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Research Article

Primer design for non-invasive genetic identification of West African threatened primates

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Abstract

The Guinean Forests are renowned for their primate diversity, with nearly 30 distinct species. These forests have been identified as some of Africa's most critical primate conservation areas. However, intensive poaching pressure and habitat degradation cause severe threats to these species. The remaining primate populations have low population densities, have secretive habitats, and are wary of humans. This can make field studies and direct observation difficult. To develop a reliable, sensitive and simple molecular identification method using Polymerase Chain Reaction (PCR), we sequenced the mitochondrial D-loop Hypervariable 1 region (HVR1) of Diana monkey (*Cercopithecus diana*) and Campbell monkey (*Cercopithecus campbelli*). From these sequences and their orthologs within Eastern black-and-white colobus (*Colobus guereza*) obtained from GenBank, we designed species-specific primers to amplify fragments of the mitochondrial D-loop HVR1 gene from faecal samples. These primers allow us to differentiate between Diana monkey (*C. diana*), Campbell monkey (*C. campbelli*), lesser spot-nosed monkey (*Cercopithecus petaurista*), Red colobus (*Piliocolobus badius*), Olive colobus (*Procolobus verus*), King colobus (*C. polykomos*), and Geoffroy's black-and-white colobus (*C. vellerosus*), demonstrating their potential for the identification of West African threatened primates.

Key words: Species-specific primer, mitochondrial D-loop HVR1, primates, West Africa

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Introduction

Because of their high level of species diversity and endemism, forest ecosystems from Guinea to Nigeria have been recognized as one of the world's 25 most threatened ecosystems [1]. The Guinean Forests are renowned for their primate diversity, with nearly 30 distinct species. These forests have been identified as some of Africa's most critical primate conservation areas.

With 22 taxa, among them 18 catarrhines, Côte d'Ivoire holds the second highest primate diversity in West Africa, after Nigeria [2]. Three of these taxa are endemic to Côte d'Ivoire and neighbouring Ghana: Miss Waldron's red colobus (*Procolobus badius waldroni*), Roloway monkey (*Cercopithecus diana roloway*) and white-naped mangabey (*Cercocebus atys lunulatus*). The major threats to primates in Côte d'Ivoire are intensive habitat degradation and poaching [2]. The situation became critical with the decline of conservation policy over the last decade because of political and social instability in the country.

This situation increases the threat to wildlife, and particularly primates, as they are particularly vulnerable to local extinction in fragmented landscapes [3, 4]. Subject to intensive human pressure, remaining primate populations have low population densities, have secretive habitats, and are wary of humans [5]. This can make field studies and direct observation difficult [6]. Practical, reliable and cost-effective indirect methods to detect primates are therefore valuable tools for ecology and conservation studies [7, 8]. Non-invasive sampling is a potentially cost-effective and efficient means of monitoring various mammal populations over large spatial scales, as has been demonstrated for a number of species [9, 10]. However, in many cases it may be impossible to distinguish between species, especially among closely related taxa. Genetic identification of mammals from non-invasive samples is a potentially useful monitoring technique for species that are difficult to survey using other methods [11].

Faeces are abundant and probably the most collectable animal product in the wild, and may be acquired without disturbance to the study animals [12]. This can be particularly important when the focal species of the study occurs in sympatry with related taxa [13]. Analyses based on faecal DNA have been applied to a broad range of taxa to address questions from occupancy and food habits to abundances, species distribution, and habitat use [14-19, 13].

Although the feasibility of employing non-invasive DNA for molecular studies has been well documented in temperate areas, few researchers have used these techniques in tropical forests [20-24]. Samples obtained in the tropics are often exposed to high humidity, warm temperatures, frequent rain, and intense sunlight, all of which can rapidly degrade DNA [25]. Moreover, insect communities that degrade faecal samples can also play an important role in scat removal in tropical areas [26, 27].

In this study, we evaluated the feasibility of performing species-level identification of West African primates from Côte d'Ivoire forest fragments, using species-specific primers (a strand of nucleic acid that serves as a starting point for DNA synthesis) to amplify a mitochondrial DNA fragment obtained from faecal samples collected in the wild.

