wolf. Numbers listed above the gel refer to the restriction enzymes used for the canids: 1 = BamH I, 2 = Dde I, and 3 = Btg I. On b), 165 is a jaguar; 253 is a puma; 1-T is a jaguarundi. Numbers listed within the gel refer to the restriction enzymes used for the large felids: 1 = Alu I, 2 = Hph I, and 3 = Rsa I. On c), 2-S is an ocelot; 118 is a margay; 284 is an oncilla; 75 is a Geoffroy's cat. Numbers listed within the gel refer to the restriction enzymes used for the small felids: 1 = Alu I, 2 = Cla I, 3 = Hpa II, and 4 = Mnl I.

Discussion

Our first objective, to develop a diagnostic molecular technique to identify canid and felid fecal samples from the Mbaracayú Reserve, was achieved, and most samples (78.2%) were successfully identified by molecular analysis. For the remaining scats, we failed to obtain a PCR product

from the extracted DNA. The majority of scats not identified were desiccated and appeared old. Rangers collected most of these scats and identified them in the field as canids (Table 3), specifically crab-eating fox, and consisted largely of palm fruit (*Butia paraguayensis*). Considering the accuracy of identifications provided by rangers for samples successfully identified by molecular analysis, we concluded that the identifications provided for samples not successfully identified by molecular analysis were also accurate. It also is important to acknowledge that both rangers and Aché were confident of all field identifications. Some other studies using DNA extracted from scats for identification have experienced greater success (Table 4), but the success of our study was exceptional because of the relatively large number of samples (238) and the diversity of species we considered.

Our results support the use of molecular analyses, and the cytochrome-*b* gene in particular, to identify feces from closely related species. This is an example of research recommended by Kohn and Wayne (1997) in their review of molecular scatology. Other researchers used molecular analyses of carnivore feces to assign identifications where multiple species, representing several families, co-existed (Foran et al. 1997) as well as where several species within a family co-exist (Paxinos et al. 1997, Hansen and Jacobsen 1999). Foran et al. (1997) used the cytochrome-*b*

Table 4. Comparison of success rates of species identifications between the present study (Paraguay, 1999–2001) and other published studies that used fecal DNA. Success refers to the ability to obtain a definitive result from the sample.

Study	Taxa : Location	Success rate	Percent
Present study	Carnivora[a] : Paraguay	186/238	(78.15)
Hansen and Jacobsen 1999	Mustelids[b] : Denmark	23/23	(100.00)
Kohn et al. 1999	Coyotes (*Canis latrans*, Canidae) : California, USA	188/238	(78.99)
Frantzen et al. 1998	Yellow baboons (*Papio cynocephalus*, Cercopithecidae) : Botswana	253/264	(95.83)
Paxinos et al. 1997	Canids[c] : California, USA	10/10	(100.00)
Reed et al. 1997	Phocids[d] : Scotland	156/173	(90.17)
Taberlet et al. 1997	Pyrenean brown bear (*Ursus arctos*) : France/Spain	21/105	(20.00)
Wasser et al. 1997	Ursids[e] : Washington, USA	105/140	(75.00)
Gerloff et al. 1995	Bonobos (*Pan paniscus*, Pongidae) : Zaïre	31/33	(93.94)
Kohn et al. 1995	European brown bears (*U. arctos*, Ursidae) : Italy	8/12	(66.67)

[a] Includes Canidae and Felidae.

[b] Includes European otter (*Lutra lutra*), American mink (*Mustela vison*), and polecat (*M. putorius*).

[c] Includes San Joaguin kit fox (*Vulpes velox mutica*), red fox (*V. vulpes*), and gray fox (*Urocyon cinereoargenteus*).

[d] Includes grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*).

[e] Fecal material primarily from free-ranging American black bears (*U. americanus*), but also includes samples from four captive Malayan sun bears (*Helarctos malayanus*).

gene, subjected to several different restriction enzymes, to differentiate feces from 14 mammalian carnivores representing the Canidae, Felidae, Mustelidae, and Procyonidae. Clearly, molecular techniques offer wildlife ecologists unique tools for enhancing studies of free-ranging animals.

The Aché and RNBM rangers who worked with us reliably identified felid and canid scats collected in the field. The virtual lack of canid samples provided by the Aché (Table 3) can be partially explained by their lack of foraging in the eastern portion of the reserve. This eastern area, the Aguara Ñu region, is farthest from the Arroyo Bandera village (Figure 1, site 4) and is primarily a cerrado habitat where maned wolves and foxes are common. Molecular tests verified the Aché and rangers' identifications. Only the identification of small spotted felids by the Aché was less precise than our identification based on molecular analyses. Their field identification, "kajamini," indicated 3 possible species options: the margay, the oncilla, and the Geoffroy's cat. It is important to emphasize that they were able to differentiate the generic "small spotted felids" from ocelots and jaguarundis.

The ability of the Aché to correctly interpret animal sign within Mbaracayú Reserve was not surprising since they led a nomadic existence within forested areas of eastern Paraguay prior to 1978 (Hill and Hurtado 1989, 1996). The ability of Aché to obtain food and avoid dangerous animals like jaguars was dependent on interpreting animal signs

(Hill and Hurtado 1989, 1996). The Aché continue to use the forest in traditional ways, even though they now live in villages (Hill and Hurtado 1989, 1996). Other local peoples, including some of the reserve rangers, also have life-long dependence on resources of the forest. Tests of Aché and rangers who have not been dependent upon wildlife should be conducted to determine whether they have skills comparable to the individuals who worked with our study team. The ability of only a few individuals was reflected in our results, and we suspect that most younger Aché and inexperienced rangers would not display the same level of accuracy.

This study provided evidence that some rangers and Aché of RNBM possess valuable TEK and can be reliable sources of data for future studies of mammalian carnivores. Our results also demonstrate that indigenous and local knowledge may help advance scientific knowledge about animals, habitats, and interactions between wildlife and their habitats. The validation of TEK by molecular techniques can help establish the credibility of using indigenous and local people to collect data in future studies. Additionally, after the credibility of indigenous and local people is validated, financial and time resources can be saved or reallocated to enhance field research and management efforts.

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