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DNA BARCODING

DNA barcoding in surveys of small mammal communities: a field study in Suriname

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Abstract

The performance of DNA barcoding as a tool for fast taxonomic verification in ecological assessment projects of small mammals was evaluated during a collecting trip to a lowland tropical rainforest site in Suriname. We also compared the performance of tissue sampling onto FTA CloneSaver cards vs. liquid nitrogen preservation. DNA barcodes from CloneSaver cards were recovered from 85% of specimens, but DNA degradation was apparent, because only 36% of sequence reads were long (over 600 bp). In contrast, cryopreserved tissue delivered 99% barcode recovery (97% > 600 bp). High humidity, oversampling or tissue type may explain the poor performance of CloneSaver cards. Comparison of taxonomic assignments made in the field and from barcode results revealed inconsistencies in just 3.4% of cases and most of the discrepancies were due to field misidentifications (3%) rather than sampling/analytical error (0.5%). This result reinforces the utility of DNA barcoding as a tool for verification of taxonomic identifications in ecological surveys, which is especially important when the collection of voucher specimens is not possible.

Keywords: biodiversity, Chiroptera, COI, *cox 1* cytochrome *c* oxidase, Didelphimorphia, mitochondrial, Rodentia

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Although molecular approaches have been a standard taxonomic tool for 20 years, protocols allowing rapid genetic assessments are much more recent. DNA barcoding (Hebert *et al*. 2003a, 2003b) has established a standardized approach across taxonomic groups, facilitating fast, accurate species identifications. This gives it the ability to aid the rapid acquisition of taxonomic assignments, especially for well-surveyed areas, and to highlight cases in need of detailed taxonomic study for poorly known and highly speciose groups (Hebert & Gregory 2005; Hajibabaei *et al*. 2007). DNA barcodes can also serve as genetic vouchers to validate field identifications made by researchers with limited taxonomic background, making them a valuable tool for ecological surveys. DNA barcoding has been instrumental in re-assessing the species diversity of regional insect faunas (Janzen *et al*. 2005; Smith *et al*. 2005; Hajibabaei *et al*. 2006) and there are early indications that it will similarly aid species inventories of vertebrate communities (Ward *et al*. 2005; Clare *et al*. 2007; Kerr *et al*. 2007).

Mammals rank among the better-studied animal groups, and their taxonomy and species diversity is relatively well documented (Wilson & Reeder 2005). However, field determinations for many small mammal species are difficult, because definitive identifications require analysis of internal morphology, that is skull or dentition. As a result, field identifications in such groups remain questionable unless voucher specimens are retained. Furthermore, molecular data suggest the frequent occurrence of cryptic mammal species overlooked by preceding morphological studies (Baker & Bradley 2006).

Until recently, the protocols for molecular diagnostics were expensive and time-consuming, making this approach justified only in cases when morphological identifications were difficult. High-throughput DNA barcoding protocols (Hajibabaei *et al*. 2005; Ivanova *et al*. 2006) have reduced this barrier, but they rely on the preservation of tissue samples with high-quality DNA.

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While tissues destined for molecular study should ideally be frozen in liquid nitrogen, this method of preservation may be inconvenient in field situations. Preservation of tissue in ethanol is a common alternative, but DNA degrades when stored in this manner for a long interval at room temperature (Kilpatrick 2002). Furthermore, shipments of ethanol are increasingly regulated, providing an incentive to evaluate the efficacy of dry preservation methods, such as FTA CloneSaver cards (Whatman, Inc).

The present study has two primary goals. It first seeks to evaluate the performance of DNA barcoding as a tool for fast taxonomic verification to complement the collecting of voucher specimens. Second, it tests the performance of CloneSaver cards, compared to liquid nitrogen, in the preservation of mammal tissues from a lowland tropical rainforest site in Suriname.

Materials and methods

Study site

Small mammal specimens were collected from January 10 to February 25, 2006 in the Bakhuis Mountains, Sipaliwini Region, Suriname. Situated in northwestern Suriname, this site is part of the Guiana Shield. Habitats surveyed ranged from tall mesic forest to low xeric forest. Sampling activities centred on four sites with a maximum distance of 40 km between localities at elevations of 50–400 m above sea level. Most of the survey coincided with peak rainfall during the second (short) rainy season.