Methods

Sampling

Faecal samples of seven primate species were collected from January 2001 to February 2001 in Taï National Park and in three forest groves (Guetitapia, Soko and Dinaoudi) (Fig. 1). These species and their respective sample size are: Campbell monkey (*Cercopithecus campbelli*) (16), lesser spot-nosed monkey (*Cercopithecus petaurista*) (12),

Diana monkey (*Cercopithecus diana*) (7), King colobus (*Colobus polykomos*) (9), Geoffroy's black-and-white colobus (*Colobus vellerosus*) (11), Red colobus (*Piliocolobus badius*) (13), Olive colobus (*Procolobus verus*) (5). Samples of approximately 0.5–2 g were placed in individual vials (50 mL size) containing silica gel beads, and stored at room temperature.

Blood samples were only collected from one individual of Campbell monkey (*C. campbelli*) and another individual of Diana monkey (*C. diana*) kept as a pet in Abidjan zoo. Laboratory work was conducted in Max Planck Institute in Leipzig (Germany) from February 2001 to August 2001.

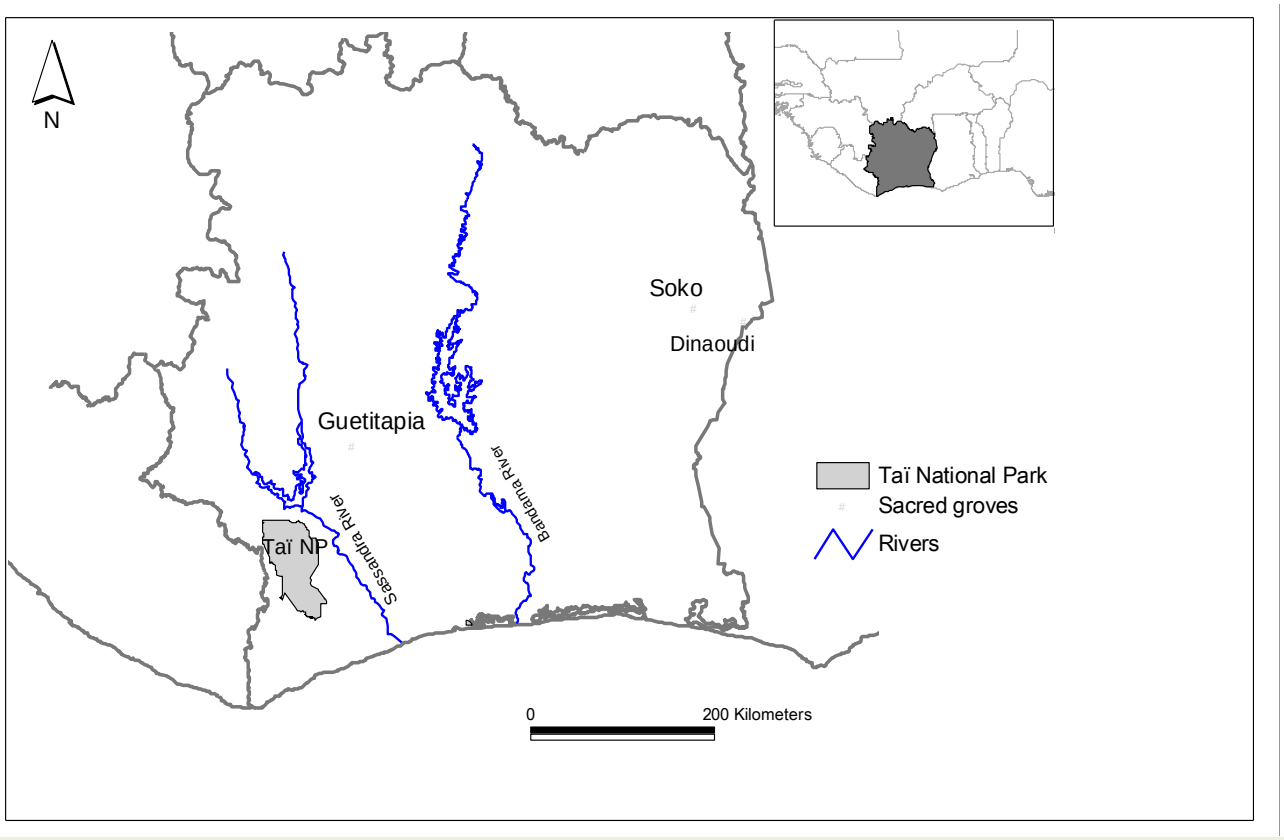


Fig. 1: Location of Faeces sampling sites (Taï NP, Guetitapia, Soko and Dinaoudi Sacred Groves) in Côte d'Ivoire. The Sassandra and Bandaman Rivers delimitating the potential hybridization zone are indicated.

