Non-invasive genetic identification confirms the presence of the Endangered okapi *Okapia johnstoni* south-west of the Congo River

DAVID W. G. STANTON, JOHN HART, ASHLEY VOSPER, NOËLLE F. KÜMPEL, JINLIANG WANG
JOHN G. EWEN and MICHAEL W. BRUFORD

SUPPLEMENTARY MATERIAL 1 Details of OJ1 and OJ2 primer amplification and species identification.

One hundred and twenty-five samples amplified at least one band. All three samples from region 1 amplified both OJ1 and OJ2. From region 2, three samples amplified OJ1 only, four amplified a band in OJ2 only, and the remaining 94 amplified both OJ1 and OJ2. All three working samples amplified OJ1 and OJ2 in region 3. From region 4 six of the samples amplified OJ1 only and the remaining six amplified both OJ1 and OJ2. All fragments from regions 1, 3 and 4 were sequenced. From region 2, 41 samples that amplified both OJ1 and OJ2 were selected for sequencing, as well as the seven that could only be amplified using one primer. All samples from regions 1, 2 and 3 aligned with okapi *Okapia johnstoni* when using Genbank (BLAST). In region 4, all six samples where a band was amplified in OJ1 but not in OJ2 aligned with bongo, whereas the five where a band was amplified in both OJ1 and OJ2 aligned with okapi.

Table S1 PCR reagents for the mtDNA PCR. PCR was carried out in a total volume of 25 µl. PCR for primers OJ1 and OJ2 was carried out in separate reactions.

PCR reagent	Final concentration/ quantity	Supplier
Bovine serum albumin	4 µg	New England Biolabs (Ipswich, USA)
PCR buffer	1x	Invitrogen (Merelbeke, Belgium)
$MgCl_2$	2.5 mM	Invitrogen (Merelbeke, Belgium)
dNTPs	0.2 mM	Invitrogen (Merelbeke, Belgium)
(each) Primer	0.5 µM	Sigma (Gillingham, UK)
GoTaq	1U	Invitrogen (Merelbeke, Belgium)
DNA	2 µl	

Table S2 PCR conditions for the mtDNA PCR. PCR was carried out in a total volume of 25 µl.

Temperature (°C)	Time
94°C	3 minutes
94°C	30 seconds*
58°C	35 seconds*
72°C	45 seconds*
72°C	5 minutes

*60 iterations

Table S3 DNA alignment of the priming site (shaded grey) and 29 bp of flanking sequence of the OJ2-reverse primer. The alignment contains five okapi haplotypes from individuals sampled in this study, and two bongo haplotypes downloaded from GenBank. Insertions/deletions are denoted by a hyphen, and bases identical to the consensus (shown in bold at the top) are denoted by a full-stop. This contig shows 11 and 12 polymorphisms, including five indels between the consensus okapi priming site and each of the two bongo haplotype priming sites.

OJ_1	**A**	**C**	**T**	**-**	**C**	**C**	**G**	**C**	**A**	**C**	**C**	**C**	**C**	**A**	**C**	**A**	**G**	**C**	**C**	**T**	**T**	**-**	**-**	**-**	**A**	**A**	**C**	**G**	**-**	**C**	**A**	**G**	**C**	**A**	**A**	**A**	**C**	**T**	**A**	**A**	**A**	**C**	T	**T**	**A**	**-**	**C**	**A**	**C**	**A**	**T**
BONGO1	.	.	.	A	A	T	T	A	.	.	.	A	.	.	.	T	.	G	T	G	.	G	G	C	.	.	G	.	A	.	.	-	T	.	.	T	A	.	G	T	.	.	A	.	.	G	T	.	.	.	.
BONGO2	.	.	.	A	A	T	T	A	.	.	.	A	.	.	.	T	A	G	T	G	.	G	G	C	.	.	G	.	A	.	.	-	T	.	.	T	A	.	G	T	.	.	A	.	.	G	T	.	.	.	.
OJ_2	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
OJ_3	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	-	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	C
OJ_4	.	.	.	-	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
OJ_5	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	G	.	.	.