Collection of specimens

Our study employed a combination of trapping methods (Lim & Engstrom 2005) to survey diversity in three mammalian orders — opossums (Didelphimorphia), rodents (Rodentia) and bats (Chiroptera). Small nonvolant mammals were captured using large (12 × 14 × 35 cm) and medium-sized (8 × 9 × 23 cm) Sherman live traps (3524 trap nights) that were set in a linear fashion at intervals of *c*. 5 m along transects cut through the forest. They were positioned on the ground, on fallen logs or on lianas up to 1.5 m above the forest floor. Additionally, medium-sized (60 × 24 × 24 cm) Tomahawk traps (136 trap nights) were set on the ground at the base of large trees that had vines extending into the canopy to target larger opossums. Bats were sampled using a combination of long (12 × 2.6 m) and short (6 × 2.6 m) mist nets, two canopy nets (30 × 10 m) and one flap trap. Mist nets were set at ground level along transects, across roads and streams, with a total number of netting hours of *c.* 1500. Canopy nets were set above roads and clearings. Nets were usually open from dusk until midnight. The flap trap (Borissenko 1999) was made with 4-m poles and a 3- × 2-m monofilament net with a mesh size of 1.4 cm. Flap trapping was conducted for 28 nights along roads and edges of clearings for a total of 150 h and 200 km of survey distance.

Captured live animals were examined and provisionally identified to species based on external morphology, measurements and visible characters of dentition. Selected individuals of common species of bats were released, while specimens kept as reference material were euthanized, following standard guidelines (American Society of Mammalogists Animal Care and Use Committee 1998), and with an approved Royal Ontario Museum Animal Use Protocol. Field identification was confirmed by postmortem examination. In all, 794 small mammals were captured, of which 526 (478 bats, 40 rodents and 8 opossums) were retained as voucher specimens (59 bat, 9 rodent, 5 opossum species) that are deposited in the Royal Ontario Museum (ROM) and the National Zoological Collection of Suriname.

Tissue sampling

Liver, heart, kidney, and spleen tissues were preserved. Because of logistical difficulties, tissues were only frozen in liquid nitrogen after the first week of the expedition, so 95% ethanol was used before this time. Long-term storage at the ROM is in an ultracold freezer maintained at –80 °C. As a comparison to ethanol- or liquid nitrogen-preserved tissue, 96 sample FTA CloneSaver cards were also used. This storage medium, originally developed for collecting and storing plasmid DNA from bacterial clones in a laboratory setting, was chosen for testing primarily because of its compatibility with the high-throughput 96-well sampling format. We investigate its limits and utility under tropical field conditions. During the dissection of each specimen, a small piece of liver was removed and blotted against one circle on the FTA card. If the liver did not provide sufficient fluid, other organs containing blood (e.g. heart) were used. Cards were air-dried in the field and stored away from light at ambient temperature in sealed zip-lock bags with silica-gel desiccant.

Molecular protocols

DNA recovery protocols for wildlife samples (Smith & Burgoyne 2004; Livia *et al*. 2006) were used, with modifications facilitating automated DNA extraction in 96-well format, using a Biomek NX robotic liquid handler (Beckman Coulter Inc.). One-millimetre disks were sampled from FTA cards into Acroprep PALL filter plate with 0.45 μm GHP membrane (Pall Life Sciences) using a Harris Micro-Punch (Whatman). Clean filter paper was punched after each sample to clear any residue off the blade.

Disks were washed with reagents for wildlife samples (Smith & Burgoyne 2004). Two hundred microlitres of first wash buffer (100 mM Tris, free base; 0.1% SDS) was added

Table 1 Summary of PCR success for different tissue sources, primer combinations and polymerases. Results for the ROM cryostorage also incorporate data for reference specimens

Tissue source	Primer cocktails	Fragment length	Polymerase	No. of PCRs	Percentage of PCR success
FTA	C_VF1di/C_VR1di	full	NEB *Taq*	233	32%
FTA	C_VF1LFt1/C_VR1LRt1	full	NEB *Taq*	515	58%
FTA	RonM/C_VR1LRt1	short	NEB *Taq*	80	96%
FTA	RonM_t1*/C_VR1LRt1	short	NEB *Taq*	187	95%
FTA	C_VF1LFt1/C_VR1LRt1	full	Platinum *Taq*	96	98%
FTA	RonM_t1*/C_VR1LRt1	short	Platinum *Taq*	190	99%
ROM	C_VF1LFt1/C_VR1LRt1	full	Platinum *Taq*	331	99%
ROM	C_FishF1t1/C_FishR1t1	full	Platinum *Taq*	40	98%

*Primer sequence: 5′-TGTAAAACGACGGCCAGTGGMGCMCCMGATATRGCATTCCC-3′.

to each of the 96 wells in the Acroprep plate set on a 36-mm collar positioned on the vacuum manifold and incubated for 30 min. Vacuum of 23 In Hg was applied for 4 min and the filtrate discarded. One hundred and eighty microlitres of second wash buffer (5 M GuSCN, 20 mM EDTA pH 8.0, 10 mM Tris-HCl pH 6.4 and 4% Triton X-100) was added to each well. The plate was incubated for 10 min and placed under vacuum for 3 min. Two hundred microlitres of ddH_2O was added to each well, incubated for 10 min and vacuum was applied for 2 min. This procedure was repeated two more times before 220 μL of 96% ethanol was added and vacuum was applied for 5 min. After removing liquid residue from the bottom of the plate, it was incubated at 56 °C for 20–30 min to evaporate residual ethanol. An empty 96-well Eppendorf plate (Eppendorf AG) was placed on top of the Acroprep plate and the disks were transferred directly into the corresponding wells by flipping the assembly upside down. Disks that remained attached to the wells of the filter plate were transferred using clean forceps.