DNA extraction

DNA was extracted from blood samples using Qiamp tissue/blood kits and from faecal samples using QIAamp DNA Stool Kit (Qiagen, Germany) following the manufacturer's instructions. In order to optimize the amount of DNA in faecal samples and reduce the time of extraction, 100mg faecal samples were rehydrated for 2 hours at 4°C followed by rehydratation overnight instead of 3 days of rehydratation as indicated by the manufacturers. Final volume of DNA extract was 200 µl for each sample. Contamination was monitored by including two extraction blanks in every extraction round.

Generation of complete D-loop Hypervariable region 1 (HVR1) sequences

Given the pervasiveness of insertions of mammalian mitochondrial DNA segments into the nuclear genome [27], including primates [28], we performed Polymerase Chain Reaction (PCR) amplifications (production of multiple copies) of the D-loop HVR1 sequence using the Universal primer pair L15996-H16498 designed for conserved regions identified from human sequences [29]. We amplified the partial HVR1 sequence (513 bp) derived from DNA extracted from blood samples of Campbell monkey (*Cercopithecus campbelli*) and Diana monkey (*C. diana*). Furthermore, since it is highly unlikely that both forward primer and reverse primer would fail to amplify the authentic target, sequence data that were consistent and unambiguous could be assumed to represent the mitochondrial DNA.

Amplifications were prepared in 20 µl reactions with 1x PCR Buffer, 2 mM $MgCl_2$, 0.25 mM of dNTP mix, 0.2 µM of each primer, 0.4 U of Gold Taq DNA Polymerase, 2 µl of genomic DNA and 16 µg bovine serum albumin (BSA). Reactions were carried out in a Peltier thermal cycler, PTC 200 (MJ Research) and thermocycling parameters consisted of an initial denaturation at 95°C for 3 min, followed by 45 cycles of 30 s at 95°C, 30 s at 50°C and 30 sec at 72°C. Blank controls were used alongside all PCR reactions. Amplification products were run in 2.8% agarose Seakam/TBE (Tris borate 0.04 M ; EDTA 0.001 M) gels containing 0.5 µg/ml ethidium bromide at 80 V during 30 to 60 minutes. PCR products showing a single band of the expected size were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using the BigDye Terminator V3.1 Cycle Sequencing Kit and a 3100 Genetic Analyser (Applied Biosystems).

To monitor for contaminations, all PCR reactions included a negative and a positive PCR control (DNA from chimpanzee), as well as the negative control from the DNA extraction.

Design and test of species-specific D-loop HVR1

Species-specific primers, amplifying a 234 to 367 bp fragment, were designed from the complete D-loop HVR1 nucleotide sequences of Diana monkey (*Cercopithecus diana*), Campbell monkey (*Cercopithecus campbelli*) and Eastern black-and-white colobus (*Colobus guereza*) using Primer Express Software version 2.0 (Applied Biosystems). The complete D-loop HVR1 nucleotide sequences of Eastern black-and-white colobus (*C. guereza*) were obtained from German Primate Center and are now accessible from GenBank accession number AY863427. Four primer pairs were respectively designed within Campbell monkey (*C. campbelli*) (MonF2/MonR2), Diana monkey (*C. diana* (DiaF2/DiaR2), King colobus (*Colobus polykomos*) / Geoffroy's black-and-white colobus (*Colobus vellerosus*) (L15996/PolyR1) and Geoffroy's black-and-white colobus (*C. vellerosus*) (PolyF2/PolyR2). To confirm the robustness of the primer sets for species identification, they were tested for amplification by screening four individuals or amplicons of each species. We used primer pairs of amelogenin (a protein of dental enamel) gene (amplifying a fragment length of 100 bp) [30] as a positive control to ensure that the DNA sample is sufficient quality for PCR. All the samples used have positive PCR amplification success with primer pairs of amelogenin gene.

PCRs were performed in 20 µl reactions with 1x PCR Buffer (Qiagen), 2 mM $MgCl_2$, 0.2 µM of each primer, 0.25 mM of dNTP mix, 0.4 U of Gold Taq DNA Polymerase (Qiagen), 16 µg bovine serum albumin (BSA) and 2 µL of DNA extract. Thermal cycling conditions for all the designed primer pairs were an initial denaturation at 95°C for 3 min, followed by 45 cycles of denaturation-annealing-extension. The final extension was 7 min at 72°C and 4°C storage in a Peltier thermal cycler, PTC 200 (MJ Research). Denaturation-annealing-extension times and temperature depending on each primer pairs are indicated (Table 1).

Table 1: Information of primers designed to amplify a species-specific fragment along mitochondrial D-loop HVR1 for Campbell monkey (*Cercopithecus campbelli*), Diana monkey (*Cercopithecus diana*), Geoffroy's black-and-white colobus (*Colobus vellerosus*) and King colobus (*Colobus polykomos*) in Côte d'Ivoire.

Primer name	Nucleotide sequences (5' – 3')	Td-Ta-Te °C	Fragment length (bp)	Target species
MonF2 MonR2	Forward GTACTATATATGCTTAACCGTACAT Reverse GAGAGTAACACTGCTTGTGC	30 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C	367	*Cercopithecus campbelli*
L15996 PolyR1	Forward CTCCACCATTAGCACCCAAAGC Reverse CTTGTAAGCATGTTTTGGACAATA	30 sec at 95°C, 30 sec at 53°C, 30 sec at 72°C	(234)	*Colobus Polykomos* *Colobus vellerosus*
PolyF2 PolyR2	Forward ACTATAAATGGTTCACTGTACAT Reverse ATTTCACGGAGGATGCTG	30 sec at 95°C, 30 sec at 49°C, 30 sec at 72°C	350	*Colobus vellerosus*
DiaF2 DiaR2	Forward GACTGTGCATAATACATATCATTG Reverse GAGGAGAGTAGCACTCTTGTG	30 sec at 95°C, 30 sec at 50°C, 30 sec at 72°C	353	*Cercopithecus diana*

Td : denaturation temperature ; Ta : anhealing temperature ; Te: extension temperature; Size: the size of the species-specific fragment amplified,

Successful PCR products were purified using QIAquick PCR purification kit (Qiagen) according to the protocol of the manufacturer, and cycle sequenced [31] in both directions using the same set of primers as for the amplification to confirm the sequence. PCR product sequencing was conducted using the BigDye Terminator V3.1 Cycle Sequencing Kit and a 3100 Genetic Analyser (Applied Biosystems).

Sequence results for each primer pair were compared with relevant sequences of Cercopithecine and Colobine available in GenBank database to check their consistency. Molecular Phylogenetic anaylsis was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model [32] in MEGA5 [33].

Results

The two analysed blood samples of Diana monkey (*C. diana*) and Campbell monkey (*C. campbelli*) amplified with primer pair L15996/H16496 were the same size (approximatively 500 bp) (Fig. 2).

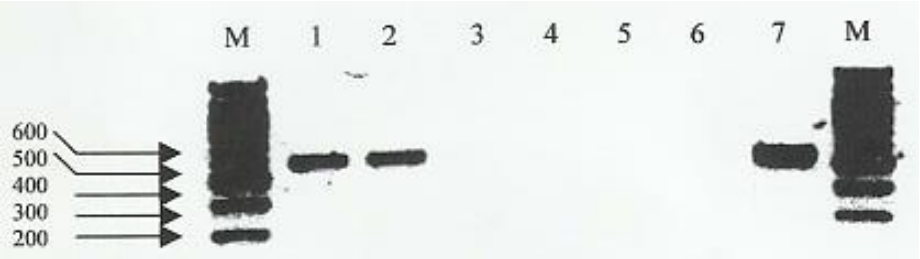


Fig. 2: Photograph of 2.8% agarose Seakam showing PCR products of three primate species. Lane 1-7 represent 600 bp amplicons obtained using primers L15996/H16496. M: size standard (100 bp DNA ladder); Lane 1: Campbell monkey (*Cercopithecus campbelli*); Lane 2: Diana monkey (*Cercopithecus diana*); Lane 7: positive control - Chimpanzee (*Pan troglodytes*); Line 3-6: negative controls.