After the wash procedure, 12.5 μL of polymerase chain reaction (PCR) master mix was added to the wells and the standard operations of PCR and sequencing with regular and M13-tailed universal primer cocktails (Ivanova *et al*. 2006; Clare *et al*. 2007; Ivanova *et al*. 2007) were followed, in order to recover 5-prime 658-bp fragments of the mitochondrial cytochrome oxidase subunit I gene (*cox1*). In cases where we were not able to recover a full length *cox1* barcode, the internal primer RonM (Pfunder *et al*. 2004) and its M13-tailed modification RonM_t1 (Table 1) was used.

The laboratory procedure was comprised of three consecutive amplification and sequencing attempts. The first sought to recover a full-length amplicon (657 bp) from FTA-preserved samples using regular *Taq* polymerase (New England Biolabs), hereafter named NEB *Taq*. The second attempt aimed to recover a shorter amplicon (421 bp) using both NEB *Taq* and Platinum *Taq* hot-start polymerase (Invitrogen) for samples that did not initially succeed; it also contributed several full-length amplicons using Platinum *Taq*. Specimens for which full DNA sequences were not recovered from FTA cards after these two attempts were resampled from the ROM frozen tissue collection and DNA was extracted. Approximately 1 mm^3 of tissue was sampled directly into 96-well plates containing 50 μL of lysis buffer with proteinase K and incubated for 12–18 h at 56 °C, followed by a robotic protocol on the Biomek NX liquid handling station (Beckman Coulter) as described in Ivanova *et al*. (2006). The procedure of PCR amplification and sequencing was the same as above. Table 1 summarizes the success of PCR attempts with different primers and amplification enzymes. The proportion of ethanol-stored tissue used during the third sequencing attempt was too small to justify treating it as a separate data set; therefore, these samples were grouped with other cryopreserved tissue.

Data analyses

Sequence data were stored and analysed using the Barcode of Life Data System (BOLD, Ratnasingham & Hebert 2007) through the online interface at www.barcodinglife.org. Neighbour-joining trees based on the Kimura 2-parameter (K2P) model were built using the 'Taxon ID tree' function of BOLD, and genetic distances between and within different taxonomic levels were summarized. After being fully assembled, sequence data were downloaded from BOLD for further analyses. MEGA version 3.1 (Kumar *et al*. 2004) was used to build compressed trees. Trees were built using the neighbour-joining algorithm with the K2P model, pairwise deletion of missing data, and the inclusion of all codon positions and substitution types; the same parameters are used for building neighbour-joining trees in the BOLD analytical module. Branch support was assessed by bootstrapping with 500 replicates.

Sequences are deposited in the National Center for Biotechnology Information (NCBI) GenBank with accession nos EU096554–EU097074. COI sequences, chromatogram trace files, and collateral specimen information are available

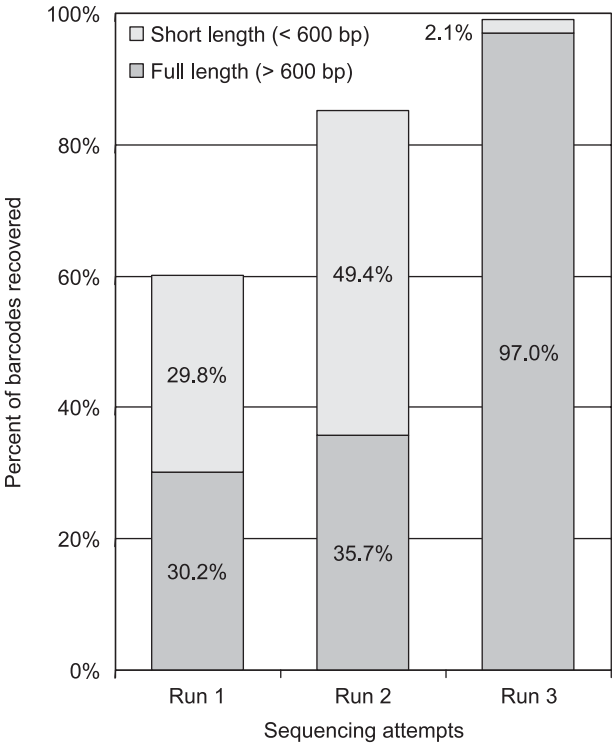


Fig. 1 Accumulation of DNA barcodes for Bakhuis mammals in three sequencing attempts. See text and Table 1 for explanations.

in the Completed Projects section of BOLD in the project 'Small mammal survey in Bakhuis, Suriname (ABSMS)'.