The partial mitochondrial D-loop HVR1 sequences produced for Diana monkey (*Cercopithecus diana*) and Campbell monkey (*Cercopithecus campbelli*) were aligned with orthologous sequences from the same species in GenBank. Each partial sequence, obtained with primer pair L15996/H16498, was compared with the GenBank database. With the sequence produced for Campbell monkey (*C. campbelli*), the most similar sequences were a Crab-eating Macaque (*Macaca fascicularis*) mitochondrial D-loop sequence (Total Score: 438 ; Query cover: 99% ; Expected Value: 1e-116 ; Identity: 83%) and Diana monkey (*C. diana*) mitochondrial D-loop sequence (Total Score: 396 ; Query cover: 97% ; Expected Value: 1e-106 ; Identity: 82%). With the sequence produced for Diana monkey (*C. diana*), the most similar sequence was a Diana monkey (*C. diana*) mitochondrial sequence (Total Score: 688 ; Query cover: 92% ; Expected Value: 0.0 ; Identity: 93%).

The positions of the designed primers on the respective partial D-loop HVR1 sequences of Campbell monkey (*C. campbelli*), Diana monkey (*C. diana*) and Black colobus (*Colobus guereza*) are indicated (Appendix 1).

An absence of multiple bands in PCR reactions, the overall consistency of the sequence data between obtained and published sequences, and the position of the obtained sequences in a phylogenetic tree along with relevant sequences of colobine and cercopithecine available in Genbank database (Fig. 3), all indicated that our data are free of nuclear pseudogenes.

Fig. 4 shows PCR amplification with each species-specific primer pair in which both target and non-target samples have been included.

Amplification success for noninvasive samples

Table 1 lists the primer sets that were designed in the present study, their sequences, and the expected size of PCR amplicons. Each primer set was species specific, only amplifying DNA of the target species when tested on four individuals or amplicons of each different primate species, except for primers L15996/PolyR1.

The primer pair designated for Campbell monkey (*Cercopithecus campbelli*) (MonF2/MonR2) provided positive sensitivity for this species (Fig.4a). The one designed for Diana monkey (*C. diana*) (DiaF2/DiaR2) is also specific to this species (Fig.4b).

Within the group of Black-and-white colobus monkeys (*C. polykomos*, *C. vellerosus*) two primer pairs were designed: L15996/PolyR1 and PolyF2/PolyR2. While the primer pair PolyF2/PolyR2 is specific to Geoffroy's black-and-white colobus (*C. vellerosus*) (Fig.4c), the primer pair L15996/PolyR1 provided positive results for King colobus (*C. polykomos*) and Geoffroy's black-and-white colobus (*C. vellerosus*) (Table 1, Fig.4d).

Of the 52 faecal samples analysed, 7.69% (4/52) showed amplifiable DNA for primers L15996/H16498.

Within Campbell monkey (*C. campbelli*), 81.25% (13/16) of the faecal samples analysed provided amplifiable DNA for primers MonF2/MonR2; 75% (3/4) faecal samples of Diana monkey (*C. diana*) amplified for primers DiaF2/DiaR2; 100% (10/10) faecal sample of Geoffroy's black-and-white colobus (*C. vellerosus*) amplified for primers PolyF2/PolyR2 and 50% (4/8) faecal sample of King colobus (*C. polykomos*) amplified for primers L15996/PolyR1.