Comparison with reference material

As part of the analytical procedure, sequences obtained in this study were compared with DNA barcodes of South American mammals currently present in BOLD as a cross-check of field identifications. Reference sequences included DNA barcodes from a study of Neotropical bats, BOLD project 'Bats of Guyana' (BCBNC) (Clare *et al*. 2007), and 74 specimens of nonvolant mammals deposited in the ROM collection, which were subsampled and analysed together with tissues for the third sequencing attempt [BOLD project 'Small mammal survey in Bakhuis reference sequences' (ABSMC); NCBI GenBank Accession nos EU095420–EU095493]. Voucher specimens were examined in all cases of discrepancy between field identifications and those inferred from their DNA barcodes.

Results

Barcode recovery

Besides relatively low PCR recovery, efforts to obtain barcode sequences from the FTA cards met with even lower success, because of low concentration of PCR products and non-specific amplification resulting in heterogeneous sequences. Only 35.7% of the samples yielded full-length DNA barcodes (Fig. 1). Higher sequencing success (85%) was obtained with the additional recovery of a shorter length PCR product (421 bp) indicating that DNA degradation had occurred, although DNA extractions from FTA cards were carried out within a month of sample collection. By contrast, after resampling from cryopreserved tissue, overall success became 97.0% for full-length PCR product and 99.1% with the addition of short-length amplicons. The five recalcitrant samples all belonged to *Saccopteryx bilineata* — a common species whose other members were sequenced (see below). Overall, the best outcomes were achieved both from FTA and from cryogenically stored tissue with a combination of M13-tailed vertebrate primers (C_VF1LFt1/C_VR1LRt1) and Platinum *Taq*.

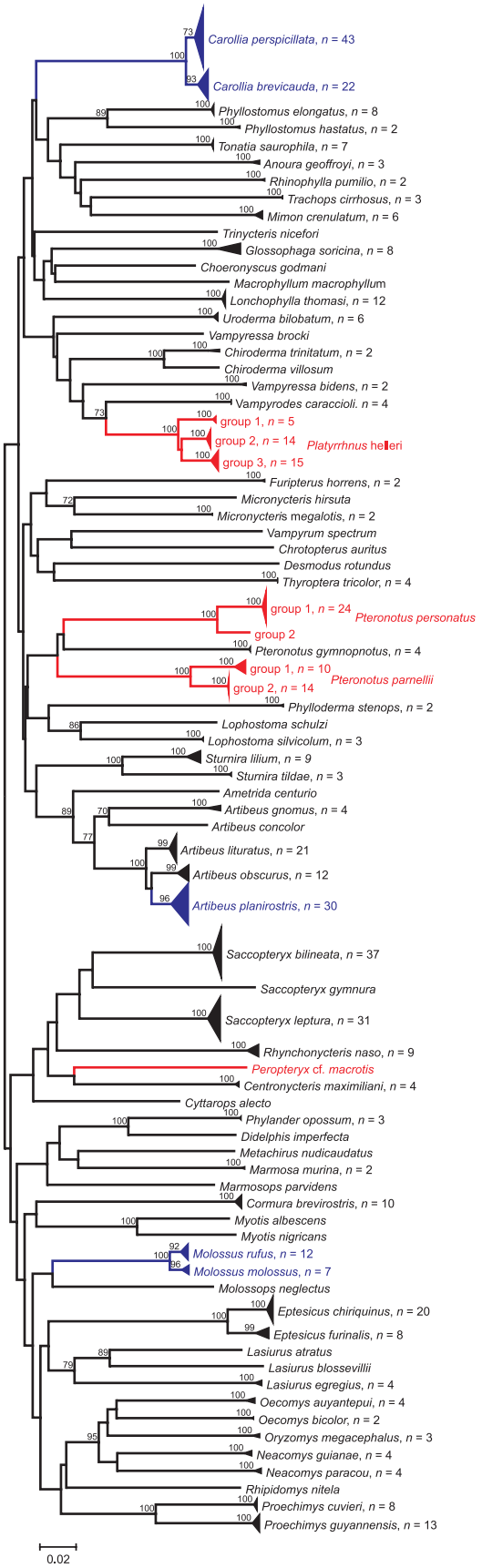
Barcode divergences between species

Distances calculated for 516 sequences exceeding 420 bp indicated a mean genetic diversity of 1.0% (0–5.3, SE = 1.23) within species and 10.1% (1.5–22.2, SE = 7.23) between species within a genus. Over 90% of genetic lineages representing known species (Wilson & Reeder 2005) showed greater than 2% genetic distance from their nearest neighbour and at least 99% bootstrap support (Fig. 2). In all cases, barcodes enabled the discrimination of known species, irrespective of the sequence length recovered.

Conflict between barcode and field identifications

The number of species provisionally recognized in the field was 74 (B. K. Lim, unpublished data) and 73 species were supported by DNA barcodes. Although the overall number of species only decreased by one, there were seven discrepancies (12 specimens) that affected the composition of species diversity (Table 2). Among the 523 specimens that were analysed, barcoding suggested that 18 specimens (3.4%) were misidentified. Examination of voucher specimens confirmed this fact in 15 cases (2.9%). The remaining three cases (0.6%) reflected one sampling error (mislabelled tissue yielding consistent erroneous results) and two cases of cross-contamination (erroneous results were not reproduced following re-extraction).

We found no cases of sequence sharing between species in our data set; although two species pairs (*Carollia brevicauda/C. perspicillata*, *Molossus molossus/M. rufus*) showed less than 2% sequence divergence (Table 3). Branches containing these four species and also *Artibeus planirostris* had < 99% bootstrap support (Fig. 2). We also noted cases of unusually deep (over 2%) intraspecific variation within *Glossophaga soricina*, *Saccopteryx leptura* and *A. planirostris* (Table 3), but no distinct mitochondrial



lineages could be defined within these species. The assignment of individuals to a given species using the BOLD online identification engine was always successful.

Additionally, DNA barcodes revealed the presence of genetically distant (> 3% divergence) and well-supported (bootstrap of 100%) lineages within three species: *Platyrrhinus helleri* (3 groups), *Pteronotus parnellii* (2 groups) and *Pteronotus personatus* (2 groups). These results are concordant in part with the findings of (Clare *et al*. 2007), who reported the presence of cryptic genetic diversity in *P. helleri* from Guyana. Thus, the overall number of genetically distinct forms in the small mammal data set studied is 77.

Discussion

Using FTA cards for field sampling

Cards with FTA technology have been widely used in forensics because of their compactness, ease of sampling and shipping (Hsiao *et al*. 1999; Vanek *et al*. 2001; Smith, Burgoyne 2004). The same reasons support their potential value in collecting trips, where logistical complications often preclude alternate preservation approaches (Edwards *et al*. 2005). In our study, FTA cards were not effective in protecting DNA from degradation in tropical field situations, compared to ethanol fixation or cryopreservation. The DNA degradation that we encountered was likely due, at least in part, to the high humidities, which are particularly inescapable in field camps during the rainy season, and also the tissue type used. Despite the tendency of DNA from liver to quick degradation (Hanner *et al*. 2005), liver remains a popular tissue type (e.g. Kilpatrick 2002), often retained by natural history collection repositories. Our study confirms that liver should not be used as the tissue source for FTA sampling. Besides high enzymatic activity, it is difficult to control the amount of blotting, which may prevent proper fixation. The results of this study contrast with successful recovery of high-quality DNA barcodes from FTA-preserved brain tissue sampled in a controlled laboratory environment (N. Ivanova, I. Kuzmin, unpublished data). This suggests that with robust protocols for humidity control and sample application from other bodily fluids such as blood, FTA cards may become an acceptable medium for ecological studies where specimen collection is not appropriate. However, further research is needed to develop tissue sampling protocols for FTA cards and other methods that are both robust and simple enough for use in the field. At

Fig. 2 Neighbour-joining tree for Bakhuis mammals. Species separated by small (< 2%) genetic distance and with less than 99% bootstrap support are marked with blue. Samples that may represent or contain undescribed taxa are marked with red. Bootstrap values less than 70% are not shown. See Methods for details on the tree-building algorithm.

Table 2 Discrepancies between identifications made in the field and those inferred from DNA barcodes that affected the overall species list

No.	No. of specimens	Field identification	DNA barcode-based identification	Comments
1	2	*Artibeus cinereus*	*Artibeus gnomus*	confirmed *A. gnomus*
2	1	*Lasiurus blossevillii*	*Lasiurus atratus*	confirmed *L. atratus*
3	1	*Peropteryx kappleri*	no match in the reference database	re-identified as *Peropteryx* cf. *macrotis*, possible undescribed species
4	1	*Vampyressa thyone*	*Vampyressa brocki*	confirmed *V. brocki*
5	4	*Neacomys paracou*	*Neacomys guianae*	confirmed *N. guianae*
6	1	*Oecomys roberti*	*Oecomys auyantepui*	confirmed *O. auyantepui*
7	2	*Oecomys rutilus*	*Oecomys auyantepui* (1 specimen)	confirmed *O. auyantepui*
			Oecomys bicolor (1 specimen)	confirmed *O. bicolor*

this stage, we cannot recommend FTA cards as a replacement for conventional methods of field preservation of tissues from voucher specimens. While liquid nitrogen storage is clearly the best option, initial preservation in 95% ethanol followed by freezer storage is an effective alternate.