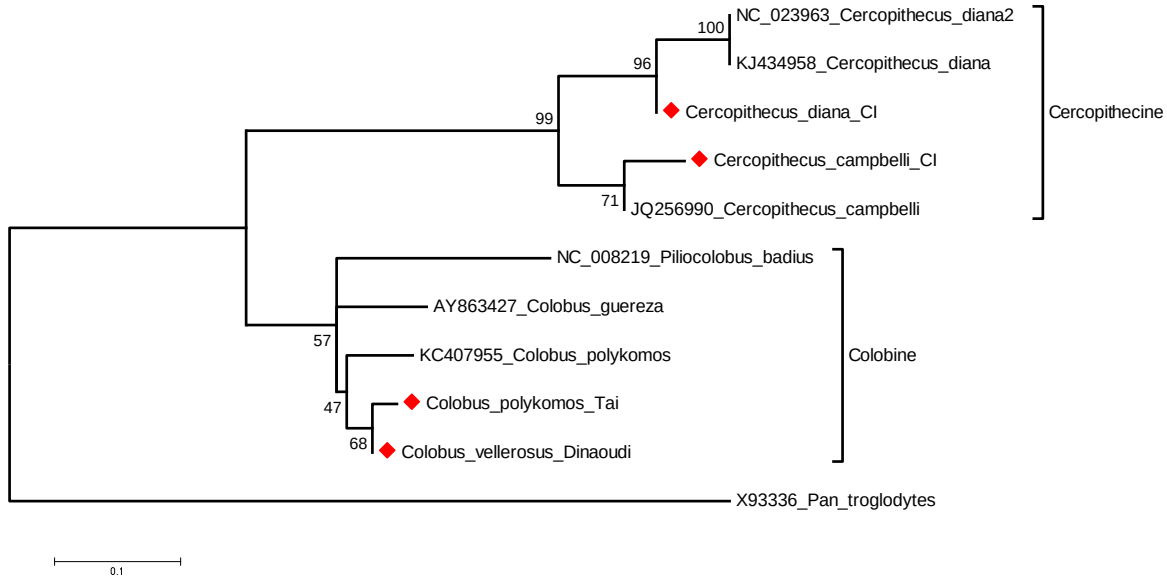


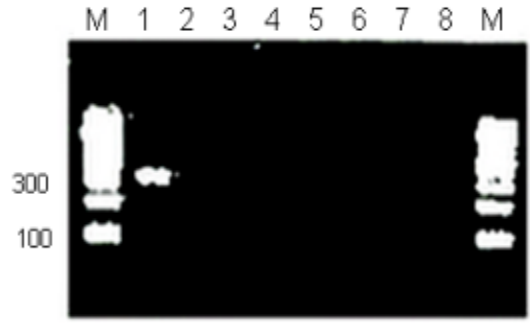
Fig. 3: Maximum Likelihood phylogenetic tree based on the Hasegawa-Kishino-Yano model [32] inferred from 11 nucleotide sequences of a portion of mitochondrial HVRI region. Sequences generated by our data are labeled in red and those obtained from GenBank are labelled with their GenBank accession number. Percent bootstrap values obtained from 500 replicates are indicated on the branches.

Discussion

The identification tools for the primate species described in this study can be used to rapidly, cheaply, and reliably assign individuals to species, thus facilitating basic biological research.

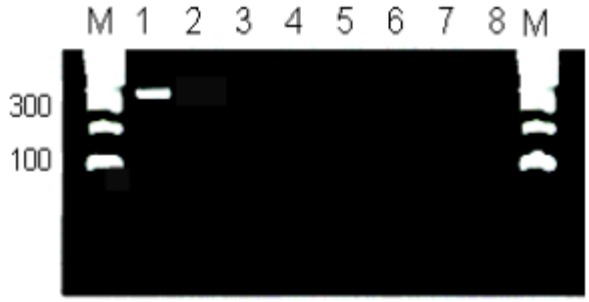
Of the four primer pairs designed, three are respectively specific to Campbell monkey (*Cercopithecus campbelli*), Diana monkey (*Cercopithecus diana*), and Geoffroy's black-and-white colobus (*Colobus vellerosus*), and another one is specific to Geoffroy's black-and-white colobus (*Colobus vellerosus*) and King colobus (*Colobus polykomos*). The fact that one of the primers could not distinguish between Geoffroy's black-and-white colobus (*C. vellerosus*) and King colobus (*C. polykomos*) suggests that both the taxa share major haplotypes as a result of possible hybridization. Geoffroy's black-and-white colobus (*C. vellerosus*) and King colobus (*C. polykomos*) share a contact zone at the interfluvial region extending between the Sassandra and Bandama rivers. Based on vocalization data and pelage feature, Gonedelé Bi et al. [34] reported a possible exchange of gene pools between Geoffroy's black-and-white colobus (*C. vellerosus*) and King colobus (*C. polykomos*) at the contact zone. Indeed the hybridization hypothesis between *C. polykomos* and *C. vellerosus* at the Sassandra - Bandama interfluvial region has been supported by Groves et al. [35]. This region has also been defined as a hybridization zone for several mammal taxa [36].