Using DNA barcoding in small mammal diagnostics

Re-examination of museum-deposited voucher specimens confirmed that most discrepancies between field identifications and those inferred from DNA barcodes were due to identification errors. There are three causes of identification mistakes that are common in field surveys: age variation, undescribed/extralimital species and lack of comparative material. Age variation obscured the diagnostic features in the species of *Oecomys* which overlap in size and morphological characters. The specimen of *Peropteryx* cf. *macrotis* (possibly an undescribed taxon) was identified in the field as *Peropteryx kappleri* — a morphologically similar species known to occur in the area. The other cases of misidentified specimens were members of genera that are simply difficult to identify in the hand. Some species of mammals, particularly many New World rodents such as *Neacomys*, *Oecomys* and *Proechimys* can only be reliably identified using characters requiring special preparation (e.g. skulls and chromosomes). In other cases, diagnostic features are vaguely defined in identification keys (e.g. fine details of pelage colouration) and their use demands examination of large series of museum specimens. In yet other cases, the diagnostic traits are only present in members of one sex. For example, species identifications in *Proechimys* often rely on the shape of the baculum which is only present in males. Aside from these limitations, the occurrence of field identification errors depends on the researcher's experience with a given fauna and is bound to increase significantly when surveys are carried out by people with limited taxonomical background. Cases such as these make clear the value of collecting small tissue samples (e.g. blood, skin biopsy) for barcode analysis to enable validation of field identifications when vouchers are not retained.

The other major cause of discrepancies between barcode-based identifications and field identifications involved cases where divergent mitochondrial lineages were observed within three species (*Platyrrhinus helleri*, *Pteronotus parnellii* and *Pteronotus personatus*), suggesting that a thorough taxonomic re-assessment of these two genera is required. The genus *Platyrrhinus* is known for its complex taxonomy. A number of new species have been recently described (Velazco 2005) and cryptic species may be present within *P. helleri* (Clare *et al*. 2007). The taxonomy of *Pteronotus* is more stable and the currently recognized species are morphologically well defined. However, considerable genetic variation has been reported for *Pt. parnellii* (Lewis-Oritt *et al*. 2001; Dávalos 2006), although it has previously been attributed to geographical variation. A comparison of the recovered patterns of genetic variation within *Platyrrhinus* and *Pteronotus* with their collecting localities shows that the genetic lineages within each taxon occur syntopically throughout the study area and reveals no pattern in habitat preference of different mitochondrial lineages.

Another potential cause for discordances between the DNA-based and field identifications is contamination events or the mislabelling/misplacement of samples either during tissue sampling/subsampling or laboratory analysis. Such errors can be easily detected when they lead to mismatching of sequences of morphologically distinct species (e.g. from different genera). We detected three such cases of inconsistencies between barcode and field identifications, and resequencing these same specimens from the ROM tissue collection produced results supporting the original field identifications. Because most of the above inconsistencies result from human error during the sampling or early analytical stages, it is expected that with the refinement of field sampling protocols and the broader use of robotic equipment in laboratory analyses, their occurrence will be minimized further.

If an ecological study does not involve the collection of vouchers, DNA barcoding remains an efficient tool for validating taxonomic assignments. This is true especially in areas where previous faunal surveys have been carried out

Table 3 Efficiency of identifying species of small mammals from Bakhuis using DNA barcodes