In primates, hybridization at contact zones occurs frequently among a number of closely related taxa, including members of both New and Old World monkeys as well as members of the clade containing apes and humans, and likely played an important role in structuring diversity in the primate order [37]. Such contact zones have been particularly well-documented for gibbons [38, 39], baboons [40-43], and macaques [44-49].



a) PCR products using primer pair MonF2/MonR2. Lane 1: *Cercopithecus campbelli* ; Lane 2: *Cercopithecus diana* ; lane 3 : *Cercopithecus petaurista* ; Lane 4 : *Colobus vellerosus*; Lane 5 : *Colobus polykomos* ; Lane 6 : *Piliocolobus badius*; Lane 7 ; *Procolobus verus* ; Lane 8 : blank sample

b) PCR products using primer pair DiaF2/DiaR2. Lane 1: *Cercopithecus diana* ; Lane 2: *Cercopithecus campbelli* ; lane 3 : *Cercopithecus petaurista* ; Lane 4 : *Colobus vellerosus*; Lane 5 : *Colobus polykomos* ; Lane 6 : *Piliocolobus badius*; Lane 7 ; *Procolobus verus* ; Lane 8 : blank sample



c) PCR products using primer pair PolyF2/PolyR2. Lane 1: *Colobus vellerosus*; Lane 2: *Colobus polykomos* ; lane 3 : *Piliocolobus badius* ; Lane 4 : *Procolobus verus* ; Lane 5 : *Cercopithecus campbelli* ; Lane 6 : *Cercopithecus diana* ; Lane 7 ; *Cercopithecus petaurista* ; Lane 8 : blank sample

d)PCR productsusing primer pair L15996/PolyR1. Lane 1: *Colobus vellerosus*; Lane 2: *Colobus polykomos* ; lane 3 : *Piliocolobus badius* ; Lane 4 : *Procolobus verus* ; Lane 5 : *Cercopithecus campbelli* ; Lane 6 : *Cercopithecus diana* ; Lane 7 ; *Cercopithecus petaurista* ; Lane 8 : blank sample

Fig. 4: Photograph of 2.8% agarose Seakam showing PCR products of Campbell monkey (*Cercopithecus campbelli*), Diana monkey (*Cercopithecus diana*), lesser spot-nosed monkey (*Cercopithecus petaurista*), Geoffroy's black-and-white colobus (*Colobus vellerosus*), King colobus (*Colobus polykomos*), Red colobus (*Piliocolobus badius*) and Olive colobus (*Procolobus verus*). Lane 1-8 represent amplicons obtained using species-specific primers. M: size standard (100 bp DNA ladder) .

Among the seven primate species analyzed, three (*C. diana, C. polykomos and Colobus vellerosus*) are listed as vulnerable on the IUCN red list. During the reconnaissance survey conducted throughout the protected areas in Côte d'Ivoire, Gonedelé Bi *et al*. [50] failed to observe these species in several of the forests where they were previously reported [51-54]. At the local level, based on presence/absence data, we reported that Geoffroy's black-and-white colobus (*Colobus vellerosus*) is critically endangered, whereas Diana monkey (*C. diana*), and King colobus (*C. polykomos*) are endangered due to the decline of their population size, their extent of occurrence and area of occupancy [50]. Since the presence/absence data are especially susceptible to erroneous or 'false' absences due to an animal's ability to disperse or hide during field surveys [55, 56], our molecular based methods could overcome these difficulties. Indeed several studies have indicated that hunting pressure affects the detectability of primate populations [6].

The four primer pairs designed within four species of West African primates prove to be a reliable species identification tool based on field-collected faeces samples.

The fragments amplified by these primers are longer than the positive control fragment amplified (100 bp). Since amplification success drastically decreased with increasing amplicon length [57], a lack of PCR product does not mean the sample is not the target species, as the lack of product could be PCR failure due to poor quality DNA. But a positive result (PCR amplification success) does mean the sample is target species DNA.

Development of DNA-based technologies to identify species has been the goal of many conservation, wildlife, and forensic biologists. In non-invasive studies, repeated PCR analyses and continuous monitoring of data quality are essential to ensure accurate data [7, 58]; therefore, it is crucial to reduce the time and cost involved in each step of data production. In this context, our designed primers can be an easy and quick approach that can be considerably useful, especially in studies of West African primates. Thus, for long-term monitoring programs, this method can be both faster and cheaper than sequencing, while much more straightforward than possible RFLP tests.

Implication for conservation

Whereas several species molecular identification tools have been designed for West African chimpanzee [8, 59], very few if any species identification tools are available for West African Catharine primates. These species are facing severe threats from intensive poaching and forest encroachment in the absence of strong protective actions [60]. The species that have survived in these habitats are now cryptic and difficult to observe. Molecular techniques represent reliable identification tools to detect such species and can help to monitor endangered primate species in West Africa.

Our method is a simple and efficient means to identify species, eliminating the need for sequencing, which is costly and requires more laboratory equipment. It allows for rapid and noninvasive assessment of primate species and is particularly useful for surveying species throughout their range of occurrence.

The species-specific PCR primer sets designed by this study are successful in amplifying DNA extracted from fecal samples of target species among seven primate species. The success of these primers can help to monitor West African primates where these species became cryptic and shy due to intensive hunting pressure and habitat deterioration. These primers could aid studies involving species identification, with implications for the conservation of West African primates.

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Appendix 1: Mitochondrial D-loop HVR1 sequences of Diana monkey (*Cercopithecus diana*), Campbell monkey (*Cercopithecus campbelli*) sequenced using primers L15996/H16496 and Eastern black-and-white colobus (*Colobus guereza*) sequence accessible from GenBank (GenBank accession number AY863427). Positions of species-specific primers designed in this study are shaded

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C_diana     CTCCACCATTAGCACCCAAAGCTGGCATTCTAGTTTAAACTACTTTCTGTATTTTAT--GTTGTACAAGCTCTGCAGTACAATTTAAACACTACCC----
C. guereza ..T....GCC.AT.......A..........TC.........C........C...AG.GG.G.A.GAACTCAA..A....CC...GT....A.TTAAT
C_campbell ..........................................................--......T..A.C..A........CCC..GT..C....TCAT

C_diana     ---------CACTATGTAATTCGTGCATTACTGCTAGCCAACATGAATAATATATAGTACTATAAGTGCTTGACTGTGCATAATACATATCATTGAACAT
AY863427_C CTCCCATGC.CT.....................................T...............A..G..C..C..A....A.....AT....C.TT.
C_campbell TACC--TGC.....................................................TA.....A..C..A.....................AC.T.C

C_diana     TTGCCCAGAAAATCCTTGGAAAGCATGCTTATAAGCAAGAACT-CTGATCGCA-AACCAAACTATGTCACATCACCTGCCCCGCTCCAAAGTCCATGGTA
C. guereza C.A..-.TT.....G.C-C...A.....C.......G.AC--.T.CT.GGT.TA.........AAT....A...--..T.T.....C.AGA....--.
C_campbell C.A.T-.TC..TCT...AC.GTA..A...........G.G.T.AT.TC...A.C..A.C....G.AAT.....T.A..T...CT......AG.GCAT...

C_diana     TTTCCACTTCAAATATCAACCGAAC-AGATCTATGTTAACCGTACAGTAGTACATTAAGTCGTTCATCGGACATAGTACAT-------------------
C. guereza ..A..C.C.GG.........A....T...AACTCAGCCGAA.....-..AA....C..A..C..TG..........C....CTAAGTCAAATCCTCCCTC
C_campbell C...AC.A...G.............-.A.CACT..CA.CT.....................................-------------------

C_diana     --------------ACCTAT-TAGAAACTTTCCTTTTCACTACGGATGACCCCCCTCACTTAGGAATCCCTTACTCACCATCCTCCGTGAAATCAGTATC
C. guereza GCAGTACATAGCAC.T...AG.CA..T.C.C...CG...AC......-.T.G......A..G..TG......GT...A.................A....
C_campbell --------------.T....-..-....C.....CC....C.......C.........G.............GT...................A....

C_diana     CCGCACAAG-AGTGCTACTCTCCTCGCTCCGGGGCCCATAATTCGTCATAATTCGTGGGGGTAGCT---------------------------------
C. guereza .........-.................A.....-.......C....GGGGG.AGC.ATAAACGA.ATCTAAAGGACATCTGGTTCTTACCTCACGGCCAT
C_campbell ..........C....T.......-----------.....-.......CC.-----------------------------------------------------
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