Species	No. of specimens	Mean distance within species, percentage	Maximum distance within species, percentage	Distance to nearest neighbour
Ametrida centurio	1	N/A	N/A	14.33
Anoura geoffroyi	3	0.63	0.95	19.16
Artibeus concolor	1	N/A	N/A	9.42
Artibeus gnomus	4	0.80	1.54	11.11
Artibeus lituratus	21	0.72	1.86	2.34
Artibeus obscurus	12	0.71	1.39	3.29
Artibeus planirostris	30	1.30	2.49	2.34
Carollia brevicauda	22	0.57	1.58	1.55
Carollia perspicillata	43	0.69	1.75	1.55
Centronycteris maximiliani	4	0.47	0.72	17.78
Chiroderma trinitatum	2	1.23	1.23	6.13
Chiroderma villosum	1	N/A	N/A	6.13
Choeroniscus godmani	1	N/A	N/A	15.46
Chrotopterus auritus	1	N/A	N/A	21.71
Cormura brevirostris	10	0.43	0.96	20.53
Cyttarops alecto	1	N/A	N/A	17.42
Desmodus rotundus	1	N/A	N/A	21.20
Didelphis imperfecta	1	N/A	N/A	12.08
Eptesicus chiriquinus	20	0.48	1.08	4.27
Eptesicus furinalis	8	0.90	1.72	4.27
Furipterus horrens	2	0.31	0.31	20.69
Glossophaga soricina	8	1.39	2.81	17.89
Lasiurus atratus	1	N/A	N/A	15.82
Lasiurus blossevillii	1	N/A	N/A	15.82
Lasiurus egregius	4	0.46	0.92	18.07
Lonchophylla thomasi	12	0.25	0.61	18.21
Lophostoma schulzi	1	N/A	N/A	14.19
Lophostoma silvicolum	3	0.10	0.15	14.19
Macrophyllum macrophyllum	1	N/A	N/A	17.36
Marmosa murina	2	0.31	0.31	17.64
Marmosops parvidens	1	N/A	N/A	17.83
Metachirus nudicaudatus	1	N/A	N/A	17.64
Micronycteris hirsuta	1	N/A	N/A	16.56
Micronycteris megalotis	2	0	0	16.56
Mimon crenulatum	6	0.36	0.93	16.38
Molossops neglectus	1	N/A	N/A	16.03
Molossus molossus	7	0.66	1.08	1.70
Molossus rufus	12	0.77	1.55	1.70
Myotis albescens	1	N/A	N/A	10.59
Myotis nigricans	1	N/A	N/A	10.59
Neacomys guianae	4	0.38	0.77	14.63
Neacomys paracou	4	0.48	0.77	14.63
Oecomys auyantepui	4	0.90	1.08	14.78
Oecomys bicolor	2	0.15	0.15	14.78
Oryzomys megacephalus	3	0.52	0.78	15.03
*Peropteryx cf. macrotis**	1	N/A	N/A	18.51
Philander opossum	3	0.10	0.15	12.08
Phylloderma stenops	2	0.31	0.31	20.62
Phyllostomus elongatus	8	0.25	0.46	12.99
Phyllostomus hastatus	2	0.46	0.46	12.99
Platyrrhinus helleri	34	2.54	5.30†	11.89
Proechimys cuvieri	9	0.26	0.46	10.66
Proechimys guyannensis	13	0.46	1.08	10.66
Pteronotus gymnonotus	4	0.08	0.15	19.82
Pteronotus parnellii	24	2.66	5.34‡	18.20

Table 3 *Continued*

Species	No. of specimens	Mean distance within species, percentage	Maximum distance within species, percentage	Distance to nearest neighbour
Pteronotus personatus	25	0.55	4.66§	19.33
Rhinophylla pumilio	2	0.31	0.31	18.80
Rhipidomys nitela	1	N/A	N/A	18.89
Rhynchonycteris naso	9	0.69	1.58	18.62
Saccopteryx bilineata	43¶	0.40	1.40	13.94
Saccopteryx gymnura	1	N/A	N/A	15.85
Saccopteryx leptura	32	0.82	2.18	13.94
Sturnira lilium	9	0.77	2.02	9.73
Sturnira tildae	3	0.2	0.31	9.73
Thyroptera tricolor	4	0	0	21.17
Tonatia saurophila	7	0.12	0.31	16.38
Trachops cirrhosus	3	0	0	17.85
Trinycteris nicefori	1	N/A	N/A	18.7
Uroderma bilobatum	6	0.36	0.92	16.89
Vampyressa bidens	2	0.61	0.61	14.99
Vampyressa brocki	1	N/A	N/A	16.03
Vampyrodes caraccioli	4	0	0	11.89
Vampyrum spectrum	1	N/A	N/A	20.36

*Possible new species; †three distinct genetic lineages present within species; ‡two distinct genetic lineages present within species; §two distinct genetic lineages present within species; ¶37 barcodes recovered.

with collection of specimens and reference sequences. Taxonomic assignments are most reliable when molecular methods are used in conjunction with morphological identification. If specimens have to be released, it is important to retain images and measurements which may provide independent evidence in case of discrepancy; although it is obvious that this information will not help when taxa are externally indistinguishable. An additional long-term benefit of retaining tissue and DNA barcodes from specimens which are subsequently released is the possibility to re-assign identifications of individuals (and their ecological collaterals), following a taxonomic revision.

This study reinforces the utility of DNA barcoding as a tool for verifying taxonomic assignments for large series of specimens of small mammals and for highlighting cases requiring systematic attention. It also illustrates the potential of DNA barcodes in discovering cryptic diversity in morphologically uniform and taxonomically understudied mammal groups. However, barcodes alone cannot be used to define taxonomic units and the collection of voucher specimens adds scientific veracity when conducting surveys in areas with insufficiently studied biodiversity and when dealing with undescribed taxa. In these cases, it is also important to archive high-quality tissue samples (e.g. frozen at –80 °C) suitable for a broad spectrum of molecular studies, including, but not limited to DNA sequencing (Edwards *et al*. 2005; Walters & Hanner 2006).

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References

American Society of Mammalogists Animal Care and Use Committee (1998) Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy*, **79**, 1416–1431.

Baker RJ, Bradley RD (2006) Speciation in mammals and the genetic species concept. *Journal of Mammalogy*, **87**, 643–662.

Borissenko AV (1999) A mobile trap for capturing bats in flight. *Plecotus et al.*, **2**, 10–19.

Clare EL, Lim BK, Engstrom MD, Eger JL, Hebert PDN (2007) DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular Ecology Notes*, **7**, 184–190.

Dávalos LM (2006) The geography of diversification in the mormoopids (Chiroptera: Mormoopidae). *Biological Journal of the Linnean Society*, **88**, 101–118.

Edwards SV, Birks S, Brumfield RT, Hanner R (2005) Future of avian genetic resources collections: archives of evolutionary and environmental history. *Auk*, **122**, 979–984.

Hajibabaei M, deWaard JR, Ivanova NV *et al*. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **360**, 1959–1967.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences, USA*, **103**, 968–971.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, **23**, 167–172.

Hanner R, Corthals A, Dessauer HC (2005) Salvage of genetically valuable tissues following a freezer failure. *Molecular Phylogenetics and Evolution*, **34**, 452–455.

Hebert PDN, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, **54**, 852–859.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 313–321.

Hebert PDN, Ratnasingham S, deWaard JR (2003b) Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, S96–S99.

Hsiao KM, Lin HM, Pan H *et al*. (1999) Application of FTA sample collection and DNA purification system on the determination of CTG trinucleotide repeat size by PCR-based Southern blotting. *Journal of Clinical Laboratory Analysis*, **13**, 188–193.

Ivanova NV, deWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, **6**, 998–1002.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, **7**, 544–548.

Janzen DH, Hajibabaei M, Burns JM *et al*. (2005) Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, **360**, 1835–1845.

Kerr KCR, Stoeckle MY, Dove C *et al*. (2007) Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes*, **7**, 535–543.

Kilpatrick CW (2002) Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. *Biochemical Genetics*, **40**, 53–62.

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Lewis-Oritt N, Porter CA, Baker RJ (2001) Molecular systematics of the family mormoopidae (Chiroptera) based on cytochrome *b* and recombination activating gene 2 sequences. *Molecular Phylogenetics and Evolution*, **20**, 426–436.

Lim BK, Engstrom MD (2005) Mammals of Iwokrama Forest. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **154**, 71–108.

Livia L, Antoniella P, Hovirag L, Mauro N, Panara F (2006) A non-destructive, rapid, reliable and inexpensive method to sample, store and extract high-quality DNA from fish body mucus and buccal cells. *Molecular Ecology Notes*, **6**, 257–260.

Pfunder M, Holzgang O, Frey JE (2004) Development of microarray-based diagnostics of voles and shrews for use in biodiversity monitoring studies, and evaluation of mitochondrial cytochrome oxidase I vs. cytochrome *b* as genetic markers. *Molecular Ecology*, **13**, 1277–1286.

Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life data system www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355–364.

Smith LM, Burgoyne LA (2004) Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. *BMC Ecology*, **4**, 4.

Smith MA, Fisher BL, Hebert PDN (2005) DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **360**, 1825–1834.

Vanek D, Hradil R, Budowle B (2001) Czech population data on 10 short tandem repeat loci of SGM Plus STR system kit using DNA purified in FTA cards. *Forensic Science International*, **119**, 107–108.

Velazco PM (2005) Systematics and phylogenetic relationships of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera, Phyllostomidae). *Fieldiana: Zoology*, **105**, 1–53.

Walters C, Hanner R (2006) Platforms for DNA banking. In: *Issues in Genetic Resources DNA Banks — Providing Novel Options for Genebanks?* (eds Vicente Cd, Andersson MS), pp. 25–35. International Plant Genetic Resources Institute, Rome, Italy.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **360**, 1847–1857.

Wilson DE, Reeder DM (2005) *Mammal Species of the World: A Taxonomic and Geographyraphic Reference*, 3rd edn. Johns Hopkins University Press, Baltimore.